FITNESS AS A MEASURABLE CHARACTER IN DROSOPHILA

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THE discussion of evolutionary problems in terms of modern genetics involves the concept of the "fitness" of different genotypes—the average ability of organisms to pass on the genes they carry to the next generation. By variations in "fitness", the array of gene frequencies is shaped from generation to generation.

As a measurable character, it is to the quantitative geneticist one extreme of the range, a character of whose function in natural selection there can be no doubt. Despite its theoretical importance, there has been surprisingly little experimental work devoted to direct measurements of fitness. There is notable work in which the relative fitnesses of different genotypes have been inferred from changes in gene frequency (for instance LUDWIN 1951; REED and REED 1948; and the work on *Drosophila* inversions by DOBZHANSKY and his co-workers) but we are here concerned with the inverse problem—the inference of changes in gene frequencies (and in some cases the stability of gene frequencies) from experimental observations of the fitness of individuals.

There are several important problems in which such measurements are of obvious value. In recent years there has been considerable interest in the effect on populations of an increase in the mutation rate (particularly by irradiation). As the genetic situation is shaped by the relative fitness of the different genotypes, so we can usefully discuss the effect of changes in the mutation rate only on fitness itself. There has been much theoretical speculation on this point which requires experimental work to check it.

As the ultimate character, fitness is of special importance to the quantitative geneticist, and its response to inbreeding, for instance, is of considerable theoretical importance. Again, the interrelationship of fitness with other characters is fundamental to a discussion of the genetic control of such characters in wild populations. LERNER has recently drawn attention to these problems in his book on *Genetic Homeostasis* (1954) in which he discusses the evidence about the mechanism by which such large amounts of genetic variation are maintained in populations.

But what is fitness? For the individual, it is simply the number of offspring in the next generation; for the genotype, it is most aptly described by FISHER's phrase (1930) the "expectation of offspring". Other terms have also been used such as survival value, selective advantage, reproductive rate, adaptive value and so on. Unfortunately, several of these have misleading connotations which have led to some controversy [see, for instance, the discussion between LI (1955) and CAIN and SHEPPARD (1956)]. "Fitness" in the sense that it will be used here refers to a property of individuals or an average value for individuals measured in a group. In so far as we shall later be discussing such groups or subpopulations, we shall solely be con-

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cerned with the individuals making up the group and in no way with the ability of the group to survive as a closed population.

It is operationally convenient to break down the total fitness into the chance of survival to maturity (measured in *Drosophila* at the point of emergence) and into the fertility after maturity. The latter will refer to the whole mature life of the animal and will therefore include longevity. We may regard these two—early survival and subsequent fertility—as the “components” of fitness. For a male, fertility consists of ability to impregnate females—male mating ability. It may be noted that though male mating ability is undoubtedly an object of natural selection within species, it contributes little to the ability of the species to survive as a unit, provided it is above a certain limit. If a *Drosophila* female is to lay eggs for three weeks, it presumably affects her output little whether she is impregnated at one hour or at five hours after emergence. As a further consequence, willingness of a female to mate will have little selective advantage within species and her fertility will then be determined mostly by the total number of eggs she lays and their hatchability, in so far as it is a property of the female. These fitness components are biologically distinct entities and must be combined to give total fitness. They are not alternative measures of fitness, as WALLACE (1952, Table 9) seems to imply. Observations on *Drosophila* populations have only too often concentrated attention on survival to emergence, often in the form of segregation ratios. There is still very little information as to which of the components are most important in controlling the variation in overall fitness and we are here concerned mostly to present a simple method of measurement which includes all components. We shall see later that it combines them in the wrong way but it is satisfactory for a preliminary attack on the problem.

If we are to count the average number of offspring left by individuals, at what stage should the counting be done? It is first of all obvious that the two generations must be counted at the same point in the generation cycle. As we are concerned with differences between genotypes, we should count at the moment the genotype comes into existence—at the moment of fertilization. This raises obvious practical difficulties. But we shall here be concerned with the average fitness of individuals forming stable breeding groups. Any point in the generation cycle will then be logically satisfactory as the separate generations will be genetically identical, and wherever in the generation cycle we start, we shall be including just the same processes but in a different order. It is convenient to count at the point of emergence and this fits in well with our breakdown of total fitness into survival to emergence and subsequent fertility. In dealing with such groups, we can also evade the problems posed by genes like fused (LYNCH 1919) and grandchildless (SPURWAY 1949) whose effects on fitness are not complete when their bearer may be dead.

The technique that we have developed does take into account all the biological components of fitness, though it has some drawbacks which we shall consider later. The basic method is to allow the stock to be tested to compete in equal numbers against a standard tester stock. The latter is chosen so that the ‘purebreds’ of either stock can be distinguished from the crossbreds. The fitness measurement is then derived from the relative numbers of the two purebred stocks which emerge. The tester stock that we have used is the well-known second chromosome lethal testing

stock, *Cy/Pm*. This contains two easily scored dominant markers balanced against each other by inversions to suppress crossing over and lethals within the inversions to eliminate the homozygotes. The process of competition with a wild type stock can be envisaged as follows

The fitness measurement (for which we shall use the noncommittal term "competitive index") will, if the tester and tested stocks have been put in in equal numbers, simply be the ratio of the numbers of *+/+* and *Cy/Pm* flies emerging.

The procedure starts with the setting-up of cultures containing a mixture of fertilized *Cy/Pm* and *+/+* females. The ratio of the two types will depend on the stock we wish to test but with outbred wild stocks we have used a *Cy/Pm*:*+/+* ratio of 2 to 1. When the progeny emerge, balanced groups of males and virgin females are collected from each emergence. In other words, if we have to set out competitive cultures in a 1 to 1 ratio, both in males and females equal numbers of the two types are taken from each hatch and the remainder discarded. From these groups (equivalent as far as the two types are concerned for age and parental culture) the competitive cultures are started, generally with 50 males and 50 females in a half pint bottle. The parents are discarded after five days and the progeny counted for the first four days of full emergence. In this period, probably about 80 percent of all flies will have emerged. The competitive index of the competing stock is given by the relative numbers of *+/+* and *Cy/Pm* flies, which obviously depends on the relative viability from egg to adult, male mating ability and female egg laying ability of the two stocks. The ratio of *Cy/Pm* to *+/+* flies in the competitive cultures can, of course, be varied. With our standard outbred stock, we have found that a 2 to 1 ratio of *Cy/Pm* to *+/+* in both sexes of the parental flies gives approximately a 1 to 1 ratio in the progeny. The resultant index has of course to be modified according to the ratio of parental types used.

As an example of the use and value of the technique, we can instance some observations on the effect of inbreeding in our standard outbred strain of *D. melanogaster*, the Kaduna strain from Nigeria. This strain has been used in the series of experiments on selection for abdominal bristles (see CLAYTON, MORRIS and ROBERTSON 1957). The competitive index of the Kaduna strain was measured using a 2 to 1 ratio in both sexes. In the series, ten replicate cultures were set up (with different samples of parents) and counts for the first four days of full emergence are given in table 1.

The proportion of wild type flies, expressed in terms of all "pure" flies, was 0.483 with a standard deviation between replicates of 0.136 and a corresponding standard error for the mean of 0.043. The competitive index of Kaduna compared to *Cy/Pm* is thus $4 \times \frac{0.483}{0.517} = 3.74$ with a standard error of 0.64.

TABLE 1
The competitive index of the Kaduna strain

Replicate	1	2	3	4	5	6	7	8	9	10
+/+	97	177	156	71	137	114	61	93	56	97
Cy/Pm	51	70	129	125	118	99	91	134	123	181
+/+										
Total "pure"	0.65	0.72	0.55	0.36	0.54	0.54	0.40	0.41	0.31	0.31

TABLE 2
The competitive index of inbred lines relative to their parent population

Line no.	Competitive index	Line no.	Competitive index
2	0.65	10	0.12
3	0.16	11	0.05
4	0.03	12	0.05
5	0.19	13	0.10
7	<0.01	14	0.01
9	0.22		
		Average	0.14

It may be felt that the accuracy is not high, bearing in mind the number of flies scored. The standard deviation between replicates corresponds to that of a binomial distribution with $n = 13$. It seems that the effective sampling unit is the first insemination of each individual female (which may not be inseminated more than once in the laying period) rather than the individual progeny scored, as was found by KNIGHT, ROBERTSON and WADDINGTON (1956) in a similar experiment on sexual isolation. It follows that increased accuracy could most easily be obtained by increasing the number of parental females in each culture.

Two series of replicates of similar size but with parental ratio 1:1 have given values for the competitive index of the Kaduna stock of 4.52 ± 0.82 and 3.21 ± 0.52 , compared to our standard Cy/Pm stock. Bearing in mind the standard errors of the estimates, the agreement is reasonable.

At the time when the method was developed, we chanced to have available a series of inbred lines made from this Kaduna wild type stock. They were the survivors of 100 lines which had been carried on by continued full-sib mating, with only one pair-mating in each line. At the eighth generation of inbreeding, they had been reduced in number to 14 and steps were taken to preserve this remainder, by keeping three pair-cultures in reserve in each line. These were thus the survivors of an intense selection pressure during the process of inbreeding. At the twelfth generation of inbreeding ($F \sim 0.90$), the competitive index of 11 of the lines was measured by the technique already described. As it was suspected that they would be low in fitness, the competition cultures contained equal numbers of inbred and Cy/Pm flies. Two replicates were taken in each line.

The results are summarized in table 2, in which the competitive index is expressed as a fraction of that of the parent outbred population.

TABLE 3

Comparison of competitive index and "adaptive value" (based on second chromosome segregation) for three inbred lines (expressed in relation to the noninbred population)

Line	Competitive index	"Adaptive value"
4	0.03	0.83
7	<0.01	0.69
14	0.01	0.76

Differences between replicates were similar to those in table 1 though a statistical analysis is difficult because of the varying ratios in the separate lines. With the exception of line 2, the inbreeding has produced a very marked decline in competitive index, in spite of the selection during the formation of the lines. However, the general relevance of these results is best left to another place, as we are concerned here in the main with the technique itself. It may be noted that, notwithstanding the low competitive index of some of the lines, none was difficult to maintain in mass cultures and the low index came as a surprise to us.

In addition to the measurements on the competitive index, we mated together the *Cy/+* flies from the competition bottles to measure the segregation ratio of *Cy/+* and *+/+* flies in the lines 4, 7, and 14, which had shown the worst competitive index. This method measures the effect of second chromosomes from the tested stock on survival from fertilization to emergence and has been used by WALLACE and KING (1951) as a measure of the "adaptive value" of their irradiated populations. The "adaptive value" is then based on the ratio of *+/+* to *Cy/+* flies segregating. The comparative results are shown in table 3.

We shall see later that our technique certainly overestimates differences between populations in fitness whereas that used by WALLACE must underestimate them, as it measures only the effect of part of the genetic material on part of the reproductive cycle. Nevertheless the discrepancy between the two measures on these three lines, which were picked out solely because they had a low competitive index, is startling.

We might also mention here some results which perplexed us at the time but which have now come more into focus. Our second test of the outbred population, on the same scale as the first, gave an index relative to the *Cy/Pm* tester of only 1.18, compared to the first value of 3.74. It was however then realized that this subpopulation had been kept in bottles for 15 generations, with a mean parental number of about 20 pairs, which would lead to an inbreeding coefficient of around 40 percent. The parent stock, in which the population size is around 5000, was then retested along with the subpopulation in bottles. The former gave a value of 4.52, as reported above, and the subpopulation again gave a value of 1.2. It seems that we can ascribe the lower index of the subpopulation to the inbreeding that it has received.

Shortcomings of the technique: The main purpose in developing this technique was to find a simple measurement involving all three components of fitness—survival to emergence, and male and female fertility. But as a method of measuring fitness itself, the technique has the drawback that the components are put together in the wrong way. The fitness of an animal (or of a group of animals) is their "expectation of

offspring" and can be split into the chance that they will survive to breeding age and into the average number of offspring of those reaching that age. In formal terms $W = SF$ where S is the chance of survival to breeding age and F the fertility of those surviving. F will naturally include longevity. If S is the same for both sexes, then for the average fitness of the population we have $W = \frac{S(F_m + F_f)}{2}$, the subscripts referring to sexes.

Now, in our tests, if we take as unity the separate components measured on the *Cy/Pm* stock, the relative numbers of eggs laid by wild type and *Cy/Pm* females, each fertilized by their own type of male, is $F_m F_f$ and, from these, the relative numbers of the two types emerging is $SF_m F_f$. Thus, in this technique, the components are combined differently from a true fitness measurement and, for this reason, we have used the term "competitive index". Consideration of the two formulae will show that differences between stocks in "competitive index" will on the whole be greater than for true fitness but that the two will in the main have a high correlation. In a sense, differences in fertility between stocks are given too much weight in the "competitive index". Perhaps the most satisfactory solution to the problem would be to measure the three components separately and to combine them in the correct manner. This would, however, be considerably more laborious than the technique presented here.

Two other important defects of the technique are obvious. The first is that, in allowing eggs to be laid over only five days, little account is taken of longevity. This can, of course, be overcome by transfer of the parents to further bottles but transfer greatly increases the time taken. We have not considered the extra labor worth while. The existence of any degree of sexual isolation between the stocks placed together in the competition bottles would wreck the technique but fortunately few cases of such isolation have been observed within species. However, if the technique is to be used in any series of experiments, other tester stocks will have to be investigated.

On a more trivial level, it is obvious that the results obtained will be greatly dependent on the precise external conditions under which the competition is carried out and therefore do not in any way give an absolute figure. The most important of these are the number and age of the females in competition, the period for which they are allowed to lay eggs, the period over which emerging flies are counted and, of course, the nature of the food supplied. The exact conditions used will depend on the particular problems being investigated. In determining the feasibility of the technique, we have been to some extent guided by experimental convenience rather than undue inflexibility. One desirable modification would be to increase the number of females laying in each bottle as this is the main factor causing deviation between replicates.

SUMMARY

A technique for examining the relative fitness of different stocks is put forward. It involves competition with a stock marked with two dominant genes and the final assessment is given the term "competitive index". Examples are given of its use in measuring the effect of inbreeding on an outbred population. Shortcomings and possible modifications of the technique are discussed.

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