the result of a recombination event between the *crossveinless* and *white-coffee* loci. However, the wild females, who first appear in any given generation, are most probably the fertile trisomic ones that have escaped dying during the pupal stage, where the lethal effect of the triplo-X condition mainly occurs, as pointed out by Brehme (1937).

Table 1. Phenotypic classes and respective number of individuals sampled from many generations (pooled together) of the non-crisscross strain, composed of *crossveinless* females and *white-coffee* males. A total of 2,103 individuals, 1,000 females and 1,103 males, were sampled. *Crossveinless* females and *white-coffee* males were the most frequent classes, and are the product of a non-crisscross inheritance. The rarer classes found in the offspring were wild females and males, *white-coffee* females and *crossveinless* males.

females			males		
+	*cv*	w^{cf}	+	*cv*	w^{cf}
9	988	3	1	4	1,098

+ = wild type; *cv* = *crossveinless*; w^{cf}= *white-coffee*

Conclusion

The unusual X-linked inheritance pattern exhibit in 100% of the offspring of a lineage of *Drosophila melanogaster,* called non-crisscross inheritance and composed of *crossveinless* females and *white-coffee* males, was a result of the occurrence of a compound (attached) X chromosome. The genetic results are similar to those obtained by Lilian V. Morgan in 1922 and were as well confirmed by the cytological analysis that proved the existence of an attached X in the female matroclinous offspring. We afford the opinion that the instability of the analyzed strain starts whenever one fertile wild type triplo-X female escapes from dying during the critical pupal stage.

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Simple high school laboratory exercise on mate attraction and reproductive isolation in *Drosophila*.

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Species are often defined as groups that fail to successfully interbreed with other groups. Behaviors that keep groups from interbreeding help maintain biodiversity on our planet, and students can explore such behaviors in K-12 biology classes. The recently retired AP Biology Laboratory 11 Part B exercise on Animal Behavior allowed students to observe the fruit fly courtship sequence. While this activity had value, it came across as somewhat minimal to most students, particularly if they had already worked with flies and had seen courtship occur. Nonetheless, many teachers and college faculty still leverage this activity for demonstrating courtship behavior, and multiple commercial kits are available for it.

Simple additions to this activity make it more engaging and allow students to witness one aspect of the principle of reproductive isolation, for both introductory biology and Advanced Placement classes. AP Biology students are required to be "able to justify the selection of data that address questions related to reproductive isolation and speciation" (College Board Fall 2012 Learning objective 1.23). Many students know that species often produce sterile hybrids after mating with other species, but they often forget that pre-zygotic mechanisms (*e.g.*, disinclination to mate with other species) are also a form of reproductive isolation that maintains the separateness of species. We reinforced this concept by having students observe same-species and different-species pairings themselves directly.

We present both a simple extension that differs trivially in effort from the original AP Biology Laboratory exercise, and an elaborate version that demonstrates the concepts more elegantly but requires additional preparation. These revised exercises keep the students engaged while reinforcing their understanding of behavior and evolution. Each version can be conducted easily within a single class period, though some advance preparation is necessary by the teacher.

Presenting the Concept

We opened by asking the students how one knows whether two groups are separate species. Students often reply that different species "can't reproduce" or that "their hybrids are sterile." Yet, students often fail to appreciate the importance of mate recognition-- animal species (*e.g.*, squirrels, pigeons, mosquitoes, etc.) encounter other animal species all the time without attempting to mate with each other. Even apparently similar species, like dogs and foxes, often exhibit little or no physical attraction to each other. In such a case, would it be relevant if these species can produce hybrid offspring when there's no inclination to try in nature?

Often, two distinct types of pre-zygotic reproductive isolation exist:

– males are disinclined to court females of the other species (species discrimination by males), and

– even if males did court females of the other species, the females would be disinclined to accept their advances (species discrimination by females).

Activity Overview

A culture of male *Drosophila simulans* (available from the *Drosophila* species stock center) is the only material the class will need besides those included in the commercially available AP Biology Laboratory 11 kits (available from Carolina Biological Supply, Ward Scientific, and other vendors). Students work in groups of two to four to observe courtship behavior in within- or between-species pairings. For the basic activity, each group should use four food-containing vials: two vials with each containing an individual *D. melanogaster* female, one vial with an individual *D. melanogaster* male, and one vial with an individual *D. simulans* male. Without anesthesia, the students pair a same-species male and female in a vial, and the different-species male and female in a separate vial. In the optional elaborate activity, students also pair the two kinds of males with *D. simulans* females.

Students document the behaviors, as described in the standard AP Biology Laboratory 11 exercise. However, they also record differences between the within- and between-species pairings. At the end of the period, the students can consolidate and present their data.

Results and Discussion

We have tried this activity with multiple classes, and we were thrilled (and relieved!) to observe striking differences between within- and between-species pairings within each class period. Courtship was very easy to identify-- the male fly would follow the female very closely from behind, periodically extending one or both wings and vibrating them. After a few minutes of following and wing vibrating, he would attempt to mount the female (whenever she stopped moving).

The first rows of Table 1 illustrate results of the basic activity. Simply, *D. simulans* males are disinclined to court *D. melanogaster* females, demonstrating species discrimination by ***males***. This is due to a cuticular hydrocarbon difference that distinguishes the females of the two species (Jallon, 1984). In contrast, *D. melanogaster* males court and mate with *D. melanogaster* females readily. With the elaborate activity, students see that the reverse is not true-- *D. melanogaster* males will readily court *D. simulans* females, but the females are unreceptive to their advances (running away or not allowing the mount), which demonstrates species discrimination by ***females***. Females rely heavily upon auditory cues produced by the male's courtship song when they decide if a mate is appropriate (Ritchie *et al.*, 1999). The students visibly enjoyed watching the different behaviors, rooting for the poor males who could never mate with their partner and cheering for those males who did mate eventually.

Table 1. Observational data from 21 student groups of within- and between-species pairings.

COURTSHIP	*D. melanogaster* male	*D. simulans* male
D. melanogaster female	100%	19% (all very short)
D. simulans female	67%	100%

MATING	*D. melanogaster* male	*D. simulans* male
D. melanogaster female	76%	0%
D. simulans female	0%	52%

This relatively simple extension on the standard AP Biology exercise increases its pedagogical value, the breadth of concepts presented, and its appeal to students. It can easily be implemented in introductory classes as well, yet it arguably touches upon all four of the "Big Ideas" associated with the AP Biology Curriculum Framework, as well as demonstrating some of the specific concepts. Detailed (and editable) step-by-step instructions are available at http://tinyurl.com/bulmcap , and a YouTube video demonstrating the fly manipulations can be viewed at: http://www.youtube.com/watch?v=8Wc01zIzfEY

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