THE EXPERIMENTAL ASSESSMENT OF FITNESS IN DROSOPHILA. II. A COMPARISON OF COMPETITIVE AND NONCOMPETITIVE MEASURES

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ABSTRACT

Twelve diverse strains of *Drosophila melanogaster* have been examined with respect to their individual fitness components and with respect to their relative performance under competitive and noncompetitive conditions. Individual fitness components included estimates of time until successful copulation (t), fecundity (f) and egg-to-adult viability (v), and a composite index of overall fitness of the form fv/t was used for comparisons among strains. Noncompetitive performance was assessed in terms of the biomass (standing crop) and productivity of equilibrium experimental populations. Competitive performance was assessed in terms of relative competitive ability vis-à-vis a standard compound-autosome-bearing strain in single-generation tests. A significant correlation was found between the composite index of individual fitness components and the competitive compound-autosome test. Although the biomass and productivity of equilibrium populations were correlated with each other, neither of these noncompetitive measures was correlated with individual fitness components or with the composite index. We suggest that the performance of strains in such noncompetitive tests may be related to what WRIGHT has called the "mean selective value" of the populations. Judging from their association with the composite index of individual fitness components, competitive tests such as the compound-autosome test seem to be related more nearly to the average Darwinian fitness of the populations.

THE central role that fitness plays in evolutionary biology has inspired numerous treatments of this concept from both theoretical and experimental points of view. Theoretical investigations have usually been restricted to defining fitness in terms of viability selection because models incorporating other components of fitness rapidly become exceedingly complex and mathematically intractable (EWENS 1979; HARTL 1980). Experimental analyses using Drosophila, on the other hand, have been quite diverse, ranging from the measurement of a single component of fitness in individual flies to estimates of overall fitness based on the long-term reproductive success of various strains.

Of the broad range of operations that fall under the heading of overall estimates of fitness, two principal categories include measures based on the reproductive success of strains tested under competitive and noncompetitive

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conditions. In the noncompetitive realm, CARSON (1961a,b) proposed that the total biomass or productivity achieved by strains grown under uniform laboratory conditions could be used to assign them relative population fitnesses. This technique has also been used by AYALA (1965) and VAN DELDEN and BEARDMORE (1968) to study fitness. Fitness measures based on competitive ability have been reviewed by HAYMER and HARTL (1982) and HARTL and HAYMER (1983). Although all of these techniques purport to measure fitness, relatively little work has been done on direct comparisons of differing techniques to determine whether they are in fact measuring the same thing (for exceptions see AYALA 1965; HAYMER and HARTL 1982).

In this study, CARSON'S (1961a) noncompetitive biomass method and the competitive compound autosome test of JUNGEN and HARTL (1979) are directly compared by subjecting a defined set of strains to both techniques. In addition, several of the "major" components of fitness have been independently estimated for these strains in the hope of determining the role that individual components might play in overall fitness estimation. The *D. melanogaster* strains used here include lines rendered homozygous for chromosome 2, a heterogeneous line derived by intercrossing several homozygous lines, and lines that were single pair sib-mated for several generations. These lines were chosen more for comparative purposes than to make inferences about natural populations.

MATERIALS AND METHODS

The strains used in these experiments were derived from isofemale lines generously provided by DR. V. FINNERTY. Several of these lines were rendered homozygous for chromosome 2 by the traditional Curly/Plum technique (WALLACE 1956) except that in the parental generation a Curly/Plum male was crossed to a wild-type female. These lines are designated by a lower case letter following the original isofemale line number designation such as AJ14a. The HET IV line was created by intercrossing several of the homozygous lines. Two lines were also single pair sib-mated for several generations such as A20-17 (line A20 sib-mated for 17 generations).

All experiments were conducted in ½ pint milk bottles containing approximately 60 ml of a standard sucrose-cornmeal-agar Drosophila medium. A 6-cm^2 piece of sterilized paper towel was inserted into the medium to provide additional pupation sites. All virgin flies were collected within 7 hr of emergence and were 4–6 days old when experiments were initiated.

Noncompetitive fitness estimate: This technique, referred to as the biomass test, was devised by CARSON (1961a,b) to estimate the relative population fitnesses of isolated strains. This involved population cages maintained on a strict cycle of change over a period of several weeks.

The population cages used in the present experiments consisted of two parts. The base of the population cage was a ½ pint milk bottle containing approximately 60 ml of the standard medium described previously. The upper portion of each cage was an inverted 500-ml polypropylene Erlenmeyer flask manufactured by the Nalgene Company. It was found that the outer edge of the neck of the flask fit precisely into the neck opening of the bottle thereby providing a sealed chamber. The flask portion of the cage provided additional airspace and a suitable holding chamber for obtaining biomass weights or for bottle changing. A small hole, ordinarily plugged with cotton, was drilled into the base of each flask for aeration and to allow for the addition of newly hatched flies.

All population cages were initiated with 25 pairs of flies, and all cage experiments were carried out simultaneously. Two replicates were carried out for each of the 12 strains tested. The cages were maintained at room temperature, 25° ± 2°. Temperature fluctuations away from 25° were relatively short term, not lasting longer than 1 day. Food bottles were changed every Monday, Wednesday and Friday. The Friday-to-Monday bottles were discarded. The Monday-to-Wednesday and Wednesday-to-Friday bottles were kept for a total of 21 days after introduction to the population cage to obtain progeny counts (productivity measures). By means of this routine, a constant total of

six productivity bottles were held for each strain. However, because of the high density of larvae in each bottle, progeny rarely eclosed before the 12th day. After each bottle to be saved was removed from the cage, two dental rolls were inserted into the medium to absorb excess moisture and to provide additional pupation sites. Progeny eclosing from bottles 12–21 days old were counted semiweekly under CO_2 anesthesia. Tabulated progeny were then added back to the appropriate cage after a suitable recovery period. The semiweekly counts were totaled into a weekly productivity census for each cage.

Biomass measurements were taken once a week, always on Monday afternoon in the following manner. First, all of the flies were forced into the bottle portion of the cage by pounding the cage on a rubber mat. The flask portion of the cage was removed and the bottle capped. A tare flask weight was taken, together with a rayon ball to plug the neck opening of the flask. The flask was then refitted to the bottle, the cage inverted, and the files pounded into the flask portion of the cage. The flask was removed, plugged with the rayon ball and a "flask + flies" weight was taken. The difference between these two weights was the biomass, or the wet weight of the "standing crop" of flies. After weighing, the flask was again refitted to the bottle portion of the cage. All weights were taken on a Mettler single pan electronic balance, accurate to 10 mg.

These cages were maintained for a total of 23 weeks, and the results are presented in terms of productivity (progeny counts) and biomass (wet weight of the standing crop) for each strain. Individual replicate values are given in the APPENDIX, but for purposes of comparison the replicate values at each sampling point were averaged so as to yield a single value for the contribution of each strain to the overall parameter estimation.

Competitive fitness estimate: The details of this procedure, referred to as the compound-autosome test, are given in HAYMER and HARTL (1982). Compound autosome-bearing strains are a "pseudospecies" in that they are completely postzygotically reproductively isolated from normal strains due to gross chromosomal rearrangements among the zygotes (for a review of compound autosomes see HOLM 1976). The particular compound strain utilized here is designated C45 = C(3*L*)RM,*ri*; C(3*R*)RM,*ry*^{2}.

The compound-autosome procedure can be briefly explained as follows. Equal numbers of virgin males and females from both the wild strain and compound autosome strain were placed in ½ pint milk bottles. Three days were allowed for oviposition, at which time the adults were transferred to a new bottle for 3 additional days before being discarded. This consititutes one replicate; five or six replicates were done for each strain. Complete progeny counts were obtained for 21 days after the initiation of any bottle. The fitness of a strain was calculated as the proportion of wild flies recovered in the total wild type and C45 hatch inasmuch as the progeny of heterogametic (compound × wild type) matings do not survive. All bottles were kept in a large incubator maintained at 25° ± 1° at about 60% relative humidity.

Fitness component estimates: The flies used in these experiments were subsets of those used in the overall fitness estimations and were all between 5 and 7 days old at the time the experiments were initiated. Data were collected from single pairs of flies in shell vials. Time to mating was measured first, representing the total time from which a male and a virgin female were combined until a successful copulation was initiated. Any pair in which no courtship activity was observed within 20 min was excluded from the mating analysis (although some pairs mated after 20 min). All mating experiments were done at room temperature between 9:00 a.m. and noon. Approximately 24 hr after the mating observations, pairs were transferred to fresh medium. Transfers were made daily for 3 additional days. Fecundity estimates were made by counting eggs immediately after transfer. Egg-to-adult viability was estimated by comparing the number of adult flies present in each vial 15–16 days later with the initial egg count.

RESULTS

The total fitness estimates obtained for the strains included in the experiments are given in Table 1. The values given for biomass and productivity both come from the biomass technique, and they represent the averages of two replicates of each strain over the last 18 weeks of the experiment. The standard errors here are between weeks (or samples). The individual replicate values for these

TABLE 1

Average fitness estimates from biomass and compound-autosome tests with standard errors

	Biomass test		Compound-autosome test
Strain	Biomass (g)	Productivity	Competitive index
AJ5a	0.31 ± 0.03	268.9 ± 39.6	0.560 ± 0.04
AJ2a	0.32 ± 0.04	370.2 ± 67.3	0.695 ± 0.05
B90a	0.44 ± 0.04	732.3 ± 63.6	0.766 ± 0.03
AJ4a	0.38 ± 0.04	408.9 ± 55.0	0.705 ± 0.02
AJ8a	0.47 ± 0.03	714.0 ± 59.1	0.648 ± 0.04
N101a	0.57 ± 0.05	675.6 ± 71.2	0.581 ± 0.04
A9a	0.34 ± 0.03	455.8 ± 70.3	0.782 ± 0.06
A12a	0.35 ± 0.02	815.1 ± 63.7	0.769 ± 0.02
AJ14a	0.37 ± 0.02	415.9 ± 47.5	0.835 ± 0.02
HET IV	0.56 ± 0.05	510.8 ± 45.7	0.950 ± 0.01
A17-17	0.69 ± 0.05	803.8 ± 85.1	0.381 ± 0.03
A20-17	0.46 ± 0.04	659.5 ± 52.2	0.567 ± 0.04

two measures of fitness are given in the APPENDIX table, and as can be seen, with one exception (strain A17-17), the individual replicates are in very good agreement. Fitness values as estimated by the compound autosome method are given as well, with the standard errors in this case being among replicates.

Table 2 gives the individual component measures available for these strains. The component data for strain A9a are unavailable, and for line A20-17 no mating activity was observed in more than 20 min of observation. The time until successful copulation is given in terms of minutes and fractions of a minute averaged for the number of pairs indicated, with the standard errors being among replicates (mating pairs). The standard errors are quite high in some cases, which is not atypical for these types of behavioral data. The fecundity estimates are given as the average per female per day, as are the hatchability data, with the standard errors again being among replicates. The composite index given in Table 3 is simply the product of the average fecundity and average egg-to-adult viability divided by the average time until successful copulation. This composite is not an all-inclusive measure of overall fitness based on individual components, as it excludes such potentially important fitness components as developmental time and longevity. Nor is it necessarily the best measure that utilizes mating time, fecundity and viability. It is, however, the simplest measure that seems to incorporate the major components of fitness in approximately the right way, and we have chosen to use it for comparative purposes (see HARTL and HAYMER 1983). The composite value for strain B90a was excluded from the correlations because its composite value (17.75) is clearly an outlier (DIXON and MASSEY 1957).

Overall fitness estimates are compared in Table 3, and the relevant product-moment and the rank correlations are given in Table 4. It can be seen in Table 4 that, although biomass and productivity correlate weakly with each other, neither of these parameters from the biomass test can be said to be correlated with fitness as estimated in the compound-autosome test. It is perhaps some-

TABLE 2

Fitness component estimates with standard errors

Strain	No. of replicates	Time until successful copulation	Fecundity	Egg-to-adult viability
AJ5a	20	8.79 ± 1.7	24.64 ± 4.1	0.56 ± 0.09
AJ2a	25	3.12 ± 0.4	18.84 ± 1.8	0.51 ± 0.05
B90a	18	2.32 ± 0.2	49.62 ± 3.6	0.83 ± 0.05
AJ4a	13	8.58 ± 1.8	23.76 ± 3.0	0.52 ± 0.06
AJ8a	11	5.55 ± 0.6	19.77 ± 2.5	0.56 ± 0.06
N101a	7	8.23 ± 1.1	31.71 ± 1.7	0.61 ± 0.06
A12a	12	5.78 ± 1.0	15.52 ± 1.5	0.69 ± 0.04
AJ14a	21	6.84 ± 0.6	31.88 ± 2.2	0.78 ± 0.03
HET IV	18	4.39 ± 0.7	24.13 ± 1.7	0.87 ± 0.04
A17-17	13	8.89 ± 1.6	16.33 ± 1.4	0.37 ± 0.03
A20-17	20		24.19 ± 1.3	0.63 ± 0.03

TABLE 3

Summary of various fitness estimates

Strain	Composite index	Compound-autosome test	Productivity	Biomass
AJ5a	1.58	0.560	268.9	0.31
AJ2a	3.12	0.695	370.2	0.32
AJ4a	1.43	0.705	408.9	0.38
AJ8a	2.00	0.648	714.0	0.47
N101a	2.35	0.581	675.6	0.57
A12a	1.86	0.769	815.1	0.35
AJ14a	3.62	0.835	415.9	0.37
HET IV	4.75	0.950	510.8	0.56
A17-17	0.69	0.381	803.8	0.69

TABLE 4

Product-moment (r) and Spearman rank (r_S) correlations between various fitness estimates

	r	r_S
Composite index and compound autosome test	0.84**	0.67*
Composite index and productivity	−0.33	−0.17
Composite index and biomass	−0.09	−0.07
Compound autosome test and productivity	0.15	−0.08
Compound autosome test and biomass	−0.39	−0.17
Productivity and biomass	0.60*	0.60*

* $P < 0.05$ for one-tailed test.
** $P < 0.01$ for one-tailed test.

what disappointing that the biomass and productivity measures correlate only weakly with each other. The biomass estimates in particular may be subject to some error in that the weights are accurate to 10 mg, whereas an average fly weighs less than 1 mg, but this source of error is relatively small. The composite index correlates well with the compound-autosome fitness estimates but not at all with the estimates based on biomass or productivity. As noted, this composite index, although clearly not the only possible formulation of fitness components, represents the simplest relationship we could think of that would incorporate what are usually considered to be the "major" components of fitness in approximately the right way.

Some striking differences in total fitness estimates are evident in Table 3. Strains such as HET IV and AJ14a, which were clearly superior in the competitive environment, performed at intermediate levels in the noncompetitive situation. The highly inbred lines A17-17 and A20-17 flourished in the noncompetitive test but fared poorly in the competitive assessment. Similar extremes are indicated in the composite indices in that the highest composite values (excluding B90a) were obtained for lines HET IV and AJ14a and the lowest value was obtained for line A17-17.

DISCUSSION

The populational attribute being measured in each of these techniques would almost universally be referred to as "fitness." Clearly though, from the results obtained in these experiments, these techniques cannot be said to be measuring the same thing. This is not to suggest that any one technique is "better" or should be rejected in favor of another. Rather, it suggests that the net parameter measured in each case must be qualified with respect to what and how it is being measured. In one of the original expositions of the biomass technique, CARSON (1961a) was careful to state that the "relative population fitness" assigned to a strain by the biomass test was something different from fitness in the sense of adaptive value. The results obtained here confirm that the biomass test is measuring something quite different from fitness as estimated in a competitive environment, despite the fact that AYALA (1970) found some agreement between the fitness rankings of a small number of strains as determined by biomass and competitive tests. The compound-autosome test employed here involves intraspecific competition, whereas AYALA'S competitive test was of an interspecific type, so the results presented here are not strictly comparable. However, HARTL and HAYMER (1983) have found no significant correlation between the results of a biomass test and several interspecific competitive assessments. Based on this finding, HARTL and HAYMER (1983) have speculated that the noncompetitive tests may be more closely related to what WRIGHT (1969) has termed the "mean selective value" of a population, which is distinct from its average fitness.

One important difference between the biomass and compound-autosome techniques is the number of generations included in the experiment. Although the inclusion of at least one complete generation cycle in both techniques presumably brings the "major" fertility and viability components into play, if

such components as developmental time and longevity are important to the fitness of a strain, they will be given greater weight in the multigeneration case. However, this cannot completely account for the lack of agreement because many of the strains in the biomass test had been previously subjected to another multigeneration fitness measure based on the work of SVED (1971). HAYMER (1982) and HARTL and HAYMER (1983) found no correlation between the Sved fitness indices and either of the biomass parameters estimated for these strains. Another potential problem for any multigenerational analysis is the question of when exactly populations achieve equilibrium. This may account, at least in part, for the weak biomass-productivity correlation, although the value obtained in this study is not greatly different from previously obtained biomass-productivity correlations (MOURAO, AYALA and ANDERSON 1972).

Close examination of some of the major fitness components that have been estimated independently for these strains reveals to some extent that there are differences in component weighting between techniques. The highly inbred lines were clearly the most sluggish in terms of mating ability. Line A17-17 required the longest average time measured to initiate copulation, and for line A20-17 no mating activity was recorded in more than 20 min of observation. Since this is largely a function of male mating activity, it is not hard to imagine how such lethargy could be devastating in the presence of intraspecific competitors as is the case in the compound-autosome test. The biomass test, on the other hand, minimizes the importance of rapid mating activity because of the lack of a competing strain. The performance ranking of the inbred lines was vastly improved in the biomass test over what it had been in the competitive fitness assessment. For all the strains, the correlation between compound-autosome fitness and time to mating was negative (Spearman rank correlation $r_S = -0.62$), whereas the biomass correlations with time to mating were either positive or essentially zero.

Few of the component *vs.* overall fitness relationships are as clear-cut as that involving mating activity. The HET IV strain, clearly superior in terms of overall fitness in the compound-autosome test, is only marginally better in terms of fecundity and viability and is somewhat inferior as far as rapid mating success. PROUT (1971) admonished that the relationship between a single fitness component and overall fitness is ill defined at best. We agree, and it is largely for this reason that we formulated the composite index from three components of fitness. This point is relevant to a discussion of what is actually being measured in these techniques. It seems to us that the compound-autosome test is closely approximating what is usually referred to as the Darwinian fitness of a genotype as discussed by DOBZHANSKY (1970), among others, namely, "the relative contribution of a genotype to the pool of genotypes in the next generation." This interpretation is in accord with that of KNIGHT and ROBERTSON (1957), who developed a competitive fitness test closely related to and the forerunner of the compound-autosome test. In the compound-autosome test, the strain being tested together with the compound strain constitutes the entire pool of genotypes in any one experiment. The strong correlation between the compound-autosome test and the composite index of fitness also confirms that the com-

petitive index is largely a function of the viability *and* fertility (including mating success and fecundity) components measured on a particular strain. Viability and fertility are considered to be the major components of fitness (ANDERSON and WATANABE 1974). The compound-autosome test also allows for rigorous experimental control of environmental variables such as density, which has been shown to be important in fitness measurements (SNYDER and AYALA 1979; MUELLER and AYALA 1981).

It is more difficult to define what is being measured in the biomass test. As CARSON (1961b) indicated, this test seems to be measuring an attribute of a population as a whole rather than the average fitness of its individual members. WRIGHT (1969 and earlier) has pointed out that significant selection occurs between demes and has emphasized that the mean selective value determining the outcome of selection among demes is a population attribute that is different from average Darwinian fitness. The biomass test may represent a technique by which mean selective value could be measured. If so, it is also conceivable that many of the predictions of WRIGHT's (1969 and earlier) shifting balance theory could become testable using these methods.

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APPENDIX

Estimates from replicate experiments of the biomass test, averaged over the last 18 weeks of the experiment

Strain	Biomass (g)	Productivity
AJ5a	0.34	285.7
	0.29	252.0
AJ2a	0.33	408.8
	0.31	331.6
B90a	0.43	739.0
	0.44	725.7
AJ4a	0.37	371.8
	0.40	445.9
AJ8a	0.43	702.9
	0.51	725.1
N101a	0.55	664.1
	0.59	687.2
A9a	0.35	482.1
	0.33	429.4
A12a	0.32	767.6
	0.37	862.5
AJ14a	0.34	402.8
	0.40	429.0
HET IV	0.52	479.6
	0.60	542.1
A17-17	0.59	639.7
	0.79	967.8
A20-17	0.48	687.9
	0.44	631.1