Inf. Serv. 96: 241-245; Zeleny, C., 1919, J. Gen. Physio. 2: 69-71; Zeleny, C., 1921, J. Exper. Zool. 34: 203-233; Zeleny, C., 1922, Genetics 7: 1-115.

Measuring narrow-sense heritability in *Drosophila melanogaster* using inbred strains.

Gittrich, Marissa Rachelle, Rachel Ann Crowl, Cameron Drew Friedman, Nathaniel P. Locke, Griffith M. Saunders, Christopher J. Schimmoeller, Kayla Christina Schwartz, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403.

For a trait to evolve by either natural or human selection, the phenotypic variation of the trait must be inherited, *i.e.* be due to genetic variation. The fraction of total variation in a trait due to genetic variation is called the heritability of the trait. In addition, the best measure of whether a trait will evolve or respond to selection is narrow sense heritability (h^2), the fraction of the total variation due to the additive effects of genes. Dominance and gene × environmental interactions also affect quantitative traits and heritability values (for discussions of heritability, see Falconer and Mackay, 1996; Roff 1997; Allendorf and Luikart 2007; Hedrick, 2011).

Three possible ways to estimate the h^2 of a quantitative trait are: 1) trait correlations between parents and their offspring, where h^2 is equal to the regression slope of mid-parent values to offspring values; 2) comparing concordance of traits in monozygotic *versus* dizygotic twins, where h^2 is equal to two times the monozygotic concordance minus dizygotic concordance; 3) and using the results of selection experiments, where h^2 is equal to the response of selection divided by the selection differential (see Falconer and Mackay, 1996).

Everett *et al.* (2016) estimated h^2 for bristle number in *Drosophila melanogaster* by comparing midparent numbers to offspring numbers and observed a h^2 of 0.05 for females and 0.04 for males. In addition, Woodruff and Thompson (2005) estimated h^2 of sternopleural bristle number by selecting for increased bristle numbers over eight generations and observed h^2 values of 0.11 for females and 0.15 for males in non-inbred lines.

In this study, we estimated h^2 for sternopleural bristle numbers using three highly inbred lines of *D. melanogaster* (see sternopleural bristles in Chyb and Gompel, 2013, and in Figure 2 of Everett *et al*., 2016). We used a modified version of the methods of Possidente and McQuade (2015), who estimated h^2 for body size using inbred lines of *D. melanogaster*. The advantage of using such highly inbred, homozygous, lines to measure h^2 is that variation among individuals within the same line is due entirely to non-genetic effects, while dominance effects are eliminated (see discussions of this topic in Falconer and Mackay, 1996; Possidente and McQuade, 2015). With inbred lines, h^2 is equal to the genetic variance (V_G) divided by the sum of genetic variance and environmental variance (V_E) (Possidente and McQuade, 2015), *i.e*.,

$$h^2 = V_G/(V_G + V_E),$$

where V_G can be calculated using half the difference in means squared of the inbred lines examined, divided by 2 ($V_G = 0.5(((Mean^1 – Mean^2)/2)^2)$), and V_E for a given inbred line can be calculated using the standard deviation squared of that line ($V_{E1} = SD_1^2$) (Possidente and McQuade, 2015). To properly estimate h^2 you need to use the pooled estimate of V_E by calculating the average V_E for two populations of the same sample size ($V_E = (V_{E1} + V_{E2}) * 0.5$). To detail this process, we will walk through the calculation of h^2 for sternopleural bristle number using two theoretical inbred lines of *D. melanogaster*, IB_1 and IB_2.

IB_1 males had a mean sternopleural bristle number of 16.00 bristles, with a standard deviation (SD) of ± 2.58, while IB_2 males had a mean of 25.31 bristles with a SD of ± 3.25. Hence,

$IB_1\ V_E = SD^2 = 2.58^2 = 6.66$ $IB_2\ V_E = SD^2 = 3.25^2 = 10.56$
Total $V_E = (V_{E1} + V_{E2}) * 0.5 = (6.66 + 10.56) * 0.5 = 8.61$
$V_G = 0.5 * [((Mean_1 – Mean_2)/2)^2] = 0.5 * [((16 – 25.31)/2)^2] = 10.84$
$h^2 = V_G /(V_G + V_E) = 10.84/(10.84 + 8.61) = 0.56$

We used these formulas to calculate narrow-sense heritability in the following three inbred lines of *D. melanogaster*:

1) yIB females and males (marked with the yellow-body, sex-linked, *y* mutant and inbred by brother sister matings for 336 generations).
2) C(1)DX, *y f* females (marked with *y* = yellow bodies and *f* = forked bristle mutants) and w^{1118} (white eyed mutant) males (inbred by brother sister matings for 136 generations).
3) and C(1)DX, *y w f* females (marked with *y* = yellow bodies, *w* = white eyes and *f* = forked bristle mutants) and Binscy males (*B* = narrow eyes mutant and inbred by brother sister matings for 12 generations).

For detailed discussions of the mutant genes and chromosome rearrangements used in this study see Lindsley and Zimm (1992). A total of 38 flies were scored for bristle numbers from each side of males and females for each inbred line.

The mean (± SD) for sternopleural bristle numbers in males and in females of each inbred line, and the h^2 values for each inbred line comparison, are given in Table 1. What is clear from Table 1 is that estimations of h^2 using comparisons of different inbred stocks is not constant (varying from 0.004 to 0.640), suggesting that heritability is influenced by differences in genetic variation present in differrent stocks, strains and populations. Yet, Falconer and Mackay (1996) state that estimates of heritability tend to be similar in different populations.

Table 1. Means (± SD) and narrow sense heritability (h^2) values for sternopleural bristle numbers in comparisons of values in males and in females of three inbred lines of *D. melanogaster*.

	Mean (± SD) of Bristles	P	h^2
Male Comparisons			
yIB vs w^{1118}	17.18 (± 2.38); 16.69 (± 1.66)	0.41	0.004
yIB vs Binscy	17.18 (± 2.38); 27.24 (± 3.55)	<0.0001	0.581
w^{1118} vs Binscy	16.69 (± 1.66); 27.24 (± 3.55)	<0.0001	0.640
Female Comparisons:			
yIB vs C(1)DX, y f	17.65 (± 1.88); 21.05 (± 2.90)	<0.0001	0.195
yIB vs C(1)DX, y w f	17.65 (± 1.88); 20.61 (± 2.41)	<0.0001	0.204
C(1)DX, y f vs C(1)DX, y w f	21.05 (± 2.90); 20.61 (± 2.41)	<0.0001	0.298

Thirty-eight flies were scored for steronopleural bristle numbers for each line. P values are from t-tests using the Prism program.

In many cases in Table 1 the lowest estimations of h^2 are for inbred lines with similar mean bristle numbers (for example in yIB *vs* w^{1118} males, where h^2 was 0.004) and the highest estimations of h^2 are observed in lines with different mean bristle numbers (for example in w^{1118} *vs* Binscy males, where h^2 was 0.640). Also notice that the highest estimations of h^2 were in comparisons of Binscy males with other males (h^2 = 0.581 and 0.640) and C(1)DX, *y w f* females compared to C(1)DX, *y f* females and yIB females (h^2 = 0.298 and 0.204). Finally, the estimations of h^2 in this study, except for yIB *vs* w^{1118} (h^2 = 0.004), were higher than those estimated by comparing midparent to offspring (h^2 = 0.05) and by selection responses (h^2 = 0.11 and 0.15) (Woodruff and Thompson, 2005; Everett *et al*., 2016). Other reported estimations of h^2 for abdominal bristle numbers from parent-offspring regressions are about 0.51 (Falconer and Mackay, 1996).

Referenes: Allendorf, F.W., and G. Luikart 2007, *Conservation and the Genetics of Populations.* Blackwell Publishing, Malden, MA; Chyb, S., and N. Gompel 2013, *Atlas of* Drosophila *Morphology*. Academic Press, New York; Everett, A.M., *et al*., 2016, Dros. Inf. Serv. 99: 92-94; Falconer, D.S., and T.F.C. Mackay 1996, *Introduction to Quantitative Genetics.* Longman Group Limited, Essex, England; Hedrick, P.W., 2011, *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, MA; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Possidente, B., and D. McQuade 2015, Dros. Inf. Serv. 98: 151-153; Roff, D.A., 1997, *Evolutionary Quantitative Genetics*. Chapman and Hall, New York; Woodruff, R.C., and J.N. Thompson, jr. 2005, Dros. Inf. Serv. 88: 139-143.

Lack of chromosome breakage and altered sex ratios by copper sulfate in *Drosophila melanogaster.*

Crowl, Rachel Ann, Cameron Drew Friedman, Nathaniel P. Locke, Griffith M. Saunders, Kayla Christina Schwartz, Michael A. Balinski and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403.

Although copper plays an important metabolic role in all organisms, high concentrations can have toxic and mutagenic effects (Pra *et al.*, 2008; Balinski and Woodruff, 2017, and references therein). One common source of excess copper concentrations in the environment is copper sulfate, which is a fungicide used to kill bacteria, fungi, and snails and is a potential producer of genetic damage in exposed humans (National Pesticide Information Center: http://npic.orst.edu/factsheets/cuso4gen.html). Copper sulfate induces chromosome breakage events in mice and increases the rate of recessive sex-linked lethal mutations in *Drosophila melanogaster* (Law, 1938; Agarwal *et al*., 1990). It is the objective of this study, therefore, to determine if copper sulfate induces chromosome breakage events in the model system *D. melanogaster*. It is our hypothesis that this chemical will significantly increase X-chromosome breakage events. Since copper can alter sex ratios (Niklasson *et al*., 2000), we also investigated the ability of copper sulfate to alter sex ratios and the recovery rate of XXX (triplo-X) female progeny.

We screened for the ability of copper sulfate to induce chromosome breakage by treating adult wild-type (Canton-S) *D. melanogaster* males with 0.5 mM of copper sulfate mixed in *Drosophila* instant food (Wards Natural Science) and mating these males to C(1)DX, *y w f* / Y females possessing two X chromosomes attached to a single centromere and the recessive genetic markers *y* (yellow body color), *w* (white eyes), and *f* (forked bristles). We have previously observed that 0.5 mM of copper sulfate is just below the toxic level for *D. melanogaster* males (Balinski and Woodruff, 2017, and unpublished results). The attached-X chromosome and visible mutants are further discussed in Lindsley and Zimm (1992).

As shown in the mating scheme below, y^+ flies have wild-type grey body color, w^+ flies have wild-type red eyes, and f^+ flies have wild-type long bristles. The Y chromosomes in females and males will be ignored, since we did not identify Y-chromosome breakage events. This assay was previously used to identify chemical-induced and gamma-ray-induced chromosomal breakage in *D. melanogaster* males (Blount and Woodruff, 1986; Woodruff and Russell, 2011).