Conservation Genetics
Population Genetics: Diversity within versus among populations

Exercise

James Gibbs

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OVERVIEW

Genetic variation at the population level is an important, although under appreciated component of biological diversity. In some species as much as half or more of the genetic variation unique to it is found only in certain of the local populations that comprise it. Preserving this genetic variation that is restricted to particular populations and not others is an important part of conservation biology. To do so we must understand something about the “structure” of genetic variation among populations. This requires uses of analysis techniques from the field of population genetics. Thus the purpose of this exercise is to learn to apply population genetics concepts to conservation planning.

PROCEDURE

For this exercise, imagine that you are working for a conservation agency and are faced with a crisis decision: a piece of land with six different wetlands hosting the only known populations of two rare orchids is about to be developed. The crisis is that you only have enough funds to purchase and protect four of these wetlands. Which of the six populations should you protect? The wetlands are of two types. Three are marshes that host the world's only known populations of *Orchis isozymus*. The other three are swamps that host the only populations of the closely related but distinct *Orchis polyzymus*. Because the populations in each wetland are large (> 1,000 plants in each) demographic issues are secondary to preserving the genetic diversity that characterizes these species. **How should you allocate your scarce funds for wetland acquisition?** Unfortunately, there is no time to raise more funds. In desperation, you send some leaf samples from these plants to a colleague in a local botany department, and a week later you receive the data below, which are in the form of protein electrophoresis gels for one allozyme locus (ORCHIS1) that is polymorphic in both these species. The locus has two alleles, Fast and Slow, that appear on the electrophoresis gel with the faster allele below the slower allele.

To complete this exercise you first need to know how to interpret enzyme electrophoresis gels and the allozyme patterns that appear on them. Allozymes are

enzyme variants with different electrical charges. These variants arise from small changes in the DNA sequence of protein coding genes, which in turn alter what amino acids are incorporated into proteins. When you make a slurry of tissue from an individual plant or animal, insert them into a starch gel, and subject the gel to an electrical current, the enzyme molecules with different electrical charges will migrate through the gel at different rates. This is the procedure known as electrophoresis. If after a period of electrophoresis you bathe the gel in a co-factor for a particular enzyme, as well as a dye that precipitates when the co-factor and enzyme react, then you can observe the relative mobility of each enzyme based on its position in the gel. The gels below exhibit typical banding patterns. The degree to which individuals share the same banding patterns of enzyme electrophoresis gels reflects the similarity of their genetic make-up. Further guidance on genetic techniques can be found in Hunter (1996:82).

To measure how genetic variation is partitioned within and among populations you first need to determine allele frequencies in each population. The particular allozyme locus assayed possesses two alternate forms. The identity of the two alleles in each individual is reflected directly by the banding patterns within each lane on a gel. For example, the first individual in the first lane of the first gel below is a heterozygote, that is, the two alleles it possesses are different and are indicated by both a fast- and slow-moving band on the gel. In contrast, the individual in the second lane is a homozygote, as indicated by the possession of a single band representing two slow alleles. To determine the allele frequencies in each population of the fast-moving allele (p) and the slow-moving allele (q) simply tally the number of alleles across individuals and divide it by the total number of alleles present in the population (always equal to two times the number of individuals). What is the observed heterozygosity of each population for the locus ORCHIS1?

Next you need a measure of genetic differentiation among populations. A useful and commonly used measure is Wright's fixation index, or F_{st}, which ranges from 0, indicating no differentiation among populations, upwards, indicating increasing subdivision. Once you have calculated the allele frequencies in each population, it is relatively straightforward to determine F_{st}. First, you need to calculate the expected heterozygosity for each species (H_s). You do this simply by multiplying $2pq$ for each population and then averaging these values over all three populations within each species. You may recall that this is the expectation of the frequency of heterozygotes in the population at Hardy-Weinberg equilibrium. Second, you need to calculate expected heterozygosity if all three populations were part of the same, extended breeding population (H_t). You do this by averaging p and q over all populations within a species, and then multiplying 2 x the average p x the average q. This would be the expected frequency of heterozygotes in the population if it acted as one large breeding

pool with no subdivision. Deviations of the frequency of heterozygotes in separate populations (H_s) from what you would expect to find if they were all part of the same larger population (H_t) provide an index of subdivision and the amount of genetic variation that is found only in local populations. Thus, $F_{st} = (H_t - H_s)/H_t$, where values of $F_{st} < 0.01$ indicate little divergence among populations, and values > 0.1 indicate great divergence among populations.

<u>To complete this exercise, for each species you need to calculate the allele frequencies in each population, the observed and expected heterozygosity for each population, and the fixation index for each species, and then compare the indices between species.</u>

***Orchis isozymus*, Population 1 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow	–	–	–	–	–	–		–	–	–		–	–	–	–
Fast	–						–				–		–	–	

***Orchis isozymus*, Population 2 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow	–		–	–	–		–		–	–		–	–	–	
Fast		–	–		–	–	–	–	–	–	–	–	–	–	–

***Orchis isozymus*, Population 3 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow		–					–							–	
Fast	–	–	–	–	–	–	–	–	–	–	–	–	–		–

***Orchis polyzymus*, Population 1 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow	–		–	–	–		–		–	–		–	–	–	
Fast		–	–		–	–	–	–	–	–	–	–	–	–	–

***Orchis polyzymus*, Population 2 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow	–		–	–	–		–		–	–		–	–	–	
Fast	–	–			–	–	–	–		–	–	–	–		–

***Orchis polyzymus*, Population 3 (individual 1..15 from left to right)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Slow	–		–	–	–			–	–	–		–		–	
Fast		–	–		–	–	–	–	–	–	–	–	–	–	–

DISCUSSION:

1. What was the observed heterozygosity at the locus ORCHIS1 for (1) each population of each species and (2) for the species as a whole? Did it differ from the expected H?
2. How will you allocate your scarce funds for wetland acquisition? Justify your decision in terms of preserving the maximum amount of genetic diversity that characterizes these two species.
3. What might be some causes of the strikingly different genetic structures you have observed in the two species? (note: you should base you answer on what you found out about the within and among population genetic diversity of this species)
4. Recall that our goal in this exercise is to capture as much of the genetic diversity that characterizes these species as is possible given a limited budget. Note that both alleles at the locus surveyed already are found in each population of both species. Why does it matter if more than a single population of each species is protected, or, for that matter, more than a single population of one of the species?

FURTHER READINGS

See Hedrick and Miller (1992) for a useful overview of conservation genetics, and Avise and Hamrick (1996) for an interesting set of case studies.

LITERATURE CITED: (to be collated at the end of the book)

Avise, J. C., and J. L. Hamrick (Eds.). 1996. Conservation genetics: case histories from nature. Chapman and Hall, New York, New York, USA.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications 2:30-46.

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