Pedigrees, fate maps, and phylogenetic trees are three widely used graphical metaphors for representing historical relationships in genetics, developmental biology, and evolution. The word pedigree emanates from the French word for a stork's leg and three-toed foot (Bradie, pers. comm.). While widely employed in genealogy, ontogeny, and phylogeny, students struggle sometimes with comprehending the inferential power of these diagrams. Therefore, we have developed: a series of elementary mathematical tools for re-representing pedigrees; pedigree generators; pedigree-driven data base management systems; and, case studies for exploring genetic relationships.

One tool for examining a pedigree is to simplify it to a genetic graph. Inbreeding is most easily seen in this simplified representation of a pedigree. In 1736, Euler, the Swiss mathematician, published a solution to an entertaining puzzle on traversing seven bridges between the two banks of a river and two islands in Konigsberg without ever traversing the same bridge twice. He demonstrated that no such solution existed by establishing a logical system henceforth known as graph theory. We believe that the principles set forth here have considerable pedagogical promise as alternative ways of presenting genetics which will hopefully simplify genetics to and/or be appealing to you. Furthermore, these principles demonstrate that graph theory as an elementary subset of modern mathematics has considerable potential for application to biological problems.

Some of the fundamental definitions of graph theory are:

1. A graph is a set of points (called vertices) and line segments (called edges) connecting pairs of vertices. We can assume for our purposes that a graph is a subset of real 2-or-3-dimensional space.

2. A directed graph (digraph) is a graph with a direction, usually indicated by an arrow, assigned to every edge. Thus, the simplest dioecious genetic graph is simply two parents and one offspring (Figure 1).

![Figure 1. Simplest genetic graph.](image)

Alternatively, the possible genotypes resulting from a mating could be represented by Figure 2.

(3) Notation: If G is a digraph, and A is a vertex of G, then the number of incoming edges to A is denoted by \( p^* (A) \) and the number of outgoing edges from A is denoted by \( p (A) \).

Every biology student can readily agree that each zygote must have two and only two biological parents, although one or both of them may be unknown. Also, any individual's parents consist of one female and one male. Finally, it is equally obvious that no one can be their own parent. Ore (1963) summarized these three biological axioms of sexual propagation in terms of graph theory:

(a) \( p^* (A) \leq 2 \) for all A: no vertex has more than two incoming edges

(b) Every circular alternating path has a number of edges divisible by 4.

(c) The graph of a genetic experiment is acyclic.
First, note that while the number of incoming edges, $p^*(A)$, (genetic material from parents) is maximally two, the number of outgoing edges from a vertex in a genetic graph, $p(A)$, can vary considerably. For instance, human females release up to 400 eggs and human males release millions of sperm. While the maximum number of progeny borne by a single human female is roughly seventy, Attila the Hun supposedly fathered 6,000 children. Therefore, we can not easily place conditions on $p(A)$.

Secondly, we can illustrate Ore's (1963) point (b) by the following illustration of an impossible genetic graph (Figure 3). If parent $P_1$ is assigned as a female, then since $P_1$ and $P_2$ are the parents of child $C_1$, $P_2$ must be a male. Similarly $P_1$ and $P_3$ are the parents of child $C_2$; therefore, $P_3$ must also be male. However, $P_2$ and $P_3$ are the parents of child $C_3$; hence, both of them cannot be males. The reader or student is invited to try other circular alternating paths before generalizing about the divisible by 4 rule. Note exceptions in plants, protists, bacteria and hermaphroditic animals.

Thirdly, while no individual is his own ancestor, we can take exception to Ore's (1963) stipulations: "For instance, since no individual may marry his own sister or brother there can be no configuration in our graph of the form given in Figure 4. Since no individual may marry one of his parents there are no configurations of the type represented in Figure 5." Unfortunately, Ore confuses both taboos and marriage with the realities of genetic relationship. Children can result both from incestuous and illegitimate matings; genetic graphs should reflect the actual biological heritage.

Crow (1976), following the work of Sewall Wright, has actually employed genetic graphs to
teach the analysis of inbreeding; however, he referred to his graphs of pedigrees as "arrow diagrams." Although his arrow diagrams implicitly follow Ore's (1963) three fundamental principles of genetic graphs, they were never explicitly stated. The pedigree in Figure 6 can be easily represented by the graph in Figure 7.

If we introduce an additional definition of graph theory, then the graph can be further simplified.

(4) A terminal edge is an edge between two vertices A and B such that there are either no other edges from A or no other edges from B. Terminal edges cannot be part of a known inbreeding pathway. Thus, if a student is making a digraph of an inbred pedigree, then they should be entreated to:

(a) ensure $p^*(A) \leq 2$ for every vertex in the digraph;
(b) ensure all circular alternating digraphs are divisible by 4;
(c) ensure the digraph is acyclic; and
d) remove all terminal edges.

This policy would result in a genetic graph of
the pedigree in Figure 6 which is illustrated in
Figure 8.

The application of this policy reduces the
arrow diagram or genetic digraph of an inbred
pedigree, with sufficient self-checks, to the point
where now subjective advice can be given for
further simplifying the digraph with little room
for confusion. At this point, the student should
remove all edges which are not on circular paths;
that is, if we want to calculate the inbreeding
coefficient of individual IV-1, then we are only
interested in retaining those directed edges for
each parent of IV-1 which emanate from a
common vertex (vertices I-3 and I-4). By applying
this further restriction, the result is illustrated very
simply by Figure 9 and Table 1.

We believe that the addition
of these few principles of graph
theory can help make the
Teaching of inbreeding analysis
more explicitly logical and thus
easier to learn. Additionally, we
would like to reiterate the fact
that you can check your arrow
diagram of a pedigree to see if it
fits the general rules of genetic
digraphs.

Another alternative for
representing inbreeding is the
"cyclogram" illustrated in Figure
10 which was developed by
Nemat Hashem (1983), a genetic
counselor in Cairo, Egypt.

Wright's formula for the inbreeding coefficient can now be readily applied:

$$F_I = \sum_{P} \left( \frac{1}{2} \right)^{n-1} (1 + F_A)$$

$F_I$ = inbreeding coefficient of individual I; or, the probability that both alleles at a given locus in
individual I came from a single ancestor

$k$ = the number of independent circular paths

$n$ = the number of directed edges in a given circular path

$F_A$ = the inbreeding coefficient of a common ancestor; or, zero, if no additional information is known
about the ancestry of the origin of a circular path

If we apply this algorithm to the pedigree in Fig. 9, then the following calculations will result:

$$F_{IV.1} = \left( \frac{1}{2} \right)^{6-1} (1 + F_{I-3}) + \left( \frac{1}{2} \right)^{6-1} (1 + F_{I-4})$$

$$F_{IV.1} = \left( \frac{1}{2} \right)^3 (1 + 0) + \left( \frac{1}{2} \right)^3 (1 + 0)$$

$$F_{IV.1} = \left( \frac{1}{32} \right) + \left( \frac{1}{32} \right)$$

$$F_{IV.1} = \left( \frac{1}{16} \right)$$

†Table 1. Inbreeding Coefficients

← Figure 9. Simplest genetic digraph of the inbreeding relationships of the pedigree shown in Figure 6.
DOUBLE  
1st COUSINS

Common ancestors.

Deleted ancestors.

Free ancestors.

**Figure 10.** Cyclograms which project the inbred components of the different inbreeding patterns which prevail among Egyptians.

**Computer Software**

We have developed two different pieces of software to help students learn how to build and interpret pedigrees. Our older software, the Pedigree Construction Kit (Calley, Soderberg, and Jungck, 1990) allows the user to see multiple pedigrees generated for autosomal dominant and recessive and X-linked dominant and recessive traits (see Figure 11). A driven data base management system called Inherit that allows users to build pedigrees from scratch and to build a variety of templates for each individual. Since this is such a powerful tool and is easily available, we will only share two aspects as shown in Figures 13 and 14.

The palette of Inherit has a number of tools for creating, editing and displaying pedigrees. These are labeled in Figure 13 which shows the palette.

**Figure 11.**

After choosing a pedigree type from the generator, multiple examples (as many as your computer memory will allow) will be generated upon your command (Figure 12). The individuals in a pedigree can be moved laterally if that helps you clean up their appearance. Generally, we find that students print out several copies of each pedigree to carry out further analysis.

More recently, we (Jones, Calley, Soderberg, and Jungck, 1994) have developed a pedigree when it is in Show Phenotype mode; there is also available a Show Genotype mode.

Nichols, Sheffield, and Stone (1993) have developed a Hypercard stack which is especially useful for linkage analysis. Also, Don Buckley at the University of Hartford is about ready to release a Supercard application on pedigree analysis that illustrates the transmission of alleles through a pedigree.
Figure 12. A PCK-generated pedigree

Figure 14. This is an example of one of dozens of templates that can be employed in Inherit to develop a clinical history for any individual in a pedigree that is being constructed.

Figure 13. The Palette of Inherit in "Show Phenotype" mode.
Using Pedigrees to Raise Social and Ethical Issues

Genetics is an ideal subject through which to explore controversial issues with students. Advances in genetic technology have raised dilemmas that have elicited strong emotional responses. The use of case studies and role playing scenarios can provide opportunities for students to acquire a better understanding of the complexities of controversial issues (see Table 2).

With PCK and/or Inherit, limitless combinations of situations can be created for classroom use. PCK facilitates flexibility in designing cases. A number of pedigrees can be generated and fit to a family history in numerous ways, or different disorders with the same mode of inheritance can be fit to the same pedigree. In either case, different issues will arise and different choices may need to be weighed. The involvement and creativity of the students make each case study unique. A case can be constructed around any genetic disorder. Pedigrees are generated by setting the mode of inheritance of a particular disorder along with preferences in regard to family size. A family history can then be written that describes the individuals represented in the pedigree. This will be illustrated with a case involving Marfan syndrome, an autosomal dominant disorder. Through this case, issues, choices and options can be examined, from a genetics counselor's position as well as from the point of view of each of the family members and their friends, colleagues and neighbors.

Table 2. Bioethical Issues that can be Raised Using Case Studies

<table>
<thead>
<tr>
<th>Issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>confidentiality</td>
</tr>
<tr>
<td>disclosure/informed consent</td>
</tr>
<tr>
<td>prenatal screening</td>
</tr>
<tr>
<td>non-paternity</td>
</tr>
<tr>
<td>quality of life</td>
</tr>
<tr>
<td>individual rights (autonomy)</td>
</tr>
<tr>
<td>issue of individuality - when does life begin and end?</td>
</tr>
<tr>
<td>eugenics - &quot;playing God&quot;</td>
</tr>
<tr>
<td>euthanasia and the prolongation of life</td>
</tr>
<tr>
<td>right to decide (competency)</td>
</tr>
<tr>
<td>intervention</td>
</tr>
<tr>
<td>allocation of medical resources: preventions vs. cures</td>
</tr>
<tr>
<td>acceptability of risk and cost-benefit analysis</td>
</tr>
</tbody>
</table>

A Sample Case Study

In 1986 an article appeared in *Sports Illustrated* magazine concerning the death of volleyball star Flo Hyman. Autopsy revealed that she had had Marfan syndrome and had died from an aortic aneurysm. Individuals who have Marfan syndrome tend to be tall and lanky. Several basketball players who had been diagnosed as having Marfan syndrome or who had died from it were discussed in the article. Anne and her parents read this article and wondered if Anne had Marfan syndrome.

**Proband Information**

Anne is 16 years old, 5'11", plays varsity volleyball and basketball, wears contact lenses to correct nearsightedness, has slight scoliosis, a concave sternum, long fingers and toes, and wide spaced eyes. Her arm span exceeds her height by 5 inches, she wears orthodontic braces, and she is slightly knock-kneed.

The first decision that will have to be made is Anne's diagnosis. The proband information was hypothetically gathered from the first part of a visit to a clinical genetics center - the physical exam. Does she or does she not have Marfan syndrome? What information do you need to gather in order to make a diagnosis? Are there any confirmative tests for this particular disorder? What additional information will you need to know?

Most students will be unwilling to commit to a diagnosis on the basis of the information provided from the hypothetical physical exam. They are typically surprised and uncomfortable with the realization that there is no definitive test to base their answer on in this particular single gene disorder. Karyotyping can only reveal chromosomal aberrations, not single gene problems, and no DNA markers are available for analysis for Marfan syndrome. The idea that their diagnosis will depend on their best decision, given the information at hand, is disquieting. Most students are used to some source of confirmation of a "right" answer. A scenario such as this can force them to re-think what it means to label an answer as correct.
**Siblings and Their Spouses**

David - Age 25, married to Jessica (age 25), one daughter named Kristi (3 mos.), David wears glasses, is 6'3", has long fingers and toes, played basketball and ran track in high school, had some knee problems then.

Jessica - Age 25, 5'8", no glasses, no health complaints, had one miscarried pregnancy in her first trimester prior to the birth of daughter Kristi.

Cheryl - Age 14, 5'9", no glasses, slight case of scoliosis, born with club feet that responded well to treatment.

**Parents**

Mary - Age 47, 5'7", wears glasses, hay fever, has been diagnosed with carpal tunnel syndrome and mild diabetes, had two miscarriages in addition to her three children.

Peter - Age 49, 6'1", wears glasses, concave chest, high blood pressure, partial dislocation of lens in right eye, long fingers and toes.

**Aunts and Uncles**

Mary's siblings:

Dorothy - Age 46, wears glasses, 5'3", no major health problems, had an ovarian fibroid tumor removed at age 40, married with four children.

Ellen - Age 50, 5'5", high cholesterol, unmarried, no children.

Eric - Age 51, 6'0", wears reading glasses, back problems from car accident, suffers from exercised-induced asthma, is married and has two children from first marriage and three from his second.

Peter's siblings:

Frank - Age 55, 6'4", wears glasses, slight hearing loss in one ear, was treated for alcoholism, is a heavy smoker and has developed chronic cough, divorced, the father of two children.

Alice - Age 56, 5'7", wears glasses, arthritis in one shoulder, married, has one daughter and a son who was born with cerebral palsy.

John - Deceased, heart attack at age 46, 6'2", had a dislocated lens in his right eye, had three children; the youngest girl is mildly mentally retarded and lives with his wife.

Larry - Age 58, 6'3", no glasses, high blood pressure, divorced twice, he now lives alone, had two children by his first marriage and one with his second wife, reported to be a heavy drinker.

**Maternal Grandparents**

Evelyn - Died at age 76 of a stroke, 5'4", arthritis in hands and feet, wore reading glasses, was said to have had as many as five miscarriages.

William - Age 81, no glasses, 5'10", no major health problems.

**Paternal Grandparents**

Martha - Age 86, 5'8", high blood pressure, some knee and ankle problems, wears glasses for distance and reading.

Charlie - Died at age 44 of a heart attack, severe vision problems, long fingers, described as tall and lanky, contracted polio at age 26 and had been wheelchair dependent since his polio treatment.

---

**Table 3. Anne's Family History**

When the students request information about Anne's family members to aid them in their diagnosis, they can be given a family history and pedigree (see Table 3). Each small group of students must come to a consensus regarding a diagnosis for each family member. Who has Marfan Syndrome and who does not? Their decisions will impact on the counseling issues they will explore next.

Despite the additional family information that has been given to the student groups, they are still often reluctant to commit to making decisions regarding diagnoses. However, faced with a case where they will have to come to consensus, the students will come to understand some of the complexities of human genetics as they learn about specific disorders. The next section will provide the instructor with the information about syndromes and pleiotropy that can be taught from this case about Anne and her family.

**About Syndromes, Pleiotropy, and the Diagnosis of Marfan Syndrome**

A syndrome represents a disorder with a range of possible characteristics and severities. Not all individuals with a given syndrome will have the
same set of symptoms, yet each will have some sort of combination from the range of symptoms common to that particular disorder. In other words, not all individuals will fit the standard textbook list of symptoms for that disorder, yet there will be many similarities among individuals with the syndrome. This is true for Marfan syndrome (see Table 4).

Most syndromes are also examples of pleiotropy, multiple effects caused by a single gene. For example, in people with Marfan syndrome, you may see myopia; long limbs, fingers and toes; spinal curvatures; and aortic root dilatation with the consequence of an aortic aneurysm. All of these symptoms seem unrelated, yet are caused by a single gene (Marfan syndrome is an autosomal dominant disorder). However, in most cases of pleiotropy, if the underlying phenomenon can be identified, then the seemingly disparate symptoms can be understood in light of the causal mechanism. In Marfan syndrome, the genetic defect results in the production of suboptimal connective tissue. Each one of the Marfan symptoms can be explained in terms of this defect. The eye lens is held in place by connective tissue. If the tissue is defective, the lens may move, causing myopia, or in severe cases, lens dislocations. Connective tissue is also the initial base material in long bone formation. This can result in the longer than average limbs and digits seen in most individuals with Marfan syndrome. The aortic root dilatation and aortic aneurysms can be explained by the defective connective tissue found in arteries. In Marfan syndrome, the aorta is the focal point of the defect--it is under great force as the blood is forcefully ejected from the ventricle and enters the curvature of the aorta. This constant pounding of the blood against the wall of the aorta can lead to minute tears and stretching. Progressive damage to the aortic wall can ultimately result in rupture, otherwise referred to as an aortic aneurysm.

When diagnosing a genetic disorder, some of the most salient information comes from the family history. It is very important to sift carefully through the information obtained about each family member - what information is pertinent to the disorder in question, and what is unrelated health information? For example, an individual with Marfan syndrome is at increased risk for aortic dilatation and subsequent aneurysm. In taking a family history, you may discover that a family member has high blood pressure. It is unlikely that this is related to Marfan syndrome. However, you may also learn that several family members wear glasses. Again, vision problems may be a result of Marfan syndrome, or they may have other causes. Syndrome diagnosis is based on the presence of several symptoms occurring together, each of which can occur independently in the general population.

Creating a Family History for a Case Study
When creating a family history from a PCK-generated pedigree, choose the "extraneous" symptoms carefully. It is desirable to have some ambiguity so that students will debate the relevance of a health problem to the disorder in question. What information offers clues or insights into the identification of individuals at risk in the family? What information is incomplete? What additional information do you need to make a diagnosis? What family and individual issues and concerns should be addressed during the counseling session?

This particular case study was created by choosing symptoms from a list of descriptions about Marfan syndrome and assigning them to individuals in the pedigree generated by PCK. Table contains a list of the spectrum of symptoms seen in individuals with Marfan syndrome. Such descriptors can be found in a variety of sources, including human genetics reference books and texts, the On-line Mendelian Inheritance in Man (OMIM), and public education information from organizations such as the March of Dimes. Once the Marfan descriptors were assigned to various individuals, other health problems were chosen and assigned to the individuals.

Once the family history has been created for
the pedigree, the case study can be enriched by creating role play statements to give to the small groups to analyze in addition to the family history information. The role play statements are meant to help students recognize and understand the emotions of guilt, blame, anger, shame and denial that surround the birth and life of a person with a genetic disorder. Additionally, they create situations in which the students must put themselves in the roles of various members of the hypothetical family that is faced with reproductive decisions. What would they do if they were facing this situation, and more importantly, why did they make the choices they did? The students will have to assess their reproductive options (e.g., prenatal testing, artificial insemination by donor, adoption, no children, etc.) and the emotional implications of each. Role play statements generally fall into two categories: those that are generalizable to most genetic disorders, and those that are disorder-specific situations. Here are three examples of role statements that can be used with the sample case study.

Anne refuses to quit playing varsity level athletics despite the fact she is at risk for an aortic aneurysm. There is a good possibility that she will receive a full college scholarship upon graduation from high school.

Mary, Anne’s mother, is terrified at the possibility that Peter may die at an early age. Mary has agoraphobia and experiences occasional panic attacks from anxieties about being in public places.

David and Jessica have planned on having two children.

<table>
<thead>
<tr>
<th>Amniocentesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- The results from your amniocentesis show a chromosome abnormality.</td>
</tr>
<tr>
<td>- The results from your amniocentesis do not show a chromosome abnormality.</td>
</tr>
<tr>
<td>- The cells taken from the amniocentesis did not grow. Would you like to have it performed again?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AFP Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>- The results of your blood serum test indicate a higher than average AFP level. Would you like us to go ahead and test your amniotic fluid values?</td>
</tr>
<tr>
<td>- The results of your AFP testing was normal.</td>
</tr>
<tr>
<td>- The results of your amniocentesis show a higher than average AFP value.</td>
</tr>
<tr>
<td>- The results of your amniocentesis show a normal level of AFP.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carrier Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>- The results of your carrier test were positive.</td>
</tr>
<tr>
<td>- The results of your carrier test were negative.</td>
</tr>
</tbody>
</table>

Table 6. Some Examples of Outcomes Cards

Additional suggestions for role play statements that can be used when creating case studies can be found in the Appendices.

Ask the students to first place themselves in the role of the individual family members. What reactions, questions, concerns, and fears will each of the family members have? What ethical dilemmas or psycho-social implications are raised by the decisions that confront both the affected individuals and the individuals who do not have the disorder? In addition, what options and decisions must be made regarding prenatal and carrier status testing (see Table 5).

The role play scenarios can be extended to include outcomes of the decisions made by the students. First, what type of testing is appropriate for this case? Then, if students decide they would opt for prenatal or carrier testing, they can find out the results of their test by drawing from a stack of Outcome Cards, such as those listed in Table 6. In any scenario that involves testing, the students should consider the possibility of false negatives and false positives. Finally, what surprising additional inferences might be drawn about the inheritance of other characteristics of the individuals in the pedigree?

Table 5. Possible Tests for Genetic Disorder

**Prenatal Tests**

1. Ultrasound (major congenital malformations or deformations)
2. Serum AFP (neural tube defects)
3. Amniocentesis
   a. amniotic AFP
   b. Chromosomal abnormalities
4. Chorionic villi sampling (CVS)
   a. chromosome abnormalities
   b. DNA analysis (some single gene disorders)

**Carrier testing**

a. by enzyme assays
b. by DNA analysis
Literature Cited


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September 28-30, 1995
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Tips on Teaching Nonmajors?
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Exciting Demonstrations?

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Email Address: _____________________________________________________________

Check One:  
____ 45 min Oral Presentation (including discussion)
____ 30 min Oral Presentation (including discussion)
____ Workshop (2-3 hours)
____ Other (specify)

Title of Presentation: _______________________________________________________

Abstract:

_________________________________________________________________________

Special equipment or facilities required:

_________________________________________________________________________

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