LABORATORY 2
Evolution: Genetic Variation within Species
OVERVIEW OF LAB
We will investigate factors that influence genetic variation within species. We address the general hypothesis:

*Environmental conditions cause changes in gene frequencies.*

You will study two demes (subpopulations) of a non-laboratory population that have presumably experienced different evolutionary histories. **After learning to identify their polymorphic traits, you will observe neighborhood cats and tabulate the phenotypes of at least 10 of these cats.** In lab we will pool the cat data collected by each individual and then discuss analysis of the data. To test the general hypothesis discussed above, we will choose a published data set collected in an area very different from our local “North Atlanta region”. This other deme of cats presumably differs from the North Atlanta region in important environmental conditions (including physical factors, but also factors such as different colonization histories and population sizes). Data will be analyzed to determine whether there is a statistical difference between demes.

We will use the same format for writing the lab report as described in the previous lab. Be sure to cite (in the introduction or Discussion) at least one other study (from a "primary" source, not a textbook, encyclopedia, or dictionary).

BACKGROUND FOR LAB
Population geneticists commonly analyze **allele frequencies** of local populations of organisms. (Aleles are alternative forms of a gene at a specific gene locus on a chromosome.) The allele frequencies can be used to measure genetic changes in the same population over time (shifts in the Hardy-Weinberg equilibrium) or to compare different demes (local populations). Demes of the same species generally do not differ in the presence or absence of particular alleles, but rather in the frequency with which particular alleles occur. In this study, we will calculate allele frequencies for certain genes for readily observable traits in domestic cats. Cats are convenient organisms for this exercise: they are relatively free of selective breeding (as long as you stay away from the cat-show "fancy" varieties and stick to street cats), but they are domesticated enough for a reasonably close-up inspection. They tend to stay close to where they were born, and there is relatively little "gene flow" between demes due to non-human causes. In addition, they show a number of polymorphic phenotypes, i.e., traits that show distinct individual variations. When a population shows genetically based polymorphism for one or more phenotypic traits, allele frequency analysis for each polymorphic trait is possible.
FIG. 1 - REPRESENTATIVE PHENOTYPES IN CATS

a. Stripes (N/N or N/n and B/B or B/b)

b. Piebald (P/P or P/p) and Non-Agouti (n/n)

c. Blotched (N/N or N/n and b/b)
INTRODUCTION

Before completing this section, read the Methods and Materials section. In the first part of the introduction discuss observations that led to the formation of the hypothesis. In a second paragraph, make a brief statement summarizing the experiment to help the reader identify the important variables. Also, state the general hypothesis to be addressed (see previous page) and formulate the specific hypotheses (precise predictions of possible results) to be tested.

METHODS AND MATERIALS

In this report, the methods section is written for you. Note that it is written in past tense, as it would appear in a lab report or published paper.

SAFETY NOTE: Unless you are very familiar with the cat (and vice-versa) do not attempt any "hands-on" observation!

You may copy the following text into your submitted report:

All the of the phenotypic polymorphisms are described below using the terms "wild-type", or the names that have been given to the various known "mutant" varieties. "Wild-type" traits are based on the appearance of the prototype "wild cat" still to be found preying on large grasshoppers and small rodents in African scrub land. This wild-type cat has prominent black and buff stripes. Wild-type alleles can be dominant (indicated by an uppercase letter) or recessive (indicated by a lowercase letter) depending on the type of trait. Any one cat in our local deme may have a mixture of wild-type (often denoted as "+" in the literature) and "mutant" features. Mutant features we will observe are:

1. **Non-agouti (n):** These variants have hairs with solid coloring. Individuals may be agouti (NN or Nn) or non-agouti (nn). Sometimes, upon close examination, or in the right light, a faint pattern of stripes can be discerned in the fur of a non-agouti cat. **Non-agouti is recessive.**

2. **Blotched (b):** There are curved whorls or blotches of pattern rather than even stripes. Individuals can have straight or even stripes (BB or Bb) or a blotched pattern (bb). **Blotched is recessive.**

3. **Long fur (l):** Wild-type cats have short fur. Individuals may have short fur that lies smoothly along their body contours (LL or Ll) or fur that fluffs out (ll). **Long hair is recessive**

4. **Piebald (P):** This dominant allele results in a varying amount of white fur - from a few (generally ventral) patches, to an almost totally white cat. Individuals may have white patches (PP or Pp) or no white patches (pp). Note that in this case, the mutant type **piebald is dominant.**

5. **Dominant white (W):** This is a dominant mutation for an all-white cat. Individuals may be totally white (WW or Ww) or have at least some color other than white (ww). Note that in this case, the mutant type **white is dominant.**

(A number of other mutant traits are known, but we did not record them in this exercise. For example, “orange” fur color is a common alternative to the gray/black color, but its genetic basis is more complex than the others (the alleles for these traits are found on the “X” chromosome). Another fur color mutant is “dilute”, which is often difficult to clearly identify.)

<table>
<thead>
<tr>
<th>Dominant Allele</th>
<th>Recessive Allele</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Agouti (N) – wild type</td>
<td>non-agouti (n)</td>
<td></td>
</tr>
<tr>
<td>Not blotched (B) – wild type</td>
<td>blotched (b)</td>
<td></td>
</tr>
<tr>
<td>Short hair (L) – wild type</td>
<td>long hair (l)</td>
<td></td>
</tr>
<tr>
<td>Piebald (P)</td>
<td>not piebald (p) – wild type</td>
<td></td>
</tr>
<tr>
<td>Solid white (W)</td>
<td>not solid white (w) – wild type</td>
<td></td>
</tr>
</tbody>
</table>

The wild type is dominant

The wild type is dominant

The wild type is dominant

The wild type is dominant

The mutant is dominant

The mutant is dominant
Each member of the class recorded the above phenotype information on at least ten cats within a single area near to where they live. An attempt was made for each class member to work in different neighborhoods to avoid recording the same cats twice. "Cat-show fancies" (Siamese, Persian, etc.) were also avoided. As much information as possible (keeping a safe distance from unfamiliar cats) was gathered on each cat. In individual showing "dominant white", genes for other fur color and pattern traits were masked. Number of dominant white cats were counted, but not added to the total pool when doing allele frequency determinations for any of the other traits.

To assess whether two demes have undergone different evolutionary histories, we calculated allelic frequencies based on these observed phenotypic frequencies. Because allelic frequencies cannot be observed directly we calculated allelic frequencies from observed phenotypic frequencies using the Hardy-Weinberg equation. The Hardy-Weinberg equation yields the genotypic frequencies of a population in which no evolutionary mechanism are acting (i.e. no selection, mutation, migration, or genetic drift). For our conclusions to be valid, we make two assumptions. 1) The present rate of evolution is zero or sufficiently low that the Hardy-Weinberg equation accurately estimates allelic frequencies. Because evolutionary change is cumulative, small changes that cannot be detected as a deviation from the Hardy-Weinberg equilibrium should manifest themselves over many generations. 2) That cat populations in different U.S. cities were derived from the same or similar cat populations in Europe, so that any significant differences in populations among U.S. cities can be attributed to evolution occurring over the many generations since these populations were established.

In Hardy-Weinberg terms, the frequency of the dominant allele = p and that of the recessive allele = q. The formula for all of the genes for a particular trait in a deme then is:

\[ p + q = 1.0 \]

Where the gene locus has only 2 alternative alleles, and p and q are expressed in decimal form.

Our observations were of whole cats, not gametes, so we worked backwards from the distribution of the genotypes of individuals in the population in order to determine allele frequencies. This was done by using the binomial expansion of the above equation, as follows:

\[ (p + q)^2 = (1.0)^2 \]

\[ p^2 + 2pq + q^2 = 1.0 \]

where:
- \( p^2 \) = the frequency of the homozygous dominant individuals
- \( 2pq \) = the frequency of the heterozygous individuals
- \( q^2 \) = the frequency of the homozygous recessive individuals

For each trait, the number of individuals expressing the recessive phenotype (the ones with the "known" genotype) was divided by the total number of cats observed for that trait. This gives \( q^2 \). Then, Hardy-Weinberg formula was used to calculate the p and q value. (For recessive mutants "q" is the allele frequency of the mutant recessive allele; for dominant mutants, "p" will be the mutant allele frequency.) Thus, \( q \) (the allelic frequency of the recessive) is the square root of the phenotypic frequency of the individuals expressing the recessive allele, or:

\[ \text{allelic frequency of recessive} = \text{square root of} \ (\text{no. of ind. expressing recessive} / \text{total no.}) \]

The "dominant white" cats were not counted in the totals for any of the other traits, because this trait masks the expression of other traits.
Individual data were pooled with the data collected by others in the class to get a good estimate for allele frequencies. The calculated allelic frequencies from each individual were grouped for comparison with published allele frequency data from a distant location. The locations of the two demes to be compared are listed below:

Location 1 - “North Atlanta Region”.

Location 2 - ____________________________________.

Chi-square contingency table tests of independence were performed for the mutant alleles “non-agouti” (n), “blotched” (b), and “long fur” (l) to determine whether allelic frequencies differed between the two demes.

**RESULTS**

Table 1. Raw data table of “North Atlanta Region” cat phenotypes by trait

<table>
<thead>
<tr>
<th>Cats</th>
<th>Location of cat w/in North Gad area</th>
<th>non-agouti (n)</th>
<th>blotched (b)</th>
<th>long fur (l)</th>
<th>piebald (P)</th>
<th>dom. white (W)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ex. C</td>
<td>Smyrna</td>
<td>nn</td>
<td>-</td>
<td>L?</td>
<td>pp</td>
<td>ww</td>
<td></td>
</tr>
<tr>
<td>ex. D</td>
<td>Smyrna</td>
<td>N?</td>
<td>bb</td>
<td>l</td>
<td>P?</td>
<td>ww</td>
<td></td>
</tr>
<tr>
<td>ex. F</td>
<td>Smyrna</td>
<td>-</td>
<td>-</td>
<td>L?</td>
<td>-</td>
<td>W?</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Cat Population Allele Frequencies (“q”) from the Midwestern U. S. A.

<table>
<thead>
<tr>
<th>Locality</th>
<th>n</th>
<th>b</th>
<th>l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omaha, NB</td>
<td>.81</td>
<td>.35</td>
<td>.37</td>
</tr>
<tr>
<td>Champaign, IL</td>
<td>.78</td>
<td>.32</td>
<td>.35</td>
</tr>
<tr>
<td>Lawrence, KS</td>
<td>.72</td>
<td>.44</td>
<td>.44</td>
</tr>
<tr>
<td>Chicago, IL</td>
<td>.72</td>
<td>.34</td>
<td>.37</td>
</tr>
<tr>
<td>Columbus, OH</td>
<td>.64</td>
<td>.46</td>
<td>.39</td>
</tr>
<tr>
<td>St. Paul, MN</td>
<td>.74</td>
<td>.47</td>
<td>.51</td>
</tr>
<tr>
<td>St. Louis, MO</td>
<td>.79</td>
<td>.51</td>
<td>.48</td>
</tr>
<tr>
<td>Cleveland, OH</td>
<td>.74</td>
<td>.62</td>
<td>.25</td>
</tr>
<tr>
<td>Reno, NV</td>
<td>.77</td>
<td>.37</td>
<td>.39</td>
</tr>
<tr>
<td>Denver, CO</td>
<td>.84</td>
<td>.26</td>
<td>.35</td>
</tr>
</tbody>
</table>


Table 3. Frequencies of phenotypes for each trait - from individual and class observations for Location 1 (“North Atlanta Region”).

<table>
<thead>
<tr>
<th>Observed phenotype frequencies</th>
<th>non-agouti</th>
<th>agouti</th>
<th>blotched</th>
<th>non-blotched</th>
<th>long hair</th>
<th>short hair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nn</td>
<td>N-</td>
<td>bb</td>
<td>B-</td>
<td>l</td>
<td>L-</td>
</tr>
</tbody>
</table>

Small Group Data

Whole Class Data

Table 4. Chi-square comparisons between Location 1 “Observed Data” (Table 3) and Location 1 “Expected Data” (based on Location 2 allele frequencies - Table 2).

<table>
<thead>
<tr>
<th></th>
<th>non-agouti</th>
<th>blotched</th>
<th>long hair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nn</td>
<td>N-</td>
<td>B-</td>
</tr>
</tbody>
</table>

Observed

Expected*

Chi-Square Probability

* To calculate “Expected” Values:
1. Find the appropriate “q” value for the Location 2 you have chosen (Table 2).
2. Calculate $q^2$.
3. Multiply $q^2 X$ (the total number of cats observed for that trait) for the Expected number of cats with the recessive (mutant) trait; subtract this result from the total number of cats observed for the Expected number with the dominant phenotype.
Your Results section should contain the following bar graphs:

*Fig. 1. Comparison of Observed and Expected non-agouti phenotypes*
*Fig. 2. Comparison of Observed and Expected blotched phenotypes*
*Fig. 3. Comparison of Observed and Expected long hair phenotypes*

Finally, your Results section should contain a short paragraph that describes the results summarized in your tables and graph.

**DISCUSSION**
Refer to previous lab reports and *A Short Guide to Writing About Biology*. For this report, focus on evolutionary mechanisms that may (or may not) differ in each of these populations. Be sure to suggest *why* you believe these evolutionary forces are likely to differ (or not to differ) between demes. If there was no difference between the demes, discuss *why* allelic frequencies could be the same in each deme. Use scientific literature on allele frequency in other cat populations to support your conclusions. In addition, identify any possible *aspects of the procedure* used in this study which may have contributed to any differences (or lack of differences) found in comparing the demes.

**LITERATURE CITED**
In your Literature Cited section be sure to include any references mentioned in this lab exercise as well as any other references requested by your instructor.