Theor Appl Genet (1988) 75:468–473

Components of fitness in a compound chromosome strain of *Drosophila melanogaster*

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Received July 2, 1987; Accepted October 13, 1987
Communicated by J. S. F. Barker

Summary. The relative net fitness of a compound chromosome strain of *Drosophila melanogaster* was about 0.05, compared with the chromosomally normal strain from which it was derived. Based on meiotic considerations alone, the expected relative fitness was about 0.25. There were no significant differences in fertility between the compound and normal strains; the compound strain produced about 28% as many offspring as the normal strain and developed faster than the normal strain in two replicates, and slower in one replicate. The low relative fitness of the compound strain was apparently due to assortative mating, in which normal females discriminated strongly against compound males. Implications for pest control projects are dicussed.

Key words: Compound chromosomes – Fitness – *Drosophila* – Assortative mating – Pest control

Introduction

Insecticides are often ineffective in controlling insect pests, and they sometimes create new problems more severe than those they are supposed to solve (DeBach 1974; Huffaker and Messenger 1976; Brattsten et al. 1986). For this reason, biologists have been searching for many years for alternative, non-chemical, means of control, such as biological control and integrated pest management (DeBach 1974; Huffaker and Messenger 1976). These methods have achieved some notable successes (Caltagirone 1981; Croft et al. 1984). Another area of recent research is that of genetic control; i.e. modifying the genetic structure of a pest population in such a way that the pest is suppressed by members of its own species (Davidson 1974; Whitten and Foster 1975). For example, sterile, lethal, or debilitating mutations are introduced into the population and maintained in such a way as to suppress or eradicate the population. The release of sterile males is the oldest of these methods, and has had several striking successes (Richardson et al. 1982; Curtis 1985). A more recent idea is the use of chromosomal mutations and the dynamics of negative heterosis to introduce such mutations into the pest population (Foster et al. 1972). The most promising kind of chromosomal mutation for this application seems to be compound chromosomes.

A compound chromosome has two homologous arms attached to the same centromere. Thus, a pair of compound chromosomes has two left arms attached to one centromere, and two right arms attached to the homologous centromere. The construction and cytogenetics of compound chromosomes have been described by Foster et al. (1972). Two aspects are important to the current study: (1) a strain carrying compound chromosomes is expected to have a relative viability of about 25% compared with a strain carrying normal chromosome, because individuals carrying compound chromosomes produce a high proportion of aneuploid gametes; (2) matings between individuals carrying compound chromosomes and individuals carrying normal chromosomes produce no offspring. Numerous experiments have verified these expectations (Fitz-Earle and Holm 1982).

A population consisting of a mixture of individuals carrying normal chromosomes and individuals carrying compound chromosomes creates a negative heterosis system (Foster et al. 1972; Prout 1981) with an unstable equilibrium point (critical point). If the initial frequency of compound chromosomes exeeds the critical point, the compound chromosome will become fixed in the popula-

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tion. In a pest population, this is beneficial for at least two reasons: (1) if the compound chromosome becomes fixed, the population may have a lower reproductive rate because of reduced viability of the compound strain; (2) it is conceivable to link some "desirable" gene which is capable of further suppressing the population (for example a conditional lethal) to the compound chromosome. Thus, compound chromosomes represent a potentially powerful tool in suppressing pest populations. All one has to do is to release individuals carrying compound chromosomes (perhaps containing a conditional lethal) into the natural pest population, so that their frequency exceeds the critical point. The dynamics of negative heterosis will then enable the compound chromosome strain to replace the natural population, resulting in suppression of the pest. This idea has been tested in both laboratory and field trials, and the results, although not completely successful, are encouraging (Cantelo and Childress 1974; McKenzie 1976; Foster et al. 1985).

The value of the critical point (i.e. the unstable equilibrium point) depends on the relative net fitness of the compound strain, compared with the normal strain. The lower the relative fitness, the higher the critical point (Prout 1981). Therefore, it is important to have an accurate estimate of the relative fitness of the compound chromosome strain if one wishes to use it in a pest control system. Several techniques for obtaining such estimates have been published (Foster et al. 1972; Barclay and Fitz-Earle 1983).

We have studied a compound chromosome strain used by Ehrman in her studies of the evolution of prezygotic reproductive isolation in *Drosophila melanogaster* (Ehrman 1971, 1973, 1979, 1983). This strain, known as the bB strain, has a compound second chromosome.

In this paper, we present estimates of relative net fitness, and of four components of fitness, for the bB strain, compared with the normal strain from which it was derived. We show that this strain has significantly lower relative fitness than predicted by the meiotic behavior of compound chromosomes, and that this low fitness is probably due solely to assortative mating. Finally, the implications for genetic pest control are discussed.

Materials and methods

General

Strains of *Drosophila melanogaster* used were those designated bB (compound chromosome II) and +B (normal chromosomes). The bB strain, derived from the +B strain in 1967, carries the recessive marker black body (b) and is easily distinguishable from the phenotypically wild type +B strain. Both strains have been maintained by Ehrman since 1970 [see Ehrman (1971) for a complete description of these strains].

All flies were reared in 1 1/4 × 4 in. plastic vials capped with foam plugs. Food was Carolina Instant Drosophila Medium, prepared according to the manufacturer's instructions. Flies were kept in an incubator at 25° ± 2°C. Relative humidity ranged from 65% to 75%.

Relative net fitness estimates

The general procedure was described by Foster et al. (1972). Several mixtures of normal and compound chromosome flies are set up at different initial frequencies of the compound strain, and the frequency of the compound chromosome is estimated over several generations. If the compound chromosome is lost from the population, then the initial frequency was below the critical point. If the compound chromosome becomes fixed in the population, then the initial frequency was above the critical point. This technique allows for fairly accurate estimation of the critical point. From this, the relative net fitness of the compound chromosome strain, compared with the normal strain, can be estimated (Proud 1981):

$$W = \frac{1 - \hat{q}}{\hat{q}} \quad (1)$$

where W = relative net fitness of the compound strain, if the fitness of the normal strain is 1 ($W < 1$)

$\hat{q}$ = the unstable equilibrium point (critical point), as estimated from the population replacement experiments.

Populations were established with initial frequencies of the compound chromosome strain of 0.40, 0.50, 0.60, 0.70, 0.75, 0.80, 0.85, 0.90, and 0.95, using equal numbers of males and females of each type. Initial population size was 32 adult flies for each frequency; subsequent generations consisted of 50 adult flies each. All females in the initial population were virgins.

On day 7, the adult flies were removed. On day 18, a random sample of 100 adult progeny was collected, and the frequency of the compound strain was estimated from that sample. The next generation was started using 50 flies, with the frequency of the compound strain as estimated from the adult sample. This was repeated for three generations. Scoring was done on day 18 to assure that all adults had emerged and matured.

Fertility, productivity, and development time

These components of fitness were compared between the normal and compound strains as follows:

Fertility. A mating was considered fertile if the female produced any larvae, sterile if she did not. There are no a priori reasons to expect either male or female fertility differences between the normal and compound strains.

Productivity–the total number of adult progeny produced by a single fertile female, from one 24 h egg collection. This actually consists of two components, fecundity (number of eggs laid), and egg to adult viability. Because of aneuploidy, we expect a relative viability of about 25% for the compound strain. Thus, productivity of the compound strain is expected to be about 25% that of the normal strain, assuming there are no differences in fecundity.

Developmental time–the mean egg to adult developmental time for all the offspring of a single fertile female. Again, there are no a priori reasons to expect differences between the normal and compound strains, although our observations of the compound strain suggested they might develop more slowly.

The culture procedure was as follows: A single virgin female was placed in a fresh vial with a single male of the same kind. After 24 h, the male was discarded, and the female placed in a fresh vial. The female was allowed to lay eggs in this vial for 24 h, after which she was removed, and the vial saved for counting of progeny. When pupae began to appear, the vials were checked every 12 h (at 07.00 and 19.00 hours). At each observa-

tion period, the number of newly emerged adults was recorded. Thus, developmental time estimates were to the nearest one-half day. Observation was continued every 12 h until no more adults emerged. Three replicate sets of single pair matings were performed: replicates 1 and 2 contained 40 pairs each of Normal and Compound flies, and replicate 3 had 50 pairs of each kind.

From each vial, the following data was obtained: total number of adult progeny produced; mean egg to adult developmental time of all progeny; and fertility (either fertile or sterile).

Assortative mating

Mating choice experiments were performed as follows: Four virgin females of each type were placed in a vial, along with four males of each type. The flies remained together and were allowed to mate for 24 h. At the end of this time, the males were discarded and each female was placed in a separate vial containing fresh medium. After 12–14 days, each vial was examined for the presence or absence of progeny. Since heterogamic matings would produce no progeny, the presence of offspring indicated a homogamic mating ($+B_{♀} \times +B_{♂}$ or $bB_{♀} \times bB_{♂}$). A female which produced no progeny was assumed to have mated with a male of the other kind. It is possible, of course, that the female mated with a male of her own kind, but one or the other was sterile, resulting in no progeny. However, fertility data (see "Results") indicate that there are no differences between the normal and compound strains; thus, we believe that occasional sterility will have no significant effect on the results of the assortative mating experiments.

These experiments were done at the same time as the population replacement (net fitness) experiments.

The degree of assortative mating was quantified using the isolation index (I) of Malogolowkin-Cohen et al. (1965). The value of I can range from -1 (complete disassortative mating) to $+1$ (complete assortative mating). Under random mating, $I = 0$.

Results

Fig 1 shows the results of one of the replacement experiments. A second replicate gave similar results. In all populations, the compound chromosome was eliminated within three generations. From Fig 1, the critical point was $\hat{q} \geqq 0.95$. This corresponds to a net fitness of the compound strain of $W \leqq 0.05$, substantially lower than the 0.25 predicted from the meiotic behavior of compound chromosomes.

The results for fertility, productivity, and development time are summarized in Table 1. There were significant differences among replicates, so results are shown for

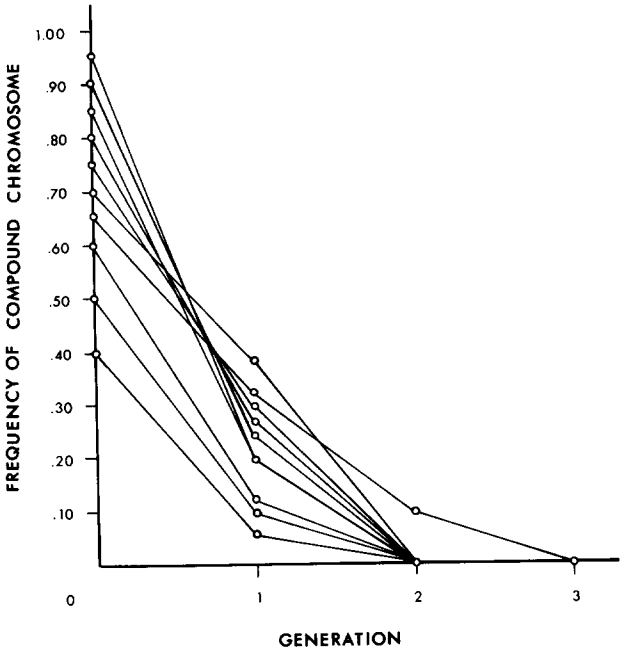

Fig. 1. Change in frequency of the compound chromosome strain (bB) in competition with the normal chromosome strain (+B). Each line represents a different initial frequency of the compound strain. The unstable equilibrium point is greater than 0.95

Table 1. Estimates (means ± SE) of three components of fitness for normal (N) and compound chromosome (C) strains of *Drosophila melanogaster*. For fertility, none of the differences between N and C are significant ($P > 0.05$). For productivity (mean offspring number) all comparisons between N and C are highly significant ($P < 0.001$); for mean development time, all comparisons are highly significant ($P < 0.001$) except for all replicates combined ($P > 0.05$)

	No. matings	Total no. offspring	Fertility	Mean productivity	Mean dev. time
Replicate No. 1					
N	40	898	0.80 ± 0.06	28.1 ± 3.72	13.6 ± 0.09
C	40	205	0.65 ± 0.08	7.9 ± 1.04	12.4 ± 0.31
Replicate No. 2					
N	40	894	0.88 ± 0.05	25.5 ± 2.37	11.9 ± 0.10
C	40	314	0.80 ± 0.06	9.8 ± 0.93	11.4 ± 0.10
Replicate No. 3					
N	50	576	0.64 ± 0.07	18.0 ± 3.46	13.4 ± 0.22
C	50	120	0.76 ± 0.06	3.2 ± 0.40	15.1 ± 0.20
Total / Avg					
N	130	2368	0.76 ± 0.04	23.9 ± 1.87	12.9 ± 0.12
C	130	639	0.74 ± 0.04	6.7 ± 0.53	13.1 ± 0.21

Table 2. Numbers of matings between + B (Normal chromosome) and bB (Compound chromosome) strains of *Drosophila melanogaster*. Thirty females of each kind were tested. Four + B and one bB died before the tests were completed. Data from G. Simonson, unpublished

$+B_{♀} \times +B_{♂}$	$+B_{♀} \times bB_{♂}$	$bB_{♀} \times +B_{♂}$	$bB_{♀} \times bB_{♂}$	I ± SD
21	5	12	17	0.38 ± 0.12

each replicate, along with results for all three replicates combined.

In no replicate was there a significant fertility difference between the normal and compound strain. The fertilities averaged over all replicates were 0.76 and 0.74 for the normal and compound strains, respectively ($\chi^2 = 0.18$, $P \cong 0.67$).

The results for productivity (offspring number) varied among replicates. The relative ratios of compound to normal were 0.28, 0.38, and 0.18. The values for the second and third replicates were significantly different from the expected ratio of 0.25, but in opposite directions. Averaged over all three replicates, the ratio was 0.28, not significantly different from the expected value.

Developmental time also varied among replicates. In replicates 1 and 2, the compound strain developed slightly, but significantly, faster than the normal strain (12.4 vs 13.6 and 11.4 vs 11.9 days, respectively). However, in replicate 3, it developed more slowly (15.1 vs 13.4 days). All of these differences are statistically significant ($P < 0.001$). The small differences between strains in replicates 1 and 2 were statistically significant because there was very little overlap in development time between the normal and compound strains; i.e. almost all compound flies eclosed before the normal flies began to emerge. Because replicate 3 varied in the opposite direction from replicates 1 and 2, the overall developmental times, averaged over all three replicates, were not significantly different (12.9 vs 13.1 days for normal and compound respectively; $P \cong 0.23$). The observed differences within replicates were not due to density effects: regression analysis and analysis of covariance showed no significant relationship between the number of offspring produced and development time ($P \cong 0.18, 0.19, 0.32$, and 0.06 for replicates 1, 2, 3, and all three combined, respectively).

The mating choice experiments (Table 2) revealed a small, but statistically significant level of assortative mating between the + B and bB strains ($I = 0.38 \pm 0.12$). This was due primarily to a deficiency of $+B_{♀} \times bB_{♂}$ matings; i.e. the + B females seemed to discriminate against the bB males.

To summarize the results, the compound strain had a net fitness of about 0.05 compared with the normal strain. There were no significant fertility differences between the normal and compound strains. The compound strain produced about 28% as many offspring as the normal strain. In two replicates, the compound strain developed faster than the normal strain and in one replicate it developed slower. Finally, there was significant assortative mating, with the + B females showing a strong preference for their own type.

Discussion

If inviability due to aneuploid gametes were the only factor affecting fitness differences between the bB and + B strains, we would expect the relative net fitness of the bB strain to be about 0.25. In fact, it was much lower than this, as estimated by the population replacement experiments (Fig. 1). Obviously, something else is affecting relative fitness of the bB strain. Considering the results for all three replicates combined, mean productivity of the bB strain was about 0.28, not significantly different from the expected value of 0.25. There were no significant differences in fertility or development time. Therefore, these three components of fitness cannot explain the low overall fitness of the bB strain. Our data suggests that nonrandom mating between the two strains contributes significantly to the lowered fitness of the bB strain.

Prout (1981) has derived the recursion equations for a negative heterosis system under random mating, and under assortative mating. From these equations, the predicted unstable equilibrium points (critical points) can easily be determined. In this discussion, we shall use his results, but with a slightly different parameterization of fitnesses. For random mating, we assume that net fitness consists of a viability component only; i.e. W = V. Therefore, the critical point is easily found from Eq. (1):

$$\hat{q}_{RM} = \frac{1}{1 + V} \tag{2}$$

where $\hat{q}_{RM}$ is the critical point under random mating, and V is the relative viability of the compound strain, compared with the normal strain.

Under assortative mating, we define

$\hat{q}_{AM}$ = critical point under assortative mating
D_{NC} = relative preference of normal females for compound males
D_{CN} = relative preference of compound females for normal males
V = relative viability of the compound strain compared with the normal strain

Here, D_{NC}, D_{CN}, and V are components of the net fitness, W, of the compound strain. Prout (1981) defined $r = p/q = (1 - q)/q$ and showed that

$$\hat{r}_{AM} = \frac{V - 1 + \sqrt{(1 - V)^2 + 4V\,D_{NC}\,D_{CN}}}{2\,D_{CN}}$$

where $\hat{r}_{AM} = (1 - \hat{q}_{AM})/\hat{q}_{AM}$. Substituting this into the left side and solving for $\hat{q}_{AM}$ gives

$$\hat{q}_{AM} = \frac{2\,D_{CN}}{2\,D_{CN} + V - 1 + \sqrt{(1-V)^2 + 4V\,D_{NC}\,D_{CN}}} \quad (3)$$

The net fitness of the compound strain is then

$$W = \frac{1 - \hat{q}_{AM}}{\hat{q}_{AM}}$$

Comparing Eqs. (2) and (3) we can see the effect of assortative mating on the value of the critical point; it is easy to show that (3) reduces to (2) if $D_{NC} = D_{CN} = 1$.

The values of D_{NC} and D_{CN} can be estimated from Table 2 as $D_{NC} = 5/21 = 0.24$ and $D_{CN} = 12/17 = 0.71$. Substituting these values into equation (3), using $V = 0.25$, we get $\hat{q} = 0.93$. This compares favorably with our estimate of $\hat{q} \geqq 0.95$ based on the population replacement experiments (Fig 1). It is probably purely coincidence that the agreement is so close, considering the large standard error of I, the effect of genetic drift during the replacement experiments, and temporal variability in the level of assortative mating (see below). However, these results suggest that the mating preferences of the females are a major component of the observed fitness differences between the +B and bB strains. The +B females seem to have discriminated strongly against the bB males ($D_{WC} = 0.24$), whereas the bB females seem to have had a much weaker discrimination against the +B males ($D_{CN} = 0.71$).

Ehrman (1983, and references therein) has performed similar kinds of mating choice experiments on these two strains several times since 1971. In general, her results have shown no significant assortative mating when the two strains are cultured separately; however, one test, performed in 1976, gave a significant isolation index of $I = 0.35 \pm 0.09$ (Ehrman 1979). Later tests have shown no significant assortative mating (Ehrman 1979, 1983). Similarly, recent tests in our laboratory (T. Wilson, unpublished) show no significant assortative mating. Clearly, the level of assortative mating has fluctuated greatly over the last fifteen years. For this reason, we consider the results in Table 2 to be the best estimate of the level of assortative mating at the time of our replacement experiments, because our assortative mating and replacement experiments were done at the same time. We recognize that this estimate is based on a very small sample size, and that the level of assortative mating apparently fluctuates greatly over time. Our calculations are primarily to illustrate the effect of non-random mating on population replacement, and we do not claim that they give precise estimates of the degree of assortative mating, or of net fitness.

It should be remembered that there are other components which may affect net fitness; for example, fecundity, longevity, and sperm competition. We have no evidence that these are important in the +B and bB strains, but they may contribute toward reduced relative net fitness in some compound chromosome strains.

We view these results primarily as a warning to those considering the use of compound chromosome strains in pest control projects. Though the standard fertility and viability components of fitness may compare favorably with the normal strain, even a small amount of non-random mating (especially if it takes the form of discrimination against compound males) can substantially raise the critical point, making it much less likely that the compound chromosome strain will replace the pest population. Furthermore, any pre-existing tendency toward assortative mating by the pest females will probably be rapidly strengthened: because postzygotic reproductive isolation is complete, the pest females will be under intense natural selection to avoid mating with the introduced males. Many experiments have documented the existence of natural genetic variation for mating preferences, and the effect of selection on strengthening those preferences (e.g. Dobzhansky and Pavlovsky 1971; Ehrman 1965; Halliburton and Gall 1981). The implications for pest control projects are ominous: over the long term, native females are likely to develop the ability to recognize and reject introduced males.

Acknowledgements. We thank Lee Ehrman for providing the *Drosophila* strains, and Victoria Diaz and Greg Simonson for technical assistance and permission to use some of their data. We also thank Natalie Humphreys for drawing the figure. Lee Ehrman and Timothy Prout critically read the manuscript and offered many insightful comments and suggestions. RH wishes to thank Timothy Prout for many stimulating discussions about compound chromosomes. Financial support was provided in part by a grant from the Connecticut State University Research Foundation.

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