Teaching Notes

Confirmation of the Bateman's principle: a sexual selection exercise.

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Females and males of most higher organisms have different reproductive and mating-behavior strategies for passing their genes to offspring. Since making eggs, growing a fetus(es), and nurturing offspring are more costly than making sperm and finding mates, in those animals where there is little, or no, paternal care of progeny, females may be more particular of their mates than males, and males, who never know that they are the true fathers of offspring, will try and mate with as many females as possible. Access to females is, therefore, a limiting resource for males, but males are not a limiting resource for females. This asymmetry in reproductive strategies between females and males can lead to sexual selection, where, for example, females are coy and choosy in selecting mates and males will compete with other males for access to females (see Darwin, 1871; Cronin, 1991; Ridley, 1995; Gould and Gould, 1997; Freeman and Herron, 2004, for discussions of this topic).

The first experiments to test that male reproductive success depends on the number of matings in a lifetime, whereas female reproductive success depends on the number of offspring produced in a lifetime (Bateman's principle), were reported by A.J. Bateman in 1948 using *Drosophila melanogaster* females and males multiply-marked with dominant visible mutations. Bateman observed that males who mated with multiple females had increased numbers of offspring, but that females had similar numbers of offspring whether they mated with one, two or three males. Subsequent behavioral and ecological genetics studies have confirmed that Bateman's principle occurs in nature in species where there is little care of offspring by males. For example, in the case of the mating behavior of the African kori bustard (*Ardeotis kori*), the husband and wife team of Tim

and Laurel Osborne reported that: "The male struts around the lek, stopping to emit their booming calls. Near sunset, the females approach the lek on foot, feeding as they go. The males advance on the females. If a female is receptive to a male, she approaches him and lies down a few feet away. He then straddles her from the back and pecks repeatedly at her head for ten to fifteen minutes. The mating itself lasts for just a few seconds, after which the couple parts and never associates again—until perhaps the next mating season" (Osborne and Osborne, 2006).

To confirm the results of the Bateman's principle, we designed a one-generation experimental protocol using two attached-X female stocks of *Drosophila melanogaster* with different visible genetic markers (w^+, red eyes, and *w*, white eyes) and two stocks of males with different visible X-linked genetic markers (sn^+, long bristles, and sn^3, singed, short bristles). Since attached-X females mated to free-X males (both also carry a Y chromosome) give rise to female progeny with the same phenotypes as the mothers and male progeny with the same phenotypes as the fathers, one can set up crosses in which females are given access to one or two males and males are given access to one or two females and multiple matings can be identified by distinct progeny types.

We set up the following eight crosses to determine if males and females have the same or increased numbers of progeny with increased numbers of mates. Twenty vials were set up for each of the crosses. In these crosses, the females also carried the yellow (*y*) body-color and forked (*f*) bristle mutations, and all parental females were virgins.

A) Crosses of Single Females and Single Males:

1) Single C(1)DX, y w f/Y females × Single sn^+/Y males

↓

Expected F1 progeny: C(1)DX, y w f/Y females with white eyes

and sn^+/Y males with long bristles

2) Single C(1)DX, y w f/Y females × Single sn^3/Y males

↓

Expected F1 progeny: C(1)DX, y w f/Y females with white eyes

and sn^3/Y males with singed bristles

3) Single C(1)DX, y f/Y females × Single sn^+/Y males

↓

Expected F1 progeny: C(1)DX, y f/Y females with red eyes

and sn^+/Y males with long bristles

4) Single C(1)DX, y f/Y females × Single sn^3/Y males

↓

Expected F1 progeny: C(1)DX, y f/Y females with red eyes

and sn^3/Y males with singed bristles

B) Crosses of Single Females with Two Males:

1) Single C(1)DX, y w f/Y females × sn^+/Y and sn^3/Y males

↓

Expected F1 progeny: C(1)DX, y w f/Y females with white eyes;

If parental females double mate,

sn^3/Y males with singed bristles

and sn^+/Y males with long bristles

2) Single C(1)DX, y f/Y females × sn^+/Y and sn^3/Y males

↓

Expected F1 progeny: C(1)DX, y f/Y females with red eyes;

If parental females double mate,

sn^+/Y males with long bristles

and sn^3/Y males with singed bristles

C) Crosses of Single Males with Two Females:

1) C(1)DX, y w f/Y and C(1)DX, y f/Y females × single sn^+/Y males

↓

Expected F1 progeny: sn^+/Y males with long bristles;

If parental males mate with two females,

C(1)DX, y w f/Y females with white eyes

and C(1)DX, y f/Y females with red eyes

2) C(1)DX, y w f/Y and C(1)DX, y f/Y females × single sn^3/Y males

↓

Expected F1 progeny: sn^3/Y males with singed bristles;

If parental males mate with two females,

C(1)DX, y w f/Y females with white eyes

and C(1)DX, y f/Y females with red eyes

In all crosses, parents were 3-4 days old at the beginning of the experiment, were subcultured to new food at day 7 from the initiation of the crosses, and progeny of each vial were scored for 21 days from initiation of the crosses or from the subcultures. The flies were raised on a standard cornmeal-molasses medium supplemented with yeast at 21-23°C. In crosses B1 and B2, progeny were recorded only if females mated with two males, and in crosses C1 and C2, progeny were recorded only if males mated with two females.

The results for each cross are shown in Table 1. The mean progeny number for crosses of single females with single males, single females with two males, and single males with two females are shown in Table 2. In addition, the number of female and male mates is plotted against the mean number of progeny in Figures 1 and 2.

Table 1. Mean number of progeny for each cross (SD = standard deviation).

Crosses	♀ × ♂	Mean ± SD
A1	C(1)DX, y w f/Y × sn^+/Y	94.40 ± 35.53
A2	C(1)DX, y w f/Y × sn^3/Y	127.88 ± 29.23
A3	C(1)DX, y f/Y × sn^+/Y	71.33 ± 41.66
A4	C(1)DX, y f/Y × sn^3/Y	70.40 ± 23.01
B1	C(1)DX, y w f/Y × sn^+/Y and sn^3/Y	64.20 ± 38.99
B2	C(1)DX, y f/Y × sn^+/Y and sn^3/Y	99.25 ± 46.54
C1	C(1)DX, y w f/Y and C(1)DX, y f/Y × sn^+/Y	145.40 ± 33.77
C2	C(1)DX, y w f/Y and C(1)DX, y f/Y × sn^3/Y	127.40 ± 39.39

Table 2. Mean number of progeny for males and females with different numbers of mates.

Crosses	♀ × ♂	Mean ± SD
A1, A2, A3 & A4	C(1)DX, y w f/Y × sn^+/Y C(1)DX, y w f/Y × sn^3/Y C(1)DX, y f/Y × sn^+/Y C(1)DX, y f/Y × sn^3/Y	89.54 ± 38.95
B1 & B2	C(1)DX, y w f/Y × sn^+/Y and sn^3/Y & C(1)DX, y f/Y × sn^+/Y and sn^3/Y	85.77 ± 45.66
C1 & C2	C(1)DX, y w f/Y and C(1)DX, y f/Y × sn^+/Y & C(1)DX, y w f/Y and C(1)DX, y f/Y × sn^3/Y	133.40 ± 37.42

The results in Figures 1 and 2 clearly show that the number of progeny per number of mates increased significantly for males ($p = 0.0005$), but not for females ($p = 0.7752$). These results are the same as observed by Bateman (1948, see Figure 1b) and confirm the Bateman's principle that *D. melanogaster* male reproductive success depends on the number of matings in a lifetime, whereas female reproductive success depends on the number of offspring produced in a lifetime.

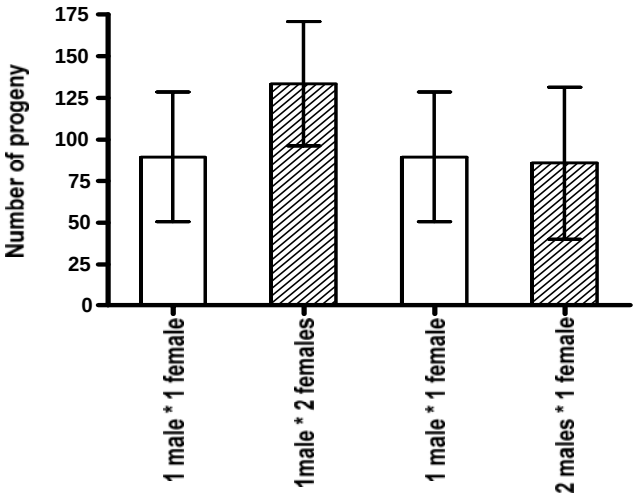

Figure 1. Mean(±SD) number of progeny for males and females with different numbers of mates. [a]$p = 0.0005$, [b]$p = 0.7752$.

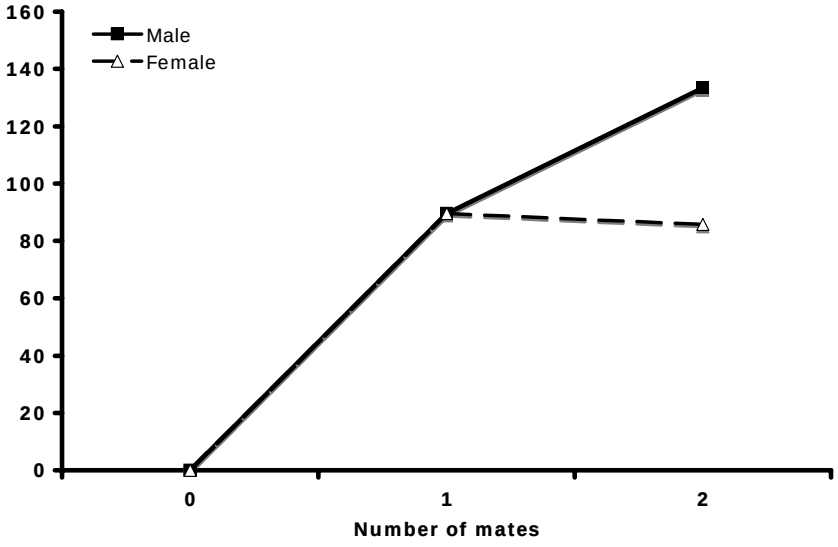

Figure 2. Relationship between the mean number of progeny and the number of mates.

A class discussion of the results of these crosses could include the following topics: 1) Bateman (1948) in his discussion stated that: "It has been demonstrated that in *Drosophila melanogaster* sexual selection is much more effective in males than in females". Do the results from this study support this statement? 2) In Figure 1b of Bateman (1948), the number of possible mates ranged from one to three. Can you think of a way to modify this experiment so that three females or three males could be added to a vial with a single male or female? You might go to FlyBase for help on possible additional stocks to use (http://flybase.bio.indiana.edu). If crosses with three females or males could be done, what would be the expected results? 3) If a similar study to this experiment could be conducted on deserted islands with humans (one female with two males and one male with two females), would you expect the results to be similar or different from this study? 4) Can you give any phenotypes of humans that may have evolved by sexual selection?

References: Bateman, A.J., 1948, Intra-sexual selection in *Drosophila*. Heredity 2: 349-368; Crowin, H., 1991, *The Ant and the Peacock*. Cambridge: Cambridge University Press; Darwin, C., 1871, *Descent of Man and Selection in Relation to Sex,* John Murray, London; Freeman, S., and J.C. Herron 2004, *Evolutionary Analysis*, Upper Saddle River, NJ, Pearson/Prentice Hall; Gould, J.L., and C.G. Gould 1997, *Sexual Selection: Mate Choice and Courtship in Nature*, New York: Scientific American Library; Osborne, T., and L. Osborne 2006, Big Bird, Natural History 115: 30-35; Ridley, M., 1993*, The Red Queen*, New York: Penguin Books.

Measuring natural selection using alcohol dehydrogenase alleles.

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The alcohol dehydrogenase locus (*Adh*) in *Drosophila melanogaster* is polymorphic in both natural and laboratory cultures, and the factors influencing this world-wide polymorphism have been studied by many researchers (*e.g*., McKenzie and McKechnie, 1978; van Delden, 1982; Karan *et al.,* 1995; van Delden and Kamping, 1997). The fast-migrating allele (Adh^F) has higher enzyme activity than the slow allele (Adh^S), but the Adh^S allele is more thermally stable (*e.g*., Gibson, 1970; McDonald and Avise, 1976; Morgan, 1975; Oakeshott, 1976; Vigue and Johnson, 1973). Thus Adh^F has a selective advantage in high alcohol environments, but Adh^S has a selective advantage at high temperatures.

A simple setup for assessing the *Adh* genotypes of your strains using cellulose acetate electrophoresis was described by Thompson *et al.* (2000). Here we describe an additional dimension, measurement of ADH enzyme activity, that can be incorporated into experiments on natural selection in alcohol-stressed environments.

ADH Activity: This experiment draws upon a study by Thompson and Kaiser (1977) to explore selection on an Adh^S allele that had especially low activity. The activity mutation was separable by recombination from the electrophoretic phenotype (Thompson *et al*., 1977) and caused the gene to produce about half as many enzyme molecules as the typical Adh^S allele. The activity assay is based on that of Ward and Hebert (1972).

1. Prepare an ice bath and a 25ºC water bath.
2. 40 mg of young adults are homogenized in 1 ml of ddH_2O.
3. The sample is microfuged to eliminate particulate matter and the supernatant is placed in an ice bath.
4. Each measurement requires 2.4 ml of phosphate buffer at 25ºC. We prepare a small amount of the stock buffer so it is fresh when used (5 g sodium pyrophosphate tetrabasic, 1.25 g semi-carbazide HCl, 0.25 g glycine, 0.4 g NaOH, and 155 ml ddH_2O).
5. Immediately before use, add 4 mg β-NAD per sample to the amount of buffer stock required for your experiment (*e.g*., 72 mg β-NAD into 43.2 ml buffer for 8 samples with 2 replicates each, plus 2 spares).
6. Combine 2.4 ml buffer/β-NAD, 0.1 ml sample supernatant, and 0.03 ml isopropanol or other alcohol substrate; vortex briefly.

7. Measure OD in a spectrophotometer at a wavelength of 340, recording the time required to change over a pre-selected range (*e.g.*, 10 divisions).

Alcohol Treatment and Sample Results: Since alcohol is not miscible in agar-based media, we used a potato flake and dextrose medium (*c.f.*, Carolina Biological Supply) prepared according to standard instructions. The alcohol sample was pipetted onto the moist surface of the medium. Amount of alcohol is one variable that can be explored in class experiments, but we found that 0.1 ml of 95% isopropanol or ethanol is a good starting treatment.

Survival can be quantified by allowing a large number of adults to lay eggs on an agar surface, such as a plastic sandwich box, and then transferring 50 eggs onto the food surface in each alcohol-treated or control tube. Adults are then counted when they eclose. Thompson and Kaiser (1977) found significantly different rates of survival as a function of genotype when comparing control tubes to those supplemented with ethanol (from n = 400 initial eggs pooled from eight replicates: 92.7% relative survival of Adh^F, but only 65.1% relative survival of Adh^S). The effect of N-butanol (0.05 ml treatments) was even stronger (67.8% relative survival of Adh^F, and 37.3% relative survival of Adh^S). Data like these on homozygous and heterozygous genotypes can be used to predict the effects of selection on allele frequencies using Hardy-Weinberg models (*c.f.,* Oakeshott *et al*., 1983).

Students can design their own experiments using other alcohols. Questions can include the differences among alcohols in their measured activity in Adh^F versus Adh^S strains and the correlation, if any, between activity and survival. Higher enzyme activity is a selective advantage in an ethanol environment, but this relationship may not necessarily hold for other alcohols. Indeed, some alcohols can be converted into a toxic product (Sofer and Hatkoff, 1972; Morrison, 1987 reprinted in 1999), placing Adh^F flies at a selective disadvantage.

References: Gibson, J., 1970, Nature 227: 959-960; Karan, D., A.K. Munjal, and R. Parkash 1995, J. Cytol. Genet. 30: 189-197; McDonald, J.F., and J.C. Avise 1976, Biochem. Genet. 14: 347-355; McKenzie, J.A., and S.W. McKechnie 1978, Nature 272: 75-76; Morgan, P., 1975, Heredity 34: 124-127; Morrison, W.J., 1999, Dros. Inf. Serv. 82: 131; Oakeshott, J.G., 1976, Genet. Res., Camb. 26: 265-27; Oakeshott, J.G., S.R. Wilson, and J.B. Gibson 1983, Genetica 61: 151-159; Sofer, W.H., and M.A. Hatkoff 1972, Genetics 72: 545-549; Thompson, J.N., jr., and T.N. Kaiser 1977, Heredity 38: 191-195; Thompson, J.N., jr., M. Ashburner, and R.C. Woodruff 1977, Nature 270: 363; Thompson, J.N., jr., R.C. Woodruff, S.B. Gray, G.S. Hendrix, and J.J. Hellack 2000, Dros. Inf. Serv. 83: 203-205; van Delden, W., 1982, Evol. Biol. 15: 187-222; van Delden, W., and A. Kamping 1997, In: *Environmental Stress, Adaptation, and Evolution*. (Bijlsma, R., and V. Loeschcke, Eds.). Experientia Supplementum, vol. 83; Vigue, C.L., and F.M. Johnson 1973, Biochem. Genet. 9: 213-227; Ward, R.D., and P.D.N. Hebert 1972, Nature New Biol. 236: 243-244.