

Gu, Sheng, Yi Gong, Matthew Smith, and R.C. Woodruff. 2002. The dominance value of the *Sb* allele in *Drosophila melanogaster*. *Dros. Inf. Serv.* 85: 143-149



The dominance value of the *Sb* allele in *Drosophila melanogaster*.

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Introduction

The genetic material of all organisms, including humans, seems remarkably stable. For example, most humans seem to develop from zygote to adult with few major morphological mistakes. There are few people with two heads or three arms. Yet this assumption of genetic stability is an illusion. For example, every cell of a human undergoes hundreds of nucleotide changes and at least ten double-strand breaks in DNA occur each day (Bernstein and Bernstein, 1991). The reason we survive this genetic insult is that most of this genetic damage is corrected by DNA repair mechanisms. However, not all of these mutations are corrected. For example, it has been estimated that at least 100 nucleotide changes occur in each human gamete, each gamete has from two to four new deleterious mutations, and each human has one or more recessive lethal mutations (Crow, 2001; Eyre-Walker and Keightley, 1999; Kondrashov, 2001). These deleterious and lethal mutations can have an important effect on our health and fitness. However, the influence of these mutations depends on their selection values (how much does each mutation reduce health and fitness) and on their genetics (are they recessive or dominant).

It is usually assumed that recessive lethal mutations also have some influence on the health and fitness of individuals when these mutations are present as heterozygotes, *i.e.*, the individual without this mutation (+/+) is assumed to be more healthy and fit (produces more children, for example) than the individual that is heterozygous (+/l) for this mutation. This reduction in health and fitness in the heterozygotes would be due to some amount of dominance for the lethal mutation. What is this level of dominance (called *h*, the dominance value) and how does one determine its value?

One way to visualize the influence of a mutation on fitness is to use the following standard population genetic model, where *s* = selection coefficient, *h* = dominance value, maximum fitness = 1, and the gene is assumed to have two alleles + and *l*.

Genotypes:	+ / +	+ / <i>l</i>	<i>l</i> / <i>l</i>
Fitness:	1	$1 - hs$	$1 - s$
With <i>s</i> = 1 (lethal)	1	$1 - h$	0

Therefore, the fitness of the +/ *l* heterozygous individual is dependent on the dominance of the *l* allele. If the *l* allele has a dominance of 1, the heterozygotes have a fitness of zero (die early or produce no progeny), whereas a dominance value of less than one will reduce fitness by that amount; for example, an *h* value of 0.4 would give a fitness of $1 - 0.4 = 0.6$ for +/l individuals. In this example, the individuals would be only 60% as fit as the ++ individuals or produce 40 percent fewer offspring.

Experiment Design

The following experiment is designed to calculate the dominance value (h) for the third-chromosome Stubble (Sb) mutation of *Drosophila melanogaster*. With the data collected from this experiment, h values can be roughly estimated through the formula provided by Chung (1967).

Two stocks are needed for this experiment: $\text{In}(3\text{LR})\text{Ubx}^{101}/Sb$ and the wild type Canton-S. Stubble (Sb) is a dominant visible mutation causing short bristles and is also a recessive lethal (*i.e.*, Sb/Sb flies die as embryos). (For details, see Lindsley and Zimm, 1992; or <http://flybase.bio.indiana.edu>). The following crosses were performed to collect virgin $Sb/+$ females and $Sb/+$ males.

To get a precise estimation of h for the Sb mutation, we set up two population cages (Cage A and Cage B), each containing 100 virgin $Sb/+$ females and 100 $Sb/+$ males. Therefore, the starting frequency of the Sb allele was 0.5.

Specifications and Details

Cage Size: $11 \times 8 \times 4.5$ inches, with 15 holes on the base for 15 vials

Vial Size: height 3.75 inches, diameter 0.88 inch

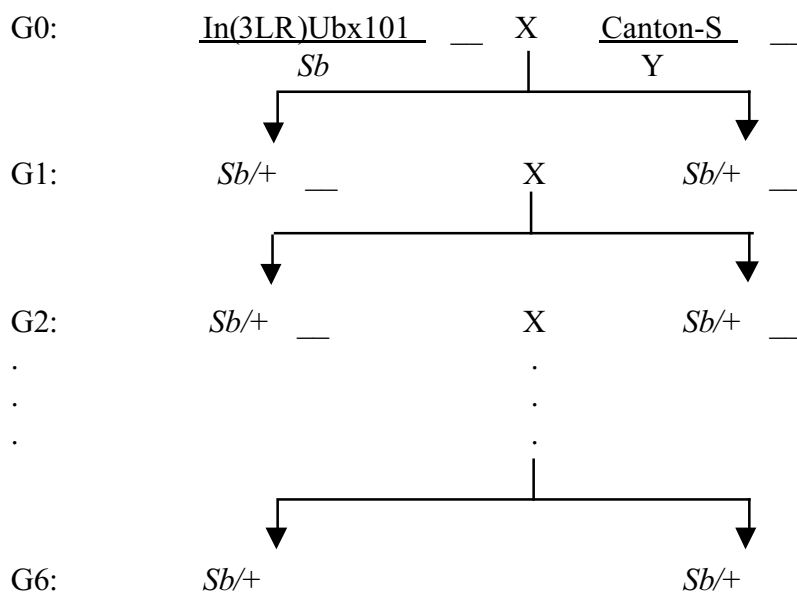
Food Change: Replacement of four new vials containing food every week for 15 weeks (six generations)

Temperature: $24 \sim 26^\circ\text{C}$

Average generation interval: 17 days

Materials and Method:

1. Each generation randomly collect 200 flies from each cage.
2. Count the number of flies with short bristles (Sb allele expression) and normal bristles.
3. Place the 200 flies back into the cages.



4. Calculate the percentage of Sb alleles out of the total 200 flies. (Example: 100 $Sb/+$ flies and 100 $+/+$ flies give the frequency of $Sb = 100/400 = 0.25$).
5. Record the data.
6. Repeat the above steps every week for 15 weeks. Finally, input data into computer and make the graph.
7. Use CA-Cricket Graph III Curve Fit function to acquire the regression line and from that line roughly estimate the

Figure 1. Mating scheme to collect $Sb/+$ females and $Sb/+$ males after six generations.

starting and ending frequency of *Sb* allele.

8. Use formula (I) below to calculate h values. We used the Mathematica 4.0 program to determine h from formula (I) (See Chung, 1967).

$$t = (1-h)/h * \ln\{[h+q_t(1-2h)]/[h+q_0(1-2h)]\} + 1/h * \ln(q_0/q_t) \quad (\text{I})$$

Derivation of Formula (I)

Genotype: +/+ *Sb*/+ *Sb*/*Sb*

Fitness: 1 1 – hs 1 – s

Since Sb/Sb is lethal, $s = 1$ and:

Fitness: 1 1 – h 0
Frequency: p^2 $2pq$ q^2

$$\Delta q = [pq(1-h) + (0)q^2] / [p^2 + 2pq(1-h) + (0)q^2] - q = [q(1-h) - qp - 2q^2(1-h)] / [p + 2q(1-h)]$$

since $p = 1 - q$,

$$\Delta q = [q(1-h) - q(1-q) - 2q^2(1-h)] / [1 - q + 2q(1-h)] = -[q(q+h-2qh)] / (1+q-2qh)$$

Since the population is changing continuously, treat Δq as approximately dq/dt .

Therefore,

$$dq/dt = -[q(q+h-2qh)] / (1+q-2qh)$$

$$dt = (1+q-2qh) / [-q(q+h-2qh)] dq$$

$$\text{Let } (1+q-2qh) / [-q(q+h-2qh)] = \mathbf{M}/-q + \mathbf{N}/(q+h-2qh)$$

Balance both sides and we get

$$\mathbf{M} = 1/h$$

$$\mathbf{N} = (2h-1)(h-1) / h$$

Hence:

$$t = \int dt = \int (1+q-2qh) / [-q(q+h-2qh)] dq = \int \mathbf{M}/-q dq + \int \mathbf{N}/(q+h-2qh) dq$$

$$t = (1-h)/h * \ln\{[h+q_t(1-2h)]/[h+q_0(1-2h)]\} + 1/h * \ln(q_0/q_t) \quad (\text{I})$$

Results

We chose the period from week 1 to week 16, covering roughly six generations (around 17 days per generation), to estimate h . Using CA-CricketGraphIII's logarithmic curve fit, we got the two curves shown in Figure 2. They are very close and have nearly the same shape. The estimation of h was made using equation I and Mathematica 4.0 (or use Maple, Matlab, or other program) as follows:

Cage A: $q_0 = 0.5$
 $q_t = -0.312\text{Log}_{10}X + 0.486 = -0.312\text{Log}_{10}16 + 0.486 = 0.11$
 $t = 6$
 $\longrightarrow h_A \approx 0.12$

Cage B: $q_0 = 0.5$
 $q_t = -0.307\text{Log}_{10}X + 0.480 = -0.307\text{Log}_{10}16 + 0.480 = 0.11$
 $t = 6$
 $\longrightarrow h_B \approx 0.12$

Table 1. Population cage experiments.

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pop A	0.5	0.32	0.36	0.31	0.27	0.26	0.25	0.22	0.2	0.16	0.18	0.16	0.11	0.12	0.12	0.08
Pop B	0.5	0.32	0.34	0.28	0.26	0.3	0.25	0.24	0.22	0.13	0.17	0.15	0.11	0.09	0.11	0.12

However, a population cage with 15 attached vials may be too expensive for each student in a laboratory class. To facilitate this experiment further, we therefore set up a parallel experiment using 1/2 pint plastic disposable milk bottles instead of population cages. The bottle size is 2.4 inches in diameter and 4.8 inches in height, which is enough for several hundred flies. Instead of counting flies each week, we collected and counted 200 flies each generation and transfer them to new bottles. In a laboratory class, each student would maintain and count the flies in one bottle. To mimic a laboratory class environment, bottles C and D were maintained and flies counted by Matthew Smith, a sophomore student at the Bowling Green High School in Bowling Green, Ohio.

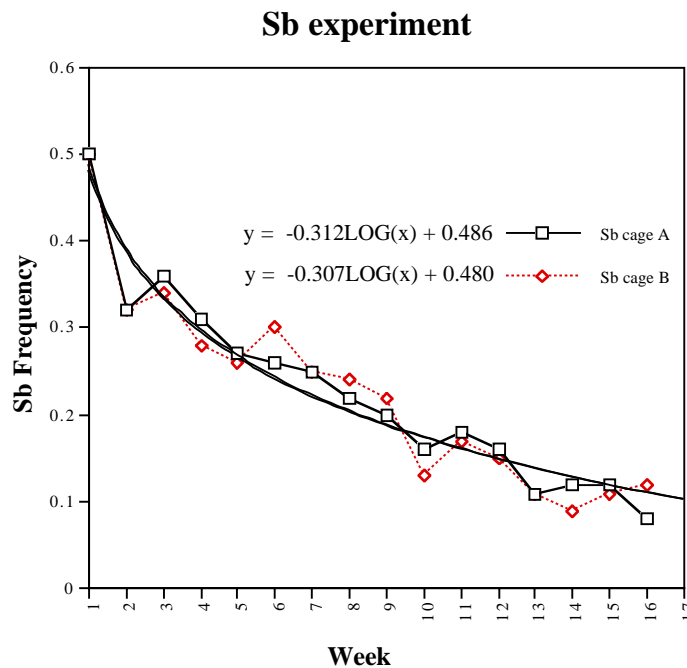


Figure 2. The change of *Sb* frequency in population cage A and B during 16 weeks.

Parallel Bottle Experiment

Table 2. Bottle experiment.

Generation	1	2	3	4	5	6	7	8
Bot A	0.5	0.33	0.27	0.26	0.20	0.16	0.20	0.16
Bot B	0.5	0.33	0.25	0.18	0.17	0.12	0.10	0.11
Bot C	0.5	0.32	0.26	0.19	0.08	0.08	0.05	0.06
Bot D	0.5	0.34	0.24	0.21	0.16	0.13	0.12	0.08

The estimation of h for each of the bottles is as follows,

Bottle A: $q_0 = 0.5$
 $q_t = -0.363\text{Log}_{10}X + 0.467 = -0.363\text{Log}_{10}8 + 0.486 = 0.139$
 $t = 7$
 $\longrightarrow h_A \approx -0.026$

Bottle B: $q_0 = 0.5$
 $q_t = -0.445\text{Log}_{10}X + 0.474 = -0.445\text{Log}_{10}8 + 0.474 = 0.072$
 $t = 7$
 $\longrightarrow h_B \approx 0.189$

Bottle C: $q_0 = 0.5$
 $q_t = -0.518\text{Log}_{10}X + 0.489 = -0.518\text{Log}_{10}8 + 0.489 = 0.021$
 $t = 7$
 $\longrightarrow h_C \approx 0.444$

Bottle D: $q_0 = 0.5$
 $q_t = -0.449\text{Log}_{10}X + 0.481 = -0.449\text{Log}_{10}8 + 0.481 = 0.076$
 $t = 7$
 $\longrightarrow h_D \approx 0.174$

Discussion

The result of the population cage experiment ($h = 0.12$) is close to 0.16 — the h value obtained from Chung's (1967) 23-week experiment. With more generations, the curve fit should provide a more accurate approximation of the Sb h value. However, for a laboratory exercise that can be performed by students during one semester, our bottle experiment is an appropriate design, for it lasts a shorter time (can be completed in one semester) but still gives satisfactory results.

The results of the bottle experiment show more fluctuations than those from the population experiment. In comparison with the population cage results and Chung's data (1967), the result from bottle A seems unreasonably low, while that from bottle C seems high. Since we counted flies each

generation and there are eight total generations, including G_0 , the curve fit from only eight data points

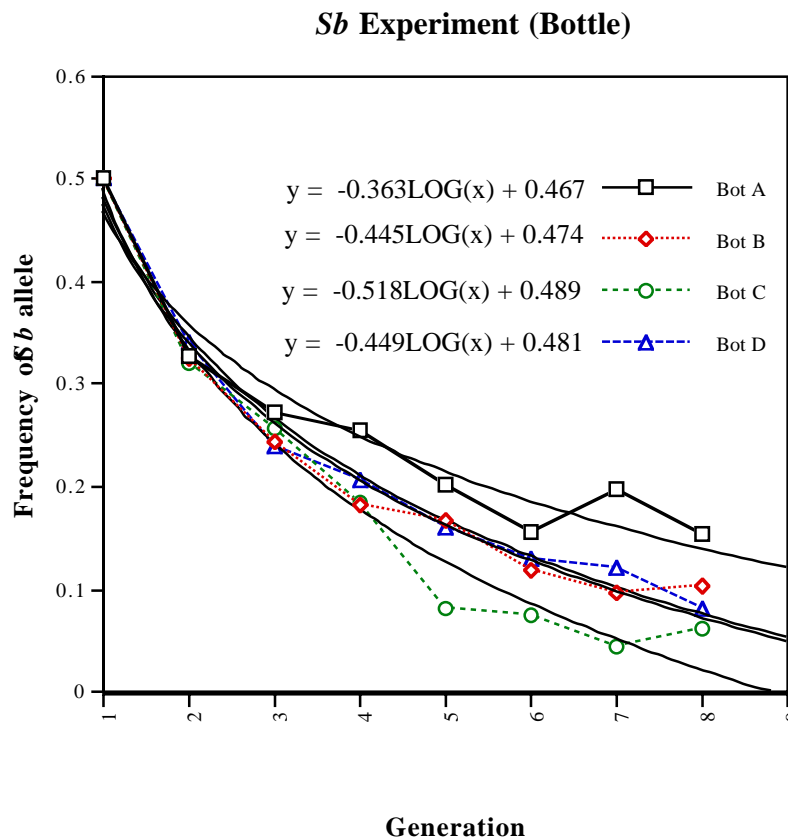


Figure 3. The change of *Sb* frequency in Bottle A, B, C and D during 8 generations.

can hardly provide an accuracy as good as that of the population experiment, which had 16 data points. Although two of the bottle results seem not to be reasonable, the other two are very close to the h value obtained from Chung's (1967) 23-week experiment. If we repeat the bottle experiment with additional bottles, we expect that the results will show a normal distribution, with most being around the normal expectation and a few outliers. Hence, compared with the population cage experiment, the bottle experiment saves labor at the cost of result accuracy. But the deviation is tolerable, and therefore it is an alternative choice for students to complete the experiment in a laboratory class during one semester. It should be noted that this technique could also be used with other dominant visible mutations that have complete penetrance and constant expression, and are also homogenous lethal, such as Plum (*Pm*, eye color) and brown dominant (*bw^D*, eye color).

From the above, it is clear that an experiment of 16 weeks or seven generations is both a good experience for students to explore a basic population genetics problem (estimation of dominance) using a traditional genetics research tool — *Drosophila*.

In a discussion session at the end of the experiment, students might be asked to go to the National Center for Biotechnology Information web page (<http://www3.ncbi.nlm.nih.gov>) and identify some human traits that have a dominant phenotype and are also homozygous recessive lethals, such as achondroplasia, and the students could be asked if h , s or fitness estimations are given for their traits and why these human mutations are not eliminated with time by selection.

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