Table 1. Assessment tools for general biology module.

<table>
<thead>
<tr>
<th>Description</th>
<th>Outcome assessed</th>
<th>Mean (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab report-summarize data and demonstrate mastery of concepts</td>
<td>1-5</td>
<td>85.7 ± 8.4</td>
<td>42-99</td>
</tr>
<tr>
<td>Punnet Square Homework showing genetic concepts</td>
<td>2</td>
<td>91.9 ± 17.2</td>
<td>0-100</td>
</tr>
<tr>
<td>Lab Exam Questions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Module concepts (cross)</td>
<td>2</td>
<td>48 ± 50</td>
<td>0-100</td>
</tr>
<tr>
<td>Basic genetic concepts</td>
<td>2</td>
<td>87 ± 10</td>
<td>3-100</td>
</tr>
<tr>
<td>Module concepts (fly model, genome)</td>
<td>1, 3, 4</td>
<td>56 ± 40</td>
<td>0-100</td>
</tr>
<tr>
<td>Experimental design</td>
<td>1, 4</td>
<td>77 ± 20</td>
<td>20-100</td>
</tr>
<tr>
<td>Genome/FlyBase</td>
<td>3</td>
<td>79 ± 25</td>
<td>0-100</td>
</tr>
<tr>
<td>Statistical test-Chi square</td>
<td>5</td>
<td>45 ± 50</td>
<td>0-100</td>
</tr>
<tr>
<td>Statistical test-t-test</td>
<td></td>
<td>50 ± 50</td>
<td>0-100</td>
</tr>
</tbody>
</table>

We intend to compile data over multiple years to assess whether we are meeting our outcomes and also to make changes in areas where we are not successful. Based on these limited assessment findings, we are successfully meeting some outcomes but not others. For example, we seem to be successfully getting across basic genetic concepts and concepts about genomics. This group of students, however, did not master concepts about the role of genetic screens and the use of model systems. Some of the student outcomes, such as using statistical tests and being able to interpret and analyze data, are also larger student outcomes we have developed for our biology majors. After one semester of biology, very few students are able to use statistical tools well, but our goal is teach this skill over the four years of the program to our majors.


**Confirmation of the Calvin B. Bridges study:** Based on nondisjunction, the white gene is located on the X chromosome of *Drosophila melanogaster*.

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In 1910, Thomas Hunt Morgan reported on the recovery of a *Drosophila melanogaster* male with white eyes, instead of the usual red eyes (Morgan, 1910; see the figures below; RCW; [http://www.cas.vanderbilt.edu/bsci111b/drosophila/supplemental](http://www.cas.vanderbilt.edu/bsci111b/drosophila/supplemental)).
After observing the F1 offspring of crosses of this white-eyed male with wild-type (red-eyed) females and the F2 offspring of subsequent crosses, Morgan proposed that the mutation (white, \( w \)) that caused this white-eye phenotype was recessive and was located on the X chromosome (was sex linked). In addition, based on previous cytogenetic work by Nettie Stevens (Stevens, 1908, see Figure 58 of that article), Morgan also proposed that the Y chromosome in males did not have a partner gene for the white gene on the X chromosome; females have two X chromosomes. “The fact is that this R [the white\(^+\) allele] and X [the X chromosome] are combined, and have never existed apart.” (Morgan 1910).

Morgan’s genetic proposal gave strong, but indirect evidence, that genes are physical objects located on (or a part of) chromosomes, giving support to the chromosomal theory of inheritance. Previously, Walter S. Sutton (1902) working on the cytogenetics of grasshoppers stated: “I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity.” This paper is considered to be the first argument that chromosomes during meiosis obey Mendelian rules (Crow and Crow, 2002).

The proposal of Morgan that genes are on chromosomes was, however, not accepted by everyone. For example, it could have been that the white gene was involved in sex determination, instead of being linked to the X chromosome. William Bateson, who first suggested the word “genetics” and was one of the rediscoverers of Mendel’s work, did not accept the chromosomal theory of inheritance until 1921 (Cock, 1983). “Do you now believe in the chromosome basis of heredity? Yes, I do – and all my life’s work has gone for nothing” (Leonard Darwin talking with William Bateson; Cock 1983).

It was Morgan’s student, Calvin B. Bridges, who gave more direct proof that the white locus of \( D. \) melanogaster was physically attached to the X chromosome (Bridges 1913, 1916a, 1916b). Bridges predicted, and observed, that exceptional progeny (about 1/1700 total) from crosses of homozygous white-eyed \( (\text{ww}) \) females and red-eyed \( (\text{w}^+\text{Y}) \) males were due to nondisjunction of the X chromosomes in females. The exceptions were F1 white-eyed females \( (\text{wwY}) \) and F1 red-eyed males \( (\text{w}^0\text{0}, \text{only had a single X chromosome}) \) (see the figure below; also see Figure 5, using the vermilion mutation, in Bridges 1913).
Bridges confirmed his genetic hypothesis by cytological observations that, for sex chromosomes, the exceptional white-eyed females had two X chromosomes and one Y chromosome (XXY) (see Plate I, Figure 5 in Bridges, 1913) and that the exceptional males had only one X chromosome (X0). “The genetic and cytological evidence in the case of non-disjunction leaves no escape from the conclusion that the X chromosomes are the carriers of the genes for the sex-linked characters.” (Bridges, 1916).

The objective of this teaching exercise is to determine if students can repeat the genetic results of Bridges. In particular, can exceptional X0 males, recovered due to nondisjunction in female parents, be shown to be sterile because of the missing male fertility factors on the Y chromosome, and do the exceptional XXY females give the expected progeny.

The following crosses were performed to isolate and test for exceptional X0 males and exceptional XXY females produced by nondisjunction in females. In these crosses, \( w^{1118}/w^{1118} \) flies have white eyes due to a deletion in the sex-linked white gene and, therefore, cannot revert to wild type; \( nub^{2} \) is linked to the second chromosome and \( nub^{2}/nub^{2} \) have very short wings; \( ve \) is also linked to the second chromosome and \( ve/ve \) have short wing veins. The symbol “+” is the wild-type allele for a gene or the symbol for a wild-type chromosome. \( w^{1118}/w^{1118} ; nub^{2}/nub^{2} \) is Indiana University stock 358; and \( ve/ve \) is Indiana University stock 628. The \( ve \) mutation is also called \( rho^{ve-1} \).
The \( w^{1118}/w^{1118} ; nub^2/nub^2 \) females × \(+/Y \); ve/ve males

F1 progeny from regular disjunction of the X chromosomes in parental females:

\[
\begin{align*}
&w^{1118}/+ ; ve \ +/+ \ nub^2 = XX \text{ females, red eyes & normal wings} \\
&w^{1118}/Y ; ve \ +/+ \ nub^2 = XY \text{ males, white eyes & normal wings}
\end{align*}
\]

F1 progeny from nondisjunction of the X chromosomes in parental females:

\[
\begin{align*}
&w^{1118}/w^{1118}/Y ; ve \ +/+ \ nub^2 = XXY \text{ females, white eyes & normal wings} \\
+0 ; ve \ +/+ \ nub^2 = X0 \text{ males, red eyes, normal wings & sterile}
\end{align*}
\]

The \( nub^2 \) and \( ve \) mutants were used in this cross to remove the possibility of accidentally counting a parental female or male as F1 exceptional progeny and to identify the progeny from non-virgin parental females that are included in the crosses by mistake. If parental bottles or vials are not completely cleared before counting F1 progeny, some parental females with white eyes may be counted as exceptional F1 XXY females, which also have white eyes, and some parental males with red eyes may be counted as F1 X0 males, which also have red eyes. In this cross, parental female have \( nub^2 \) wings and can be clearly distinguished from F1 females that have wild-type wings, and parental males have \( ve \) wings and can be clearly distinguished from F1 males that have wild-type wings. Progeny from non-virgin parental females will have white eyes and nubbed wings. Also note that if students score some F2 progeny by mistake, one fourth of these flies will be \( ve/ve \) and will have veinlet wings (none of the F1 flies have veinlet wings) and one fourth will be \( nub^2/nub^2 \) and have nubbed wings (F1 have wild-type wings).

Considering only the sex chromosomes, the F1 exceptional, XXY females (\( w^{1118}/w^{1118}/Y \), with white eyes) will in most cases have mated with their XY brothers (\( w^{1118}/Y \), white eyes). If one can collect virgin XXY females, they can be mated with wild-type males (+/Y), giving the following possible progeny.

\[
\begin{align*}
&w^{1118}/w^{1118}/Y \text{ females} \times +/Y \text{ males} \\
\end{align*}
\]

\[
\begin{align*}
\text{F1} \ w^{1118}/+ &= XX, \text{ red eyed, normal females} \\
\text{F1} \ w^{1118}/w^{1118}/+ &= XXX, \text{ red eyed, weak females} \\
\text{F1} \ +/Y &= XY, \text{ red eyed, fertile males} \\
\text{F1} \ w^{1118}/Y &= XY, \text{ white eyed, fertile males} \\
\text{F1} \ w^{1118}/Y/Y &= XYY, \text{ white eyed, fertile males} \\
\text{F1} \ Y/Y &= \text{ die as embryos}
\end{align*}
\]
Considering only the sex chromosomes, the F1 presumptive X0 males (+/0, with red eyes) were mated with five C(1)DX, y f virgin females. These females came from a stock of C(1)DX, y f /Y females, which have two X chromosomes attached to a single centromere, and sn3/Y males (singed, very small bristles). Hence, if by mistake a mated C(1)DX, y f female is used in the above cross, the progeny males are sn3 with short bristles. If the presumptive nondisjunction F1 males are X0, they will be sterile due to the absence of male fertility factors on the Y chromosome (see a discussion of this topic in Carvalho et al., 2000).

In a pilot run, where w1118/w1118 females were mated with Canton-S (wild-type) males, we recovered one presumptive X0 male out of 2,175 total males and this male was sterile. We also recovered one presumptive XXY female out of 2,782 total females, but we were not sure if this female was a parental female that was scored as an exceptional F1 progeny (they have the same white-eyed phenotype). This is the reason that we included the nub2 and ve markers in the crosses of this teaching exercise.

To confirm that X0 males and XXY females recovered in this study are due to nondisjunction, female parents (w1118/w1118; nub2/nub2) were placed at 5°C (in a refrigerator) for three or four days, removed and mated with untreated parental males (+/Y; ve/ve) (Woodruff and Thompson, 2002). Since cold has been reported to induce nondisjunction in D. melanogaster (Leigh, 1979; Foureman, 1988; Woodruff and Thompson, 2002), it is our hypothesis that the frequency of X0 and XXY nondisjunction events will be significantly higher in the cold-treated females compared to females maintained at room temperature (about 22°C).

Results and Discussion

The identification of extraneous flies: We recovered a few parental flies and F2 flies among F1 progeny. In all cases we were able to identify these flies as not being F1s, because of the presence of nub2 or ve phenotypes.

Nondisjunction: The nondisjunction events recovered from parental females were as follows:

<table>
<thead>
<tr>
<th>X0 Males</th>
<th>XXY Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/11,272 (0.0004)⁴</td>
<td>2/12,719 (0.0002)⁴</td>
</tr>
</tbody>
</table>

⁻χ² (1 df) = 0.31, P = 0.58 (Fisher’s exact test)

All four of the X0 males were sterile due to the lack of a Y chromosome. Hence, they arose from nondisjunction in female parents. The two recovered XXY females were not virgins; they had mated with their sibling males. Hence, we were not able to test these XXY females for extra sex chromosomes by matings to wild-type males, as discussed above. The frequency of nondisjunction leading to X0 males and to XXY females was not significantly different (P = 0.58). Yet, the rate of nondisjunction events in this study (6/23,991 = 0.0003, or about one event in 3,333 progeny) was lower than that observed by Bridges (about 1/1700) (Bridges, 1916a). This may mean that the rate of nondisjunction is influenced by the genetic background. There are known mutants that increase the frequency of nondisjunction (see Szabad et al., 1995, and references therein).

Cold treatment: As an additional confirmation that the X0 and XXY events recovered in this study were from nondisjunction, we treated w1118/w1118; nub2/nub2 females for one day at 5°C and then mated them with +/Y; ve/ve males. We recovered one XXY female and two X0 males out of 1,944 total flies. The X0 males were sterile. The comparison of the frequency of nondisjunction at room temperature (about 22°C) and in cold are as follows.

Cold treatment: As an additional confirmation that the X0 and XXY events recovered in this study were from nondisjunction, we treated w1118/w1118; nub2/nub2 females for one day at 5°C and then mated them with +/Y; ve/ve males. We recovered one XXY female and two X0 males out of 1,944 total flies. The X0 males were sterile. The comparison of the frequency of nondisjunction at room temperature (about 22°C) and in cold are as follows.
Hence, as previously reported (Leigh, 1979; Foureman, 1988; Woodruff and Thompson, 2002), cold treatment did significantly increase the frequency of XXY and X0 progeny in this study, confirming that these exceptions were due to nondisjunction in female parents.

A class discussion of the results of this teaching exercise could include the following topics:

1) Bridges (1916b) stated that the exceptional offspring that he observed were caused by nondisjunction in female parents: “Evidence has been presented which proves that the occasional (1 in 1,700) matroclinous daughters or patroclinous sons produced by females is known to be XX in composition is due to primary non-disjunction, that is, the X’s fail to disjoin and are both included in the egg or both extruded to the polar cell.” (Bridges, 1916b). Why did Bridges rule out nondisjunction occurring in parental males? Does nondisjunction occur in parental males? If it does, what F1 progeny are expected from the cross of this study? The answer is that nondisjunction does occur in males, but the X0 and XXY progeny from nondisjunction in parental males have the same phenotypes as normal XY and XX progeny. “If primary non-disnuction occurred in the male, XY and zero sperm would be formed, but the zygotes from them would not differ in their sex-linked characters from regular offspring, so that such an occurrence could not be detected immediately.” (Bridges, 1916a).

2) Nondisjunction can occur at division I or II of meiosis. Is there a difference in the expected frequency of nondisjunction events among other gametes in these two divisions? Nondisjunction in division I of meiosis will give rise to one-half of gametes that have two X chromosomes and one-half that have no X chromosomes. Nondisjunction in division II of meiosis give rise to one-fourth of gametes with two X chromosomes, one-fourth with no X chromosomes, and one-half with one X chromosome. Would one be able to identify nondisjunction events that occurred in division I and II in the cross of this study? No, since nondisjunction in division I or II give rise to exceptional progeny with the same phenotypes.

3) Does nondisjunction occur in humans? Yes it does. Students might discuss that it is important to have a model system (such as Drosophila) to identify aneuploidy (loss or gain of chromosomes) that is caused by nondisjunction, because aneuploidy occurs in at least five percent of human pregnancies, is a leading cause of pregnancy loss, and is the most common cause of mental retardation in humans (Hassold et al., 1996; Hassold and Hunt, 2001; Hassold et al., 2007). For example, it has been reported that irradiation, oral contraceptives, fertility drugs, alcohol, and smoking may increase aneuploidy in humans (Hassold and Hunt, 2001).