MEASUREMENT OF EFFECTIVE GENERATION LENGTH IN DROSOPHILA POPULATION CAGES

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MUCH of the discussion in population genetics is in terms of discontinuous models with discrete generations, whereas most real populations change continuously with overlapping generations and reproduction at various ages. The usual procedure to measure selection coefficients in a *Drosophila* cage population is to estimate an average generation length from knowledge of the life cycle or from other experiments and apply this to the analysis of measurements made from the cage population, using a discrete model as an approximation. We give here a procedure that is sometimes applicable, whereby the effective generation time in a continuous population can be measured directly from the rate of change in genotype frequencies in the cage.

With discontinuous populations we write equations for gene frequency changes in the form, $\Delta q = f(q, w_1, w_2, \dots)$, where q is the gene frequency, the w 's are fitnesses of the various genotypes, and f is a function of these and perhaps other quantities. For a continuously changing population we can write the rough equivalent, $dq/dt = \Delta q$, where t is time measured in units of generations. The generation time is a complex function of the age-distribution and the age-specific reproduction rates of the populations. However, when the population reaches a stable age distribution, the gene frequency will change so that, with a suitably chosen measure of generation time, the change per generation corresponds to that in a population with discrete generations.

We therefore think of the *effective* generation length as a period of time such that the gene or genotype frequency change during this time corresponds to that in one generation with a discrete model. If the w 's are known, we can plot the change in gene or genotype frequency and then determine the effective generation length from the time that has elapsed. The calculation will be simpler if some transformation of the gene or genotype frequency can be found that is expected to change linearly with time; then the frequency can be plotted against time and the generation length will be a simple function of the slope.

It is not ordinarily possible to measure the w 's independently of the rate of gene frequency change. However, some experimental situations afford this opportunity. One such is a dominantly marked chromosome that is advantageous when heterozygous but lethal when homozygous. Thus the homozygous fitness is known to be

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zero and the relative fitness of the other two genotypes can be determined from the equilibrium frequency that is finally attained.

EXPERIMENTAL PROCEDURES

The details of the experiments are given in the following paper by CHUNG (1967). *Drosophila melanogaster* were grown in small plastic cages which maintain a population of several hundred adults. The food was in eight shell vials with the oldest replaced every 2 days, so that each vial remained 16 days. Samples of 200 adults were counted weekly.

The bristle mutant, Stubble (Sb^w), was used as a marker. This dominant mutant was collected several years ago from a "wild" population in a Madison, Wisconsin, fruit warehouse. It is usually associated with one or more inversions and some combinations are heterotic. FRYDENBERG (1963, 1964) has published a detailed analysis of the associative overdominance in this system. Sb/Sb homozygotes are lethal in the larval stage.

The Stubble chromosome was put in various backgrounds and one set of data is shown in Figure 1. There were two initial frequencies of $Sb/+$ flies, 100% and 20%. Each curve represents the average of two experiments that gave similar results; this was done in an effort to smooth out some of the irregularities. Figure 1 shows the curves for a population in which the Sb chromosome had a relatively slight heterozygous advantage (the average of EAI-1 and EAI-2, and of EAII-1 and EAII-2, in CHUNG's experiments).

The populations were clearly reaching an equilibrium; this is especially convincing since the same value was approached from opposite directions.

METHOD OF ANALYSIS

Let Q be the proportions of $Sb/+$ in the adult population and $P = 1 - Q$ be the proportion of $+/+$. Assume that $Sb/+$ and $+/+$ zygotes survive to adulthood in ratio $s:1$ and that the fertility of $Sb/+$ and $+/+$ adults is in the ratio $f:1$. We then have, with random mating and discrete generations:

Generation	Ratio of frequencies		
	$+/+$	$+/Sb$	Sb/Sb
t adults	P	Q	0
$t + 1$ zygotes	$(P + (Qf/2))^2$	$(P + (Qf/2))Qf$	$(Qf/2)^2$
$t + 1$ adults	$(P + (Qf/2))^2$	$(P + (Qf/2))Qfs$	0

FIGURE 1.—Proportion of Stubble flies (Q) at various times (T) measured in weeks. Each curve is the average of two experiments. The estimated relative fitnesses of the three genotypes, $+/+$, $Sb/+$, and Sb/Sb , are 0.57 : 1.00 : 0.00.

Thus γ' , the ratio of $+/+$ to $+Sb$ in $t+1$ adults is

$$\gamma' = \frac{P + (Qf/2)}{Qfs} = \frac{\gamma}{sf} + \frac{1}{2s}, \quad (1)$$

where $\gamma = P/Q$, and

$$\Delta\gamma = \gamma' - \gamma = \gamma \left(\frac{1-sf}{sf} \right) + \frac{1}{2s} \quad (2)$$

If there is an equilibrium, $\Delta\gamma = 0$, and the equilibrium value of γ is

$$\hat{\gamma} = f/2(sf - 1). \quad (3)$$

Substituting from (3) into (2) leads to

$$\Delta\gamma = K(\gamma - \hat{\gamma}), \quad (4)$$

where

$$K = (1-sf)/sf = -1/2\hat{\gamma}s. \quad (5)$$

Equation 4 is approximately the same as the continuous equation

$$d\gamma/dt = K(\gamma - \hat{\gamma}) \quad (6)$$

where t is time measured in units of effective generation length. This integrates into

$$(1/K) \ln(\gamma - \hat{\gamma}) = t + C \quad (7)$$

or $Z = t + C$, where $KZ = \ln(\gamma - \hat{\gamma})$ and C is a constant determined by the initial gene frequency.

We now let T be the time measured in absolute units (weeks, in these experiments). Then $T = t\Delta T$ where ΔT is the effective generation time. Thus

$$Z = (1/\Delta T)T + C \quad (8)$$

If Z is plotted as the ordinate against T as abscissa there will be a linear relationship and the generation time, ΔT , is given by the reciprocal of the slope. A significant departure from linearity indicates that the assumptions (e.g. constant genotypic fitness throughout the duration of the experiment) are not met, or that K or $\hat{\gamma}$ is incorrectly estimated.

Two quantities are needed, $\hat{\gamma}$ and s . The former can be determined from the equilibrium ratio of $+/+$ to $Sb/+$ in the experimental population. To measure s we take advantage of the fact that in weeks 1 and 2 of the populations starting with 100% $Sb/+$ all the flies are descendants of identical parents; hence fertility selection cannot influence their ratios. The expected proportion of Stubble progeny from $Sb/+$ parents is

$$Q = 2s/(1+2s) \quad (9)$$

from which s can be estimated.

RESULTS

The results of plotting Z against T are shown in Figure 2. These are the transformation of the points from the upper line in Figure 1. The lower line is nearly useless for this purpose because of the very slight change in Stubble frequency. The equilibrium value, $\hat{Q}$, was taken as 0.3, so that $\hat{\gamma} = (1 - \hat{Q})/\hat{Q} = 2.33$. The average Q for $T = 1$ and 2 was .62. Substituting this into (9) leads to $s = .816$. From these values $Z = -2\hat{\gamma}s \ln(\gamma - \hat{\gamma})$ was computed for each experimental point and plotted as shown in Figure 2.

Using equation (3), we can obtain f from s and $\hat{\gamma}$. In this case $f = 2.137$. The fitness of the heterozygote, relative to the $+/+$ homozygote, is sf or 1.74. In more conventional terminology, the relative fitnesses of the three genotypes $+/+$, $Sb/+$, and Sb/Sb are 0.57 : 1.00 : 0.00.

The large fluctuations in the points at the right side reflect the magnification of small differences near the equilibrium value with this transformation. This makes it important to weight each point by its amount of information. The line was fitted by least squares with each point weighted by the expected value of the

FIGURE 2.—The data from the upper part of Figure 1 with a linearizing transformation. The line is fitted by least squares weighting each point by its invariance. The generation length, estimated by the reciprocal of the slope, is 2.93 weeks.

reciprocal of its variance. The large sample variance of $Z (= -2s\hat{y} \ln (\gamma - \hat{y}))$ is given by

$$V(Z) = 4 \left(\frac{s\hat{y}}{\gamma - \hat{y}} \right)^2 V(\gamma) \quad (10)$$

where $\gamma = (1 - Q)/Q$. If Q is binomially distributed, this becomes

$$V(Z) = 4 \left(\frac{s\hat{y}}{\gamma - \hat{y}} \right)^2 \frac{1 - Q}{NQ^3} \quad (11)$$

where Q is the proportion of Stubble flies and N is the number counted. The weights were determined from the expected values of Q and γ , which were obtained from a line drawn by eye. It can be seen from (11) that the weights become very small near the equilibrium. The least squares line was used to get new weights and the whole process was repeated until further iterations gave no improvement. The final fitted line is shown in Figure 2. The point corresponding to $T = 0$ was not used in the calculations.

As the population gets close to the equilibrium value, Z becomes highly variable, but as mentioned earlier the weights in this region are very small. If the frequency fluctuates across the equilibrium value the transformation becomes meaningless, since it involves the logarithm of a negative number.

The effective generation time, given by the reciprocal of the regression of Z on t , is 2.93. We have not tried to give a standard error for this, since we think it better to do repeated experiments and determine the precision of the estimate from the reproducibility of the value in different experiments.

Equation 4 is appropriate for discrete generations whereas we used the same form in equation 6 as if it were appropriate for continuous change. If the population is at equilibrium with regard to age distribution and the gene frequency

change per generation is small the difference between the two equations is slight. On the other hand, with rapid change the discrepancy could be quite large. Therefore this procedure is appropriate only when changes are relatively slow.

These experiments could have been improved by starting all population cages at or near the equilibrium age distribution. Departures from such demographic equilibrium probably account for most of the irregularities in the early generations. The experiments were not originally designed for this purpose and the emphasis of this note is on the method rather than the numerical value obtained.

This, and other estimates made by the same method, are in fair agreement with the value of 15 days which FRYDENBERG (1962) obtained by a totally different method. Until more and better data are obtained, we take as a provisional estimate of generation length in this type of population cage about two and a half weeks.

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SUMMARY

When one homozygote is lethal and the other is less fit than the heterozygote, it is possible to measure the effective generation length in a population cage with overlapping generations and variable age of reproduction. The generation length is defined as a period of time such that the gene frequency change during this period equals that in one generation with a discrete model.—Using a transformation making the expected change linear with time, the generation time can be found from the slope of the regression line. From *Drosophila* population cage experiments involving the dominant mutant, Stubble bristles, the generation length was estimated to be about $2\frac{1}{2}$ weeks.

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