" A STUDY OF SEX-LINKED POLYMORPHISM IN LABORATORY POPULATIONS OF DROSOPHILA MELANOGASTER. "

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SUMMARY.

Twelve laboratory populations of Drosophila melanogaster were maintained under constant environmental conditions for over a year, each population containing two of a series of pseudo-allelic sex-linked mutants at the white locus in an inbred background genotype. Regular sampling of adult flies from the cages allowed gene frequency changes to be followed, and tests were made of the hypotheses that random mating and no gametic selection occurred within the populations.

Several of the populations appeared to achieve a stable equilibrium for the sex-linked pseudoalleles, and attempts were made to determine the relative selective values responsible for these equilibria. Two methods of analysis previously developed for data from population cages containing autosomal alleles in competition were modified for application to a sex-linked system. The relative selective values were also calculated directly from the equilibrium gene and genotype frequencies. The analyses yielded conflicting results as to the values of the equilibrium gene frequencies, the magnitude of the relative selective values, and the stability of the established equilibria. Possible reasons for these contradictions are discussed, and criteria suggested for an ideal method of analysis.

Significant heterogeneity was found in all populations

for the sex ratio of different samples from the same cage. The deviations in sex ratio occurred in both directions, and showed no relationship to gene frequency changes in the populations. Further experimental work to elucidate the causes of this phenomenon is suggested.

STATEMENT.

This thesis contains no material submitted for any other degree in any University. To the best of my knowledge, due credit has been given in the text for the authorship of all material published previously by some other person.

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1. INTRODUCTION.

For many years there have been studies made of both natural and laboratory populations which show apparently stable polymorphisms. These have ranged from the polymorphisms found in man for various blood groups and haemoglobin characteristics to the chromosomal inversion polymorphisms in many Drosophila species and to more complex situations such as are found in snail and grasshopper colour patterns. Most of these are controlled by genes exhibiting autosomal inheritance, although some authors have reported appreciable frequencies of inversions of the X chromosome in natural populations of a few Drosophila species. Little attention, however, has been given to the problem of polymorphisms for sex-linked allelic systems, and the conditions for the existence of such polymorphisms have only recently received consideration. The selective forces required to maintain such a system have been shown to be subject to different restrictions from those required for the maintenance of an autosomal polymorphism, allowing species with a chromosomal determination of sex to exhibit polymorphisms with more than one possible system of controlling selective forces.

It seemed that a study of laboratory populations containing two sex-linked alleles might determine whether a selectively balanced sex-linked polymorphism was possible

under artificial conditions. Drosophila melanogaster was chosen as a convenient species, as it has a short average generation time and large populations can readily be maintained. Estimations of the relative selective values of the different genotypes in the populations would allow deductions to be made as to the possible existence of stable balanced polymorphisms. If such were found comparisons could then be made between the selective forces responsible for these polymorphisms and those maintaining polymorphisms for autosomally inherited characters.

2. REVIEW OF LITERATURE.

(a) THEORETICAL.

The necessary and sufficient conditions for the existence of a stable polymorphism for a pair of alleles at an autosomal locus were established many years ago (Fisher, 1922). More recently attention has been given to the problem of a stable polymorphism with multiple alleles at an autosomal locus (Owen, 1954; Kimura, 1956; Penrose, Smith, and Sprott, 1956; Mandel, 1959(a)). The simplest case of only two alleles at a locus involves a "principle of heterozygosity", (Mandel) by which the heterozygous genotype is required to have a greater fitness than either of the homozygotes for a stable polymorphism to occur. With a greater number of alleles at an autosomal locus the same "principle" is expressed by stating that each homozygote has a viability less than the average viability of the zygotic population, but this is a sufficient as well as a necessary condition for stability in certain special cases only. A situation of some interest has also been examined by Owen (1953), who showed that if the genotypes have differing selective values in the two sexes more than one stable equilibrium can result.

The investigation of stable polymorphism for alleles at a sex-linked locus has only recently been undertaken, the

first paper being by Bennett (1957). He considered the relation between the gene frequencies in both sexes of two sex-linked alleles A and a, and the relative selective values required to maintain a polymorphism. The relative selective values in the homogametic sex (taken as female) were defined for the genotypes AA, Aa, and aa as S_{AA}: 1: S_{aa}, and in the heterogametic sex (taken as male) for the genotypes A and a as t_A: 1, where $t_A = 1 + h$ and h is positive. The frequency PA was taken to be that of gene A in the gametic output, and the equilibrium gene ratio $\frac{PA}{Pa}$ was expressed for each sex in terms of the relative selective values as follows:-

$$\text{in females, } \left(\frac{PA}{Pa}\right)f = \frac{1 + t_A - 2\,Saa}{1 + t_A - 2\,t_A.\,S_{AA}}$$

$$\text{in males, } \left(\frac{PA}{Pa}\right)m = t_A.\left(\frac{PA}{Pa}\right)f.$$

It is clear that at equilibrium the frequencies of A and a will not be the same in the two sexes unless $t_A = 1$, i.e. unless there is no relative selective difference between the two male genotypes. The assumption of equal gene frequencies in the two sexes has often been made for naturally occurring sex-linked polymorphisms, as for example in Allison's (1960) study of glucose-6-phosphate dehydrogenase deficiency in man. The gene frequency of this enzyme deficiency in

East African males was used to calculate the expected number of female homozygotes and heterozygotes, and thus a figure obtained for the percentage of female heterozygotes detectable with the technique employed. As selective factors such as anaemia and immunity to malaria are known to affect persons with this enzyme deficiency, the assumption of no relative selective difference between the two male genotypes would be hard to justify, and in consequence the assumption that the male gene frequency could be applied to the females was not valid.

Bennett's paper also gave the necessary and sufficient conditions for stability of a sex-linked polymorphism as $S_{aa} < 1 - \frac{1}{2} h$ and $S_{AA} < 1 - \frac{1}{2} \frac{h}{1+h}$. It can be seen that the autosomal "principle of heterozygosity" no longer applies, as only in the special case of $t_A = 1$ are both female homozygotes required to have relative selective values less than that of the female heterozygote as a necessary and sufficient condition for stability. Bennett illustrated this fact with a theoretical example in which one female homozygote had a relative selective value greater than unity and yet a stable polymorphism existed. He gave another example where both S_{AA} and S_{aa} were less than unity but no stable polymorphism was possible, as the existence of an equilibrium depends on the relative selective values in the male as well as in the female sex.

An illustration was provided by Mandel (1959(b)) of a "principle of heterozygosity" for the case of sex-linked polymorphism. Using a different notation from that of Bennett, he assigned selective values to each of the five possible genotypes, taking none as a standard. He then showed that if certain combinations of these selective values were taken as applying to the homogametic sex, the stability conditions would be that the mean viability of the female population must be greater than that of each female homozygote, and that each female homozygote must have a selective value less than that of the heterozygote. The modifications of the selective values amounted to a reiteration of Bennett's stability conditions, and unfortunately have led to a certain confusion as to the conditions for stable equilibria (see Allison, 1960). When Mandel stated that female heterozygotes are present in excess of Hardy-Weinberg expectations as a condition for stability, he was considering the female part of the population only as analagous to the whole of a population showing autosomal polymorphism, and then applying a formula to it which is not strictly applicable in the sex-linked case. He was not implying disagreement with Bennett's illustration of the fact that an excess of female heterozygotes over the sum of female homozygotes is neither a necessary nor a sufficient condition for stable sex-linked plymorphism.

In a later paper, Bennett (1958) elaborated on the formulae presented in his first note, and applied his results to data published by Wallace (1948) for laboratory populations of Drosophila pseudo-obscura containing a sex-linked condition known as "Sex-Ratio",(SR). Males with an SR-bearing X chromosome produce mainly daughters, but the same average number of offspring as normal males, so that counter-selection must be present if the SR-bearing chromosome is not to replace the normal one in the population. Wallace claimed that a laboratory population reached an equilibrium with about 6% of SR when maintained at 16.5°C, and he presented relative selective values for both male and female genotypes. Bennett showed that although these relative selective values would maintain a stable equilibrium, the resulting frequencies would not be those observed by Wallace. He then presented the gene and genotype frequencies which would occur if Wallace's relative selective values were the correct ones.

It should be noted that it is impossible to estimate the relative selective values from Wallace's equilibrium data. The frequencies were determined from egg samples alone, so that only the zygotic frequencies before selection and the frequencies of gametes uniting at random can be deduced from the experimental results. Bennett's formulae allow estimation of t_A in such circumstances, but can only indicate a relationship between s_{AA} and s_{aa}. A complete solution requires knowledge of the genotypic frequencies giving rise to the

gametes, which would be tedious to achieve experimentally for the populations studied by Wallace.

In a recent paper Edwards (1961) suggested that Bennett's formulae are inapplicable to any sex-linked polymorphism where gametic selection occurs, such as the "sex-ratio" condition studied by Wallace. Edwards developed formulae specifically for the SR population data, separating the effects of gametic selection from those of zygotic selection. In his model the "equilibrium gene frequency" is taken to be that occurring in mature zygotes, and not, as taken by Bennett, that occurring in the gametes uniting at random to form the next generation. This latter definition does of course allow for gametic selection as well as zygotic selection to occur before the "equilibrium frequency" is attained, and Bennett's model is not in consequence restricted to certain types of sex-linked polymorphism.

Edwards considered the genotypic frequencies as summing to unity over both sexes, thereby allowing the calculation of the frequency of one sex in the population. This is impossible in applying Bennett's model, where the genotypes were summed to unity within sexes, although Edwards has erroneously assumed that a 1:1 male:female ratio was implied by so doing. Edwards has furthermore assigned the same relative selective value to the normal males and the homozygous normal females without any experimental justification. His attempt to consider zygotic and gametic selection separate-

ly is perhaps unnecessary even for the "sex-ratio" populations, where only the zygotic frequencies before selection occurs (i.e. frequencies from egg sampling) are experimentally determined.

Edwards also presented a formal proof of the stability conditions for a two-allele sex-linked polymorphism, and the results of a simulation of Wallace's experiment on an electronic digital computer. The latter illustrated that it is possible to assign relative selective values which result in approximately the observed changes in chromosome frequency.

One further theoretical study of sex-linked polymorphism is that by Sprott (1957). He presented a model for a multiple allelic sex-linked polymorphism, defining the equilibrium gene frequencies as those in mature zygotes of both sexes. He stated in his introduction that fitness could be considered to have two components, f_1 the chance of mating at all (zygotic viability) and f_2, the contribution to fertility of a pair after mating. The f_2 factor was originally introduced by Penrose for autosomal loci to consider separately the possibility of reproductive overcompensation maintaining a polymorphism, but as Sprott took f_2 as equal to unity he has considered only zygotic selection. He assigned the selective values $1 + K_{ij}$ to the female genotypes $A_i A_j$, and $1 + k_i$ to the male genotypes A_i, and derived the equilibrium gene frequencies in terms of these K- and

k- values. He also derived the necessary and sufficient conditions for an equilibrium to be a stable one, but these were not presented as simple inequalities of the selective values. When reduced to the two-allele case these led to a necessary condition for stability being $K_{12} > \frac{-(k_1 + k_2)}{2 + k_1 + k_2}$, which was stated to be sufficient also only if the deviations from unity of the female selective values were "small". In Bennett's terminology this necessary condition is equivalent to $0 > \frac{-h}{2+h}$, but greater precision than this can be obtained from Bennett's analysis for both male and female relative selective values compatible with stability.

Sprott considered several examples of two-allele sex-linked polymorphisms as illustrations of his results. One was the hypothetical case of a rare recessive gene lethal in the female homozygote only, for which he derived a condition for equilibrium in the form of a relationship between the selective values of the female heterozygote and the male hemizygote. Another example presented numerically a situation of a stable polymorphism being achieved without selective superiority of the female heterozygote, though this fact was not commented on. One three-allele situation was also examined numerically, using special assumptions as to equality of selective values of the three female heterozygotes. Sprott did not further explore the three-allele situation to see whether the general conditions for stability differed from

those of the two-allele case, and indeed it seems difficult to do so in general terms from his formulae.

(b) EXPERIMENTAL.

Very few experimental data have been published for laboratory populations containing competing sex-linked alleles. The most extensive experiment reported was undertaken by Wallace (1948), who placed the SR-bearing X chromosome of Drosophila pseudoobscura in competition with a standard (ST) X chromosome. Although not alleles the two types of X chromosome may be regarded as such for analytical purposes, as the SR-chromosome contains three inversions and crossing over is consequently very rare. Wallace maintained populations of average size 4,000 under constant environmental conditions, and followed the changes in frequency of the two types of X chromosome by removing egg samples at regular intervals and cytologically examining the resultant larvae. He detected no influence of initial chromosome frequencies on final chromosome frequencies, and reported that at 25°C SR was eliminated but at 16.5°C an equilibrium resulted with about 6% SR. He then attempted to obtain estimates of the selective values of the genotypes within the cages by experimentally analysing fitness differences at different stages of the life cycle, and presented the product of these fitness values as an overall selective value for each

genotype. The heterozygous females proved superior in overall selective value to both homozygous females, and the ST/ST females superior to the SR/SR females, while for the males ST was superior in overall selective value to SR at 25°C but inferior at 16.5°C. Wallace claimed these selective differences accounted satisfactorily for his population data. However, he admitted the inadequacy of examining arbitrarily chosen components of the life cycle, under different environmental conditions from those in a population cage, as a method for determining selective values applicable within a population.

That his selective values did not adequately account for his observed results was demonstrated theoretically by Bennett, and also "experimentally" by Barker (1958). Barker simulated Wallace's populations at 25°C on an automatic digital computer, using Monte Carlo procedures to allow random fluctuations at each of four stages of the life cycle. His programme was designed to allow for four types of selection, namely, zygotic selection, genotypic reproductive selection, selection between gametes at meiosis in heterozygotes, and gametic selection at fertilization. He adapted to his programme the fitness values given in Wallace's paper for the different stages of the life cycle, and his "experimental" populations differed markedly from Wallace's in achieving a stable equilibrium. He also ran a series of populations using Wallace's overall selective values for 25°C, applied

as zygotic selection only, and obtained the same results of an equilibrium, both series of simulated populations agreeing with the theoretical equilibrium demonstrated by Bennett for Wallace's selective values. Barker concluded that either specific or overall selective values may be used to study this genetic situation, and although this was challenged by Edwards on the grounds that sex ratio would differ in the two approaches, Barker was only concerned with relative chromosome frequencies within sexes as measured from egg samples, and for this the population sex ratio is irrelevant.

A series of experiments was reported by several authors on competition between sex-linked alleles of Drosophila melanogaster in small population-bottle units (Reed and Reed, 1948; 1950; Ludwin, 1951; Merrell, 1953a, 1953b; Merrell and Underhill, 1956). These populations varied considerably in size throughout their history, and at their smallest were reported by Reed and Reed to average 161 individuals. With such small numbers random drift effects might be expected to contribute to gene frequency changes, and certainly great fluctuations in gene frequency were common in all these experiments. Reed and Reed (1948) demonstrated a relationship between size of population and change in genotype frequency between successive samplings, so although the results were averaged over a number of population units to eliminate drift effects, the changes in gene frequency cannot safely be attributed to selective forces alone.

In many cases a sex-linked mutant was placed in competition with its wild-type allele, and not surprisingly elimination of the mutant was observed. Reed and Reed reported this for white eye colour, Merrell for yellow body, cut wing, raspberry eye colour and forked bristles, and Ludwin for the same genes as Merrell but with the four loci studied in all possible combinations. Reed and Reed, and Merrell attempted to account for the observed gene frequency changes on the basis of selective mating alone, using results from ancillary experiments which demonstrated differences in mating performance of mutant and wild type genotypes. To adequately compare the observed results with those expected on this hypothesis, an average generation time had to be assigned to their populations, and the different authors used a different number of days for this parameter. While non-random mating may well be important in such experiments other selective forces should perhaps also have been considered, if only to account for the observed elimination of the forked gene which did not differ from its wild type allele in its affects on mating performance. Merrell always estimated the gene frequency in females as the square root of the observed frequency of female homozygous recessives, which is only correct for a sex-linked gene on the assumptions of no zygotic selection and the same gene frequency in the two sexes, and this error must throw doubt on some of his conclusions.

One experiment by Reed and Reed (1948) showed an apparently stable equilibrium being achieved despite the large fluctuations in frequency in individual population units. This experiment involved competition between a Muller-5 X chromosome (inversion, Bar eye, apricot eye) and a normal X chromosome marked with white eye (w) miniature wing (m) and forked bristle (f), and for many generations the authors observed only two adult female genotypes, M-5/wmf and wmf/wmf, in the ratio .6:.4, and the two adult male genotypes wmf and M-5 in the ratio of .93:.07. Reed and Reed assumed the M-5 males did not mate, as they were known to be semi-sterile and of poor viability, and concluded that the M-5 chromosome was therefore maintained in the population by superiority of the female heterozygote. Wright calculated the relative selective values of the wmf/wmf and M-5/wmf females to be .27:1 for such an equilibrium. If one assumes that the observed zygotic frequencies are those of the individuals which give rise to gametes without further selection occurring, the relative selective values in Bennett's terminology must be $S_{AA} = .34$, $S_{aa} = 0$, $t_A = 5.69$, assigning A to the normal X chromosome marked with w, m, f. These relative selective values satisfy the stability conditions, and such considerable selective differences presumably prevent the detection of drift effects in small populations. Reed and Reed's figures also indicated that

the equilibrium populations contained only 30% of males, although they did not comment on this fact. Such a result could fairly well be accounted for by assuming that the male larvae with the M-5 chromosome rarely reached maturity.

Merrell and Underhill (1956) introduced a single male wild type fly into small populations of either yellow body or raspberry eye flies. They reported that in 3 out of 9 yellow populations and 5 out of 10 raspberry populations the mutant was eventually replaced by its wild type allele, although there was considerable variation between populations in the rate at which this occurred. In the remaining populations the wild type allele was presumably lost by chance. Ancillary experiments on larval viability and "female choice" mating tests led them to conclude that selection had exerted its major effect through mating performances, and this was roughly borne out by Barker (1958), who simulated the series of populations with yellow on an electronic computer. Merrell and Underhill's data on viability and mating performances were adapted for use in Barker's programming, and in 8 out of 10 "populations" the introduced wild type was lost. The rate at which the wild type replaced the yellow in the other two "populations" showed less variability than in the laboratory populations, but the simulation was a reasonable approximation to the original experiment.

Another group of experiments on competing sex-linked alleles was conducted by Merrell and Underhill, using

pseudoalleles at the white locus. Their small populations contained the white allele and one of eosin, apricot, coral and satsuma, and were maintained for approximately a year. Very large fluctuations in gene frequency were observed in all bottles, but the graphs of gene frequency changes in males suggested that eosin appeared to be replacing white, while white was perhaps superior to apricot, coral, and (less obviously) satsuma. It was impossible to predict whether elimination of one allele or a balanced polymorphism would have resulted for larger populations maintained for a longer time.

As all the female heterozygous genotypes, except those for satsuma and white, were distinguishable by eye from the two homozygous female genotypes of the same population, the gene frequencies for both sexes could readily be estimated. These were only presented averaged over all counts of one bottle rather than over all bottles at one time, so that changes in gene frequency with time cannot be examined. The authors claimed that the gene frequencies differed in the two sexes for eosin-white and coral-white populations, but their statistical analysis appears to have been inadequate for the appropriate null hypothesis. They furthermore claimed to have demonstrated the existence of heterosis for all the populations, by showing an excess of observed female heterozygotes over those expected, but they stated that the expected values were obtained using the Hardy-Weinberg

formula and the female gene frequencies only. As these are sex-linked alleles this procedure would have led to an incorrect estimate of expected female heterozygote numbers if indeed the gene frequencies did differ in the two sexes. In consequence, little value can be attached to the authors' suggestions of the occurrences of heterosis with pseudo-alleles, and its possible relation to theories of overdominance.

These same authors also reported an excess of males in the pseudo-allelic populations, although they indicated that there were considerable fluctuations in sex ratio from sample to sample, as perhaps expected with small populations. The percentages of males from each population are themselves significantly heterogeneous within each group of populations, except that of satsuma and white. Consequently the figures given for the overall percentage of males in a population series have little meaning. It is perhaps difficult to evaluate the authors' suggestion that differential viability of males and females is related to the proportions of pseudo-alleles in the population, without examining the original data to see whether there was a relationship between gene frequency changes and sex ratio changes.

A recent publication by Thomson (1961) discussed a repetition of Merrell and Underhill's experiments with white and satsuma, using both small and large populations. In

contrast to the former results, satsuma appeared to replace its white allele, the rate depending on the genetic background of the stocks used, but not on the temperature, the size of the population, or the illumination received by the cage. Selective mating experiments demonstrated a superiority of the satsuma males over the white males, and the extent of this superiority in mating performance was shown to depend on the illumination in which the test was conducted, but not on the background genotype of the stocks used. Consequently Thomson concluded that selective mating could not by itself be the principal determinant of gene frequency changes in the cages, and suggested that his data on reduced larval viability of the female homozygous white genotype were relevant in accounting for the observed population results. The number of observed female heterozygotes was not significantly greater than the number expected. Sex ratio was found to be very variable in both large and small populations, with no apparent relation to gene frequency. It was, however, dependent on the genetic background of the stocks used, and the author postulated that this was an intrinsic characteristic of each population.

(c) STATISTICAL ANALYSIS OF EXPERIMENTAL DATA.

No statistical analysis exists in the literature for confirming the apparent establishment of an equilibrium for two or more sex-linked alleles in a laboratory population.

Neither is there an analysis for determining the relative selective values of the genotypes in such a population from data obtained by observing gene frequency changes over a period of time. Consequently any review of the literature of statistical analyses of laboratory population data can only refer to techniques developed for populations containing two or more autosomal alleles.

The first such analysis was presented by Wright (Wright and Dobzhansky, 1946) for application to laboratory populations containing an inversion of the third chromosome in Drosophila pseudoobscura. The frequencies of the normal (ST) and inversion (CH) chromosomes had been recorded for several generations, and an apparent equilibrium established without about 70% ST. Wright attempted to estimate the relative selective values of the different genotypes in the population, and also the equilibrium chromosome frequencies, by expressing the expected change in chromosome frequency per generation in terms of the relative selective values and the chromosome frequencies. This was equated to the observed changes in frequency. As there were a number of observations of both chromosome frequencies and changes in frequency per generation, the method of least squares was used to estimate the unknown relative selective values. The expression for change in chromosome frequency was not linear with respect to the relative selective values, so a solution was determined by iteration. The analysis assumed constancy of the

relative selective values throughout the existence of the population, no selective differences between the sexes for the same third chromosome genotype, discrete generations, and random mating. It furthermore required an estimate of the average generation time within the population, and this was not experimentally determined but chosen empirically.

The analysis was then extended to allow for differing selective values in males and females by assigning to a genotype the average of its relative selective values in the two sexes. This modified analysis did not allow estimation of differing relative selective values in the two sexes which could best fit the data. It merely illustrated how these values could vary considerably without altering the agreement between observed and expected frequencies, provided the average for each genotype remained the same as determined in the first analysis.

A further modification consisted in allowing the relative selective values to be dependent on chromosome frequencies, ignoring possible sex differences. A particular relationship between relative selective values and chromosome frequencies was chosen, and very approximate methods used to find solutions for the parameters expressing this relationship. This refinement again amounted to little more than an illustration of how different hypotheses and varying relative selective values could account for the observed data, without indicating any preference between the solutions obtained.

The method of analysis was criticised by Levene (Levene, Pavlovsky and Dobzhansky, 1953) on the grounds that only the magnitude of the observed changes in chromosome frequency were taken into consideration, and not the order in which they occurred. This meant that the early changes in frequency per generation, when the population was far from equilibrium, contributed to the estimates of relative selective values far more than did later changes, which were necessarily smaller as the population approached an apparent equilibrium. Levene suggested an alternative analysis using a minimum χ^2 method of estimation. Relative selective values were chosen empirically to calculate expected chromosome frequencies, and the χ^2 of goodness of fit between observed and expected results determined. Improvement in the fitting was then achieved by altering the relative selective values, until no further corrections were considered necessary. The calculations required were extensive, and the corrections to the relative selective values chosen by trial and error alone, so this analysis can perhaps be considered impractical where extensive data exist for each of many populations, unless recourse can be had to an electronic computer.

A different approach to the problem was presented by Cavalli (1950), who assumed selection to be a continuous process which could best be represented by means of a

differential equation. He expressed a small change in gene frequency dp as a function of selective values which were defined in terms of an element of time dt. On integration he obtained a transformation of gene frequencies which would give a function linear with time, and he showed how estimation of the parameters of this linear function would lead to estimation both of the equilibrium gene frequency and relative selective values per time dt. The required estimation was achieved by an extension of Fisher's method of scoring, applied to maximum likelihood equations. A χ^2 for goodness of fit of the transformed gene frequencies to the fitted linear function then tested the hypothesis that the differential equation did in fact adequately describe the selective process.

This analysis did not assume discrete generations, but did presuppose constancy of the selective values throughout the existence of the population. It was not applicable if the only equilibrium possible in a particular case were a trivial one, and one possible cause of a bad fit could have been the population not being large enough to reduce the chances of drift occurring.

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3. MATERIALS AND METHODS.

No naturally occurring sex-linked polymorphism is known in Drosophila melanogaster, either for chromosomal re-arrangements or for characters which can be scored without causing the death of the individual in the process. In constructing an artificial population containing two competing sex-linked genes, therefore, it was necessary to use mutant phenotypes found in laboratory stocks. Furthermore, as none of the common laboratory mutants are found in natural populations, it was felt that if such a mutant were placed in competition with its wild-type allele the mutant would be eliminated, i.e. no balanced polymorphism would result (Merrell, 1953). It was therefore decided to arrange for competition between two allelic or pseudo-allelic mutants in a population, and three sex-linked pseudo-alleles at the white locus were chosen - white (w), apricot (w^a) and blood (w^{bl}). As the heterozygous apricot-white and blood-white females can easily be distinguished by eye from the homozygous apricot and blood females, a complete classification of any sample of flies could be achieved without the necessity of progeny testing.

Prior to establishing the populations containing competing pseudo-alleles, lines were set up each segregating for white and either apricot or blood, and maintained by single-pair sib matings for 14 - 19 generations. This

should have ensured relatively high homozygosity for all loci except those closely linked to the w locus. Any selective differences deduced from the experiment could then be validly assigned to the w locus itself, and not to heterozygosity of background genotypes. The method of propagating the white-apricot lines was to cross an heterozygous female to either a white or apricot sib, repeating the same cross each generation, i.e. always using a white male or an apricot male within a line. In this way the w^a gene was introduced into a w stock, and conversely, the w gene into a w^a stock. A similar process was followed for blood-white lines, incorporating the w^{bl} gene into a w stock, and the w gene into a w^{bl} stock. This method was followed to determine whether the background genotype of stocks used would influence the subsequent behaviour of the two pseudoalleles in competition.

The laboratory stocks from which the lines were set up contained two stocks of each of the three mutants. Consequently a further comparison was possible between the background genotypes of the two stocks of each mutant in their effects on competition between the pseudoalleles. No great differences were expected here, however, as in all cases the two stocks had been separated only fifteen months prior to the establishment of the lines.

A summary of the lines used in the experiment is given

in Table 3.1, together with the number of generations of sib mating in each line.

TABLE 3.1

Line No.	Original cross.	Genotype of male parent of subsequent generations.	No. of generations of sib mating.
1 A	W^a Stock 1 x W Stock 2	W^a	16
1 B	" " " " " "	W	17
11 A	W^a Stock 2 x W Stock 1	W^a	14
11 B	" " " " " "	W	19
111 A	W^{bl} Stock 1 x W Stock 2	W^{bl}	14
111 B	" " " " " "	W	18
IV A	W^{bl} Stock 2 x W Stock	W^{bl}	19
IV B	" " " " "	W	16

The design of the cages used for maintaining the populations was a modification of that described by Thomson (1957), and is illustrated in Figure 1. Each cage was constructed from a twelve-inch glass funnel, held in a ring on a retort stand. A lid made of $\frac{1}{8}$" perspex had a circular groove $\frac{1}{2}$" from its perimeter, into which the ground edge of the funnel fitted closely, allowing no flies to escape, and permitting easy rotation of the lid. In the centre of the lid was a circle of 2" diameter of fine copper mesh to allow

FIGURE 1

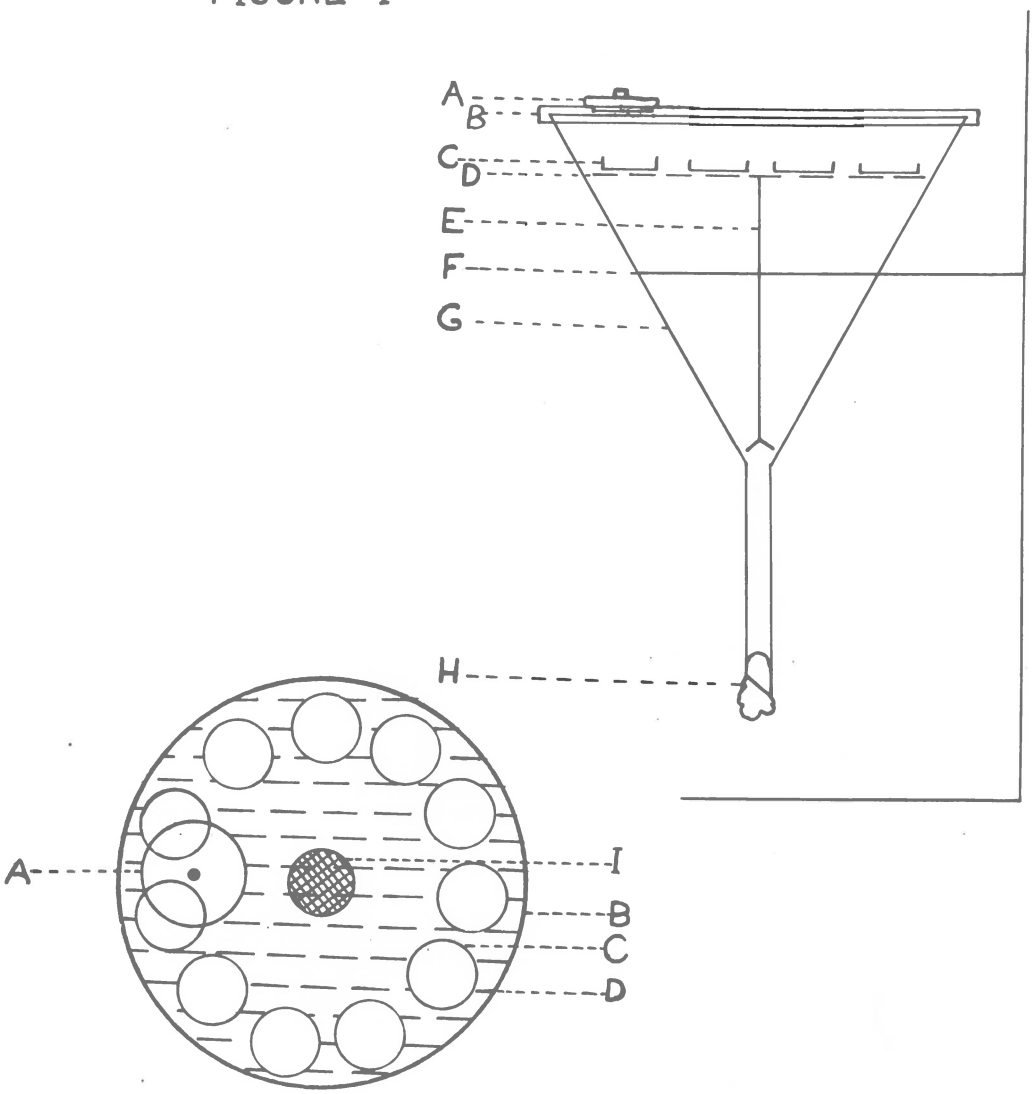

FIGURE 1

DIAGRAMMATIC REPRESENTATION OF POPULATION CAGE, SHOWING ELEVATION AND VIEW FROM ABOVE. SCALE: 1" = 4"

A.	Secondary lid	F.	Ring from retort stand
B.	Main lid	G.	12" glass funnel
C.	Food container	H.	Cottonwool plug
D.	Open metal grid	I.	Copper mesh ventilation screen
E.	Support for metal grid		

for ventilation, and a small lid of 2⅜" diameter was cut into the large lid with its centre 3⅜" from the centre of the latter to allow for removal of old food containers and their replacement by fresh ones. A rubber stopper was fitted into this secondary lid so that carbon dioxide could be blown into the cage, temporarily anaesthetizing the flies, before the change of food containers was effected.

Within the cage, a circular piece of galvanized metal lattice was placed to rest against the sides of the funnel at a vertical distance of 1½" from the lid. This was prevented from tilting by a vertical rod attached to its underside, which had a three-pronged foot resting against the sides of the funnel near the latter's point of minimum diameter. The lattice was sufficiently open to prevent restriction on free movement of the flies, and on it were placed eleven food containers in a circle round the cage. These food containers were identical bakelite screw-top jar lids of 2" diameter, capable of being autoclaved. They each held 25 mls of a standard treacle-semoline-agar food medium, seeded with .5 mls of a solution of live yeast when solid. A small quantity of sodium benzoate was included in the medium to hinder fungal growth.

Each cage was first set up with a single dish of medium, and another added every two days. After twenty-one days the circle of eleven dishes was complete, and from then till the end of the experiment the oldest container was

replaced by a fresh one every two days. This might have resulted in selection for rapid development, but very few larvae and pupae were ever observed in the 22-days old medium when it was removed, Drosophila melanogaster usually taking ten to fourteen days from egg to adult at 25°C.

The main advantage of using the glass funnel as the body of the cage was that dead flies and old medium accummulated above the cottonwool plug at the base of the funnel and could be removed periodically (every 14 days) without disturbing the population.

The populations were sampled every 14 days by anaesthetizing all the flies with carbon dioxide, collecting them in a beaker from the base of the funnel, and removing a random teaspoonful before returning the bulk of the population to the cage. A total of 300 flies was examined using a binocular microscope, scoring being done for sex and eye-colour genotype. The sampled flies were then returned to the cage, having been out of it for no longer than half an hour.

In the early stages of the experiment an estimate was also made at regular intervals of the total number of flies in each cage, using a photographic technique (Thomson, 1957). When removed for sampling purposes, the flies from each cage were anaesthetized with carbon dioxide and spread in darkness on a 12" x 10" sheet of Ilford Glossy Bromide B5 photographic paper. A light was then briefly switched on, and the flies

returned to the cage. When the paper was developed a permanent record of the number of flies present was obtained, and a count made using a glass graticule and hand counter.

Twelve cages were set up during the fortnight beginning 26/2/1958. Nine of these were maintained until 28/4/1959, a total of 60 weeks, until a mite infestation occurred and the experiment had to be terminated. The other three cages became contaminated by wild-type flies or another mutant, and were discontinued after 16, 22, and 24 weeks respectively. The cages were kept in a constant temperature room at 25°C± ½°C. On 10/6/1958 and 8/2/1959 the temperature rose to 28°C and 27.5°C respectively for a period of 6 - 8 hours, but no detectable effect on gene frequency changes could be attributed to the temporary temperature fluctuations.

The humidity was not controlled in the constant temperature room, but was maintained by a large sinkful of water. A hydrothermograph placed in the room from 22/4/1958 to 29/4/1958 showed temperature fluctuations of only 1°C. The room humidity varied from 56% to 62%, but within the cages it was probably much higher due to the presence of moist food surfaces.

The initial composition of each cage is shown in Table 3.2, which gives the original number of flies, the initial genotype numbers, and consequent gene frequencies. In all cases the number of males and females was the same.

TABLE 3.2
(a) Apricot-white competition.

Cage designation	Inbreeding Line.	Date of Establishment.	Total No. of flies.	Genotypes $w^a w^a$	$w^a w$	ww	w^a W	w^a gene frequency.	
K	1A	4/3/1958	24	-	12	-	6 6	.5	.5
L	1B	26/2/1958	40	-	20	-	10 10	.5	.5
M	11A	26/2/1958	28	-	14	-	7 7	.5	.5
N	11B	28/2/1958	32	-	16	-	8 8	.5	.5
O	1A	12/3/1958	48	12	12	-	6 18	.75	.25
P	1B	10/3/1958	24	-	6	6	9 3	.25	.75
Q	11A	10/3/1958	24	-	6	6	9 3	.25	.75
R	11B	10/3/1958	24	6	6	-	3 9	.75	.25

(b) Blood-white competition.

Cage designation	Inbreeding Line.	Date of Establishment.	Total No. of flies.	Genotypes $w^{bl} w^{bl}$	$w^{bl} w$	ww	w^{bl}	w^{bl} gene frequency	
S	111A	28/2/1958	16	-	8	-	4 4	.5	.5
T	111B	28/2/1958	32	-	16	-	8 8	.5	.5
U	IVA	6/3/1958	20	-	10	-	5 5	.5	.5
V	IVB	18/3/1958	24	-	12	-	6 6	.5	.5

It can be seen that cages K, L, M, AND N differed from cages O,P, Q, R respectively only in the initial gene frequencies in the two sexes. With respect to the derivation

of the lines used, then, the pairs K-O, L-P, M-Q, and N-R can be considered as replicates. Similarly, in considering whether the two genes are competing in a W^a or W background, cages K, M, O, and Q can be compared with L, N, P, and R. Again, cages K, L, O, and P originate from one set of laboratory stocks, and cages M, N, Q, and R from another, allowing another comparison to be made. Within the set of four cages with W^{bl} and W in competition, fewer groupings can be made. However, S and U compared with T and V reflect differences in background genotypes, and the pairs S-T and U-V are from different laboratory stocks. No cages were set up in this series with gene frequences differing from .5 in either sex, as it was felt that 12 cages was the maximum number that could be maintained and scored.

A more detailed analysis of the type of selection occurring within the cages was attempted by two ancillary experiments based on cage L. On two occasions (24/9/1958 and 15/1/1959) a test was made to detect departures from random mating within the cage. The females scored for eyecolour genotype in the routine sampling of the cage were isolated in individual creamers, and from their progeny the mating of the female could be determined unambiguously. The first time 139 females were isolated, of which 108 gave sufficient progeny, and the second time 123 matings were determined from 148 females isolated. The 19% of females

who were not scored does not necessarily indicate high sterility in the population, as both old and virgin females were included in the sampling.

The mating that had occurred could be inferred from the genotypes of the female offspring, and at the same time the genotypes of the male progeny afforded a check on the classification of the female parent. A small percentage of misclassification was detected, but as will be shown when discussing the results of the experiments, it did not significantly affect gene frequency estimates.

All regular scoring was carried out with adult flies, and in subsequent analyses it was assumed that the estimated gene frequencies were those after selection had operated in the life cycle of the flies. This entailed the three assumptions of no selective differences in fertility, random mating, and absence of gametic selective differences. The second assumption was tested as above. In an attempt to detect the presence of gametic selective differences a random sample of eggs was removed from cage L at the time of each of the random mating tests, and the larvae permitted to develop under optimal conditions of ample food supply and no crowding. As nearly 100% of the eggs were scored as emerging adults, selection in the larval and pupal stages of the life cycle was thus virtually removed. A comparison could then be made between the gene frequencies estimated from the egg sample, and those of the adults in the cage at

the time the sample was taken, to detect the presence of selective differences between the mature zygote stage of the life cycle and the formation of the new zygotes. If no such selective differences were found, a further comparison could be made between the gene frequencies of the egg sample and those among the adults of the next generation in the cage, to detect any selective differences during the larval and pupal stages of development within the cage.

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4. RESULTS AND ANALYSIS.

(a) PRESENTATION OF DATA.

The data from the samplings of the twelve population cages are presented in Tables 4.1 to 4.12. Each table shows the age of the population in weeks, the numbers of each genotype found in the total of 300 randomly selected individuals, and the gene frequencies for both sexes with their standard errors calculated for each sample. The error for p_2, the frequency of gene W^a (or W^{bl}) in the males, was calculated from the binomial variance $V_{(p_2)} = \frac{p_2(1-p_2)}{n_2}$, where n_2 was the number of males in the sample. The error for p_1, the frequency of gene W^a (or W^{bl}) in the females, was obtained using Kempthorne's (1956) formula, $V(p_1) = \frac{P+Q/2 - (P+Q)^2}{n_1}$, where P is the observed proportion of W^aW^a (or $W^{bl}W^{bl}$) homozygotes, 2Q the proportion of heterozygotes, and n_1 the number of females in the sample.

The tables also show the sex ratio for each sample, expressed as the observed proportion of males. The error is again calculated from the binomial variance, $V(r) = \frac{r(1-r)}{300}$, and the values of r differing significantly from .5 at the .05 level of significance are indicated by an asterisk. As a large number of the samples shows this significant deviation from r= .5, homogeneity of the sex ratio was tested for each cage using the Brandt and Snedecor χ^2 formula. In all cases a significant χ^2 was obtained, the probability of the χ^2 being 1 - 2% for cages T and V, and <.001 for all the other cages.

Such heterogeneity is hard to account for. If it accurately reflects changes in sex ratio within the cages, there may be an extreme sensitivity of sex ratio control to small environmental fluctuations in such highly homozygous mutant populations. Alternatively, it may indicate a non random sampling technique, with the bias not constant from one sampling to another, but this seems unlikely as the observed gene frequencies show no large or erratic changes corresponding to those of the sex ratio.

Population S showed the most extreme deviations in sex ratio, 17 out of 24 samplings having significantly more than 50% males, and one sample reaching 74% males. It was thought that selection for female inviability might have been associated with the observed selection for the W^{bl} gene, but before this suggestion could be experimentally tested a reversal in the direction of gene frequency changes was noted which continued until the experiment was terminated. Whether this reduction was due to a mutation affecting genotypic selective values, or to contamination by, say, W flies with a different genetic background, is not known. In subsequent analyses only the initial rise in W^{bl} gene frequency is considered.

The tables also show the population sizes at the time of the first six samplings from each cage. These show that although large fluctuations in total count were common in the early stages of each population the minimum number once the population had become established was always greater than 1000 flies.

TABLE 4.1

DATA FROM POPULATION K.

Age in weeks.	Total Count	Females W^aW^a	W^aW	WW	Males W^aW	WW	p_1=freq.W^a in ♀♀		p_2=freq.W^a in ♂♂		r=proportion of males	
0	24		12		6	6	.5		.5		.5	
2	460	48	58	40	84	70	$.5247^{\pm}$	.0320	$.5455^{\pm}$	.0401	$.5133^{\pm}$	.0289
4	1380	57	77	24	64	78	$.6044^{\pm}$	.0272	$.4507^{\pm}$	.0418	$.4733^{\pm}$	.0288
6	2240	52	81	14	94	59	$.6293^{\pm}$	.0255	$.6144^{\pm}$	.0394	$.5100^{\pm}$	.0289
8	2690	76	64	20	100	40	$.6750^{\pm}$	.0273	$.7143^{\pm}$	.0382	$.4667^{\pm}$	.0288
10	2240	65	64	12	113	46	$.6879^{\pm}$	.0268	$.7107^{\pm}$	.0360	$.5300^{\pm}$	.0288
12	3290	86	53	4	129	28	$.7867^{\pm}$	.0229	$.8217^{\pm}$	.0305	$.5233^{\pm}$	.0288
14		90	52	8	123	27	$.7733^{\pm}$	.0243	$.8200^{\pm}$	.0314	$.5000^{\pm}$	.0289
16		104	47	3	119	27	$.8279^{\pm}$	.0197	$.8151^{\pm}$	.0321	$.4867^{\pm}$	.0289
18		106	44	4	118	28	$.8312^{\pm}$	.0211	$.8082^{\pm}$	.0326	$.4867^{\pm}$	.0289
20		112	44	4	113	27	$.8375^{\pm}$	.0205	$.8071^{\pm}$	.0333	$.4667^{\pm}$	.0288
22		108	42	1	132	17	$.8543^{\pm}$	.0191	$.8859^{\pm}$	.0260	$.4967^{\pm}$	.0289
24		111	32	1	122	34	$.8819^{\pm}$	.0184	$.7821^{\pm}$	.0330	$.5200^{\pm}$	.0288
28		91	24	1	162	22	$.8879^{\pm}$	.0203	$.8804^{\pm}$	.0239	$.6133^{\pm}$	.0281*
30		122	35	2	130	11	$.8774^{\pm}$	.0182	$.9220^{\pm}$	.0226	$.4700^{\pm}$	.0288
32		124	29	1	133	13	$.8994^{\pm}$	.0168	$.9110^{\pm}$	.0236	$.4867^{\pm}$	.0289
34		107	21	1	156	15	$.9109^{\pm}$	.0177	$.9123^{\pm}$	.0216	$.5700^{\pm}$	.0286*
36		135	24	0	120	21	$.9245^{\pm}$	.0142	$.8511^{\pm}$	.0300	$.4700^{\pm}$	.0288
38		124	26	2	136	12	$.9013^{\pm}$	.0174	$.9189^{\pm}$	.0224	$.4933^{\pm}$	.0289
44		140	29	0	122	9	$.9142^{\pm}$	.0145	$.9313^{\pm}$	.0221	$.4367^{\pm}$	.0286*
46		129	28	1	134	8	$.9051^{\pm}$	.0162	$.9437^{\pm}$	.0193	$.4733^{\pm}$	.0288
48		118	21	0	147	14	$.9245^{\pm}$	.0152	$.9130^{\pm}$	.0222	$.5367^{\pm}$	.0288
52		135	23	1	128	13	$.9214^{\pm}$	.0151	$.9078^{\pm}$	.0244	.4700	.0288

TABLE 4.2

DATA FROM POPULATION L.

Age in weeks.	Total Count	Females $w^s w^s$	Females $w^s w$	Females ww	Males w^s	Males w	p_1=freq.w^s in ♀	p_2=freq.w^s in ♂	r = proportion of males
0	40	54	20		10	10	.5	.5	.5
2	700	54	68	41	65	72	.5399 ± .0297	.4745 ± .0427	.4567 ± .0288
4	2930	51	82	30	71	66	.5644 ± .0386	.5182 ± .0427	.4567 ± .2288
6	5790	49	66	28	81	76	.5734 ± .0301	.5159 ± .0399	.5233 ± .0288
8	6920	64	81	27	78	50	.6076 ± .0265	.6094 ± .0431	.4267 ± .0286*
10	2260	65	60	18	85	72	.6643 ± .0287	.5414 ± .0398	.5233 ± .0288
12	2170	100	43	9	107	41	.7993 ± .0243	.7230 ± .0368	.4933 ± .0289
14	1730	105	54	13	104	24	.7674 ± .0241	.8125 ± .0345	.4267 ± .0286*
16		105	51	6	111	27	.7747 ± .0280	.8043 ± .0338	.4600 ± .0288
18		105	29	8	128	30	.8415 ± .0241	.8101 ± .0312	.5267 ± .0288
20		102	37	6	129	26	.8310 ± .0230	.8323 ± .0300	.5167 ± .0289
22		91	30	4	139	36	.8480 ± .0235	.7944 ± .0306	.5833 ± .0285*
24		123	38	3	103	33	.8659 ± .0188	.7575 ± .0368	.4533 ± .0287
28		97	35	9	128	31	.8121 ± .0253	.8050 ± .0314	.5300 ± .0288
30		94	38	7	116	45	.8129 ± .0246	.7205 ± .0354	.5367 ± .0288
32		113	47	5	113	22	.8273 ± .0208	.8370 ± .0318	.4500 ± .0287
34		95	30	9	138	28	.8209 ± .0261	.8313 ± .0291	.5533 ± .0287
36		116	43	7	110	24	.8283 ± .0216	.8209 ± .0331	.4467 ± .0287
38		109	41	4	125	21	.8409 ± .0209	.8562 ± .0290	.4867 ± .0289
42		113	37	4	121	25	.8539 ± .0204	.8288 ± .0310	.4867 ± .0289
44		110	39	5	123	23	.8409 ± .0214	.8425 ± .0301	.4867 ± .0289
46		105	39	4	130	22	.8412 ± .0214	.8553 ± .0285	.5067 ± .0289
48		100	38	3	136	23	.8440 ± .0213	.8553 ± .0279	.5300 ± .0288
52		117	46	1	116	20	.8537 ± .0183	.8529 ± .0304	.4533 ± .0287

TABLE 4.3

Age in weeks	Total Count	Females $w^a w^a$	Females $w^a w$	Females ww	Males w^a	Males w	DATA FROM POPULATION M. p_1=freq.w^a in ♀♀	DATA FROM POPULATION M. p_2=freq.w^a in ♂♂	r= proportion of males
0	28	14			7	7	.5	.5	.5
2	650	52	76	41	64	67	.5325 ±.0284	.4885 ±.0437	.4367 ±.0286*
4	2190	50	74	25	88	63	.5839 ±.0282	.5828 ±.0401	.5033 ±.0289
6	1440	43	67	28	84	78	.5543 ±.0302	.5185 ±.0393	.5400 ±.0288
8	2290	44	67	50	84	55	.4814 ±.0301	.6043 ±.0415	.4633 ±.0288
10	2280	49	60	35	79	77	.5486 ±.0316	.5064 ±.0400	.5200 ±.0288
12	1120	46	37	13	151	53	.6719 ±.0359	.7402 ±.0307	.6800 ±.0269*
14	1150	56	92	31	73	48	.5698 ±.0255	.6033 ±.0445	.4033 ±.0283*
16		79	56	22	80	63	.6815 ±.0285	.5594 ±.0415	.4767 ±.0288
18		91	63	19	69	58	.7081 ±.0259	.5433 ±.0442	.4233 ±.0285*
20		78	66	26	70	60	.6529 ±.0276	.5385 ±.0437	.4333 ±.0286*
22		69	61	36	80	54	.5994 ±.0299	.5970 ±.0424	.4467 ±.0287
24		77	69	48	59	47	.5747 ±.0283	.5566 ±.0482	.3933 ±.0276*
28		64	53	25	89	69	.6373 ±.0312	.5633 ±.0395	.5267 ±.0288
30		56	74	39	75	56	.5503 ±.0286	.5725 ±.0432	.4367 ±.0286*
32		89	52	25	82	52	.6928 ±.0285	.6119 ±.0421	.4467 ±.0287
34		87	51	19	102	41	.7166 ±.0279	.7133 ±.0378	.4767 ±.0288
36		78	38	20	119	45	.7132 ±.0315	.7256 ±.0348	.5467 ±.0287
38		89	51	18	107	35	.7247 ±.0274	.7535 ±.0361	.4733 ±.0288
44		89	32	8	125	46	.8140 ±.0263	.7310 ±.0339	.5700 ±.0286*
46		58	24	4	155	59	.8140 ±.0308	.7243 ±.0305	.7133 ±.0261*
48		94	41	14	109	42	.7685 ±.0271	.7219 ±.0365	.5033 ±.0289
52		91	36	11	126	36	.7900 ±.0270	.7778 ±.0327	.5400 ±.0288

TABLE 4.4

Age in weeks	Total Count	Females w^sw^s	Females w^sw	Females ww	Males w^s	Males w	p_1=freq.w^s in ♀♀	DATA FROM POPULATION N. p_2=freq.w^s in ♂♂	r = proportion of males
0	32		16		8	8	.5	.5	.5
2	610	56	81	31	76	56	.5744 ±.0272	.5758 ±.0430	.4400 ±.0287*
4	1790	69	71	20	85	55	.6531 ±.0269	.6071 ±.0413	.4667 ±.0288
6	3750	60	62	25	110	43	.6190 ±.0298	.7190 ±.0363	.5100 ±.0289
8	5600	50	65	19	119	47	.6157 ±.0293	.7169 ±.0350	.5533 ±.0287
10	3710	70	61	8	110	51	.7230 ±.0255	.6832 ±.0367	.5367 ±.0288
12	3180	55	37	9	118	81	.7277 ±.0325	.5930 ±.0348	.6633 ±.0273*
14		63	49	11	127	50	.7114 ±.0293	.7175 ±.0338	.5900 ±.0284*
16		76	42	11	130	41	.7519 ±.0285	.7602 ±.0327	.5700 ±.0286*
18		57	57	9	135	42	.6951 ±.0280	.7627 ±.0320	.5900 ±.0284*
20		74	41	5	132	48	.7875 ±.0261	.7333 ±.0330	.6000 ±.0283*
22		71	33	9	140	47	.7743 ±.0300	.7487 ±.0317	.6233 ±.0280*
24		86	49	8	126	31	.7727 ±.0251	.8025 ±.0318	.5233 ±.0288
28		82	26	2	138	52	.8636 ±.0227	.7263 ±.0323	.6333 ±.0278*
30		78	35	6	139	42	.8025 ±.0267	.7680 ±.0314	.6033 ±.0282*
32		95	33	8	124	40	.8199 ±.0253	.7561 ±.0335	.5467 ±.0287
34		77	26	12	156	29	.7826 ±.0314	.8432 ±.0267	.6167 ±.0281*
36		63	26	4	174	33	.8172 ±.0292	.8406 ±.0254	.6900 ±.0267*
38		99	33	3	132	33	.8556 ±.0215	.8000 ±.0311	.5500 ±.0287
44		99	26	3	146	26	.8750 ±.0214	.8488 ±.0273	.5733 ±.0286*
46		81	28	2	158	31	.8559 ±.0233	.8360 ±.0269	.6300 ±.0278*
48		113	29	4	135	19	.8733 ±.0204	.8766 ±.0265	.5133 ±.0289
52		107	32	7	130	24	.8425 ±.0231	.8442 ±.0292	.5133 ±.0289

TABLE 4.5

DATA FOR POPULATION O.

Age in Weeks	Total Count	Females $w^a w^a$	Females $w^a w$	Females ww	Males w^a	Males w	p_1=freq.w^a in ♀♀	p_2=freq.w^a in ♂♂	r=proportion of males
0	48	12	12		6	18	.75	.25	.5
2	370	55	78	15	117	35	.6351 ± .0260	.7697 ± .0341	.5067 ± .0289
4	1680	41	62	15	134	48	.6102 ± .0300	.7363 ± .0327	.6067 ± .0282
6	4040	70	79	7	95	49	.7019 ± .0230	.6597 ± .0395	.4800 ± .0288
8	2170	100	57	8	83	52	.7788 ± .0228	.6148 ± .0419	.4500 ± .0287
10	1970	91	39	9	122	39	.7950 ± .0258	.7578 ± .0338	.5367 ± .0287
12	1060	102	57	7	102	32	.7861 ± .0223	.7612 ± .0368	.4467 ± .0287
14		119	44	6	104	27	.8343 ± .0208	.7939 ± .0353	.4367 ± .0286

TABLE 4.6

DATA FOR POPULATION P.

Age in Weeks	Total Count	Females $w^a w^a$	Females $w^a w$	Females ww	Males w^a	Males w	p_1=freq.w^a in ♀♀	p_2=freq. w^a in ♂♂	r = proportion of males
0	24		6	6	9	3	.25	.75	.5
2	610	26	61	31	83	99	.4783 ± .0319	.4560 ± .0369	.6067 ± .0282*
4	2040	71	79	21	87	42	.6462 ± .0257	.6744 ± .0413	.4300 ± .0286*
6	2440	57	75	19	99	50	.6258 ± .0270	.6644 ± .0387	.4967 ± .0289
8	2630	83	58	15	100	44	.7172 ± .0265	.6944 ± .0383	.4800 ± .0288
10	2630	67	52	16	119	46	.6889 ± .0398	.7212 ± .0349	.5500 ± .0287
12	1950	75	76	14	86	49	.6848 ± .0247	.6370 ± .0414	.4500 ± .0287
14	3200	85	62	21	78	54	.6905 ± .0269	.5909 ± .0428	.4400 ± .0286*
16	3720	79	58	14	102	47	.7152 ± .0267	.6846 ± .0331	.4967 ± .0289
18	4250	90	50	8	105	47	.7770 ± .0245	.6908 ± .0375	.5067 ± .0289
20		97	66	12	96	29	.7429 ± .0235	.7680 ± .0377	.4167 ± .0285*
22		114	46	10	103	27	.8059 ± .0228	.7923 ± .0356	.4333 ± .0260*
26		117	47	12	101	23	.7983 ± .0231	.8145 ± .0349	.4133 ± .0284*
28		87	35	1	150	27	.8496 ± .0215	.8475 ± .0270	.5900 ± .0284*
30		114	35	5	126	20	.8539 ± .0210	.8630 ± .0285	.4867 ± .0289
32		70	12	1	196	21	.9157 ± .0222	.9032 ± .0201	.7233 ± .0258*
34		112	27	4	132	25	.8776 ± .0205	.8408 ± .0292	.5233 ± .0288
36		104	24	1	146	25	.8992 ± .0185	.8538 ± .0270	.5700 ± .0286*
38		118	18	1	147	16	.9270 ± .0160	.9018 ± .0233	.5433 ± .0288
42		141	14	0	140	5	.9548 ± .0115	.9655 ± .0152	.4833 ± .0289
44		101	19	2	168	10	.9057 ± .0195	.9438 ± .0173	.5933 ± .0284*
46		123	24	0	139	14	.9184 ± .0152	.9085 ± .0233	.5100 ± .0289
50		128	19	3	143	7	.9167 ± .0173	.9533 ± .0172	.5000 ± .0289
56		134	22	0	133	11	.9295 ± .0139	.9236 ± .0221	.4800 ± .0288

TABLE 4.7

DATA FROM POPULATION Q.

Age in weeks	Total Count	Females w^Bw^B	Females w^Bw	Females ww	Males w^B	Males w	p_1=freq.w^B in ♀♀	p_2=freq.w^B in ♂♂	r = proportion of males
0	24		6	6	9	3	.25	.75	.5
2	1010	31	131	0	23	115	.5957 ± .0155	.1667 ± .0317	.4600 ± .0288
4	1860	28	92	66	60	54	.3978 ± .0250	.5263 ± .0468	.3800 ± .0280*
6	1970	36	89	72	54	49	.4086 ± .0256	.5243 ± .0492	.3433 ± .0274*
8	2300	53	62	53	60	72	.5000 ± .0306	.4545 ± .0433	.4400 ± .0287*
10	2390	36	45	31	91	97	.5223 ± .0365	.4840 ± .0364	.6267 ± .0279*
12	1360	63	61	43	72	61	.5599 ± .0305	.5414 ± .0432	.4433 ± .0287
14		61	65	40	70	64	.5633 ± .0299	.5224 ± .0432	.4467 ± .0287
16		48	60	28	95	69	.5735 ± .0314	.5793 ± .0386	.5467 ± .0287
18		63	55	22	101	59	.6464 ± .0305	.6313 ± .0381	.5333 ± .0288
20		70	55	28	100	47	.6373 ± .0304	.6803 ± .0385	.4900 ± .0289
22		71	61	14	97	57	.6952 ± .0271	.6299 ± .0389	.5133 ± .0289
26		74	64	22	111	29	.6625 ± .0278	.7929 ± .0342	.4667 ± .0288
28		94	57	11	103	35	.7562 ± .0244	.7464 ± .0370	.4600 ± .0288
30		88	48	13	100	51	.7517 ± .0267	.6623 ± .0385	.5033 ± .0289
32		71	38	10	109	72	.7563 ± .0296	.6022 ± .0364	.6033 ± .0282*
34		98	44	12	113	33	.7792 ± .0256	.7740 ± .0346	.4867 ± .0289
36		97	45	10	104	44	.7862 ± .0249	.7027 ± .0376	.4933 ± .0289
38		92	35	9	130	34	.8051 ± .0261	.7927 ± .0317	.5467 ± .0287
42		108	42	10	111	29	.8063 ± .0238	.7929 ± .0342	.4667 ± .0288
44		81	30	6	137	46	.8205 ± .0267	.7486 ± .0321	.6100 ± .0282*
46		79	38	5	126	52	.8033 ± .0256	.7079 ± .0341	.5933 ± .0284*
50		95	37	8	123	37	.8107 ± .0250	.7688 ± .0333	.5333 ± .0288
56		77	24	5	156	38	.8396 ± .0271	.8041 ± .0285	.6467 ± .0276*

TABLE 4.8

DATA FROM POPULATION R.

Age in Weeks	Total Count	Females $w^a w^a$	$w^a w$	ww	Males w^a	w	p_1 = freq. w^a in ♀♀		p_2 = freq. w^a in ♂♂		r = proportion of males	
0	24	6	6		3	9	.75		.25		.5	
2	740	19	90	36	128	27	.4414	± .0251	.8258	± .0305	.5167	± .0289
4	550	71	75	16	80	58	.6698	± .0255	.5797	± .0420	.4600	± .0288
6	287	20	39	9.	131	88	.5809	± .0384	.5982	± .0331	.7631	± .0251*
8	550	54	66	17	98	65	.6350	± .0285	.6012	± .0384	.5433	± .0288
10	2680	59	55	15	110	61	.6750	± .0298	.6145	± .0364	.5967	± .0283*
12	2050	61	57	22	104	56	.6393	± .0303	.6500	± .0377	.5333	± .0288
14		75	53	10	114	48	.7355	± .0267	.7037	± .0359	.5400	± .0288
16		76	62	14	105	43	.7039	± .0265	.7095	± .0373	.4933	± .0289
18		84	63	13	92	48	.7219	± .0253	.6571	± .0401	.4667	± .0288
20		86	36	8	122	48	.8000	± .0264	.7176	± .0345	.5667	± .0286*
22		82	44	15	122	37	.7376	± .0286	.7673	± .0335	.5300	± .0288
26		101	41	8	121	29	.8100	± .0239	.8067	± .0322	.5000	± .0289
28		106	33	11	137	13	.8167	± .0251	.9133	± .0230	.5000	± .0289
30		133	30	2	124	11	.8970	± .0169	.9185	± .0235	.4500	± .0287
32		70	17	0	180	33	.9023	± .0212	.8451	± .0248	.7100	± .0262*
34		110	26	2	142	20	.8913	± .0190	.8765	± .0258	.5400	± .0288
36		125	20	0	133	22	.9310	± .0143	.8581	± .0280	.5167	± .0289
38		127	22	0	133	18	.9262	± .0146	.8808	± .0264	.5033	± .0289
42		141	19	0	129	11	.9406	± .0128	.9214	± .0227	.4667	± .0288
44		91	17	0	173	19	.9213	± .0175	.9010	± .0216	.6400	± .0277*
46		137	21	0	134	8	.9335	± .0135	.9437	± .0193	.4733	± .0288
50		134	11	1	147	7	.9555	± .0127	.9545	± .0168	.5133	± .0289
56		129	13	0	145	8	.9388	± .0135	.9477	± .0180	.5100	± .0289

TABLE 4.9

DATA FOR POPULATION S.

Age in Weeks	Total Count	Females $w^{bl}w$	Females $w^{bl}w^{bl}$	Females ww	Males w^{bl}	Males w	p_1=freq.w^{bl} in ♀♀	p_2=freq.w^{bl} in ♂♂	r = proportion of males
0	16		8		4	4	.5	.5	.5
2	310	82	46	15	137	20	.7343 ± .0283	.3726 ± .0266	.5233 ± .0288
4	380	96	66	6	103	29	.7679 ± .0287	.7803 ± .0360	.4400 ± .0287*
6	1070	60	57	2	135	46	.7437 ± .0244	.7459 ± .0324	.6033 ± .0282*
8	1460	77	30	2	170	21	.8440 ± .0240	.8901 ± .0226	.6367 ± .0278*
10	1700	102	25	1	161	11	.8945 ± .0189	.9360 ± .0187	.5733 ± .0286*
12	3500	86	25	1	178	10	.8795 ± .0212	.9468 ± .0164	.6267 ± .0279*
14		104	15	0	173	8	.9370 ± .0152	.9558 ± .0153	.6033 ± .0282*
16		72	6	0	217	5	.9615 ± .0151	.9775 ± .0100	.7400 ± .0253*
18		95	6	0	197	2	.9703 ± .0117	.9899 ± .0022	.6633 ± .0273*
20		90	1	0	209	0	.9945 ± .0055	1.0000 ± .	.6967 ± .0265*
22		99	4	0	197	0	.9806 ± .0095	1.0000	.6567 ± ,0274*
24		78	1	0	221	0	.9937 ± .0062	1.0000	.7367 ± .0254*
28		78	18	0	186	18	.9063 ± .0199	.9117 ± .0199	.6800 ± .0269*
30		67	32	7	162	32	.7830 ± .0298	.8351 ± .0266	.6467 ± .0276*
32		74	39	6	141	40	.7857 ± .0271	.7794 ± .0308	.6033 ± .0282*
34		45	30	8	130	87	.7229 ± .0364	.5991 ± .0333	.7233 ± .0258*
36		69	50	16	91	74	.6963 ± .0297	.5515 ± .0387	.5500 ± .0287
38		64	47	19	85	85	.6731 ± .0316	.5000 ± .384	.5667 ± .0286*
44		53	45	27	81	94	.6040 ± .0345	.4629 ± .0377	.5833 ± .0285*
46		43	39	29	92	97	.5631 ± .0377	.4868 ± .0364	.6300 ± .0278*
48		62	54	42	79	63	.5633 ± .0319	.5563 ± .0417	.4733 ± .0288
52		71	59	24	64	82	.6526 ± .0292	.4384 ± .0411	.4867 ± .0289

TABLE 4.10. DATA FROM POPULATION T.

Age in Weeks	Total Count	Females $w^{bl}w^{bl}$	Females $w^{bl}w$	Females ww	Males w^{bl}	Males w	p_1=freq.w^{bl} in ♀♀	p_2=freq.w^{bl} in ♂♂	r = proportion of males
0	32		16	40	8	8	.5	.5	.5
2	970	72	57	40	74	57	.5947 ± .0304	.5649 ± .0433	.4367 ± .0286*
4	2380	54	63	39	58	86	.5481 ± .0308	.4023 ± .0409	.4800 ± .0286
6	2250	49	66	31	68	86	.5616 ± .0302	.4416 ± .0400	.5133 ± .0289
8	1570	69	75	31	76	49	.6086 ± .0274	.6080 ± .0437	.4167 ± .0285*
10	1590	53	60	34	94	59	.5646 ± .0313	.6144 ± .0393	.5100 ± .0289
12	1170	53	61	26	90	70	.5964 ± .0307	.5625 ± .0392	.5333 ± .0288
14		64	58	29	99	50	.6159 ± .0305	.6644 ± .0387	.4967 ± .0289
16		63	50	34	97	56	.5986 ± .0325	.6340 ± .0389	.5100 ± .0289
18		63	42	25	109	61	.6462 ± .0337	.6412 ± .0368	.5667 ± .0286*
20		82	56	16	99	47	.7143 ± .0271	.6731 ± .0387	.4867 ± .0289
22		94	34	12	96	64	.7929 ± .0272	.6000 ± .0387	.5333 ± .0288

TABLE 4.11.

Age in Weeks	Total Count	Females $w^{bl}w^{bl}$	Females $w^{bl}w$	Females ww	Males w^{bl}	Males w	p_1=freq.w^{bl} in ♀♀	p_2=freq.w^{bl} in ♂♂	r = proportion of males
		DATA FROM POPULATION U.							
0	24		12		6	6	.5	.5	.5
2	570	54	70	43	76	57	.5327 ± .0294	.5714 ± .0429	.4433 ± .0287
4	520	47	71	21	97	64	.5935 ± .0286	.5025 ± .0386	.5367 ± .0288
6	2290	50	70	25	93	62	.5862 ± .0291	.6000 ± .0393	.5167 ± .0288
8	1530	68	67	23	75	67	.6424 ± .0280	.5282 ± .0419	.4733 ± .0288
10	1460	74	59	30	74	63	.6350 ± .0295	.5401 ± .0426	.4567 ± .0288
12	760	65	71	14	93	57	.6700 ± .0262	.6200 ± .0396	.5000 ± .0289
14		66	57	27	102	49	.6309 ± .0305	.6755 ± .0381	.5033 ± .0289
16		75	49	16	95	65	.7107 ± .0290	.5938 ± .0388	.5333 ± .0288
18		51	65	22	85	77	.6051 ± .0296	.5247 ± .0392	.5400 ± .0288
20		53	49	14	116	68	.6681 ± .0316	.6304 ± .0356	.6133 ± .0281*
24		66	67	21	93	53	.6461 ± .0279	.6370 ± .0398	.4867 ± .0289
26		71	48	24	103	54	.6643 ± .0312	.6561 ± .0379	.5233 ± .0288
28		79	40	31	94	56	.6600 ± .0324	.6267 ± .0395	.5000 ± .0289
30		101	35	28	81	55	.7226 ± .0299	.5956 ± .0421	.4533 ± .0287
32		63	51	25	90	71	.6367 ± .0317	.5590 ± .0391	.5367 ± .0288
34		73	46	32	93	56	.6358 ± .0321	.6242 ± .0397	.4967 ± .0289
36		90	43	21	95	51	.7240 ± .0291	.6507 ± .0395	.4867 ± .0289
42		78	49	25	75	73	.6743 ± .0303	.5068 ± .0411	.4933 ± .0289
44		74	54	26	81	65	.6553 ± .0299	.5548 ± .0411	.4867 ± .0289
46		91	47	21	83	58	.7201 ± .0276	.5887 ± .0414	.4700 ± .0288
50		95	49	21	85	50	.7242 ± .0276	.6296 ± .0416	.4500 ± .0287

TABLE 4.12

DATA FROM POPULATION V.

Age in Weeks	Total Count	Females $w^{bl}w^{bl}$	Females bl w	Females w w	Males w^{bl}	Males w	p_1=freq.w^{bl} in ♀♀	p_2=freq.w^{bl} in ♂♂	r =proportion of males
0	24		12		6	6	.5 .	.5 .	.5
2	410	46	82	25	67	80	.5686 ±0270	.4558 ±0411	.4900 ±0289
4	1110	44	60	23	99	74	.5827 ± .0314	.5723 ± .0376	.5767 ± .0285*
6	2610	67	65	25	95	48	.6338 ± .0286	.6643 ± .0395	.4767 ± .0288
8	3240	53	67	20	112	48	.6179 ± .0288	.7000 ± .0387	.5333 ± .0288
10	2100	75	45	7	119	54	.7677 ± .0266	.6879 ± .0352	.5767 ± .0285*
12	2080	99	64	3	104	30	.7892 ± .0206	.7761 ± .0360	.4467 ± .0287
14		67	43	7	156	27	.7964 ± .0281	.8525 ± .0262	.6100 ± .0282*
16		96	40	1	132	31	.8467 ± .0204	.8098 ± .0307	.5433 ± .0288
18		78	40	6	151	25	.7903 ± .0262	.8580 ± .0263	.5867 ± .0284*
20		69	48	5	163	15	.7623 ± .0261	.9157 ± .0208	.5933 ± .0284*

A considerable fluctuation in numbers is expected in such populations, with an eventual size approximately midway between the extremes of the fluctuations (Thomson, 1957). The total counts were not continued long enough in the present experiments to determine the eventual population number, as it was merely desired to establish that the numbers present were great enough to ignore random drift effects on gene frequency changes.

The data are illustrated graphically in Figures 2 to 4, where the frequency of the w^a (or w^{bl}) gene in a cage is plotted against time, the sexes being treated separately. The estimated frequencies for males are subject to greater error than those for females, due to the smaller number of sex-linked genes sampled in the heterogametic sex. It can be seen that the frequencies differed little in the two sexes of any one population. The graphs also illustrate the fact that populations started with gene frequencies differing between the sexes rapidly achieved the same frequency in both sexes, and thereafter behaved similarly to those started with the same gene frequency in each sex. In all cases, it is obvious that the w gene was at a disadvantage compared to its colour-producing allele, regardless of the breeding history of the lines used in establishing the cages. The frequency of w^a or w^{bl} rose markedly, in some cases appearing to reach an equilibrium with 10 - 20% of w genes, and in others tending to establish a monomorphic population of the colour-producing gene.

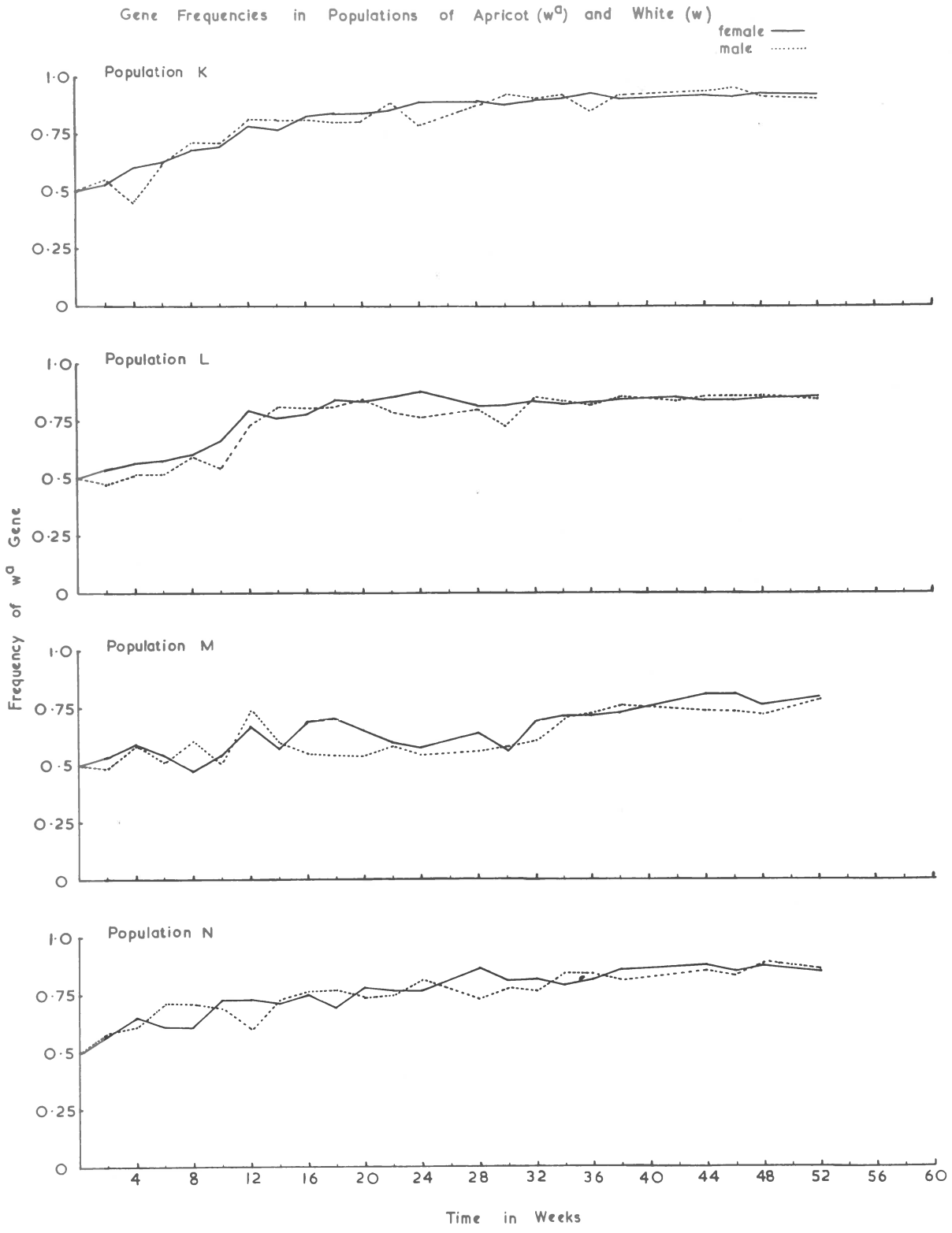

Gene Frequencies in Populations of Apricot (w^a) and White (w)
female
male
Population K
Population L
Population M
Population N
1·0
0·75
0·5
0·25
0
Frequency of w^a Gene
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
Time in Weeks

Fig. 2.

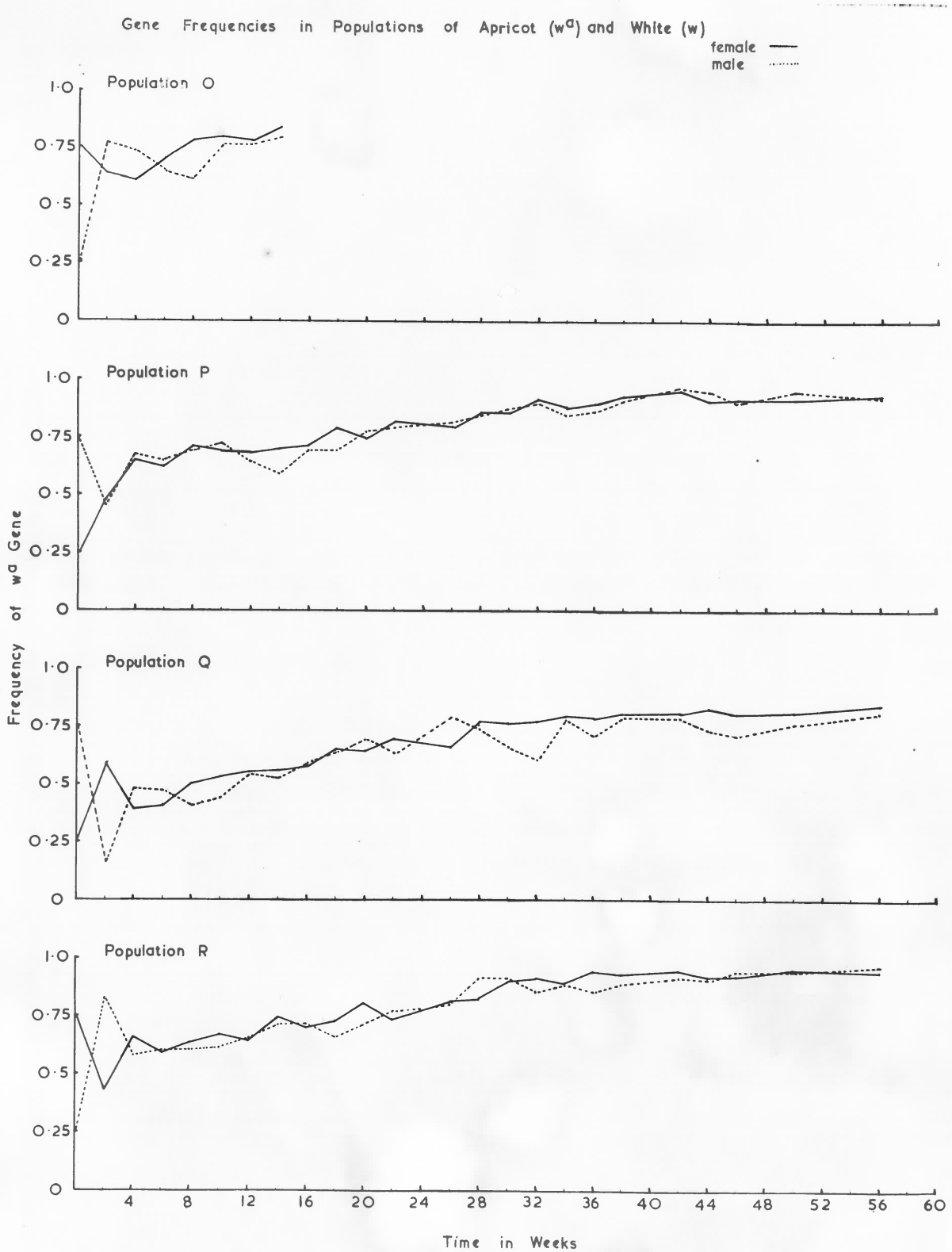

Gene Frequencies in Populations of Apricot (w^a) and White (w)
female
male
Population O
Population P
Population Q
Population R
1·0
0·75
0·5
0·25
0
Frequency of w^a Gene
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
Time in Weeks

Fig. 3.

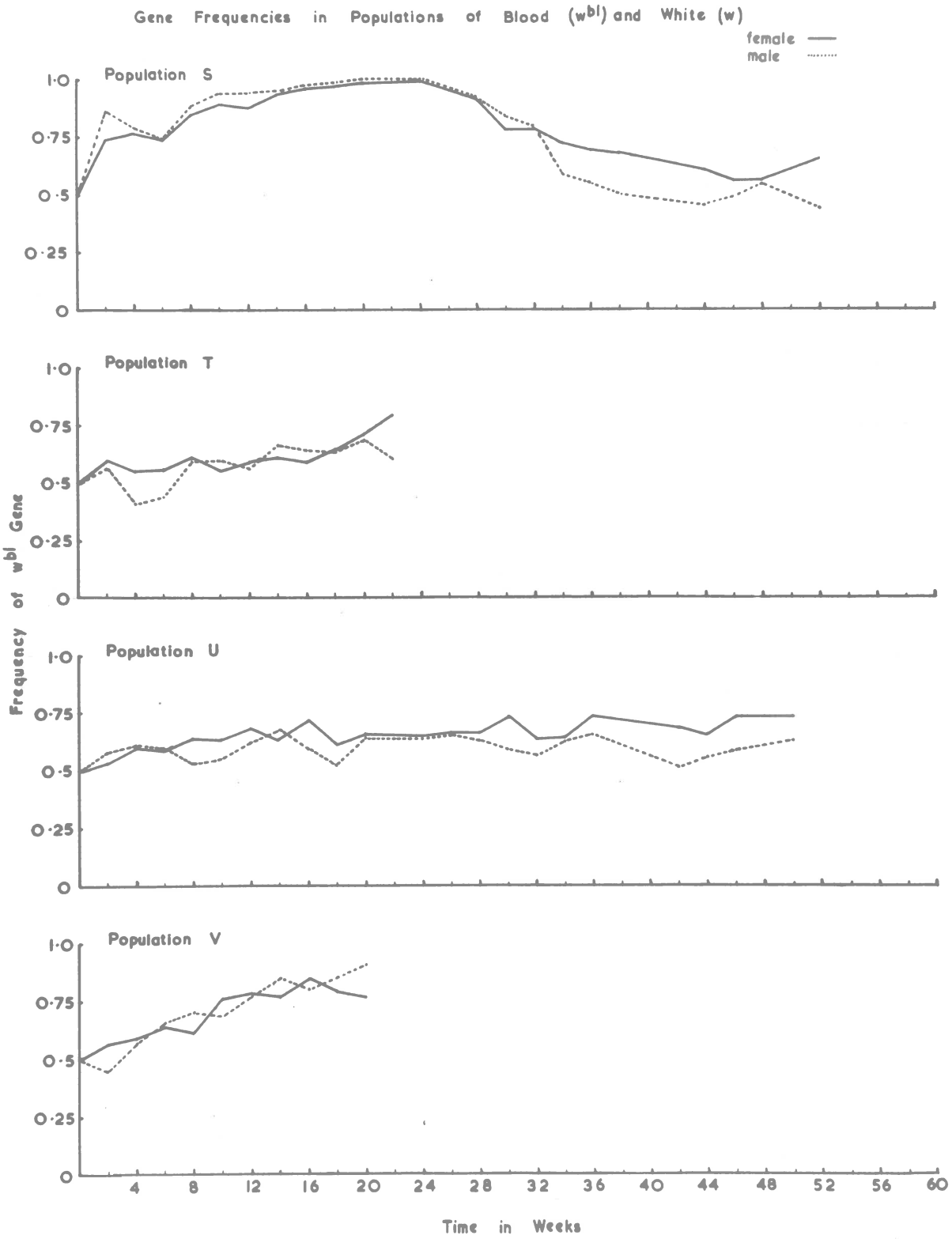

Gene Frequencies in Populations of Blood (w^{bl}) and White (w)
female
male
Population S
Population T
Population U
Population V
1·0
0·75
0·5
0·25
0
Frequency of w^{bl} Gene
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
Time in Weeks

Fig. 4.

(b) RESULTS AND ANALYSIS OF ANCILLARY EXPERIMENTS.

The results of the ancillary experiments need only a brief consideration. Two tests to determine whether random mating was occurring in Population L were carried out, when the cage had been maintained for 30 and 46 weeks respectively, using the technique outlined in the previous section. On the first occasion 139 females were isolated, but as some gave no progeny, or so few that the mating that had occurred could not be unambiguously deduced, only 108 matings could be determined. In the second test 148 females were isolated, and of these 125 yielded sufficient progeny for analysis. The male offspring provided a check on the classification of the female parent, and the female progeny gave information as to the mating that had occurred in the cage. Matings by males of more than one genotype were inferred when the different genotypes among the female offspring did not conform to expected Mendelian ratios, and such double matings were assigned equally to the two male genotypes. Double matings by males of the same genotype could not of course be deducted, and introduce an element of uncertainty into the results.

Table 4.13 shows a comparison of the effects of misclassification of $w^a w^a$ and $w^a w$ females in the two tests, and the χ^2 calculated to test the independence of the 2 x 2 tables formed by the $w^a w^a$ and $w^a w$ females yielding progeny as scored visually and by progeny analysis.

TABLE 4.13

Effects of misclassification in random mating tests.

	Test 1 Genotype				Test 2 Genotype			
	$w^a w^a$	$w^a w$	ww	Total	$w^a w^a$	$w^a w$	ww	Total
Visual classification of sampled females.....	94	38	7	139	105	39	4	148
Visual classification of females yielding progeny..	76	27	5	108	89	34	2	125
Classification by progeny analysis..	63	40	5	108	87	36	2	125
	$\chi^2 = 3.43$	$.05 < P < .10$			$\chi^2 = 0.02$	$.80 < P < .90$		

Neither of these χ^2 was significant at the 5% level, showing that although misclassification did occur, it did not significantly affect the classification of the samples in these two cases, and presumably did not therefore have serious consequences in the other samplings of the cage. The w^a gene frequencies estimated from the females classified by progeny testing ($p_1 = .7685 \pm .0281$, and $\hat{p} = .8400 \pm .0223$) did not differ significantly from those estimated from the females classified visually ($d = 1.19$, $P = 23\%$ and $d = .04$, $p = 96\%$).

The results of the random mating tests are shown in Table 4.14, where the few double matings detected have been allocated as indicated above. The χ^2 values testing the hypothesis of independence of association of male and female genotypes were both non-significant, so that no departures from random mating were apparent.

TABLE 4.14

Results of random mating tests in Cage L.

Male genotype	Test 1. Female genotype. $w^g w^g$	$w^g w$	ww	Total	Test 2. Female genotype. $w^g w^g$	$w^g w$	ww	Total
w^g	56	34	5	95	77	27	1	105
w	12	8	0	20	10	9	1	20
	68	42	5	115	87	36	2	125

$\chi^2_2 = 1.14$, $.3 < P < .5$ $\chi^2_2 = 5.21$, $.05 < P < .1$

The results of the two tests were homogeneous, as shown by applying the Brandt and Snedecor test to a 6 x 2 table formed with the results above, ($\chi^2_5 = 7.62$, $.10 < P < .20$), and when the results were pooled into one table, again no departure from independence of association of male and female genotypes was detected ($\chi^2_1 = 2.13$, $.30 < P < .50$). No significant non-random mating could therefore have been occurring in the population, or its presence would have been detected in the present sample of 240 analysed matings.

Egg samples taken from cage L at the time of the above tests were allowed to develop under optimal conditions of plentiful food supply. Two half-pint milk bottles were used for each sample, and as the results were not significantly heterogeneous between bottles of a sample, only the totals for each sampling are presented in Table 4.15.

TABLE 4.15.
Adult flies scored from isolated egg samples.

Genotype.	$w^a w^a$	$w^a w$	ww	w^a	w	Total.
Sample 1	65	29	3	83	17	197
Sample 2	147	54	7	147	21	376

On the assumption that there were no selective differences in female fertility, that mating was random with respect to eye colour genotype, and that no gametic selection occurred within the cages, the gene frequencies in mature adults at the time the egg samples were removed should be those of gametes uniting at random to form the next generation. A χ^2 test for agreement between the observed numbers of each genotype resulting from the egg samples, and those expected on the above hypothesis, gave a nonsignificant result for both samples ($\chi^2_3 = 3.29$, $.3 < P < .5$ for sample 1, $\chi^2_3 = 2.52$, $.3 < P < .5$ for sample 2. This experiment does not therefore invalidate the assumption made in subsequent analyses that the gene frequencies in adult flies were those after selection had completed its action in the life cycle.

A further χ^2 was calculated to test the homogeneity of the observed numbers of each genotype resulting from the egg samples and those observed in the cage at the time the adults emerged from the egg samples. During the time between removal of the egg sample and emergence of the flies from it, differential larval and pupal selection had presumably been operating in the cage, but not in the bottles where the samples developed.

significant differences between the two sets of results would have demonstrated the action of selection at these stages of the life cycle within the cage. No significant differences were found ($\chi^2_3 = 1.7$, $.5 < P < .7$ for sample 1, $\chi^2_3 = 4.9$, $.1 < P < .2$ for sample 2, the ww females being pooled with the w^8w genotype), but this does not necessarily mean that differential selection did not occur in the cage during these developmental stages. The sampling technique allowed the inclusion of flies which could have been sampled previously - i.e. the sample from the cage did not consist entirely of individuals which were at the egg stage when the egg sample was removed, and had it been possible to obtain such a sample, significance might well have been established.

(c) ANALYSES OF EQUILIBRIA.

An analysis was developed to determine whether an equilibrium had been attained in each cage, and how many sampling results could be regarded as those from a population in equilibrium. For each cage the last two readings were compared by means of a contingency χ^2, and if a nonsignificant result were obtained the two sets of results were summed and tested against the third-to-last reading. The procedure was repeated until a significant χ^2 was obtained, and the sample which thus differed significantly from the sum of all subsequent samples was taken to be the one before an equilibrium had been established, all later readings being samples of the equilibrium population. In this test the sexes were treated separately.

to avoid significance due to variation in the sex ratio alone. For the females the ww numbers were added to those of the heterozygotes, as the former were too small to allow separate treatment, and the test therefore consisted of a series of 2 x 2 contingency tables for each sex. As an example the results of this analysis for Population K are presented in Table 4.16.

TABLE 4.16.

Contingency χ^2_1 values for determining the number of samples forming the equilibrium in Population K (see Text)

Females	Probability	Males	Probability
0	1.00	.03	.8 - .9
.8	.3 - .5	.5	.3 - .5
.1	.7 - .8	.2	.5 - .7
.3	.5 - .7	.2	.5 - .7
.3	.5 - .7	4.4	.02 - .05
.1	.7 - .8	.3	.5 - .7
.7	.3 - .5	.1	.7 - .8
3.7	.05 - .1	.5	.3 - .5
1.0	.3 - .5	.1	.7 - .8
2.6	.1 - .2	20.6	.01
8.9	.01		

It can be seen that the last eleven samples of females could be considered as being from an equilibrium population, whilst only the last ten samples of males gave nonsignificant values, and consequently the population was considered to be in equilibrium during the time of the last ten samplings. For the males, the sixth-to-last reading when tested against

the other five gave a χ^2_1 with probability of 2 - 5%, but as the next χ^2_1 was nonsignificant the former was taken to be the 1 in 20 chance expected using the 5% significance level, and was not taken as indicating a sample prior to the establishment of the equilibrium.

The number of samplings considered to be those from the equilibrium populations for all the cages with w^a and w in competition are shown in Table 4.17.

The above method of determining the number of samples from the population which could be considered as being from an equilibrium might mean that a few samples were included which had been taken before the establishment of the equilibrium. However, the numbers involved in the test were such that only a slight deviation would be significant, and for all cages a clear distinction occurred between the equilibrium samples and the one prior to the equilibrium. A χ^2 test for homogeneity of the equilibrium samples was then carried out, using the Brand and Snedecor formula, and in all cases the result was non-significant. Thus had a homogeneity test alone been used to determine the number of samples from the equilibrium, at least the same number of samples, and in many cases more samples, would have been included as being those from a population in equilibrium.

The results of all the samples from the equilibrium population were then summed for each genotype and the following statistics calculated for each cage:-

Equilibrium frequency of $w^a w^a$ genotype in adult females

$= x = \frac{\text{number of } w^a w^a \text{ females}}{n_1}$, where n_1 = total number of females.

$$V_x = \frac{x(1-x)}{n_1}.$$

Equilibrium frequency of $w^a w$ genotype in adult females

$$= y = \frac{\text{number of } w^a w \text{ females}}{n_1},$$

$$V_y = \frac{y(1-y)}{n_1}.$$

Equilibrium frequency of ww genotype in adult females

$$= z = \frac{\text{number of ww females}}{n_1},$$

$$V_z = \frac{z(1-z)}{n_1}.$$

Equilibrium frequency of gene w^a in female adults = $p_1 = x + \frac{1}{2}y$.

$$V_{p_f} = \frac{x + y/4 - (x + y/2)^2}{n_1}.$$

Equilibrium frequency of gene w^a in male adults

$= p_m = \frac{\text{number of } w^a \text{ males}}{n_2}$, where n_2 = total number of males.

$$V_{p_m} = \frac{p_m(1-p_m)}{n_2}.$$

$$\text{Cov}(x, y) = -\frac{xy}{n_1}$$

$$\text{Cov}(z, y) = \frac{-zy}{n_1}$$

$$\text{Cov}(x, p_f) = \text{Cov}(x, x + \tfrac{1}{2}y) = V_x + \tfrac{1}{2}\text{Cov}(x, y).$$

$$\text{Cov}(y, p_f) = \text{Cov}(y, x + \tfrac{1}{2}y) = \tfrac{1}{2}V_y + \text{Cov}(x, y).$$

$$\text{Cov}(z, p_f) = \text{Cov}(z, -(z + \tfrac{1}{2}y)) = -V_z - \tfrac{1}{2}\text{Cov}(z, y).$$

Values of p_f, V_{p_f}, p_m, and V_{p_m} are shown in Table 4.17 for all cages with w^a and w in competition.

Of major interest are the relative selective values responsible for maintaining a population in equilibrium, and

these were estimated as follows from the equilibrium gene and genotype frequencies.

On the assumption that no differential selection occurred between the adult zygote stage of the life cycle and the formation of gametes which united at random to form the next generation, p_f = equilibrium frequency of w^a gene in the female gametic output, and p_m = equilibrium frequency of w^a gene in the male gametic output.

The relative selective values of the female genotypes w^aw^a, w^aw, and ww were taken to be S_{AA}:1:S_{aa}, and those of the male genotypes w^a and w to be t_A:1 or 1:t_a, so that t_A or t_a = 1 + h, where h was positive.

Then random union of the gametes would have resulted in the relative frequencies of the genotypes in the next generation being:-

Before selection:

females $p_f\ p_m\ w^aw^a$, $(p_f\ (1-p_m) + (1-p_f)\ p_m)\ w^aw$, $(1-p_f)\ (1-p_m)\ ww$

males $p_f\ w^a$, $(1-p_f)\ w$,

After zygotic selection:

females $p_f\ p_m\ S_{AA}\ w^aw^a$, $(p_f\ (1-p_m) + (1-p_f)\ p_m)\ w^aw$, $(1-p_f)(1-p_m)S_{aa}\ ww$,

males $p_f\ t_A\ w^a$, $(1-p_f)\ w$, or $p_f\ w^a$, $(1-p_f)\ t_a\ w$.

As the populations were assumed to be in equilibrium, the relative selective values were estimated by

$$S_{AA} = \frac{x}{y}\left\{\frac{p_f + p_m - 2p_f\ p_m}{p_f\ p_m}\right\},$$

$$S_{aa} = \frac{z}{y}\left\{\frac{p_f + p_m - 2p_f\ p_m}{(1-p_f)(1-p_m)}\right\},$$

$$t_A = \frac{p_m(1-p_f)}{p_f(1-p_m)} \quad \text{or} \quad t_a = \frac{p_f(1-p_m)}{p_m(1-p_f)}$$

Values of S_{AA}, S_{aa}, and t_A or t_a for the cages with w^a and w in competition are given in Table 4.17.

To obtain variances for the estimates of the relative selective coefficients the following formulae were developed:

$$S_{AA} = \frac{x}{y}\left(\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right)$$

$$= f(x, y, p_f, p_m)$$

$$= f(x^*, y^*, p_f^*, p_m^*) + \frac{\partial f}{\partial x}(x - x^*) + \frac{\partial f}{\partial y}(y - y^*) + \frac{\partial f}{\partial p_f}(p_f - p_f^*) + \frac{\partial f}{\partial p_m}(p_m - p_m^*)$$

where x^*, y^*, p_f^*, and p_m^* are particular values of x, y, p_f, and p_m.

$$\therefore V_{S_{AA}} = \left(\frac{\partial f}{\partial x}\right)^2 V_x + \left(\frac{\partial f}{\partial y}\right)^2 V_y + \left(\frac{\partial f}{\partial p_f}\right)^2 V_{p_f} + \left(\frac{\partial f}{\partial p_m}\right)^2 V_{p_m} + \left(\frac{\partial f}{\partial x}\right)\left(\frac{\partial f}{\partial y}\right)\mathrm{Cov}(x,y)$$

$$+ \left(\frac{\partial f}{\partial x}\right)\left(\frac{\partial f}{\partial p_f}\right)\mathrm{Cov}(x, p_f) + \left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial f}{\partial p_f}\right)\mathrm{Cov}(y, p_f),$$

the other covariance terms being zero as p_m is independent of x, y, and p_f.

Therefore -

$$V_{S_{AA}} = \frac{1}{y^2}\left[\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right]^2 V_x + \frac{x^2}{y^4}\left[\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right]^2 V_y + \frac{x^2}{y^2 p_f^4} V_{p_f} + \frac{x^2}{y^2 p_m^4} V_{p_m}$$

$$- \frac{x}{y^3}\left[\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right]^2 \mathrm{Cov}(x,y) - \frac{x}{y^2 p_f^2}\left[\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right]\mathrm{Cov}(x, p_f)$$

$$+ \frac{x^2}{y^3 p_f^2}\left[\frac{p_f + p_m - 2p_f p_m}{p_f p_m}\right]\mathrm{Cov}(y, p_f).$$

Similarly, it was shown that -

$$V_{S_{aa}} = \frac{1}{y^2}\left[\frac{p_f + p_m - 2p_f p_m}{(1-p_f)(1-p_m)}\right]^2 V_z + \frac{z^2}{y^4}\left[\frac{p_f + p_m - 2p_f p_m}{(1-p_f)(1-p_m)}\right]^2 V_y + \frac{z^2}{y^2(1-p_f)^4} V_{p_f}$$

$$+ \frac{z^2}{y^2(1-p_m)^4} V_{p_m} - \frac{z}{y^3}\left[\frac{p_f + p_m - 2p_f p_m}{(1-p_f)(1-p_m)}\right]^2 \mathrm{Cov}(z,y)$$

$$+ \frac{z}{y^2(1-p_f)^2}\left[\frac{p_f + p_m - 2p_f p_m}{(1-p_f)(1-p_m)}\right]\mathrm{Cov}(z, p_f) - \frac{z^2}{y^3(1-p_f)^2}\left[\frac{p_f + p_m - 2p_f p_m}{(1-p_f)(1-p_m)}\right]\mathrm{Cov}(y, p_f).$$

$$V_{t_A} = \frac{(1-p_f)^2}{p_f^2(1-p_m)^4} V_{p_m} + \frac{p_m^2}{p_f^4(1-p_m)^2} V_{p_f}$$

or,

$$V_{t_a} = \frac{p_f^2}{(1-p_f)^2 p_m^4} V_{p_m} + \frac{(1-p_m)^2}{(1-p_f)^4 p_m^2} V_{p_f}.$$

The results of applying these formulae to the data from Populations K-R are given in Table 4.17.

For an equilibrium to be a stable one, the relative selective values must conform to the following inequalities (Bennett, 1957).

$S_{AA} < 1 - \frac{1}{2}\frac{h}{(1+h)}$, and $S_{aa} < 1 + \frac{1}{2}h$ when $t_A = 1 + h$ (h positive)

or $S_{AA} < 1 + \frac{1}{2}h$ and $S_{aa} < 1 - \frac{1}{2}\frac{h}{1+h}$ when $t_a = 1 + h$ (h positive)

When h = 0, i.e. there is no differential selection in the males, then $p_f = p_m$, and both S_{AA} and $S_{aa} < 1$ are necessary for stability. This is the familiar condition of superiority of the heterozygote, here applying only in the homogametic sex.

Considering the results of the analyses summarized in Table 4.17, it could be seen that the number of samples from the equilibrium population varied from 3 to 16, and although little reliance could be placed on an equilibrium existing for as short a time as 6 weeks, the analysis was carried through for populations M, O, and R for purposes of comparison.

The equilibrium gene frequencies varied from .80 to .94 w^a for the females, and from .74 to .95 w^a for the males, and in all cases but two, p_f and p_m did not differ significantly at the 5% significance level. The exceptions were Populations M and Q. For population M a greater number of equilibrium samples might have resulted in smaller variances for p_f and p_m, and consequently the significance of the difference between $p_f = .794$ and $p_m = .738$ might have been more clearly established ($t_6 = 2.59$, $.02 < P < .05$). Population Q gave $t_{14} = 2.86$, $.01 < P < .02$, for the comparison between $p_f = .805$ and $p_m = .762$. These two populations were also the only ones where t_a (or t_A) differed significantly from unity, as expected when the equilibrium gene frequencies were not the same in the two sexes.

With regard to the origins of the lines used for the populations, comparisons were made between the equilibrium gene frequencies within pairs of cages. The pairs K-L, M-N, O-P, and Q-R reflected differences due to a w^a or a w background, yet in only the first of these pairs did a w^a genetic background result in a higher equilibrium frequency of w^a in both sexes. The other three pairs showed a significantly lower equilibrium frequency for w^a in the w^a as compared to the w background genotype.

TABLE 4.17

RESULTS OF ANALYSES OF EQUILIBRIA FOR POPULATIONS WITH w^a AND w IN COMPETITION.

Cage	No. samples from equilibrium		No. equilibrium samples assumed	p_f	V_{p_f}	p_m	V_{p_m}	s_{AA}	$V_{s_{AA}}$	s_{aa}	$V_{s_{aa}}$	t_A	t_a	V_{t_A} or V_{t_a}
K	11	10	10	.9059	$.2740\times10^{-4}$	.9084	$.5525\times10^{-4}$	.9589	.00453447	.6779	.05169438	1.0181	-	.01225451
L	16	9	9	.8392	$.5048\times10^{-4}$	.8424	1.0058×10^{-4}	1.0287	.00404136	1.2309	.04045883	1.0242	-	.00889387
M	4	6	4	.7939	1.9392×10^{-4}	.7378	2.7715×10^{-4}	1.5355	.02133060	1.8546	.11273608	-	1.3689	.02745209
N	9	7	7	.8455	$.8402\times10^{-4}$	.8409	1.0912×10^{-4}	1.1885	.00852588	1.8807	.11665776	-	1.0354	.01181433
O	4	3	3	.8059	1.7421×10^{-4}	.7700	4.1573×10^{-4}	1.2022	.02080210	1.1780	.05167766	-	1.2402	.03133805
P	7	6	6	.9262	$.4026\times10^{-4}$	.9325	$.6746\times10^{-4}$	.9766	.01012215	1.3596	.32753689	1.1008	-	.03107086
Q	10	8	8	.8045	$.8227\times10^{-4}$	.7616	1.3828×10^{-4}	1.3702	.00850838	1.6106	.04685389	-	1.2331	.01247840
R	7	3	3	.9424	$.5877\times10^{-4}$	.9438	1.0819×10^{-4}	.9204	.02851851	.6922	.49565072	1.1326	-	.08443127

For explanation and methods of calculation see text.

The pairs of cages K - O, L - P, M - Q, and N - R differed within themselves only in the initial gene frequencies in the two sexes, and were therefore replicates with regard to the breeding history of the lines used. However, within the pairs K - O, L - P, and N - R the equilibrium gene frequencies differed significantly, for both males and females, which suggested that initial gene frequencies could influence the resulting equilibrium. The pair M - Q, on the contrary, showed no significant differences when the two p_f^s and two p_m^s were compared, and therefore in this case at least the initial gene frequency did not affect the equilibrium frequencies.

Comparisons within the pairs K - M and L - N reflected differences in the two laboratory stocks of both w^a and w used in setting up the lines. The first of these pairs showed significant differences in the equilibrium frequencies, the second did not. Comparisons of equilibrium frequencies within the pairs O - Q, P - R, reflected the same differences in the two laboratory stocks of w^a and w, and also a complication due to differing initial gene frequencies, but neither pair showed a significant difference in either sex. This was surprising in view of the effects of differing initial gene frequencies alone considered above.

To sum up the results of these comparisons, no general conclusions could be drawn as to the effects on the equilibrium gene frequencies of genetic background or initial gene frequencies in the cages, or the possible differences

between the two laboratory stocks of each allele.

Turning now to consider the stability of the equilibria for populations K - R, Table 4.18 shows the 95% confidence limits for the female relative selective coefficients, and also the maximum value possible for each of them for a stable equilibrium to exist. These maxima were calculated from Bennett's inequalities, using the estimated values of t_A or t_a.

TABLE 4.18.

95% CONFIDENCE LIMITS AND MAXIMA FOR STABILITY FOR FEMALE RELATIVE SELECTIVE COEFFICIENTS.

Cage	S_{AA}	95% Confidence limits.	Maximum value for stability.	S_{aa}	95% confidence limits	Maximum value for stability.
K	.9539	(1.0908,.8270)	.9911	.6779	(1.1236,.2322)	1.0091
L	1.0287	(1.1534,.9040)	.9882	1.2309	(1.6251,.8367)	1.0121
M	1.5355	(1.8217,1.2493)	1.1845	1.8546	(2.5128,1.1964)	.8653
N	1.1885	(1.3694,1.0076)	1.0177	1.8807	(2.5600,1.2114)	.9829
O	1.2022	(1.4848,.9196)	1.1201	1.1780	(1.6235,.7325)	.9032
P	.9766	(1.1738,.7794)	.9542	1.3596	(2.4813,.2379)	1.0504
Q	1.3702	(1.5509,1.1895)	1.1441	1.6106	(2.0349,1.1863)	.8882
R	.9204	(1.2514,.5894)	.9415	.6922	(2.0720,-.6876)	1.0663

It can be seen that only for Populations K and R did both female relative selective coefficients satisfy the conditions for stability of the equilibrium. In both cases the error term for S_{aa} was so large that little weight could be

attached to the estimate of this relative selective coefficient. However, as the frequency of the w^a gene rose markedly from its initial values for males and females in both populations, the w gene was at some selective disadvantage, and the true values for S_{aa} in consequence were probably both less than unity. The confidence limits for the estimates of S_{AA} for the two populations included values greater than the maximum permissible for the stability of the equilibrium, so it could not be concluded that the existence of two stable equilibria had been unambiguously established.

For the other populations the situation was more confusing. None of the relative selective values satisfied the inequalities for stability of the equilibria, although for populations L, O, and P, values within the confidence limits of both S_{AA} and S_{aa} did fall below the necessary maxima. The analysis of Population L suggested that an equilibrium had existed for a longer period of time than was the case for Populations O and P, but for all three cages the magnitude of the relative selective coefficients made the existence of stable equilibria very much open to question. In Population N the confidence limits of S_{AA} contained values conforming to the stability requirement, but no possible value of S_{aa} fell low enough to suggest the existence of a stable equilibrium. Finally, for Populations M and Q no values within the confidence limits of either relative selective value fulfilled the stability requirements.

It can be seen, then, that this analysis established no unambiguous case of an attained stable equilibrium. Cages K and R satisfied the conditions reasonably well, and cages L, O, and P may perhaps have been in stable equilibrium, while cages M, N, and Q could not have remained in equilibrium if their selective coefficients were those estimated above.

It should be noted that the calculations of the inequalities used the estimated value of t_A or t_a. Recalculation using the upper and lower limits given by the 95% confidence limits for t_A or t_a, while giving different maxima for the female selective coefficients in the inequalities, in no way altered the conclusions as to which cages might have been in stable equilibrium and which ones could not have been with the calculated selective coefficients

This analysis was not carried out for the four cages with w^{bl} and w in competition, as it had given such inconclusive results for those containing w^a and w.

(d) ANALYSIS USING AN ADAPTATION OF WRIGHT AND DOBZHANSKY'S METHOD.

It seemed desirable to apply a method of analysis which used all the data obtained from each cage, and not only the last few readings, in determining whether an equilibrium had been reached and what the selective values were for each population. This was first done by using a modification of the analysis employed by Wright and Dobzhansky (1946) for populations containing an autosomal

inversion in Drosophila pseudoobscura. In this method, the observed change in gene frequency per generation was expressed in terms of the (unknown) selective values, and the method of least squares adopted to estimate these selective coefficients. As the expression was not linear with respect to the parameters, a solution had to be obtained by iteration.

In adapting Wright's derivation of the analysis for autosomal genes to the case of sex-linked ones, let p_1 be the frequency of gene A in the female gametic output of the nth generation, and p_2 the frequency of the same gene in the male gametic output of the same generation. If A and a are the genes competing in the population, let the selective values of the genotypes present be:-

Genotype:	AA	Aa	aa	A	a
Selective value:	S_{AA}	S_{Aa}	S_{aa}	S_A	S_a

Then the mean fitness of the female population in the $(n+1)^{th}$ generation, after selection has operated,

$$= \bar{S}_f = p_1 p_2 S_{AA} + \{p_1(1-p_2) + p_2(1-p_1)\} S_{Aa} + (1-p_1)(1-p_2) S_{aa}.$$

Similarly, the mean fitness of the male population in the $(n+1)^{th}$ generation after selection has operated

$$= \bar{S}_m = p_1 S_A + (1-p_1) S_a.$$

The change in gene frequency in one generation in females = Δp_1 can be shown to be

$$= \left[p_1 p_2 (1-p_1) S_{AA} + \left\{ \tfrac{1}{2} - p_1 \right\} \{ p_1(1-p_2) + p_2(1-p_1) \} S_{Aa} - p_1 (1-p_1)(1-p_2) S_{aa} \right] \Big/ \bar{S}_f$$

and the same change in males =

$$\Delta p_2 = \left\{ p_1(1-p_2)\, s_A - p_2(1-p_1)\, s_a \right\} \Big/ \bar{s}_m .$$

The data consisted of readings of p_1 and p_2 at different times. Using an average generation time of 21 days (Reed and Reed, 1950), though this seemed an overestimate, appropriate values of Δp_1 and Δp_2 could be obtained from the observations. The method of least squares was then used to minimise the sum of the squared deviations between observed and expected values of Δp_1 and Δp_2.

Let $x_{11} = p_1 p_2 (1-p_1) \Big/ \bar{s}_f$

$$x_{12} = (\tfrac{1}{2}-p_1)\left\{ p_1(1-p_2) + p_2(1-p_1) \right\} \Big/ \bar{s}_f$$

$$x_{13} = p_1(1-p_1)(1-p_2) \Big/ \bar{s}_f$$

and let $x_1 = p_1(1-p_2) \Big/ \bar{s}_m$

$$x_2 = p_2(1-p_1) \Big/ \bar{s}_m$$

Let observed $\Delta p_1 = y_1$ and observed $\Delta p_2 = y_2$.

For any one pair of simultaneous readings, the deviation

$$\delta = (y_1 - \Delta p_1) + (y_2 - \Delta p_2).$$

It was desired to minimise $\Sigma \delta^2 = \Sigma(y_1 - \Delta p_1)^2 + \Sigma(y_2 - \Delta p_2)^2$, for as selection was assumed to operate independently in the two sexes, Δp_1 and Δp_2 could be considered as independent.

Therefore $$\Sigma \delta^2 = \Sigma(y_1 - x_{11} s_{AA} - x_{12} s_{Aa} + x_{13} s_{aa})^2 + \Sigma(y_2 - x_1 s_a + x_2 s_a)^2$$

Five normal equations were obtained by putting -

$$\frac{\partial \Sigma \delta^2}{\partial s_{AA}} = \frac{\partial \Sigma \delta^2}{\partial s_{Aa}} = \frac{\partial \Sigma \delta^2}{\partial s_{aa}} = \frac{\partial \Sigma \delta^2}{\partial s_A} = \frac{\partial \Sigma \delta^2}{\partial s_a} = 0$$

This resulted in:-

(1) $S_{AA}\Sigma x^2_{11} + S_{Aa}\Sigma x_{11}x_{12} - S_{aa}\Sigma x_{11}x_{13} = \Sigma x_{11}y_1$

(2) $S_{AA}\Sigma x_{11}x_{12} + S_{Aa}\Sigma x^2_{12} - S_{aa}\Sigma x_{12}x_{13} = \Sigma x_{12}y_1$

(3) $S_{AA}\Sigma x_{11}x_{13} + S_{Aa}\Sigma x_{12}x_{13} - S_{aa}\Sigma x^2_{13} = \Sigma x_{13}y_1$

(4) $S_A\Sigma x^2_1 - S_a\Sigma x_1 x_2 = \Sigma x_1 y_2$

(5) $S_A\Sigma x_1 x_2 - S_a\Sigma x^2_2 = \Sigma x_2 y_2$,

which form two groups of equations, a set of three for the female selective coefficients, and a set of two for the male selective coefficients.

The analysis was first applied to the data from Population K, letting A represent the w^a gene, and its allele a the w gene. Trial values for the five selective coefficients were chosen, using values obtained by a preliminary application of the method of analysis of the previous section (C) to the data from Population K, when the cage had been maintained for only 38 weeks. (It should perhaps be pointed out that the analysis of this section(D) was undertaken before that of section (C); otherwise the selective coefficients in Table 4.17 could have been used as trial values.) $\bar{s}_f$ and $\bar{s}_m$ were calculated for each set of p_1 and p_2, and from these $x_{11}, x_{12}, x_{13}, x_1$ and x_2 obtained, using the definitions above. The two sets of equations were then solved for the selective coefficients, and the corrected values used to obtain trial values for a further iteration. The selective coefficients used in the

second trial were chosen as being ones which would satisfy the stability conditions for an equilibrium, without deviating too markedly from the calculated values from Trial 1, which did not themselves satisfy these conditions. The selective values chosen for both Trials 3 and 4 were in the same ratio as the corrected values resulting from the previous trial, using the corrected values of S_{aa} and S_a as standards. The results obtained in the successive iterations are shown in Table 4.19.

It can be seen that convergence did not occur, and the analysis was not further continued. In all cases the calculated selective value for the $w^a w^a$ female was greater than that for the heterozygote, and the w^a male had a greater selective value than the w male. Under these conditions equilibrium could not be established, and this might explain why the analysis, based on the assumption of an equilibrium, did not give more intelligible results. However, other assumptions were also implicit in the analysis. It had been assumed that the selective values were constant throughout the existence of the population, that the population reproduced in discrete generations, and that the observed gene frequencies were those after selection had completed its action in the life cycle. Failure of one or more of these assumptions might have been responsible for the observed results of applying the analysis, rather than the absence of a nontrivial equilibrium.

TABLE 4.19

RESULTS OF ADAPTATION OF WRIGHT'S ANALYSIS APPLIED TO POPULATION K.

Selective Coefficient.	Trial 1			Trial 2			Trial 3			Trial 4		
	Tried	Calculated	Ratio	Tried	Calculated	Ratio	Tried	Calculated	Ratio	Tried	Calculated	Ratio
S_{AA}	.99	.69	1.13	.70	.48	1.17	.37	.27	1.35	.25	.16	1.23
S_{Aa}	1.00	.61	1	.80	.41	1.	.33	.20	1.	.20	.13	1
S_{aa}	.80	.06	.10	.20	.06	.15	.05	.05	.25	.05	.04	.31
S_A	1.00	.75	1	.80	.58	1	.63	.45	1	.45	.32	1
S_a	1.02	.57	.76	.65	.46	.79	.50	.35	.78	.35	.25	.78

This method of analysis was not used for the other cages, as considerable labour was required for each iteration. Apart from the fact that it was inconclusive when applied to the data from Population K, the method suffered from the same defects as Wright's analysis of the autosomal case. These have been mentioned by Levene, (Levene, Pavlovsky & Dobzhansky, 1953). Basically, each Δp was considered as being independent of all the others. Hence the early changes in gene frequency, which would be greater in value than later ones, were given too great a weight in the fitting process. The order, as well as the magnitude, of successive Δps would need to be considered in a satisfactory analysis.

(E) ANALYSIS USING AN ADAPTATION OF CAVALLI'S METHOD.

The third method of analysing the data was a modification of that proposed by Cavalli (1950) for populations with two autosomal alleles in competition. It was supposed that selection was a process continuous in time, and its effects on gene frequency could therefore best be represented by a differential equation.

In adapting Cavalli's analysis to the sex-linked case, let the frequency of gene A in the female gametic output at time t be p_1, and that of the same gene in the simultaneous male gametic output be p_2. Then $q_1 = 1-p_1$ and $q_2 = 1-p_2$, are the frequencies of gene a in the female and male gametic outputs respectively at time t.

The genotypic frequencies resulting from these gametes are:

Genotypes:	AA	Aa	aa	A	a
Frequency:	p_1p_2	$p_1q_2+p_2q_1$	q_1q_2	p_1	q_1
Relative selective Values:	1-Adt	1	1-Cdt	1+hdt	1

Where dt is a small time interval, and A, C, and h the unknown relative selective coefficients.

In the time interval dt the change in female gene frequency dp_1, and the change in male gene frequency dp_2, will not depend solely on the magnitude of the relative selective values of the different genotypes. If no differential selection occurs in the population, and $p_1 \neq p_2$, the values of p_1 and p_2 will oscillate with decreasing amplitude about an average gene frequency $f = \frac{2p_1+p_2}{3}$, and hence dp_1 and dp_2 are functions of (p_1-p_2). In wishing to consider changes in gene frequency due to differential selection alone, therefore, it is advisable to consider changes in the average gene frequency f.

$$\text{Then } df = \frac{2dp_1+dp_2}{3}$$

$$= \frac{2}{3}\left\{\frac{p_1p_2(1-Adt)+\frac{1}{2}(p_1q_2+p_2q_1)}{1-Ap_1p_2dt-Cq_1q_2dt} - p_1\right\} + \frac{1}{3}\left\{\frac{p_1(1+hdt)}{1+p_1hdt} - p_2\right\}$$

$$= \tfrac{1}{3}\,dt\left\{q_1q_2(p_1+p_2)\,C - p_1p_2(q_1+q_2)\,A + p_1q_1h\right\}, \text{ ignoring terms in } (dt)^2.$$

This equation, unfortunately, is not very amenable to mathematical treatment.

A less general situation, but one which appeared to apply to most of the present experimental data, is where $p_1 = p_2$ at all times.

At equilibrium, Bennett's (1957) formula gives -

$\left(\frac{p_e}{q_e}\right)$ males $= (1+hdt)\left(\frac{p_e}{q_e}\right)$ females, so that for the gene frequencies to be the same in males and females h must = 0. This is equivalent to stating that there is no differential selection between the two male genotypes, and that differential selection occurs solely among the female genotypes of the population.

Then $df = \frac{1}{3}f(1-f)\{2C(1-f) - 2Af\}dt$

$$\frac{df}{dt} = \frac{1}{3}f(1-f)\{2C(1-f) - 2Af\}$$

which on integration gives

$$\frac{1}{2C}\log f + \frac{1}{2A}\log(q-f) - \frac{A+C}{2AC}\log\left|\frac{C}{A+C} - f\right| = \frac{1}{3}t + \text{const.} \quad (1)$$

At equilibrium $\frac{df}{dt} = 0$,

Therefore

$$p_e = \frac{C}{A+C}, \quad q_e = \frac{A}{A+C}$$

Equation (1) can then be rewritten as -

$$\frac{1}{p_e}\log f + \frac{1}{q_e}\log(q-f) - \frac{1}{p_e q_e}\log|p_e - f| = \frac{2}{3}(A+C)t + \text{const.} \quad (2).$$

This is of the form $Y = a+bt$, where $b = \frac{2}{3}(A+C)$

This is similar to Cavalli's equation for autosomal selection curves, and provides a transformation for the experimental data which will be linear with time. The problem of estimating the various unknown parameters can then be resolved by applying the same maximum likelihood method.

In practice, a trial value was chosen for p_e, by inspection of the data. For each observed f a transformed value y was calculated, using equation (2), and then a straight line $Y = a+bt$ fitted by eye to the graph of y against time. In the present data, unlike Cavalli's, the initial gene frequency was known exactly, and the 'a' of the straight line (Y_o = a at t=o) was therefore known. It remained to find corrections to the trial values of p_e and 'b' which would improve the fit of the theoretical model to the observations, and the following formulae were employed.

If n = number of genes observed in each sample,

f = $\frac{2p_1+p_2}{3}$ for each sample; p_1 and p_2 were not weighted by inverse variances, as in the case of the greatest differences in Population K f was altered by .7% only, and the extra labour was not considered to be justified by such a small correction.

y = transformed value of f, from equation (2).

Y = expected value of y, depending on value of 'b' chosen graphically.

P = expected value of f, obtained by means of the transformation formula, corresponding to each Y.

$s = (f-P)(p_e-P)$

$w = P(1-P)(p_e-P)^2$

$z = \frac{-1}{p_e q_e}\left\{\log\left(\frac{1-P}{P}\right) - \frac{1}{p_e-P} - (q_e-p_e)\,Y\right\}$

δb = correction to estimate of b,

δp_e = correction to estimate of p_e.

Then $\Sigma nst = \delta b \Sigma nwt^2 + \delta p_e \Sigma nwtz$
$\Sigma nsz = \delta b \Sigma nwtz + \delta p_e \Sigma nwz^2$
} give the scores in respect of the parameters and the matrix of the coefficients of adjustment, which is the matrix of information in respect of the estimates of the parameters.

These equations were developed in an identical fashion to those of Cavalli, the only difference being that only two parameters (b and p_e) needed to be considered. By inversion of the information matrix adjustments to b and p_e were calculated. Then the calculations could be repeated using corrected values for the parameters, until the corrections obtained were smaller than their standard errors (obtained from the diagonal of the inverted information matrix).

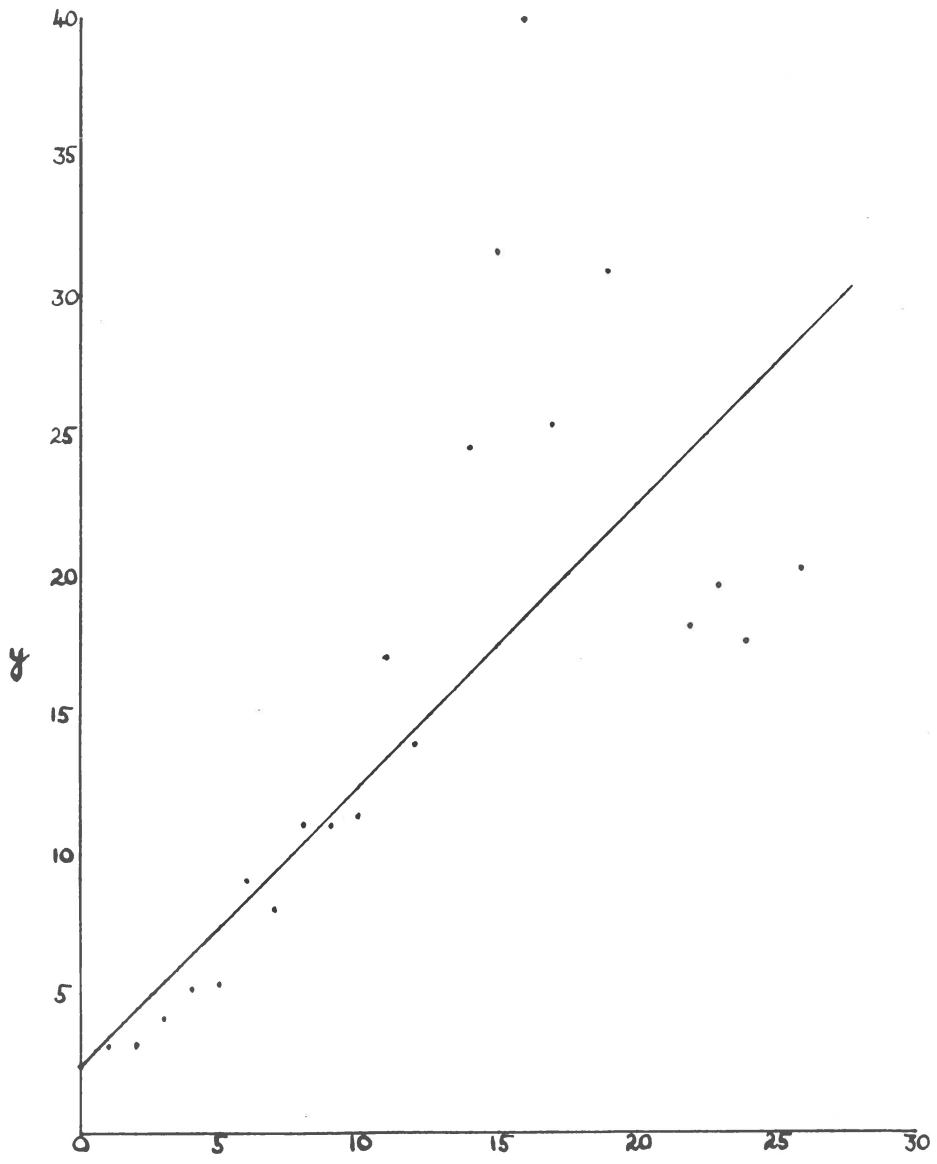
The goodness of fit could be tested after each correction had been made, by comparing observed and expected gene frequencies. The degrees of freedom of the χ^2 equalled the number of samplings less two, as two parameters had been estimated.

This method of analysis was first applied to Population K, taking w^a as the A gene and w as its a allele. Table 4.20 is an illustration of the calculations required, and shows the working in obtaining the first corrections for p_e and b. p_e was chosen to be .90, and figure 5 is the plot of y against time for the same cage, from which the first estimate of b = 1.00 was made.

FIGURE 5

PLOT OF y AGAINST TIME FOR POPULATION K.

p_e chosen as .90 b = 1.00



TIME in units of fourteen days

TABLE 4.20

WORKING FOR OBTAINING FIRST CORRECTIONS TO THE PARAMETERS FOR POPULATION E.

t	f	n	y	Y	P	e	w	z
0	.5	36	2.479	2.479	.5	0		
1	.5334	446	3.041	3.479	.5357	-.08379	.03300	1.16478
2	.5532	458	3.052	4.479	.6520	-.02450	.01395	11.96554
3	.6243	447	4.003	5.479	.7007	-.01523	.00833	16.49965
4	.6881	460	5.174	6.479	.7352	-.00776	.00530	21.17709
5	.6955	441	5.341	7.479	.7588	-.00894	.00364	24.94520
6	.7984	443	9.153	8.479	.7742	.00304	.00276	26.64575
7	.7889	450	8.042	9.479	.8024	-.00132	.00151	45.15629
8	.8236	454	11.010	10.479	.8126	.00096	.00116	50.28273
9	.8235	454	11.000	11.479	.8228	.00005	.00087	58.95116
10	.8274	460	11.364	12.479	.8330	-.00038	.00063	72.76893
11	.8648	451	17.011	13.479	.8433	.00122	.00042	94.84957
12	.8486	444	13.918	14.479	.8509	-.00011	.00030	116.94511
14	.8854	416	24.573	16.479	.8563	.00127	.00023	127.74021
15	.8923	459	31.662	17.479	.8590	.00137	.00021	135.71153
16	.9033	454	40.012	18.479	.8617	.00159	.00018	146.17674
17	.9114	429	25.373	19.479	.8644	.00167	.00015	159.54417
18	.9000	459	103.680	20.479	.8671	.00108	.00013	176.52771
19	.9072	452	30.937	21.479	.8699	.00112	.00010	199.32736
22	.9199	469	18.185	24.479	.8780	.00092	.00005	309.38825
23	.9180	458	19.532	25.479	.8807	.00072	.00004	371.43630
24	.9207	439	17.648	26.479	.8834	.00062	.00003	456.47488
26	.9169	459	20.365	28.479	.8888	.00031	.00001	762.01102

p_e chosen to be = .90
b chosen to be = 1.00
t is expressed in units of 14 days.
n varies, due to differing numbers of males and females in each sample of 300 flies.

$\Sigma nst = -16.48677$
$\Sigma nwt^2 = 514.35455$
$\Sigma nwtz = 3833.31224$
$\Sigma nwz^2 = 34616.46541$
$\Sigma nsz = 651.72416$

$$I^{-1} = \begin{pmatrix} .01112779 & -.00123226 \\ -.00123226 & .00016534 \end{pmatrix}$$

Therefore $\delta b = -.9866 \pm .1055$
$\delta p_e = .1231 \pm .0129$

For explanation and derivation of symbols, see text.

The calculated corrections led to the impossible value of p_e = 1.0281, preventing a further iteration being carried out. As an empirical approach, the goodness of fit between observed and expected gene frequencies was then found for a range of arbitrarily chosen values of p_e and b. The best fit obtained was for p_e = .9999, b = .57 (χ^2_{20} = 29.96, .05 < P < .10), which corresponded to A = .0001 and C = .8549, or selective values of .9999 and .1451 per unit time for the $w^a w^e$ and ww homozygotes respectively. The population therefore appeared incapable of establishing a stable non-trivial equilibrium, due to lack of significant superiority of the heterozygous females and low selective value of the ww homozygotes.

It was noticed during the empirical testing of various p_e and b values that the last seventeen readings seemed to form a group distinct from the first five readings in their contributions to the χ^2 for goodness of fit. This suggested that the hypothesis of constant selective values did not apply for this population, and that the early changes in gene frequency might perhaps be explained by a set of relative selective values which did not apply later in the history of the population.

A similar conclusion was reached when the analysis was applied to the data from Population L. The original trial values of p_e = .85, b = 1.1, resulted in a very poor fit of observed and expected gene frequencies, (χ^2_{21}=40.76, .001 < P < .01) but when calculated corrections were applied the agreement between observed and expected became worse (χ^2_{21}=62.96, P < .001).

The major contributions to the χ^2 values came from the last eighteen sampling results when using the corrected parameters, but from the first five results when using the uncorrected parameters, and it was decided that it would be impossible to reconcile the data with a single set of parameters. The two groups of readings were then treated as if they came from two different cages, and each rapidly led to a satisfactory result in the iteration procedure. The first group of five readings indicated an eventual equilibrium frequency p_e = .845 ± .059, with .912 ± .070 and .518 ± .220 as the relative selective values per unit time of the $w^a w^a$ and ww homozygotes respectively. In analysing the second group of eighteen readings the method of analysis had to be modified, as the "original" gene frequencies were no longer known, and three parameters had therefore to be estimated. The iteration procedure led to the conclusions of p_e = .848 ± .013, with .736 ± .179 and -.196 ± .929 as the selective values of the $w^a w^a$ and ww homozygotes respectively. Although such grouping of the data was purely empirical, it was of interest that the same equilibrium gene frequency resulted for the two groups, maintained by different relative selective values. It was concluded that the selective values had probably altered with time, and that any analysis based on constancy of the selective values would be inadequate for these data.

The same conclusion was drawn from the analysis of the data from Population R. p_e was first chosen as .95, and the resulting graph of y against time was curvilinear rather

than linear. Calculated corrections did not alter the lack of agreement between observed and expected gene frequencies, so the first nine sampling results were empirically chosen for separate treatment, and by testing of various p_e and b values a fit obtained for $p_e = .99$ ($\chi^2_7 = 12.30$, $.05 < P < .10$) with 1-A = .9973 and 1-C = .7327 as female selective values per unit time. These coefficients are equivalent to a trivial equilibrium only, as previously found for Population K. The fact that these two cages appeared to have achieved stable nontrivial equilibria when the equilibria when the equilibria alone were considered in Section C is perhaps further evidence that the selective values were not constant throughout the existence of the populations.

When the method of analysis was applied to the data from Population Q the results of the first two samplings had to be omitted in order to obtain a satisfactory conclusion. This exclusion was perhaps justified by the fact that the assumption of equal gene frequencies in the two sexes could not be expected to apply in the early stages of a population started with differing gene frequencies. The remainder of the data led to the conclusion of an eventual equilibrium gene frequency $p_e = .901 \pm .049$, with $.945 \pm .035$ and $.501 \pm .084$ as the relative selective values per unit time of the $w^a w^a$ and ww homozygotes respectively. This conclusion of a stable nontrivial equilibrium was in marked contrast to that obtained using the analysis of Section C, where the estimated relative selective values were incapable of satisfying the stability conditions.

It proved impossible to apply the modification of Cavalli's analysis to the data from Population M, as the f values showed no regular trend. It became apparent that no values of p_e and b would result in a satisfactory fit of observed and expected gene frequencies, even for empirically selected portions of the data, so the results had to be left unanalysed by this method.

The method of analysis also proved unsatisfactory when applied to the data from Populations N and O. In both cases the calculated corrections led to a poorer agreement of observed and expected gene frequencies, and although this suggested the failure of one of the assumptions on which the analysis was based, inspection of the data did not reveal an obvious cause. Empirical testing of a large range of values of the parameters p_e and b led to good fits being obtained for both populations. The best for Population N was for p_e = 88, with resulting relative selective values per unit time of .892 ± .034 and .208 ± .118 for the $w^s w^s$ and ww female respectively, and such an equilibrium should be stable non-trivial one. For Population O the best fit was for p_e = .98, with .983 ± .058 and .147 ± .261 as the two female relative homozygote selective values, but the stability of this equilibrium was more open to question due to the large error terms.

For the data from Population P, the analysis again proved incapable of estimating parameters which would give a satisfactory fit of observed and expected gene frequencies.

When the last seventeen sampling results were considered alone the iteration procedure led to $p_e = .99 \pm .02$, with female homozygote relative selective values of $.988 \pm .019$ and $-.233 \pm .074$, but as for Population O such selective coefficients were not likely to result in a stable nontrivial equilibrium.

Summarizing the results of applying this method of analysis to the data from cages with apricot and white in competition, in no case could the entire body of data be analysed satisfactorily. Empirical selection of portions of the data led to suggestions of varying selective values for Populations K, L, and R, and to conclusions of trivial equilibria for Populations O and P. The validity of the stable equilibrium for Population N was doubtful, as the result could only be obtained by empirical testing of various values of the parameters. Only for Population Q, by omitting the first two sampling results, did the method of analysis indicate a stable non-trivial equilibrium.

Similar results were obtained when the same analysis was applied to the data from the four cages with blood and white in competition. No analysis was possible for Population S, as extinction of the white allele occurred in males, and probably would have in females, and the existence of a non-trivial equilibrium was assumed in obtaining the original transformation of f values to y values. With the data from Population U, two applications of the iterative procedure led to estimated corrections for the parameters p_e and b which were smaller than their standard errors. However, the resulting

$p_e = .66 \pm .01$ corresponded to $\chi^2_{19} = 34.18$ $(.01 < P < .02)$ for agreement between observed and expected gene frequencies, and consequently little weight could be attached to the relative selective values per unit time of $.292 \pm .115$ and $-.376 \pm .204$ for the $w^{bl}w^{bl}$ and ww females respectively.

For Populations T and V, calculated corrections for the trial values of the parameters led to larger χ^2 values and consequently poorer agreement between observed and expected gene frequencies. For the data from Population V a range of values of p_e and b was tested, and the best fit resulted in $p_e = .99$, with the $w^{bl}w^{bl}$ females having a relative selective value of .99. As this was equivalent to a trivial equilibrium only, the original application of the analysis was not valid, which was perhaps the cause of the observed non-convergence of the iterative procedure. If a similar explanation accounted for the nonconvergence found for Population T, it could be concluded that three of the four populations with blood and white in competition either led or would have led to extinction of the white allele, while the fourth population might perhaps have established a stable nontrivial equilibrium.

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5. DISCUSSION AND CONCLUSIONS.

Of major interest in an experiment consisting of competition between different genotypes in a controlled environment are the relative selective values under such conditions. A knowledge of the magnitude and nature of these values can lead to a better understanding of selective processes under natural conditions, and can help explain how genetic material is organised to deal with its environment with maximum efficiency. It is not of great value merely to demonstrate that differential selection has occurred, as for theoretical reasons one may assume it does unless conclusive evidence is presented to the contrary. In the present series of experiments the gradual but definite changes in gene frequency are evidence that selection was in fact operating. However, attempts to estimate relative selective values led to a number of contradictions, and it seems worth considering whether these were due to failure of technical or analytical procedures, and whether the inferences that could be drawn from the experimental results were of more than superficial interest.

With regard to the design of the cages, it was desired to maintain populations large enough to ignore random drift effects, so that changes in gene frequency could be attributed mainly to selection. The total counts of flies made in the early weeks indicated that sufficiently large populations were soon established, so that in this respect the cages seemed satisfactory.

Competition between two pseudo-alleles resulted as expected in slow changes in gene frequency, from which one would suppose that the selective differences between genotypes were small. Although no two populations were exact replicates they all agreed in showing a reduction in the frequency of the white pseudo-allele. Environmental variations cannot therefore have been of major importance, although complete environmental control could not be achieved. Drosophila melanogaster is known to be susceptible to humidity variation (Spencer, 1950 and regulation of this environmental constituent would be desirable in experiments of this type. Even had it been controllable, however, complete equality of environment between cages, and even within different areas of the one cage, is probably a practical impossibility. The circular structure of the present cages, and the regular replacement of food every two days, should have reduced wide fluctuations in internal environment such as occurred in the population-bottle units designed by Reed and Reed (1948). This is surely important when any analysis of selective values is attempted. If cyclic variations in food supply, for instance, are spread over a period of time greater than the average generation time of the organism concerned, the calculated selective value for a genotype will not apply in each generation, but will be the average of the values applicable to a number of generations.

It is important that any technique for sampling a population should be a random one, and it is difficult to see in what manner any bias could have been introduced by the

sampling method described for the present experiment. It is also important that sampling variation should be kept to a minimum, which means that the number in each sample should be as large as practicable. The present sample size of 300 was larger than that selected by Wright and Dobzhansky (1946), who regularly analysed a total of 300 autosomal chromosones, whereas the number of sex-linked genes here classified varied about a mean of 450.

The effects of misclassification also need consideration in discussing experimental techniques, and data from the random mating tests are relevant in this connection. It was noted that although neither of the two tests showed a significant difference between the classification of females as scored visually and as scored by progeny testing, in each case the visual misclassification did result in a deficiency of recorded heterozygotes. This deficiency was more marked in the first of the two tests than in the second, conducted sixteen weeks later. However, as the population had been sampled regularly during the thirty weeks prior to the first test, the reduced misclassification of the second test is not likely to be due to increased proficiency in scoring with time on the author's part. Despite lack of significance of the two χ^2 values (Table 4.13), the suggestion remains that the frequency of female heterozygotes may have been underestimated throughout the experiment, and that consequently the frequency of the w^s gene as calculated may be greater than the true value, although not significantly so.

Many authors have analysed sexual preferences in mating by placing two genotypes of one sex with only one genotype of the other sex (e.g. Merrill, 1949; Thomson, 1961). It is difficult, however, to extrapolate from the resulting male and female preference data to the situation found in any natural or artificial population, where in a different environment from that of the mating tests both sexes are simultaneously able to exercise a preference in mating partner. The isolation of females taken from the cage, and the determination by progeny analysis of the mating they had undergone when in the cage, avoided the criticism outlined above. To prevent affecting the subsequent history of the cage, however, the sample of isolated females had to be a random one, with the result that the rarer genotypes were poorly represented and little information could be obtained as to their matings. Even had they exhibited strong mating preferences these would not have been detected. This deficiency in the technique of detecting non random matings could have been avoided had the tests been conducted earlier in the history of the population, when the genotypes would have been more equally represented in any random sample, but as it is the conclusions must be viewed with caution.

Turning to a consideration of the egg sampling experiments, scoring was carried out on the adults resulting from the egg samples taken from the cage to a more favourable environment. These genotypic frequencies were shown not to differ significantly from those expected from the gene frequencies among the adults within the cage at the time of

removal of the eggs, on the hypothesis of random mating and no gametic selection. As no departure from random mating was indicated in the previous experiment, this test was then one to detect significant gametic selection. The lack of significance in both experiments lends support to the assumption, made in subsequent analyses of the population cage data, of there having been random mating and no gametic selection within the cages, so that the observed gene frequencies among adults could be taken as those among gametes uniting at random to form the next generation. However, it is clear that the numbers involved in the test for the existence of gametic selection were too small to enable detection of very small selective differences, although no calculation was made to determine the limit of detection. Too large a sample of eggs could not of course be removed from the cage at any one time without adversely affecting the population, so this technique is probably better adapted to situations where large gametic selective differences might be expected, as for example with the "sex-ratio" condition in Drosophila pseudoobscura.

The same experiment was not able to demonstrate the existence of differential zygotic selection within the cage, although selection must presumably have operated at some stage of the life cycle to account for the observed changes in gene frequency. Comparisons were made between the genotype frequencies observed among adults in the cage at the time of emergence of the adults from the egg samples, and the genotype

frequencies of these emerging adults. The latter were presumed to have developed without differential zygotic selection occurring, the reverse being true for individuals developing within the cage. The lack of significance found for the comparisons does not mean that no differential zygotic selection in fact occurred within the cage, as the adults sampled from the cage would not all have been at the egg stage at the time the egg sample was removed, and this would add difficulty to making the desired comparisons. It had been hoped, however, to demonstrate the existence of differential selection within the cage by experimental as well as by analytical techniques.

Turning from consideration of experimental procedures to those of analysis, the situation is less satisfactory. Given a population in a condition of apparently stable equilibrium, the relative selective values of the different genotypes can readily be calculated from the equilibrium genotype frequencies, and this has been done for natural populations showing polymorphism for alleles at an autosomal locus (e.g. Allison, 1954). The same technique can of course be applied to any experimental population which achieves an apparently stable equilibrium. However, in most laboratory populations the gene frequency changes are recorded as an equilibrium is approached, and in many cases it is difficult to determine when a true equilibrium has been achieved, or even whether elimination of one allele would occur if the population were maintained for a longer period of time. When selective

differences are small the final elimination of one allele is expected to be very slow in terms of the number of generations required (Fisher, 1930). It is tempting to speculate whether this may be an explanation for the results obtained by Lewontin (1958), when he maintained Drosophila pseudoobscura populations containing two third-chromosome gene arrangements for over three years in a constant environment. Lewontin recorded a gradual change in chromosome frequency, towards elimination of the rarer gene arrangement, from that which he had considered as an equilibrium value. He attributed this change to a change in relative selective values, due to a stable environment reducing the evolutionary advantage to the population of a polymorphic system. The present author attempted to analyse the results from Lewontin's cage C by the method of Cavalli (1950), using the data recorded both before and after the establishment of the "equilibrium". Using a trial value of $p_e = .95$, the calculated corrections led to a value of the equilibrium chromosome frequency greater than unity, implying complete elimination of the rarer inversion. An attempt to discover empirically chosen values of the parameters which would lead to agreement between observed and expected chromosome frequencies was unsuccessful, the best fit obtained being for $p_e = .99$ ($\chi^2_{13} = 23.39$, $.02 < P < .05$) which cannot be considered as establishing the existence of a stable nontrivial equilibrium. Although the method of analysis is not applicable unless a nontrivial equilibrium is possible, the above result of its application does suggest that even without changing

selective values no stable non trivial equilibrium was possible for this population. An alteration to the original selective values would not then need to be postulated, as the observed "equilibrium" was perhaps a spurious one.

This example illustrates the difficulty of determining whether an experimental population has in fact achieved a stable equilibrium. The method used for the present data was not entirely satisfactory, particularly when only a small number of samples could be regarded as coming from the equilibrium population. The statistical procedure employed allowed the size of the sample to influence the number of readings considered as equilibrium ones, and therefore the estimates of the equilibrium gene frequency were affected by the arbitrarily chosen number of individuals examined at each sampling. The estimated frequency of the more common allele may in any case be lower than the true equilibrium frequency, if such exists, either from the fact that equilibrium had not been quite attained, or that earlier samplings prior to the establishment of the equilibrium were included as being from the equilibrium population, so lowering an overall estimate of gene frequency. Considering the magnitude of the error terms for the present calculated gene frequencies, however, a slight variation in equilibrium value would not have significantly altered estimates of the relative selective coefficients.

The advantage of working only with equilibrium gene and genotype frequencies in determining relative selective values

was, apart from ease of computation, that the sole assumption necessary was constancy of these relative selective values as long as the equilibrium was maintained. The disadvantages, however, in experiments like the present one, were that the results of a large number of early samplings were not considered in the analysis, and that the populations had to be maintained for a long period of time to satisfactorily establish the existence of an equilibrium. These two disadvantages did not occur in other methods of analysis developed for populations polymorphic for alleles at an autosomal locus, nor in the present modifications of these analyses for the case of a sex-linked locus. Wright's approach to the problem, as well as that of Cavalli, used all the data from the samplings of the population, and did not require an equilibrium to have been attained before relative selective values could be determined. Against this, however, is the fact that many more assumptions had to be made in applying these analyses, some of which might not have been justified.

Wright's theoretical model assumed discrete generations, and this is obviously untrue in a population cage. It is difficult to estimate the importance of this particular lack of correspondence between the model and the population to which it is applied. Generally it has been assumed to be of little significance (e.g. Edwards, 1961), but this assumption has been made when considering cases of populations either near or at equilibrium, when the gene frequency will be subject to slow changes per time unit. It may well be more

important in an analysis of populations far from equilibrium, when larger changes in gene frequency are expected in each selected time interval. In a population with overlapping generations a particular set of relative selective values will result in smaller observed changes in gene frequency per time unit than would be observed with the same relative selective values in a population with discrete generations. This is due to the fact that the techniques of sampling a population allow inclusion of individuals which have contributed to an earlier estimate of gene frequency in the former case, but not in the latter. With large changes in gene frequency expected from one sampling to another, this may lead to considerable differences between the gene frequency estimates from the two populations. Hence the equating of a model assuming discrete generations and an actual population having overlapping generations may well lead to erroneous estimates of the relative selective values.

The analyses of both Wright and Cavalli also assumed constancy of relative selective values throughout the history of the population, and, more serious perhaps, were not capable of indicating when this assumption alone was not valid. It will obviously be difficult to determine relative selective values if these are not constant, but it would seem desirable to be able to detect the existence of changing relative selective values when such occur. Another assumption common to both analyses was that the gene frequencies could be estimated after selection had completed its action in the life cycle, so that

in any application of the models the estimated gene frequencies could be taken as those in the gametes which united at random to form the next generation. It is difficult to justify the assumption that no further differential selection occurred during the lifetime of the sampled individuals. Tests could be made for the absence of gametic selection and the existence of random mating in the population. However, if either of these tests had been significant, the gene frequencies estimated from adult zygotes would not have been those in the gametes uniting at random; even if these latter frequencies could then have been deduced, it would have been difficult to interpret the calculated relative selective values, for the effects of nongenotypic selection would then have been included in a value assigned to a genotype.

Cavalli's analysis also presupposed the existence of a non trivial equilibrium, and could not be used to determine the relative selective values responsible for the elimination of one allele. In its present adaptation to the sex-linked case, it also suffered from the particular restriction of the assumption of $t_A = 1$, implying no differential selection in males and consequent equality of male and female gene frequencies at equilibrium. Wright's approach considered only the magnitude and not the order of gene frequency changes. This could result in absurd conclusions from an application of the analysis, as shown by Levene, Pavlovsky, & Dobzhansky (1954). A hundred generations with no chromosome frequency changes were theoretically added to existing data from a

population polymorphic for an autosomal inversion. However, when Wright's analysis was applied to this situation of a very stable equilibrium, the resulting relative selective values were such that no stable equilibrium could have been maintained. This contradiction was due to the emphasis given in the analysis to the initial large changes in chromosome frequency, and the relatively small weight given to the many successive zero changes in chromosome frequency.

With the above assumptions and criticisms in mind, a few suggestions can be made as to the requirements of ideal methods of analysis for data from laboratory populations containing either autosomal or sex-linked genes in competition. Such an analysis should make use of all the sampling data, and consider order as well as magnitude of observed gene or genotype frequency changes. It should be capable of application when no equilibrium can be established, perhaps deducing relative selective values from the rate at which one allele is eliminated, and should preferably give an estimate of the equilibrium gene frequencies, if such exist. Overlapping generations should perhaps form the basis of the model from which the analysis is derived, and the possible detection of changing relative selective coefficients would be a practical advantage. Similarly, separate estimation of gametic and zygotic relative selective values could be of advantage in understanding the method of selection in some populations. Other subdivisions of the overall selection could perhaps be informative also, when considering particular populations.

This set of requirements will be difficult to satisfy, even for the case of populations allowing competition between alleles at an autosomal locus, and the difficulties will be increased in the sex-linked case where more parameters are required to describe the population. The present data are the most extensive available for large laboratory populations with sex-linked alleles in competition and can serve for the application of a satisfactory method of analysis when one is developed.

The conclusions which could be drawn from the present analyses of the data contain a number of contradictions. When the equilibrium gene frequencies alone were considered, the existence of stable equilibria was indicated for two populations, K and R, although for the latter population it had not long been established. These were the only populations for which the relative selective values of both female homozygous genotypes were less than unity, and when the equilibrium gene frequencies do not differ in the two sexes this is a necessary and sufficient condition for a stable equilibrium. All the populations but M and Q showed no significant differences between the equilibrium gene frequencies in males and females, so with these exceptions the female homozygote relative selective values should all have been less than unity. Populations L, O, and P could perhaps be considered to satisfy this requirement, but Populations M, N, and Q could not be in stable equilibrium with the calculated relative selective values

for the homozygotes, as all these values were too great in magnitude. The possible effects of misclassification are relevant in this connection. As the frequency of the female heterozygous genotype appears in the denominator of the formulae for estimating S_{AA} and S_{aa}, an increase in this frequency, and consequent decrease in the frequency of the W^aW^a genotype, would have resulted in lower values for the relative selective coefficients of the female homozygotes. Misclassification did occur in this experiment, and, although not statistically significant, was such that the estimated relative selective values for the female homozygotes would have been greater than the true values for the populations.

Comparing these conclusions with those drawn from the modification of Cavalli's analysis, the results from the latter approach gave no indication of stable equilibria for Populations K and R, and seemed to indicate that the W gene would have been eliminated had these populations been maintained for a longer period of time. There was also a suggestion of the selective values having varied with time, which could not have been detected in an analysis considering only equilibrium frequencies. The analysis of the data from Population L also suggested changing selective values, and when applied to Populations O and P the method led only to the conclusion of eventual elimination of the W allele. Population N might perhaps have achieved a stable equilibrium, as might Population Q. However, as Populations M and Q had been shown to have different equilibrium gene frequencies in the two sexes, the

application of this modification of Cavalli's analysis was not valid, as the simplifying assumption of $t_A = 1$ (which implies equality of the gene frequencies in the two sexes) had had to be made. There were a number of other assumptions made for this method of analysis, so that the conclusions must be considered as being very tentative.

An experiment might well be conducted to determine whether relative selective values do alter with time in such a laboratory population. A large sample of flies could be removed from a cage after changes in gene frequency had been observed, and used to start another cage, with gene and genotype frequencies adjusted to the original values of the first cage. Changes in the second cage paralleling those of the first would then indicate no change in selective coefficients. Faster (or slower) changes in gene frequency would suggest that the relative selective values had altered in the first cage from the time of its inception to the time of establishment of the second cage. This difference in the selective values could be accounted for by mutational or recombinational events. Further work might also investigate the possible dependence of relative selective values on gene or genotype frequencies in a population. This question should preferably be examined using population cages, rather than by analysing selection at different stages of the life cycle outside a cage, as it is difficult either to enumerate or investigate all possible stages of the life cycle where differential selection may operate.

One final matter which emerged from the present experiment was the remarkable heterogeneity of the sex ratio observed in all the cages. A total of 35% of the samplings had a proportion of males differing significantly from .5, whilst for individual cages the percentage of significant samplings varied from 5% for Population U to 82% for Population S. There were more significant excesses of males than of females, mainly due to two populations, N and S, which showed 12 and 17 significant excesses of males respectively to only one significant excess each of females. Even without these two cages there were 29 significant excesses of males and 21 of females in a total of 185 samplings from ten populations. No possible association could be detected between these significant departures from a 1:1 sex ratio and changes in either gene frequency or population size (the latter for the early stages of each population only, as counts were not continued beyond twelve weeks).

If the sampling technique used throughout the experiment had been responsible for a non random selection of the two sexes, the similarity of the method used in obtaining each sample should have resulted in homogeneity of the sex ratios, even if these latter had differed significantly from the expected 1:1 ratio of males: females. It would therefore seem that the observed heterogeneity of the sex ratios could not be ascribed to deficiencies in experimental techniques, and in consequence a genetical or environmental explanation of the significant deviations must be considered.

The presence at a particular locus of an allele capable of influencing the relative proportions of the sexes is not very likely. Not only would such a gene have had to be maintained by chance through many generations of single-pair matings, in twelve different lines, prior to the establishment of the populations, but it is hard to suggest a possible mode of action for either an autosomal or sex-linked gene which could result in the observed fluctuations of the sex ratio. If such a gene affected the viability of one sex alone, fluctuations in its frequency could explain the recorded data on the frequencies of the sexes; however, the necessary variation with time in the frequency of the gene would imply the existence of random drift effects, which could only occur if the effective mating population were much smaller than the total population. If this were true, similar random drift effects should have been detected for the eye-colour gene frequencies, but no such evidence of drift was observed.

The suggestion arose earlier that the relative selective values of the eye-colour genotypes had altered with time. Genotypic selective values which varied so as to result in changing average fitnesses of the male and female subpopulations could lead to varying sex ratios. In this case, as above, one would then expect a relationship to exist between the significant excesses of one sex and the changes in gene frequency at the W locus, and no such association could be detected.

Deviations from equality in numbers of the sexes have been reported for laboratory population studies employing Reed

and Reed's (1948) small population-unit technique. In these cases, however, the populations were sampled when environmental conditions were at their most adverse in terms of food supply, and any viability differences between the sexes might then be expected to lead to an excess of one sex. Usually an excess of females has been reported, although Merrill and Underhill (1956) found an overall frequency of males greater than .5 for some populations with competing sex-linked alleles at the W locus. These authors did not present their original data, but stated that the sex ratio was very variable, as were the frequencies of the eye-colour genes. Since fluctuations ascribable to random chance are to be expected in these small populations, the observed sex ratios might have been due to chance effects alone.

However, Thomson (1961) reported variable sex ratios in large laboratory populations containing the same sex-linked pseudo-alleles as those studied by Merrill and Underhill. Here random chance could not account for the data, and the author stated that the excesses of one or the other sex appeared to be an intrinsic character of each population. As there seems to be no association between the sex ratio and changes in gene frequency at a sex-linked locus, either in Thomson's experiment, or in the one reported by the present author, it seems probable that such variable sex ratios may occur in other laboratory populations where sex-linked alleles are not segregating. However, the published data for large laboratory populations with competing autosomal

alleles have not been presented with the sexes recorded separately, so it is not at present possible to verify this suggestion.

Apart from the fact that alleles at the same locus were studied, there was another similarity between the experiment reported by Thomson and the present one, which may be relevant in explaining the data on sex distribution. In both cases the populations were believed to be homozygous at all loci, except perhaps those closely linked to the W locus. If an homeostatic mechanism for sex ratio control were postulated, with a predominantly autosomal inheritance of this control system, disturbances in sex ratio independent of changes in gene frequency at a sex-linked locus could be accounted for. Accepting Lerner's (1954) theory of heterozygosity per se conferring balance in a normally outbreeding species, very small fluctuations in the environment could lead to large responses by the sex ratio control mechanism in these autosomally homozygous populations.

An experiment might well be conducted to test the above hypothesis, by maintaining large laboratory populations with varying degrees of autosomal homozygosity, and recording any variations with time of the sex ratio. The effects of the presence of mutant genes on the homeostatic system could also be examined, by comparing the variations of the sex ratio in wild type populations with those in populations either homozygous or segregating for different mutants. There is also the theoretical problem to be considered of how a system of

gene balance in individuals of a population could react to environmental fluctuations so as to affect a population characteristic such as sex ratio. This general question of sex ratio variation in laboratory populations is of great interest, and warrants a detailed investigation.

Considering the results of the analyses of the population cage data, it would appear that the relative fitnesses of the different genotypes did not depend on the amount of pigment present in the eye of the fly. In the two populations which achieved an equilibrium, not only did the heterozygous female genotype have a greater selective value than the darker-eyed homozygous female genotype, but no selective differences existed between the male genotypes, one with an eye-colour pigment and the other completely without. Furthermore, as there was no evidence of differential gametic selection within the populations, it would appear that differential selection occurred during the zygotic stages of the life cycle of females alone. For much of this time, and particularly during the intense larval competition for food, no eye-colour pigments are present. It would therefore seem that these alleles have effects on their carriers other than the production of adult eye-colour pigments, and are to this extent pleiotropic.

With regard to the maintenance of sex-linked polymorphisms, it has been shown that stability can be achieved by two different possible mechanisms. Either one allele can be at an advantage in one sex and at a disadvantage in the other sex, or no selective differences need occur in the

heterogametic sex, together with a selective superiority of the heterozygotic genotype in the homogametic sex. The latter mechanism is theoretically a rather special case, for it results from taking $t_A = 1$, instead of allowing the parameter to vary. In practice, however, this may be the more common method of maintenance, for sex-linked polymorphisms are likely to occur in species which also exhibit autosomal polymorphisms, and which have therefore evolved mechanisms for obtaining the necessary heterozygotic superiority. The equilibria found in the present experiment were certainly of this second type, and it will be of interest to see which mechanism of control occurs with the greater frequency as more sex-linked polymorphisms are studied.

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