Drosophila melanogaster as a model system to measure the effect of inbreeding depression on the viability of offspring of first cousin matings.

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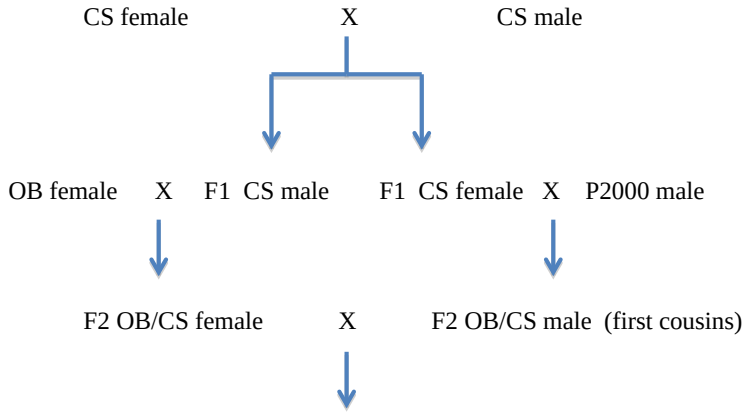
The offspring of first cousin matings in humans have a significant increase in infant mortality and congenital anomalies in comparison to offspring of matings between nonrelatives (Bennett *et al.*, 2002; Bittles and Neel, 1994; Stoltenberg *et al.*, 1999a, b; Helgason *et al.*, 2008). These decreases in fitness of the progeny of first cousin matings are caused mainly by homozygosis of deleterious recessive alleles that are present in close kin (inbreeding depression) (Charlesworth and Willis, 2009; Hedrick, 2011).

Inbreeding depression, and evidence to support it, was described by Charles Darwin in a chapter entitled "On the Good Effects of Crossing and on the Evil Effects of Close Interbreeding" in volume two of his 1868 book, *The Variation of Animals and Plants under Domestication* (Darwin, 1868). Darwin reported that the progeny of cross-fertilized plants were more vigorous that the progeny of self-fertilizing plants (Pannell, 2009; Berra *et al.*, 2010). These observations caused Darwin to be concerned that his offspring with his first cousin, Emma Wedgwood, would have reduced health (Berra *et al.*, 2010). Emma and Charles shared their grandparents Josiah and Sarah Wedgwood. Three of Darwin's ten children died before the age of ten.

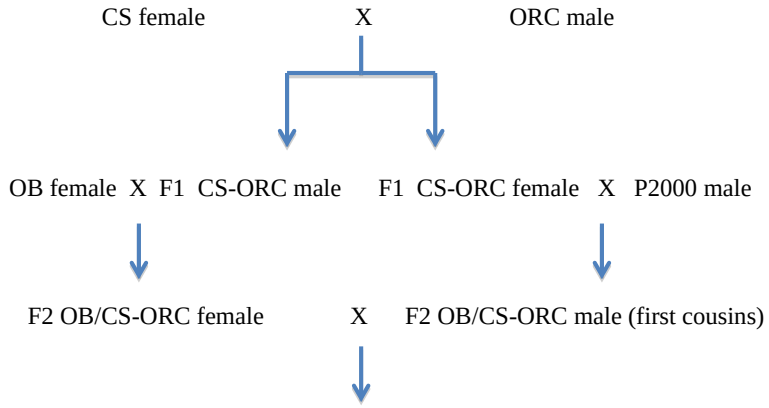
It is the objective of this teaching exercise to determine if *Drosophila melanogaster* can be used as a model system to identify the expected reduction in fitness (measured here by the reduction in viability; the average number of progeny per matings) of the progeny of first cousin marriages. We observed that *D. melanogaster* can be used to observe inbreeding depression in these consanguineous matings.

We performed two first cousin matings: one (Mating Scheme A) with the original, parental cross between flies of the same genetic stock and one (Mating Scheme B) with the original, parental cross between flies of unrelated genetic stocks. We observed inbreeding depression in Mating Scheme A, but not in Mating Scheme B. In the description of these crossing schemes below, the following wild-type stocks were used: Canton-S (CS) is a long-term, wild-type stock collected in Canton, Ohio, in the 1920s (Lindsley and Zimm, 1992); OBL1&2 (OB) was initiated by mixing six mated females from a Perrysburg, Ohio, population that were captured by sweeping bananas in 2010; Perrysburg 2000 (P2000) was initiated by mixing six mated females from a Perrysburg, Ohio, population in 2000; Perrysburg 2013 (P2013) was initiated by mixing six mated females from a Perrysburg, Ohio, population in 2013; and Oregon-R-C (ORC) was obtained from the University of Indiana Stock Center. Each stock was maintained in a half-pint milk bottle on standard cornmeal, molasses, and agar medium. In these mating schemes, single parents were crossed in vials, females were virgins, and the F2 OB/CS flies in Mating Scheme A and the OB/CS-ORC flies in Mating Scheme B were first cousins.



Mating Scheme A:

Count the number of F3 progeny per first cousin mating. As non-cousin controls, F2 OB/CS females were mated with P2013 males (non-cousin control I), F2 OB/CS males were mated with P2013 females (non-cousin control II), and F3 progeny per mating were counted.



Mating Scheme B:

Count the number of F3 progeny per first cousin mating. As non-cousin controls, the F2 OB/CS-ORC females were mated with P2013 males (non-cousin control I), F2 OB/CS-ORC males were mated with P2013 females (non-cousin control II), and F3 progeny per mating were counted.

It was our hypothesis that inbreeding depression would be higher in the cousin crosses of Mating Scheme A than in Mating Scheme B, since the parental cross in Mating Scheme A was between flies from the same CS bottle, whereas in Mating Scheme B the parental cross was females and males from two separate stocks, CS and ORC. This hypothesis was supported by the following results.

Results

Mating Scheme A: A total of 48 F2 vials were scored for the cousin crosses, with a mean ±s standard error of 39.90 ± 1.812 progeny per vial; a total of 45 F2 vials were scored for the non-cousin control I, 46.20 ± 1.721; and a total of 45 vials were scored for the non-cousin control II, 46.22 ± 1.978 (Figure 1). An analysis of variance showed that these results are significantly different (P = 0.02). By t tests, both of the non-cousin crosses were also significantly different from the cousin cross (I, P = 0.01; II, P = 0.02), whereas the two non-cousin crosses were not significantly different (I *vs*. II, P = 0.99). Hence, the viability (progeny count) for first cousin matings is significantly lower than for non-cousin crosses in Mating Scheme A.

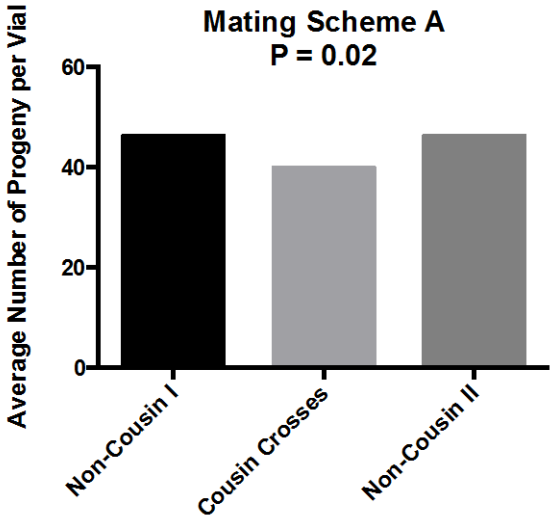


Figure 1. Average number of progeny per vial in first cousin and non-cousin matings. Parental females and males were from the CS stock.

Mating Scheme B: A total of 34 F2 vials were scored for the cousin crosses, with a mean ± standard error of 45.29 ± 3.082 progeny per vial; a total of 34 F2 vials were scored for the non-cousin control I, 46.38 ± 3.172; and a total of 35 vials were scored for the non-cousin control II, 51.83 ± 2.829 (Figure 2). An analysis of variance showed that these results are not significantly different (P = 0.26). By t tests, both of the non-cousin crosses were not significantly different from the cousin cross (I, P = 0.81; II, P = 0.12) and were not significantly different from each other (I *vs*. II, P = 0.20). Hence, the viability (progeny count) for first cousin matings is not significantly lower than for non-cousin crosses in Mating Scheme B.

The significant decrease in progeny numbers in the cousin crosses of Mating Scheme A is probably due to the increased chance of homozygosis of deleterious alleles present in the CS stock used in the parental cross. In Mating Scheme B, the parental cross was from two different stocks (CS and ORC), which will have fewer common alleles that could become homozygous in the F3 progeny. Mating Scheme A would be similar

to parental crosses in humans from Darwin's village of Downe (where individuals are related at some level), whereas the parental crosses of Mating Scheme B would be similar to crosses from individuals from Downe mating with individual from elsewhere in England (where individuals are unrelated).

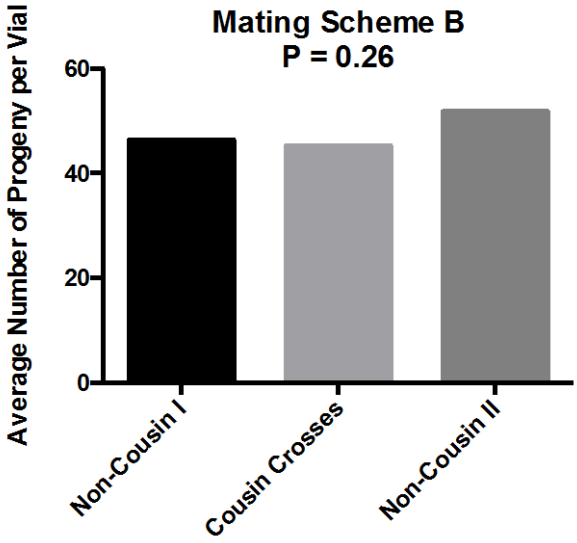


Figure 2. Average number of progeny per vial in first cousin and non-cousin matings. Parental females were from the CS stock, whereas parental males were from the ORC stock.

A class discussion of the results of this teaching exercise could include: 1) Twenty-two states of the USA prohibit marriages between first cousins (Relethford, 2012). Students could read Berra *et al*. (2010) and discuss if these state restrictions are scientifically appropriate. Why in Wisconsin are first cousin marriages allowed if the woman is over 55 years of age? Also ask the students to determine if their state or country allows first cousin marriages. 2) Students could be asked to determine the inbreeding coefficients (F) of first cousin matings (F values go from zero for no inbreeding to one for self-fertilization; see discussions of this topic in Hedrick, 2011; Relethford, 2012; http://www.ihh.kvl.dk/htm/kc/popgen/genetics/4/2.htm). The F value for first cousin matings is 0.0625, whereas the average F value for humans is usually below 0.05, but can be much higher in some parts of the world (Bittles, 2001; Relethford, 2012). 3) Students could be asked to discuss the consequences of inbreeding in a self-fertilizing organism, such as the whiptail lizards. Have them begin with all heterozygous organisms for a gene with two alleles (*A* and *a*) and observe that there is a decrease in heterozygotes (*Aa*) and an increase in homozygotes (*AA* and *aa*) with generations. For example, the frequency of heterozygotes in *n* generations will be $1/2^n$. In four generations, the frequency of heterozygotes will be $1/2^4 = 1/16 = 0.0625$, whereas the frequency of the *AA* or *aa* homozygotes will be $(1-1/2^n) / 2 = 0.9375/2 = 0.46875$ each. The homozygous individuals are the ones that will show inbreeding depression. Students could also be asked if the *A* and *a* alleles change in frequencies over generations of self-fertilization. They do not change frequencies; the *A* and a alleles remain at 0.5 (Hedrick, 2011). For example, after four generations the frequency of the *A* allele is 0.46875 + 0.0.3125 = 0.5. 4) Students could be asked to go to the internet and find other first cousin matings between famous people, such as Queen Victoria and Prince Albert; Albert Einstein and his second wife, Elsa; Saddam Hussein and Sajida Talfah; Jesse James and Zerelda Mimms; Jerry Lee Lewis and Myra Gale Brown; Igor Stravinsky and Katerina Nossenko; Martin Van Buren and Hannah Hoes; H.G. Wells and Isabel Mary Wells; and many more in the royal families of Europe.

References: Bennett, R.L., *et al*., 2002, Journal of Genetic Counseling 11: 97-119; Bittles, A.H., 2001, Clinical Genetics 60: 89-98; Bittles, A.H., and J.V. Neel 1994, Nature Genetics 8: 117-121; Charlesworth, D., and J.H. Willis 2009, Nature Reviews Genetics 10: 783-796; Darwin, C., 1868, *The Variation of Animals and Plants Under Domestication*. Volume II, John Murray, London; Hedrick, P.W., 2011, *Genetics of Populations*. Jones and Bartlett Publishers, Sundbury, MA; Helgason, A. *et al*., 2008, Science 319: 813-816; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Pannell, J.R., 2009, Biol. Lett. 5: 332-335; Relethford, J.H., 2012, *Human Population Genetics*. John Wiley and Sons, Hoboken, New Jersey; Stoltenberg, C., P. Magnus, A. Skrondal, and R.T. Lie 1999a, American Journal of Medical Genetics 82: 423-428; Stoltenberg, C., P. Magnus, A. Skondal, and R.T. Lie 1999b, American Journal of Public Health 89: 517-523.