In A World of Flies…..

Introduction:

L.B. Slobodkin defines population genetics as a "complex of mathematical models in which selection, mutation rates of various kinds, linkage patterns and assumed population distributions and mating systems produced changes in gene-frequency distribution with the passage of time". (2003)

The following background information is a synopsis of the article *Theories of Speciation,* by Hope Hollocher.

Understanding how one species splits into two (speciation) is key to understanding evolutionary biology. First, however, one must define *species*. It must be noted that there is no single, universally accepted definition, and that most are a function of the types of questions being pursued. The inherent problem of trying to categorize groups of organisms undergoing a continual process of change must also be considered. It must also be acknowledged that speciation is not caused by a single mechanism, but by a collection of different mechanisms of divergence affecting different traits at potentially different rates. As a result, boundaries that encircle these natural groups are often blurry. Depending on which process is being emphasized, the actual definition may vary based on circumstance. In most cases, conflicting boundaries are not so much a problem of competing species definitions, as they are a reflection of the biological processes that govern speciation.

One criterion used to determine species is the Morphological Species Concept, which simply states that groups that look different are different species. This is typically used as a starting point for identification; however, a problem arises when you are working with cryptic species (those that look the same but have different gene pools due to reproductive isolation, or those with the ability to change phenotype based on environmental conditions). The Phenetic Concept, which states that morphological difference are usually accompanied by genetic differences, is typically considered along with the Morphological Species Concept

A second species concept is the Biological Species Concept (BSC) put forth by Dobzhansky and reiterated by Mayr (1937 and 1982, respectively). The BSC states that speciation is the development of biological barriers to gene flow between the multiple gene pools (interbreeding groups are reproductively isolated from other groups). Barriers include both the premating (seasonal, sexual, and mechanical isolations) and postmating (inviable or sterile offspring) sort.

While there are other species concepts to consider, for practical purposed I will limit the content to these two. Within the context of these, however, one must also consider evolutionary forces, modes of speciation, and genetic patterns of differentiation.

Evolutionary forces, such as natural and sexual selection, genetic drift, and mutation, can work independently or in concert within specific geographical, ecological, behavioral and genetic contexts, the extent to which can significantly impact variation. For example, natural selection can serve to both cause and prevent change if one part of a coadapted gene complex is not exactly the most fit. Any change here requires a population to experience a decline, both in fitness and population, before obtaining a better optimum fitness. While genetic drift is random, without immigration or mutation, variation may actually decrease. Populations may actually respond differently to genetic drift in the absence of natural selection, resulting in an altogether unique divergence. With sexual selection, adaptations tend to revolve around mating purposes rather than environmental conditions. And finally, mutations are the primary source of genetic variation. Most spontaneous mutations decrease fitness and/or are bound by DNA properties making some changes more common. As a result, one needs to consider whether the mutation has an impact on phenotype and/or fitness, since allele frequency changes depend on whether or not the allele is already at a low frequency.

Various combinations of the above four forces give rise to numerous modes of speciation (such as allopatric v. peripatric speciation), most of which incorporate the action of all the evolutionary forces discussed above, accentuating the operation of certain forces over others.

Allopatric speciation considers the physical barriers that prevent gene flow between populations (gene pools) which may diverge and be different on either side of the barrier, but may still be under the influence of mutation, genetic drift, and sexual selection. Rates of divergence caused by these various forces, and which traits may most easily be affected can be explored. The outcome depends on the circumstances of the isolation.

Peripatric speciation (founder's effect) focuses on actions between genetic drift and natural selection or sexual selection early in speciation. It is possible that peripatric speciation may occur more rapidly than allopatric speciation due to large population size; however, the actual size of the population does not affect probability of changing fitness as much as the underlying genetic architecture may. Genetic drift and natural selection may work to affect divergence when traits with high epistasis (interaction between different alleles at two or more loci such that the phenotype differ from what would be expected if each locus acted independently) are involved. Also, reproductive isolation can be significantly affected by rapid change because of male/female coevolution when genetic drift and sexual selection are both driving divergence.

Ultimately, analyzing the genetic basis of traits having diverged between species could be useful in determining whether genetic patterns of change can tell us about the evolutionary forces that had a role in divergence. Although very early in speciation research based on genetics, some models have already begun to take form. One such pattern is Haldane's Rule. In 1922, Haldane's observations led him to conclude that "When in the F1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterogametic sex." The rule is true for males as well as females, and appears to be fundamental to speciation in all taxa. Explanations, however, depend on whether or not hybrid inviability or sterility is being considered.

In conclusion, the study of speciation can no longer be considered a singular entity. It must be considered along with and in terms of population genetics, molecular and developmental biology. They need to be fused coherently in order to offer possible explanations where no simple ones exist.

Purposes:

1) To apply both concepts above to different species of *Drosophila*. By examining the flies for morphological similarities and differences, as well as testing for premating isolation, you will define how many species you have in your group sample. You will then construct an hypothesis about the evolutionary relationship between your different species and come up with ideas about what forces may be working on speciation.

2) To use phenotype to determine relatedness, and to examine its correlation to genotype relatedness. Why should we consider both phenotype and genotype when trying to determine their relatedness and evolutionary history? Delineate which factors may have been important in determining the pattern of speciation for one *Drosophila* species.

Indiana Academic Standards Addressed:

B.1.15: Understand and explain that, in biological systems, structure and function must be considered together.

B.1.21 Understand and explain that the information passed from parent to offspring is transmitted by means of genes which are coded in DNA molecules.

B.1.30: Understand and explain that molecular evidence substantiates the anatomical evidence for evolution and provides additional detail about the sequence in which various lines of descent branch off from one another.

B.1.26 Demonstrate how the genetic information in DNA molecules provides instruction for assembling protein molecules and that this is virtually the same mechanism for all life forms.

B.1.32 Explain how natural selection leads to organisms that are well suited for survival in particular environments, and discuss how natural selection provides scientific explanation for the history of life on Earth as depicted in the fossil record and in the similarities evident within the diversity of existing organisms.

B.1.35: Explain that the degree of kinship between organisms or species can be estimated from the similarity of their DNA sequences, which often closely matches their classification based on anatomical similarities. Know that amino acid similarities also provide clues to kinship.

B.1.36 Trace the relationship between environmental changes and changes in the gene pool, such as genetic drift and isolation of sub-populations.

Additional background information:

The *Drosophila dunni* subgroup of fruit flies displays a near perfect pigmentation cline throughout the Caribbean, from Puerto Rico to Grenada, which is highly unusual (Heed and Krishnamurthy 1959) and which is likely the result of selective forces acting in the habitat of each species during speciation. Analysis indicates that natural selection has caused overall intensity of pigmentation among the northernmost species of the cline to converge.

The regularity of changes in pigmentation does not correspond to the evolutionary history of the species, as indicated through chromosomal inversion patterns (Heed and Russell 1971) and mitochondrial DNA sequence analysis (Hollocher 1998) and strongly suggests the action of natural selection in shaping the cline. (Hollocher, Hatcher and Dyerson 2000) Moreover, genes affecting pigmentation tend to affect reproduction physiology and/or mating behaviors, which may then contribute to reproductive isolation. It can be concluded then that, based on pigmentation, natural and sexual selection have possibly been affecting abdominal morphology divergence among these species (Hollocher, Hatcher and Dyerson 2000).

D. dunni is a subgroup of the larger *D. cardini* group, which exhibit pigmentation patterns on the mainland of South America. While there is a large variety of phenotypic variability within the confines of a single mainland species, those on the islands exhibit partitioned phenotypic variability among the allopatric species (da Cuhna 1949; Heed 1963; Heed and Blake 1963).

Instructor will need the following information:

1. Mitochondrial (COII gene) DNA and 16S ribosomal RNA sequences, included or accessible from GenBank.
2. Abdominal illustration, numbered, not labeled with names, included.
3. Map of Caribbean Islands.
4. Actual mtDNA phylogeny, included. **teacher needs to remove names from images and sequences, and establish a key correlating the images and sequences
5. Student lab hand-outs, included.

6. Supporting articles, see bibliography.

Part A-1: Expected answers may state that the northern abdomens are lighter in color, and get darker as they go south.

Part A-2: Expected student results based on abdominal phenotype alone:

PR—D. dunni dunni
ST—D. dunni thomasensis
SK—D. arawakana kittensis
GU—D. arawakana arawakana
SL—D. antillea
SV—D. similis
MA—D. caribiana
BA—D. nigrodunni

The actual distribution, however, is as follows:

PR—D. dunni dunni
ST—D. dunni thomasensis
SK—D. arawakana kittensis
GU—D. arawakana arawakana
MA—D. caribiana
SL—D. antillea
SV—D. similis
BA—D. nigrodunni

Part B-1: Students will need to analyze the gene sequences for each fly. First the ribosomal RNA, then the mitochondrial DNA sequences. Careful examination reveals that the rRNA sequences identify two basic groups of flies. Further examination of the mtDNA sequences allows further separation into a distinct phylogeny. It is reasonable to expect minimal variation with the rRNA sequences because of the inherent nature of ribosomal function. However, mitochondrial DNA allows for greater genetic variety, establishing then a more specific picture of relatedness among the various species.

Part B-2: The phenotypic distrubution does not agree with the genotypic distribution. It is likely that the Caribbean flies descended from two mainland lines, one north and one in the south. Observations do not support the statement, which is from Indiana Biology Standard B.1.30.

Wrap-Up: Students should note that the pigmentation distributions they created are more similar to the actual distribution than the sequences are. Darwin's principal of natural selection is/was at work on these flies. Both phenotype and genotype need to be considered in order to more accurately identify kinship. For this example, examining genotype alone would not have explained accurately the distribution across the islands. Articles supporting the same patterns in other animals can be found in the bibliography. Data supports divergent evolution.

Literature Cited:

da Cunha, A.B. 1949. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. Evolution 3:239-251.

Eizirik, E., N. Yuhki, W.E. Johnson, M. Menotti-Raymond, S.S. Hannah, and S.J. O'Brien. 2003. Molecular genetics and evolution of melanism in the cat family. Current Biology 13: 448-453.

Heed, W.B. 1963. Density and distribution of *Drosophila polymorpha* and its color alleles in South America. Evolution 17:502-518.

Heed, W.B., and P.R. Blake. 1963. A new color allele at the *e* locus of *Drosophila polymorpha* from northern South America. Genetics 148:217-234.

Heed, W.B., and N.B. Krishnamurthy. 1959. Genetic studies on the *cardini* group of *Drosophila* in the West Indies. Univ. of Texas Publ. 5914:115-179.

Heed, W.B., and J.S. Russell. 1971. Phylogeny and population structure in island and continental species of the *cardini* group of *Drosophila* studied by inversion analysis. Univ. Texas Publ. 7103:91-130.

Hoekstra, H.E., and M.W. Nachman. 2003. Different genes underlie adaptive melanism in different populations of rock pocket mice. Molecular Ecology 12: 1185-1194.

Hollocher, H. 1998. Island hopping in *Drosophila*: genetic patterns and speciation mechanisms. Pp. 124-141 *in* P. Grant, ed. Evolution on islands. Oxford Univ. Press, Oxford, UK.

Hollocher, H. 2000. Theories of speciation. Pp. 383-396 *in* S. Levin, ed. Encyclopedia of biodiversity. Academic Press, New York.

Hollocher, H., J.L. Hatcher, and E.G. Dyerson. 2000. Evolution of abdominal pigmentation differences across species in the *Drosophila Dunni* subgroup. Evolution 54: 2046-2056.

Mundy, N.I., and J. Kelly. 2003. Evolution of a pigmentation gene, the melanocortin-1 receptor, in primates. American Journal of Physical Anthropology 121: 67-80.

Nachman, M.W., H.E. Hoekstra, and S.L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. PNAS 100: 5268-5273.

Theron, E., K. Hawkins, E. Bermingham, R. Ricklefs, and N. Mundy. 2001. The molecular basis of an avian plumage polymorphism in the wild: A *melanocortin-1 receptor* point mutation is perfectly associated with the melanic plumage morph of the bananaquit *Coereba flaveola*. Current Biology 11: 550-557.

Wilder, J.A., and H. Hollocher. 2003. Recent radiation of endemic Caribbean *Drosophila* of the *Dunni* subgroup inferred from multilocus DNA sequence variation. Evolution 57: 2566-2579.

In A World of Flies….

Adapted from: “Where There Was One, There Will Be Two: Speciation in Drosophila”
By H. Hollocher, UND.

Part A-1: Fly Backsides

Imagine you have just been to the major of the islands in the Caribbean where you spent your time wandering the woodlands collecting *Drosophila* flies. You have just gotten back and wish to observe your collection more closely. You begin by anesthetizing your flies and separating them into rough categories based on overall appearance, generally dark ones from light ones. As you are sorting, you notice that the dark ones AND the light ones could be subdivided even further into darker and lighter groups and by the patterns you are observing on the back of their abdomens. Eventually you determine that you collected eight different types of flies but you did not note from what island they came. You made larger representations of them, and now they are in the envelope at the teacher’s desk, please collect one envelope per pair.

Being the astute observer you are, you search the literature for possible explanations of these abdominal differences. You come across a notation in a journal that reads:

> *The Drosophila subgroup displays a nearly perfect latitudinal cline in abdominal pigmentation that likely resulted from selective forces acting in the habitat…during speciation. And that natural selection has caused an overall intensity decline in pigmentation in the northernmost species.(Hollocher, Hatcher, Dyerson 2000)*

What does this information mean? Your own words, please.

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____

____

Part A-2: Flies On the Map

Armed with this new information, you set out to determine where your flies were likely captured. Using the map obtained from your teacher, and the information stated above, identify where the flies were likely collected, and record your information (fly number). Do not move your fly/abdomen map, we will be referring back to it before the end of the lab.

Barbados:	_____	Puerto Rico:	_____	St. Thomas:	_____
Guadaloupe:	_____	St. Kitts:	_____	St. Vincent:	_____
Martinique:	_____	St. Lucia:	_____		

Your rationale for placing your flies where you did (do not restate the professional literature, put it in your own words):

____
____
____

Search through copies of articles in the folders on the main desk for any information that may help you explain what you saw (phenotype). In other words, what would be a reasonable explanation (hypothesis) for such a distribution?

____
____
____

Part B-1: Fly Sequences

In an effort to further understand the cause behind the observation, you decide to pursue a genetic analysis of the results to confirm that the flies come from the islands you have indicated. You collected and sequenced mitochondrial DNA from each of your types of flies. The sequence results can be found in a folder at your teacher's desk. Remember, the more similar the sequences, the closer the evolutionary relationship between the flies. You again pursue the literature and find the following information helpful:

> *…our study compares…interisland genetic variation…we test the hypothesis that divergence in these species has occurred through a pattern of "island hopping" in which new islands were successfully colonized by older island populations, with a loss of variation at each hop…(Wilder, Hollocher 2003)*

Based on this new data (genetic variation), and identify which flies go with which island:

Barbados:	_____	Puerto Rico:	_____	St. Thomas:	_____
Guadaloupe:	_____	St. Kitts:	_____	St. Vincent:	_____
Martinique:	_____	St. Lucia:	_____		

Your rationale for placing your flies where you did (do not restate the professional literature, put it in your own words):

____

____

____

Part B-2: So What?

Does the phenotype organization agree with the genotype organization? ____________

Make a hypothesis about why this is so:

In this case, do your observations support the statement that "molecular evidence substantiates the anatomical evidence for evolution"? Explain.

Wrap-Up: The Real McFly

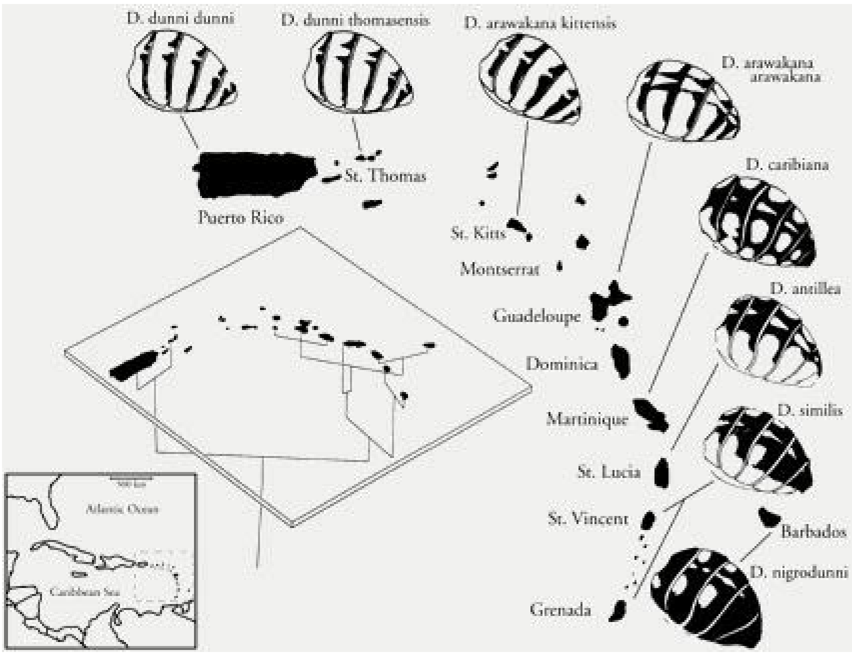
Obtain the top-secret dossier from your teacher containing the known distribution of flies among the Caribbean islands. Compare your results with the known, indicate similarities and differences—along with a possible explanation of why.

So, which of Darwin's principals is responsible for the known distribution?

Why do we need to consider both the phenotype and genotype of the flies?

Further research indicates that similar pigment "effects" occur also in deermice, wild cats and humans. Although the mechanism is the same from the fly to this group of animals, the protein responsible is different (but the same within this group of animals). Why do you think this is so?

Make an argument supporting either convergent or divergent evolution, based on what you have observed: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D. dunni thomasensis
D. arawakana kittensis
D. arawakana arawakana
D. caribiana
D. antillea
D. similis
D. nigrodunni
Puerto Rico
St. Thomas
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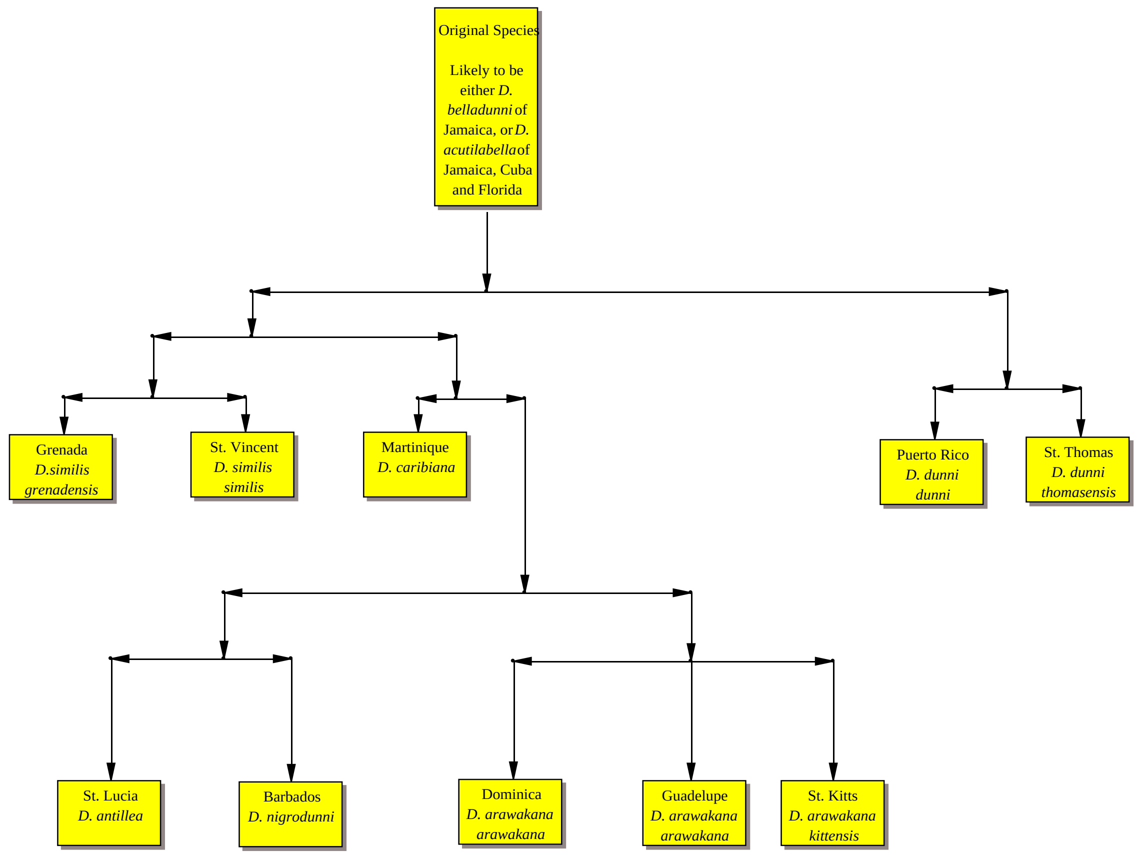

GenBank Sequences

Dunni Subgroup
Distribution: Caribbean Islands

16S ribosomal RNA (rRNA) mitochondrial gene

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Cytochrome oxidase subunitII (COII) mitochondrial gene

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