backgrounds with the results from the stock control. We predict that there will be significant differences in male to total progeny ratios for the five backgrounds.

Results

A total of 121 crosses were set up, including 24 w^{1118} control crosses (with the w^{1118} autosomal background) (mean = 0.51; variance = 0.17), 25 crosses with the CS autosomal background (mean = 0.59; variance = 0.13), 21 crosses with the OBL1&2 background (mean = 0.63; variance = 0.24), 31 crosses with the Per+(2000) background (mean = 0.58; variance = 0.13), and 20 crosses with the Per+(2013) background (mean = 0.60; variance = 0.13). The results of these crosses are shown in Figure 1. All four of the crosses with new autosomal genetic backgrounds had significantly higher male/total progeny means compared to the w^{1118} control (P values were 0.0005 for the CS autosomal background, 0.0004 for OBL1&2, 0.002 for Per+(2000), and 0.0007 for Per+(2013).

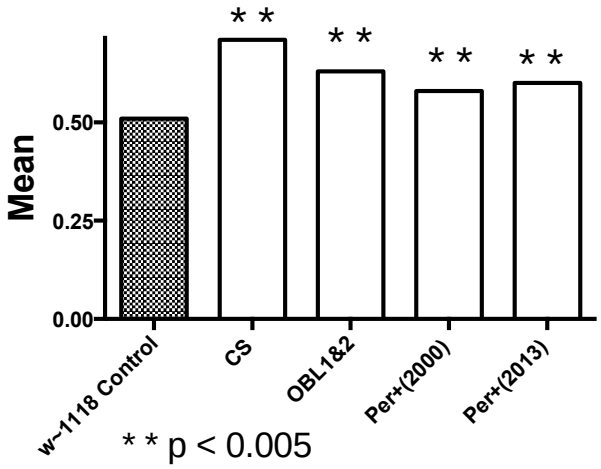

Figure 1. Comparison of the means of male progeny to total progeny in lines with different autosomal genetic backgrounds.

Hence, the effect of the w^{1118} mutant, and its X-linked genes, on viability (male progeny to total progeny) does depend on epistasis with genes on the autosomal genetic background.

A class discussion of the results of this study might include the role of single genes *vs*. multiple-genes in the evolution of adaptive traits. An example of an adaptive trait caused by a single gene is coat color in deer mice (Linnen *et al.*, 2009), whereas an example of a trait associated with selection caused by multiple genes is corn kernel oil content (Laurie *et al.*, 2004).

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Establishment of double mutant strains of *Drosophila melanogaster* (Diptera, Drosophilidae) for teaching purposes.

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Abstract

Drosophila melanogaster is a valuable model organism that has been used in genetics research since the beginning of the last century, as well as for teaching genetics concepts in the classroom. However, in the latter case, we have noticed the internet can negatively influence the learning process by making experimental outcomes easy to find and students do not need to think over their own observations and results. To overcome such a drawback, the present project aimed to establish six unusual double-mutant strains of *Drosophila melanogaster*, with little to no online information, encouraging students to reach conclusions by their own observations, not only during project's execution but also while collecting data from crosses proposed by the professors. Each double-mutant strain (*yellow brown, lozenge singed*, *scute sepia*, *crossveinless eyeless*, *lozenge sepia* and *crossveinless singed*) was established by crossing two single-mutant strains, provided by the Drosophilidae Stock Center of the *Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo*, with neither the mutant's name/symbol nor its inheritance pattern being revealed. Some of the strains obtained in this project have already been used during basic genetics practical classes for freshmen of Biological Sciences major at the referred university.

Key Words: Basic Genetics, Didactic, Exceptional Flies, Inheritance pattern.

Introduction

References to the vinegar-fly, not necessarily under the binomen *Drosophila melanogaster* Meigen, 1830, are ancient. It is possible to find 358 citations to these flies prior to 1900 (Drosophila Information Service, 1994). The first documented reference is from 1684, and includes China ink drawings of real-sized flies and one magnified puparium and flies as seen through one of the first microscopes (Mentzel, 1684). Probably the most historically influential person to use this organism for research in the 20th Century was William E. Castle (Allen, 1975), a Harvard University professor who initiated a key-project in 1901, published in 1906, entitled "The Effects of Inbreeding, Cross-breeding, and Selection upon the Fertility and Variability of *Drosophila*". Curiously, its publication year matches the year William Bateson coined the term "Genetics" to name this emergent field of study. Thanks to that article, Thomas H. Morgan, a University of Columbia professor, felt motivated to start using *Drosophila melanogaster* as his research material (Allen, 1975). In 1910, he [or most probably his student Calvin Bridges] found a male that, instead of having typical red eyes, presented white eyes (Sturtevant, 2001). Studies upon this male led to his renowned article "Sex Limited Inheritance in *Drosophila*", published in the same year. Since then, thousands of researchers started studying these flies (Dos Santos *et al.*, 2015), because they are easily bred in laboratories, present a short life-cycle, have conspicuous sexual dimorphism, only four chromosomes pairs, a myriad of described mutations, and produce numerous offspring (Demerec and Kaufmann, 1967; Lindsley and Zimm, 1992).

Due to subsequent inclusion of a Genetics course in Natural History and Medicine majors in universities worldwide, *Drosophila melanogaster* started being largely used for teaching, since all of the advantages of using it for research also make it outstanding material for practical classes. It is worth noting that, in less than two months, it is possible to perform projects that elucidate the concepts of segregation, independent assortment, linkage, recombination, and linkage mapping (Strickberger, 1962; Marconi and Vilela, 2013). On the other hand, access to class-based experimental outcomes has become too simple and immediate, since students can go online to conclude projects without any need of intense intellectual effort, even though they should enjoy the opportunity to learn through introspection and heuristics.

In this project, we established six unusual double mutant strains of *Drosophila melanogaster*, for which online information is not frequently available, challenging the students to reach conclusions on their own.

Materials and Methods

Our study material is *Drosophila melanogaster*, popularly known as the vinegar-fly, and the most common species indoors all over the world. Approximately one hundred wild and mutant strains belonging to this model organism are currently (2015) being maintained by the Drosophilidae Stock Center of the

Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo. The collection was assembled over a six-decade period by several curators, who received most of the strains from different stock centers, mainly from USA.

The establishment of six double-mutant strains was performed by one of us (ASR), from whom the name/symbol and inheritance pattern of mutations present in single-mutant lineages (parental generation) (Table 1) were unrevealed.

Table 1. Single-mutant strains of *Drosophila melanogaster* used to establish double-mutant lineages. Male adults were collected from an unknown α strain, and the female pupae, from an unknown β strain.

Cross	Unknown α	Unknown β
1	*yellow*	*brown*
2	*lozenge*	*singed*
3	*scute*	*sepia*
4	*crossveinless*	*eyeless*
5	*lozenge*	*sepia*
6	*singed*	*crossveinless*

After identifying the affected phenotypes, three to nine random couples were crossed in cylindrical vials (height: 7.5 cm, diameter: 2 cm) (Shorrocks, 1972) containing a small amount (ca. 5 ml) of banana-agar culture medium with foam plug enclosures (Goldstein and Fyrberg, 1994). Posteriorly, small pieces of fresh bakers' yeast (*Saccharomyces cerevisiae*) were added to feed the couples. For this and subsequent generations, all females were virgins, identified and isolated during pupal stage.

The vials were placed in a chamber at constant temperature (25±1°C), and, eventually, in chambers at lower temperatures (22±1°C and 18±1ºC). Every 3-5 days, flies were transferred to new vials containing culture medium. Larvae remained in previous vials, and more of the same fresh bakers' yeast was added (Shorrocks, 1972) with 10×2 cm V-shaped strips of filter paper (one per vial) inserted into the culture medium (Freire-Maia and Pavan, 1949). Nine days after larvae hatched from eggs it was possible to find F_1 emerged flies, which were anesthetized by triethylamine fumes (Fuyama, 1977) and analyzed under a stereomicroscope.

Results and Discussion

The first author (ASR) determined each mutation's inheritance pattern through phenotypic analysis of emerged flies from F_1 and F_2 generations. In all parental crosses, females exhibited one mutation (called **m1**) and males another (called **m2**). Only two inheritance patterns were identified. In one case, F_1 males presented the **m1** mutation, which indicated its allele was located on the X chromosome, since it exhibited crisscross inheritance, meaning the affected character observed in parental females was transmitted to F_1 males. In contrast, all F_1 female flies were phenotypically wild-type. In the F_2 generation, six phenotypic classes were observed: **m1** females, wild-type females, **m1** males, **m2** males, wild-type males and double-mutant males. The absence of both **m2** and double-mutant females in the F_2 generation suggested this mutation (present in parental males) was also X-linked. In order to produce the two last-cited male phenotypes, a crossing over must have occurred between the two genetic markers, once they presented two mutated alleles, or two wild-type alleles, in *cis* position, therefore, located in the same X chromosome. Moreover, one could expect that, in addition to the recombinant males, there would also be heterozygous **m2** females among the phenotypically **m1** female specimens, which would produce recombinant gametes for both genes. In order to detect them, individual test-crosses were performed between F_2 **m1** females and F_2 double-mutant males. In some of those crosses, it was possible to observe that part of their offspring was constituted by double-mutant males and females. To establish the desired strain, recently emerged (less than 4 h) double-mutant females were isolated and then crossed to double-mutant males.

In the other case, all F_1 flies were wild-type. Lack of crisscross inheritance (from parental females to F_1 males) demonstrated the **m1** mutation was autosomal. In the F_2 generation, six phenotypic classes were observed: **m1** females, wild-type females, **m1** males, **m2** males, wild-type males, and double-mutant males. The absence of both **m2** and double-mutant females in the F_2 generation females suggested this mutation was X-linked. It was necessary to perform test-crosses as well, between F_2 **m1** females and F_2 double-mutant males, to verify which females were heterozygous for **m2** mutation. Once identified, as detailed in the preceding paragraph, their double-mutant offspring were intercrossed.

The six established double-mutant strains are listed in Table 2, and are candidates to be used in the basic Genetics course, offered to ca. 120 students enrolled in the Biological Sciences major at *Universidade de São Paulo* per year.

Table 2. Phenotypes of six established double-mutant strains of *Drosophila melanogaster*.

Strain	Phenotypes
1	*yellow brown*
2	*lozenge singed*
3	*scute sepia*
4	*crossveinless eyeless*
5	*lozenge sepia*
6	*crossveinless singed*

As detailed by Marconi and Vilela (2013), the students are organized in groups of mostly four people and must perform a project during part of the four-month (15 week) semester. They are requested to investigate the inheritance pattern of four conspicuous mutations, two being present in the parental male (sampled from an unknown α *Drosophila melanogaster* strain), crossed to parental female flies (sampled from an unknown β strain), which also bear two different mutations. Combinations vary from year to year. The experiment requires the dedication of 105 min per week, for six weeks. First, each group must isolate twelve male pupae from a strain called unknown α, and twelve female pupae from an unknown β strain. Sex identification of pupae is based on presence (male)/absence (female) of sexual combs on front legs' first tarsomeres. Pupae are more easily sexed when they rest over a wet filter paper strip placed on a white stage plate under a stereomicroscope illuminated with white LED ring light, which is not hot and does not kill them by overheating. Upon emergence, five random mating couples must be established and crossed by each group, and kept in vials containing banana-agar culture medium. Next, aiming to identify the genetic markers of both strains, students must analyze a few of the remaining flies (regarding their sexes and the presence/absence of genetic markers), anesthetized with triethylamine fumes (Fuyama, 1977), under a stereomicroscope. Ideally, the female parental strain must exhibit at least one X-linked mutation, and the mutations must always be recessive. Finally, students analyze the next two generations, F_1 (n = 13 randomly sampled flies per student) and F_2 (n = 11 males and 11 females per student). In the F_1 generation, non-crisscross inheritance may be detected. This rare and unusual event is an exciting manner to stimulate students to treasure exceptions, as stressed by Marconi and Vilela (2013). At the end of the project, groups are requested to map the X-linked genes. They should reach by themselves to the conclusion that is more convenient to use only male offspring frequencies of the F_2 generation, without any need of test crossing.

Table 3. Phenotypes of parental and F_1 *Drosophila melanogaster*, and total of F_1 e F_2 flies sampled during the projects made in three consecutive years. F_2 sampled flies belong to 16 different male phenotypes and 4 different female phenotypes. Exceptional flies were intentionally excluded from this table (see Table 4). F_1 flies were analyzed randomly regarding the sexes, whereas F_2 flies, in equal number of males and females.

Year	Sex	Parental generation	F_1 generation	F_2 generation
2012	Male	*lozenge singed*	615 *yellow*	1287
	Female	*dumpy yellow*	983 wild-type	1287
2013	Male	*crossveinless forked*	690 *scute*	1243
	Female	*scute sepia*	868 wild-type	1243
2014	Male	*eosin hedgehog*	501 *crossveinless singed*	1265
	Female	*crossveinless singed*	825 wild-type	1265

As of 2015, three of the six strains established during this project have already been used in the basic Genetics practical classes, ministered by the second author (CRV) and colleagues. In 2012, 128 students crossed *lozenge singed* males (from unknown α strain) with *dumpy yellow* females (from unknown β strain). In 2013, 125 freshmen performed their project based on *crossveinless forked* males (unknown α strain) crossed with *scute sepia* females (unknown β strain). A total of 116 students crossed *eosin hedgehog* males (unknown α strain) with *crossveinless singed* females (unknown β strain) in 2014. The parental generation and the total

of flies sampled by students throughout F_1 and F_2 generations and their respective phenotypes are listed in Table 3.

Table 4. Phenotypes of exceptional *Drosophila melanogaster* observed among F_1 generation in three consecutive years. Students tested and verified that all exceptional males were sterile, whereas females were fertile.

Year	Sex	Phenotype
2012	Male	1 *yellow scute*
	Female	0
2013	Male	1 *crossveinless forked*
	Female	1 *scute*
2014	Male	1 *eosin*
	Female	1 *crossveinless singed*

It should be pointed out exceptional flies were found in every of the three cited years (Table 4).

All exceptional flies were investigated in extra class experiments, performed by the groups who detected them. At the end, all enrolled students have access to their results, and are requested to include them in a simulated manuscript, in which they must hypothesize how these organisms could have been produced. It is worth noting one mutation observed among 2013 exceptional flies was not present in the parental generation, providing the students the opportunity to generate additional hypothesis, which could have been tested, if there was enough time for additional experiments.

Results of linkage mapping obtained by freshmen from 2012 to 2014 using double-mutant strains established during this project are represented in Figure 1.

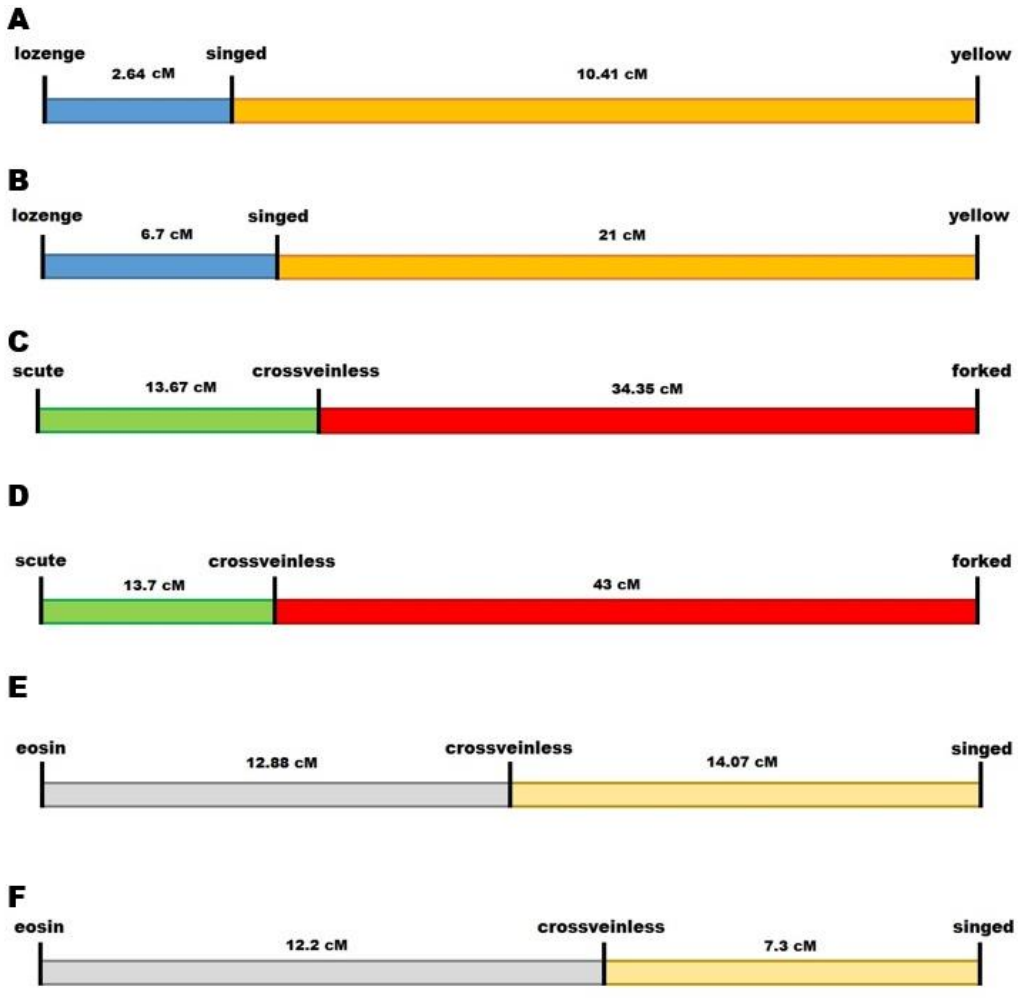

Figure 1. Linkage mapping obtained by freshmen in 2012 (A), 2013 (C), and 2014 (E); compared to respective chromosomal distances detailed in Lindsley and Zimm, 1992 (B, D, F).

Conclusions

Strains established in this project are highly recommended for developing similar projects. As those combinations are unusual, students will not be able to easily find expected results online, which contributes to the development of their own observation, data collection, and analysis, and awakens their curiosity, which may increase their interest in the challenging scientific activities.

Acknowledgments: We are grateful to the basic Genetics team and enrolled freshmen in 2012, 2013, and 2014, which included 369 Biological Sciences students, four additional Professors (Drs. L. Mori, L.E.S. Netto, D.S. Sheepmaker, and T.T. Torres), monitor students of 2012 (C.R.R. Arantes, B.L.B. Argiona, V.Q. Pretti, and S.C. Vaz), 2013 (J.A. Bachur, C.S. Faria, and P. Perre), and 2014 (C.I.S. Costa, C. Kaid, I.L. Teixeira, and T.I. Yassumoto), two technicians (C.E. Lopes and F. Flauzino) for numerous reasons, Dr. Gerhard Bächli for gently sending us a copy of Mentzel (1684)'s paper, and Dr. Maxi Polihronakis Richmond for kindly reviewing the English version.

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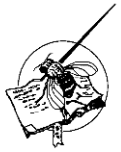

Using DGRP sequenced genomes to map heterozygous modifier effects on cell death in *Bar* eye of *Drosophila.*

Thompson, James N., jr., Daniel Tinney, Jacob Khoussine, Gary Cox, J. Ross Ogden, Grayson Audette, Saba Bingabr, Danielle Branesky, Andres Gomez, Jason Lauderdale, Cassandra Long, Jacob Mitchell, Sonya Narula, Dennis Nguyen, Thuc-Vi Nguyen, Daniel Pons, Cameron Steele, Garrett Sutton, Tyler Tallman, and Barbara Safiejko-Mroczka. Department of Biology, University of Oklahoma, Norman, OK.

The *Drosophila* Genetic Reference Panel (DGRP) lines developed by Trudy MacKay and her colleagues (Mackay *et al*., 2012) offer a powerful resource for analyzing multi-gene influences on development, behavior, and physiology of *Drosophila melanogaster*. Rather than trying to isolate genes that influence a trait of interest using chromosomal substitutions, recombination mapping, or other approach, mapping of relevant loci begins with known genomes. By correlating specific trait expressions with the extensive database of SNPs for each sequenced line in the DGRP set, regions of the genome that consistently associate with a targeted phenotypic expression can be identified and explored in additional detail. But many of the traits our group is interested in studying require an additional element. We want to know about genes that act as modifiers of a mutation's expression, such as wing vein length mutations like *plexus*, with extra vein fragments, and *veinlet* with wing vein gaps in *Drosophila* (*e.g*., Thompson, 1974, 1975a, 1975b). A