RESEARCH ARTICLE

Genotype–environment interaction for total fitness in *Drosophila*

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Abstract

A fundamental assumption of models for the maintenance of genetic variation by environmental heterogeneity is that selection favours different genotypes in different environments. Here, I use a method for measuring total fitness of chromosomal heterozygotes in *Drosophila melanogaster* to assess genotype–environment interaction for fitness across two ecologically relevant environments, medium with and without added ethanol. Two-third chromosomes are compared, one from a population selected for ethanol tolerance, and the other from a control population. The results show strong crossing of reaction norms for outbred, total fitness, with the chromosome from the ethanol-adapted population increasing fitness on ethanol-supplemented food, but decreasing fitness on regular food, relative to the chromosome from the control population. Although I did not map the fitness effects below the chromosome level, the method could be adapted for quantitative trait locus mapping, to determine whether a substantial proportion of fitness variation is contributed by loci at which different alleles are favoured in different environments.

[Fry J. D. 2008 Genotype–environment interaction for total fitness in *Drosophila. J. Genet.* **87**, 355–362]

Introduction

Models of both single loci (Levene 1953; Haldane and Jayakar 1963) and polygenic characters (e.g., Slatkin 1978; Gillespie and Turelli 1989) show that when selection varies across environments, genetic variation can be maintained. A fundamental assumption of these models is that selection favours alternative alleles in different environments. Although there are examples of loci where this appears to be the case (e.g., Clarke *et al*. 1963, reviewed in Hedrick *et al*. 1976; Hedrick 1986; Eanes 1999), it is not clear whether such loci contribute substantially to genetic variation for fitness in populations. Families or clones usually show different fitness rankings when measured in different environments, but such crossing of reaction norms can also result from loci where the magnitude of allelic effects differs between environments, without differences in the direction of the effects (Fry 1993). Quantitative trait locus (QTL) mapping provides a promising approach for distinguishing these possibilities. If polymorphisms at which different alleles are favoured in different environments are common, then QTL associated with high fitness in one environment should often be associated with low fitness in other environments (see Fry *et al*. 1998).

There are two challenges in using QTL mapping to detect loci showing antagonistic pleiotropy across environments. Because fitness traits usually have a low heritability (Mousseau and Roff 1987; Roff and Mousseau 1987), it is desirable to measure them on replicated genotypes, which requires creating recombinant inbred or isogenic lines (RILs). In outbreeding species, however, such lines will suffer from inbreeding depression, and therefore may give results that are not representative of noninbred genotypes. The RILs can be crossed to unrelated strains to produce outbred hybrids, but fitness traits of such hybrids may still be affected by the inbreeding of their parents, particularly early-life-fitness traits, which often show a strong maternal influence (Bowman 1974; Roach and Wulff 1987). The best method, then, would be to measure fitness of outbred progeny of outbred individuals, but such progeny will normally consist of many genotypes due to segregation and recombination, thus conflicting with the need to measure traits on replicated genotypes.

A second challenge is the need to measure total fitness, not just individual fitness components. In theory, different

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Keywords. antagonistic pleiotropy; balancer chromosomes; environmental heterogeneity; maintenance of genetic variation; trade-offs.

components of fitness can be measured and combined into a single comprehensive measure, but this is laborious, and even then may not capture all of the relevant fitness variation. For example, an individual's genotype may influence not only its own probability of survival, but that of its offspring (e.g. Kerver and Rotman 1987); such transgenerational effects cannot be detected in single-generation fitness assays.

Drosophila melanogaster is one organism where it is possible to circumvent these difficulties. In this species, one can measure total fitness of replicated outbred genotypes by taking advantage of specially constructed chromosomes called balancers (Falk 1967; Fowler *et al*. 1997; Gardner *et al*. 2005). These contain inversions that suppress recombination with normal-sequence homologues, visible markers that allow flies with the balancer to be distinguished, and one or more recessive lethals. With appropriate care, balancers can be used to establish populations in which visibly distinguishable genotypes that either contain or lack a given wild-type chromosome (in heterozygous form) segregate without recombination. The rate of frequency change of the genotypes can then be used to provide a comprehensive measure of their relative fitness. By applying these methods to recombinant chromosomes, one could in principle map QTL affecting total fitness, to determine how often alleles that increase total fitness in one environment decrease fitness in another environment. At the least, under the hypothesis that environmental heterogeneity maintains variation, it should be possible to identify pairs of chromosomes in which the chromosome with higher relative fitness in one environment has lower relative fitness in a second environment.

Here, I report the first application of a balancer-based method to study genotype–environment interaction for total fitness. I estimated total heterozygous fitness of two *D. melanogaster* third chromosomes in each of the two environments, normal medium and medium supplemented with ethanol, a natural component of *D. melanogaster's* breeding sites (McKenzie and McKechnie 1979; Gibson *et al*. 1981; Merçot *et al*. 1994). One chromosome came from a laboratory population that had been selected for ethanol tolerance by being maintained on ethanol-supplemented medium for > 100 generations (the 'HE1' population of Fry *et al*. 2004), and the other chromosome came from a corresponding unselected population ('R1' population). I focussed on chromosome three, because a preliminary study showed that roughly one-half of the substantial difference in larval-ethanol resistance (survival and development rate on 16% ethanol) between the HE and R populations mapped to this chromosome (J. D. Fry unpublished data; see Chakir *et al*. 1996). The chromosome from the ethanol-adapted population had higher relative fitness on ethanol-supplemented food, but lower relative fitness on regular food, than the chromosome from the control population, thus confirming the presence of genotype–environment interaction for total fitness in the predicted direction. Although I did not map the fitness effects below the chromosome level, the method used here could be readily adapted for that purpose. The results also cast light on recent results of Gardner *et al*. (2005) that were interpreted as giving evidence for extraordinarily high levels of genotype–environment interaction for fitness in a single *D. melanogaster* laboratory population; I suggest a different interpretation.

Materials and methods

Overview of method

Suppose that '+' is a wild-type chromosome whose heterozygous fitness we wish to measure, and '*Bal m leth1*' is a balancer with recessive visible marker *m* and a recessive lethal at locus 1. The first step of the method described here is to cross a second recessive lethal, *leth2*, onto one end of the wild-type chromosome; the resulting chromosome, '+ *leth2*', is then placed over the balancer. The genotype *Bal m leth1/+ leth2* is a true-breeding, balanced lethal stock; neither homozygote can survive, and recombination that could produce nonlethal-bearing chromosomes is suppressed. A similarly true-breeding competitor strain of genotype *Bal m leth1/m leth2* can also be constructed; unlike the first genotype, this genotype is homozygous for *m* and therefore will show the marker phenotype. If these two genotypes mate, they will produce the same two genotypes in equal proportions among the zygotes; no other viable genotypes will be produced. The relative fitness of the two genotypes in a mixed population can be measured by monitoring the change in frequency of flies with the visible marker over time. By measuring the fitness of different + chromosomes relative to the same marked competitor chromosome, the fitness of the + chromosomes can be compared.

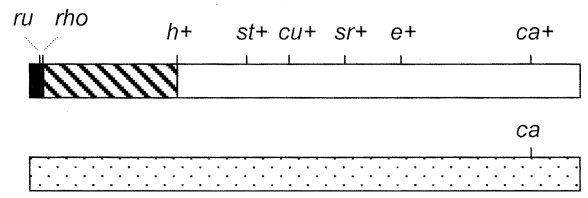
Rearing conditions, strains and crosses

Flies were maintained on medium containing cornmeal, dead brewer's yeast, molasses, and agar, under continuous light at 25°C. Flies were handled under light CO_2 anesthesia.

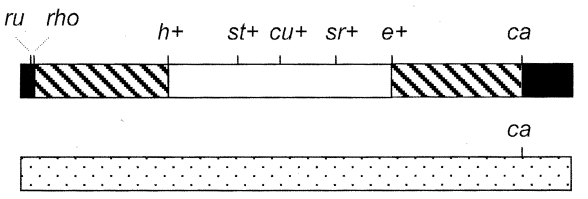
The experimental populations were made using the following stocks (Lindsley and Zimm 1992; FlyBase Consortium 2003): (1) Raleigh; *TM3, ru Sb e/H:* a third-chromosome balancer stock with outbred genetic background from a Raleigh, North Carolina population. (2) *rucuca:* a stock homozygous for the third chromosome recessive markers (map position in cM) *ru* (0), *h* (26.5), *st* (44), *cu* (50), *sr* (62), *e* (70.7), and *ca* (100.7). (3) Raleigh; *TM3, ru Sb e/ru* rho^{7M43} *h st cu sr* e^s *ca:* a stock heterozygous for a chromosome with the same markers as *rucuca*, with the addition of the recessive lethal *rho*, very tightly linked (0.1 cM) to *ru*. This stock had recently been made isogenic for the *ru* rho^{7M43} *h st cu sr* e^s *ca* chromosome. (4) HE1-8 and (5) R1-1: stocks isogenic for third chromosomes extracted from an ethanol-adapted (HE1) and control (R1) population, respectively, of Fry *et al*. (2004), using stock 1. Several chromosomes were extracted from each population; HE1-8 and R1-1

were chosen because they were relatively healthy as homozygotes, indicating that they did not contain major deleterious mutations. These stocks were maintained as homozygotes by mass transfer for approximately 10 generations before being used for the experiments reported here. (6) *TM6C, cu Sb ca/+* and (7) *TM6B, Tb ca/+:* stocks with third chromosome balancers bearing the recessive marker *claret* and dominant markers *Stubble* and *Tubby*, respectively, maintained by crossing *Sb* or *Tb* males with females from the R1 and R2 unselected populations (Fry *et al*. 2004).

The above stocks were used to construct stocks containing the *ru* marker and *rho* lethal crossed onto the left end of the wild-type HE1-8 and R1-1 chromosomes (figure 1,a). For each progenitor chromosome, three such stocks were made, each derived from a different recombinant male (and thus likely to have different break points in the *ru-h* interval; see figure 1,a). In addition, two marked competitor stocks were constructed so as to have *ca* on the right end of each of HE1-8 and R1-1, as well as *ru* and *rho* on the left end (figure 1,b). The experimental chromosome stocks (figure 1,a) were maintained over *TM6C, cu Sb ca*, and the competitor chromosome stocks (figure 1,b) were maintained over *TM6B, Tb ca*.



a) Experimental genotype (wild-type eye color).



b) Competitor genotype (*claret* eyes).

Figure 1. The two third chromosome genotypes in each experimental population. The lower bar in each figure represents a balancer chromosome bearing the recessive marker *ca (claret)*. In the other chromosome, unshaded areas are derived from one of the progenitor wild-type chromosomes, black areas are derived from the multiply-marked *rucuca* chromosome with the recessive lethal *rho* (stock 3; see text for details), and hatched gray areas denote regions of recombination between the wild-type and *rucuca* chromosomes.

The sequence of crosses were: (i) stocks 4 and 5 females × stock 3 males; (ii) wild-type female progeny × *rucuca* males; (iii) single recombinant males (*ru* or *ru* ca phenotypes) × stock 1 females; (iv) phenotypically *ru Sb e+* males (that received the recombinant chromosome) × stock 1 females; (v) phenotypically *H Sb+* males × stock 6 or stock 7 females; (vi) *TM6C, cu Sb ca/ru rho* or *TM6B, Tb ca/ru rho ca* flies crossed *inter se* to establish experimental and competitor stocks, respectively. These crosses ensured that all stocks had similar, outbred genetic backgrounds derived mostly from unselected Raleigh chromosomes.

Experimental populations

A total of 48 experimental populations were established, comprising every possible combination of the two progenitor chromosomes, three replicate experimental chromosomes within progenitor chromosome, two competitor chromosomes, two balancers (*TM6C, Sb* and *TM6B, Tb*), and medium with and without added ethanol. As a first step, females of each of the six experimental chromosome stocks (with the *TM6C, Sb* balancer) were crossed to males of both of the competitor chromosome stocks (with the *TM6B, Tb* balancer). To establish the experimental populations with the *TM6C, Sb* balancer, *Sb* male progeny of this cross, which inherited the *ru rho ca* competitor chromosomes from the *Tb* parents, were crossed to females of each of the experimental chromosome stocks. To establish the experimental populations with the *TM6B, Tb* balancer, the *Tb* male progeny, which inherited the *ru rho* experimental chromosomes from the *Sb* parents, were crossed to females of both of the competitor chromosome stocks. In both cases, the viable zygotes produced by these crosses should have consisted of equal proportions of experimental (figure 1,a) and competitor (figure 1,b) genotypes.

Each founding cross (n = 24) was performed in 10 25-mm diameter vials containing regular medium, with five pairs per vial. After three days, flies were turned over to vials containing medium supplemented with 10% ethanol and allowed to lay eggs for four more days to establish the ethanol-food populations. The ethanol concentration was increased to 12% in subsequent generations. Although the HE populations are kept on 16% ethanol, the experimental populations were not able to survive on this concentration, and 12% was sufficient to reveal significant differences between the selected and unselected populations (Fry 2001). Each of the experimental populations was maintained thereafter on 14-day discrete generations, following essentially identical procedures as are used to maintain the R and HE populations. On the 14th day, all flies in each vial were anesthetized, and the number of *claret* and wild-type (normal-eyed) flies were counted. Flies from the 10 vials per population were then pooled and mixed, and approximatly 25 flies were placed in each of 10 new vials. The resulting population size of ~250 should have been large enough to allow selection to discriminate between chromosomes differing in fitness by a few per

cent. Flies were removed from the vials after laying eggs for two days on regular food, and after three or four days on ethanol-supplemented food. The experiment was continued for six generations.

Data analysis

Two effectively clonal genotypes + and m with initial frequencies p_0 and q_0 will change in frequency according to,

$$p_n/q_n = w^n p_0/q_0, \quad (1)$$

where w is the relative fitness of + to m, and n is the number of generations (Hartl and Clark 1997). This relationship holds with fertility as well as viability selection, as long as the male and female fertilities combine in multiplicative fashion, and selection acts similarly in both the sexes. With these assumptions, it is easy to show that equation (1) holds regardless of whether frequencies are monitored in zygotes or in adults. Therefore, the change in frequencies of wild-type and *claret* flies in the experimental populations can be used to estimate the fitness of the experimental chromosomes relative to the *claret* competitors. From equation (1), if the logarithm of the ratio of wild-type to *claret* flies is regressed against generation number, the slope estimates $\log(w)$. Regression slopes were calculated for each of the experimental populations, and used as the dependent variable in a mixed-model factorial analysis. Fixed effects were progenitor chromosome, balancer, medium, and competitor chromosome, as well as all possible two-way, three-way, and four-way interactions. Random effects were replicate chromosome within progenitor chromosome, and all possible interactions of replicate chromosome with the fixed effects. Analysis was performed using PROC MIXED in SAS (Littell *et al.* 1996), with the 'SATTERTHWAITE' option. With this option, SAS eliminates from the model any random effects with estimated variance of zero before performing F-tests for the fixed effects. To obtain 95% confidence intervals (CI) for the difference in $\log(w)$ between HE1-8 and R1-1 derived chromosomes on each medium, additional MIXED analyses were performed for each medium separately, using the 'ESTIMATE' and 'CL' options. The upper and lower limits of the CI were transformed into upper and lower limits for the ratio of w between HE1-8 and R1-1 experimental chromosomes by taking the exponent.

To determine whether it was necessary to count and score flies for every generation, the above analyses were repeated using only the final generation's data. For this purpose, regression slopes were estimated as $\log(p_n/q_n)/(n-1)$, where n is the number of generations (this assumes that $p_1/q_1 = 1$, i.e. that viability selection in the progeny of the founding crosses was negligible).

Results

Changes in the ratio of wild-type experimental chromosomes to *claret*-eyed competitors in each of the 48 populations are shown in figure 2. Wild-type chromosomes increased in frequency in all instances, probably due to deleterious effects of the *claret* marker. Table 1 shows the results of the mixed-model analysis, where the dependent variable was the slope of the regressions of log (no. of wild-type flies/no. of *claret* flies) on generation number. Notably, there was no significant effect of either wild-type progenitor chromosome (P) or medium (M), but there was a highly significant interaction between the two. In particular, on ethanol-supplemented medium, wild-type chromosomes derived from HE1-8, the chromosome from the ethanol-adapted population, increased in frequency more quickly than those derived from R1-1, the chromosome from the control population (figure 2), with the former having a 16.7% (95%CI: 7.2–27.2%) fitness advantage over the latter. In contrast, on regular food, the differences were reversed, with R1-1 having 17.8% (4.9–32.4%)

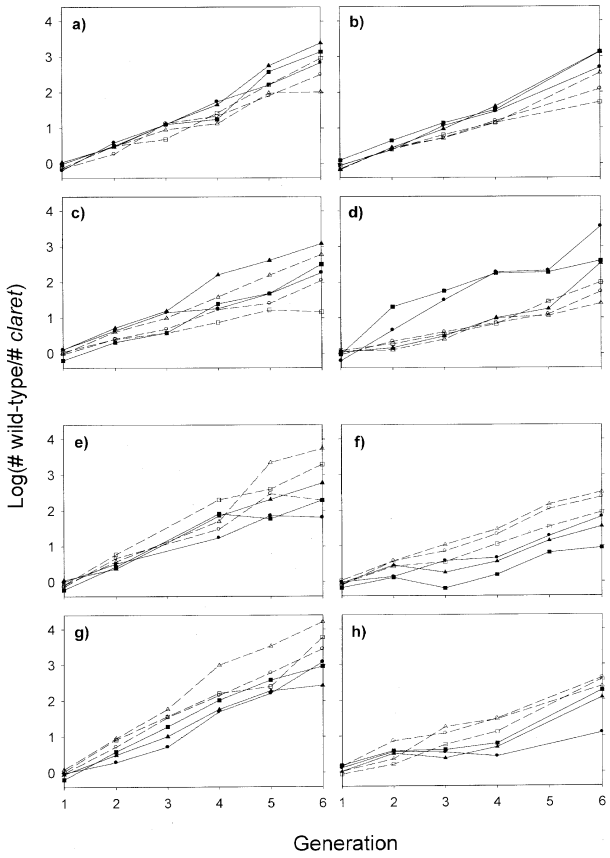


Figure 2. Changes in frequency of wild-type and *claret* flies in each of the 48 experimental populations. Solid lines with filled symbols: experimental chromosomes derived from ethanol-selected population chromosome, HE1-8. Dashed lines with open symbols: experimental chromosomes derived from control population chromosome, R1-1. (a–d), Populations on ethanol-supplemented medium. (e–h) Populations on normal medium. (a,b,e,f), Populations with *TM6C, Sb* balancer. (c,d,g,h), Populations with *TM6B, Tb* balancer. (a,c,e,g), Competitor chromosome derived from HE1-8. (b,d,f,h), Competitor chromosome derived from R1-1.

Table 1. Mixed-model analysis of regression slopes; F-tests for fixed effects are shown. The only random effects with nonzero point estimates were replicate chromosome within progenitor chromosome (estimated variance = 0.00011, S.E. = 0.00092), and the residual variance (estimate = 0.0090, S.E. = 0.0024).

Effect	F[1]	Prob.
Balancer (B)	0.50	0.49
Competitor chromosome (C)	24.03	< 0.0001
Medium (M)	0.11	0.74
Progenitor wild-type chromosome (P)	0.03	0.88
$B \times C$	0.07	0.79
$B \times M$	8.28	0.0076
$B \times P$	0.09	0.77
$C \times M$	12.25	0.0016
$C \times P$	0.19	0.67
$M \times P$	33.89	< 0.0001
$B \times C \times M$	1.20	0.28
$B \times C \times P$	0.00	0.99
$B \times M \times P$	0.02	0.88
$C \times M \times P$	0.73	0.40
$B \times C \times M \times P$	0.22	0.65

[1]All mean squares except MS(P) were tested over the residual mean square (28 df); MS(P) was tested over MS(rep. chromosome(P)) (4 df).

higher fitness than HE1-8. The relative fitness of HE1-8 and R1-1 was not affected by balancer (B) or competitor chromosome (C), as indicated by the nonsignificance of the $B \times P$, $C \times P$, and higher-order interactions (table 1). Wild-type chromosomes increased faster against the HE-derived competitor than against the R-derived competitor, especially on regular food (figure 1); this accounts for the significant C and $C \times M$ effects (table 1). The only other significant effect in the model was the $B \times M$ interaction: on ethanol-supplemented food, wild-type chromosomes increased faster when over the *TM6C, Sb* balancer than when over the *TM6B, Tb* balancer, whereas on regular food, the trend was reversed (figure 2). Although this result is difficult to interpret, it does not affect inferences about the relative fitness of the two progenitor chromosomes, which was not affected by balancer. Finally, the effect of replicate chromosome within progenitor chromosome was nonsignificant (table 1), indicating that heterogeneity of recombination breakpoints in the *ru-h* region (figure 1) had little effect on the results.

Although flies were counted and scored for every generation (with a small number of exceptions; figure 2), analysis using data only from only the final generation gave nearly identical results to those above. Using just the final generation's data, the estimated fitness superiority of HE1-8 over R1-1 on ethanol-supplemented food was 18.0% (95%CI: 8.8–28.0%), while the fitness superiority of R1-1 over HE1-8 on regular food was 18.3% (95%CI: 9.1–28.3%).

Discussion

In this experiment, two *D. melanogaster* third chromosomes showed different rankings for outbred, total fitness depending on whether fitness was measured on medium with or without added ethanol. The fitness differences were robust to changes in the balancer chromosome, giving evidence that they did not result from interactions with the dominant markers on the balancers (but see caveats below). The fitness differences were also unaffected by whether the competitor chromosome was related or unrelated to the experimental chromosome, thus giving no evidence for frequency-dependent selection. Although it is possible that interactions with the recessive lethal *rho* were responsible for some of the fitness differences, this seems unlikely, given that the average heterozygous fitness effect of lethals is only about 2% (Simmons and Crow 1977). This possibility could nonetheless be tested by repeating the experiment with a different lethal.

There are two possible explanations for the results. First, there may have been one or more loci at which the allele conferring higher fitness in the presence of ethanol conferred lower fitness in its absence. Such trade-offs could help maintain genetic variation for environment-specific fitness within populations, and could also contribute to the maintenance of the latitudinal cline in ethanol tolerance in *D. melanogaster* (e.g. David and Bocquet 1975; Anderson 1982), which maps primarily to the third chromosome (Chakir *et al*. 1996). In a previous study (Fry 2001), I found no evidence that selection for ethanol tolerance reduced larval survival or development rate in the absence of ethanol, but the method used here measures total fitness, not just juvenile components. In addition, the HE1 population used as the source of the ethanol-adapted third chromosome in this study was selected on medium with 16% ethanol, as opposed to 12% for the 'E' populations of Fry (2001). An alternative possibility is that the changes in fitness ranking were caused by differences in the magnitude of allelic effects across environments, without differences in the direction of the effects. An extreme version of this hypothesis would be that the fitness difference on each medium was caused by loci that were neutral on the other medium.

The method described here, with only minor modification, could be used to distinguish these possibilities, and to map loci giving rise to genotype–environment interaction for fitness in other instances. The key requirement would be to create chromosomes that are recombinant for the two wild-type chromosomes being mapped, and that also bear the known lethal. The easiest method would be to first introduce the lethal onto one of the wild-type chromosomes, and then create F_1 hybrids heterozygous for the wild-type chromosomes and the lethal. Recombinant F_2 chromosomes bearing the lethal could then be recovered with the assistance of the linked visible marker, measured for fitness by the method described here, and scored for molecular markers that discriminate the two parental chromosomes. The amount of labour required for scoring fitness in a large number of recombi-

nant lines could be made manageable by counting flies at intervals of several generations, rather than every generation, which appears to result in negligible loss of information. Improvements in power over the current experiment could be made by using larger population sizes to reduce the effect of drift, using more replicate chromosomes, and starting the experimental populations with a lower proportion of wild-type flies than the initial frequency of 0.5 used here, which would enable more generations to elapse before the competitor became rare. Simulations (J. D. Fry, unpublished data) also show that power would be maximized by using a competitor chromosome whose fitness is close to the average of the wild-type chromosomes. Because all easily-scoreable visible markers are likely to reduce fitness, an alternative would be to use a molecular marker, the frequency of which could be estimated in DNA pools from the experimental populations. Although this study focussed on the *ca* 80% of the third chromosome to the right of *h* (figure 1), the general method could be applied to the X and second chromosomes, as well as to the left end of the third chromosome, by appropriate choice of lethals. As a result, the method could be used to quantify and map virtually any fitness difference in *D. melanogaster*.

The results reported here do not give information on how much genetic variation for fitness is present within the HE1 and R1 populations, because only one experimental chromosome derived from each population was studied. A larger study would be needed to measure genetic variation for fitness, and to determine whether the crossing of reaction norms for total fitness seen in the two experimental chromosomes is a general property of third chromosomes from the populations. For such a study, it would be preferable to use a random sample of third chromosomes, not just ones that are relatively healthy as homozygotes, as were used in the "proof-of-principle" experiment reported here.

Balancers have been used extensively for fitness estimation in *Drosophila*, but rarely for multigeneration estimation of variation in total fitness among outbred genotypes (Falk 1967; Mackay 1985; Fowler *et al*. 1997; Gardner *et al*. 2005). Sved and Ayala (1970) and Sved (1971) introduced a multigeneration balancer-based method for measuring total fitness of chromosomal homozygotes. The 'Sved' method has been used primarily to document the existence of substantial inbreeding depression for total fitness (Sved and Ayala 1970; Sved 1971; Mackay 1985, and references therein). The method also yields estimates of the variance in fitness among isochromosomal lines, but this is of less interest than the variance among outbred genotypes. The most extensive use of balancers in *Drosophila*, however, has been to create replicated genotypes for single-generation estimation of the genetic variance of individual fitness components. Several large studies using this approach have documented the presence of significant additive genetic variance for egg-to-adult viability of chromosomal heterozygotes (Mukai *et al*. 1974; Mukai and Nagano 1983; Tachida *et al*. 1983). There appears to have been only one study, however, to simultaneously measure preadult, adult male, and adult female fitness of chromosomal heterozygotes, yielding the interesting discovery that male and female fitness are negatively correlated (Chippindale *et al*. 2001). As noted in the Introduction, all single-generation methods have the limitation of overlooking possible transgenerational fitness effects. Another advantage of multi-generation methods is that small fitness differences become amplified over time, thus potentially yielding higher power than single-generation methods.

The method used here contrasts somewhat with the balancer-based method used by Fowler *et al*. (1997) and Gardner *et al*. (2005) to document substantial genetic variation for total outbred fitness among third chromosomes from a single, long-established laboratory population. In these studies, each experimental population has two balancer chromosomes, *TM1* and *TM2*, and a single wild-type chromosome, resulting in four possible genotypes: *TM1*/+, *TM2*/+, *TM1/TM2*, and +/+ (the original method was restricted to lethal-bearing wild-type chromosomes, resulting in the absence of the last genotype, but Gardner *et al*. (2005) found that chromosomal homozygotes had such low fitness that their presence had little effect on the results). The *TM1/TM2* genotype is the same in all experimental populations, and the fitness of heterozygotes for different wild-type chromosomes relative to this common standard can be estimated from the changes in frequency of the genotypes. With this method, it is necessary to monitor zygotic frequencies to estimate fitness; this necessitates an extra step compared to the method used here (which is similar to one used by Falk (1967) for comparing heterozygous fitness of irradiated and nonirradiated second chromosomes). The two-balancer method nonetheless has the advantage of not requiring crosses to introduce a known lethal onto the experimental chromosomes, which potentially introduces random fitness variation among the recombinant chromosomes. In this study, no fitness variation among the replicate chromosomes was detected, but a clear outlier was found in a preliminary experiment using other progenitor chromosomes (J. D. Fry, unpublished data). Ideally, more replicate chromosomes should be used in future experiments; alternatively, molecular markers could be used to select recombinant chromosomes with a more precisely defined breakpoint.

Interestingly, Fowler *et al*. (1997) and Gardner *et al*. (2005) found evidence for genotype–environment interaction involving unknown environmental changes over the course of their 300-day experiments, even though they attempted to maintain constant environmental conditions. The most convincing evidence was that in both studies, genotype frequencies showed modest short-term departures from the fitted long-term trends that were correlated between replicate cages containing the same wild-type chromosome, suggesting that the cages were responding in similar fashion to environmental fluctuations. Less convincing as evidence for genotype–environment interaction, Gardner *et al*. (2005) observed that for a subset of wild-type chromosomes, the rela-

tive fitness of *TM1*/+ and *TM2*/+ changed substantially over the course of the experiments. The experiments were initiated by introducing a small number of *TM2*/+ flies in cages containing *TM1*/+ flies. For about half of the wild-type chromosomes ('invaders'), *TM2* rapidly increased in frequency, eventually eliminating *TM1*. For some chromosomes ('transients'), however, *TM2* at first increased only slowly, then decreased again and was eventually eliminated. A model fit to the data from these chromosomes indicated that the relative fitness of *TM1*/+ to *TM2*/+ increased by orders of magnitude over the course of the experiment. Considering that the authors made no effort to manipulate environmental variables, this would be an extraordinary amount of genotype–environment interaction; for comparison, in the experiment reported here, the change in relative fitness across environments of the chromosomes derived from the ethanol-selected and control populations was well under two-fold.

A more likely explanation for the increase in relative fitness of *TM1*-bearing flies in Gardner *et al*. (2005) 'transient' lines is selection for modifiers of the deleterious effects of the balancer. Chromosomes other than the third were genetically variable in the cages of Gardner *et al*. (2005); one would expect any allele on these chromosomes that increased fitness of the most common balancer to be favoured, regardless of its effect on fitness of the rare balancer. In lines in which *TM2* did not have an initial strong fitness advantage and therefore remained rare, selection for modifiers would lead to a deterministic increase in the fitness of *TM1* relative to *TM2*. In the 'invader' lines, in contrast, *TM2* increased rapidly, leaving little time for selection to favour modifiers that increased the fitness of *TM1*. Consistent with the modifier hypothesis, the relative fitness of *TM2* increased in these lines over the course of the experiment, although not as dramatically as the fitness increase of *TM1* in the transient lines. This interpretation invokes multiple levels of fitness epistasis involving the balancers: different wild-type third chromosomes modify the relative fitness of *TM1*/+ and *TM2*/+, and this in turn affects the outcome of selection for epistatic modifiers on the other chromosomes. That there should be an extensive epistasis involving balancers is not surprising. Modifiers of deleterious mutations are well known (e.g. Lindsley and Zimm 1992); not only do balancers contain dominant visible mutations, but the inversions on them were generated by multiple rounds of X-ray mutagenesis, which likely generated many cryptic deleterious mutations.

In the experiment reported here, selection for modifiers of the fitness effects of the balancers was unlikely to have affected the results. Not only was the experiment much shorter than those of Gardner *et al*. (2005) (six generations vs. ~20), but all flies in the experimental populations were heterozygous for the same balancer, so that selection for modifiers of deleterious alleles on the balancers would not be expected to change the relative fitness of the experimental and competitor chromosomes. Nonetheless, the likely existence of modifiers of the fitness effects of balancers raises the question of whether balancer-based methods can produce artifactual fitness differences between chromosomes, or at least exaggerate existing differences. In the experiments of Fowler *et al*. (1997) and Gardner *et al*. (2005), fitness of *TM1*/+ and *TM2*/+ (relative to *TM1/TM2*) was correlated across wild-type chromosomes (although the variance in *TM1*/+ fitness was greater than that of *TM2*/+ fitness), giving evidence that the qualitative fitness differences were not dependent on the balancer. Similarly, in the experiment reported here, there was no evidence that relative fitness of the wild-type chromosomes was affected by balancer (although the two balancers used are related; Lindsley and Zimm 1992), and the pattern of genotype–environment interaction was consistent with the selection history of the populations from which the chromosomes were derived (Fry *et al*. 2004). These observations suggest that, if cautiously applied, balancer-based methods provide a useful tool for assessing genetic variation and genotype–environment interaction for total fitness.

Acknowledgements

I thank T. Coleman, K. Donlon, J. Klopotowski and A. Vasicek for assistance with the experiment, and the anonymous reviewers for helpful comments on the manuscript. This work was supported by grants from the National Science Foundation.

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Received 5 June 2008, in revised form 19 August 2008; accepted 21 August 2008
Published on the Web: 23 December 2008