Molecular Analysis of Spotted Salamander Populations: Amplified Fragment Length Polymorphism and Genetic Diversity's Correlation to Breeding Success

http://www.cortland.edu/herp/keys/images/salamand/amacullg.jpg

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http://www.pbase.com/tmurray74/image/96446643

Abstract

Spotted Salamanders (*Ambystoma maculatum*) breed in vernal pools during the spring months. Females lay fertilized egg masses on structures within their breeding pool. We have counted these egg masses in two vernal pools as an index of a population's breeding success rate. We have developed protocol for molecular analysis of these populations, and found data that is highly suggestive of a correlation between breeding success and intra-population genetic diversity. DNA extraction was successfully performed on developed embryos and adult toe clippings. This DNA was used to measure genetic variability of two breeding populations with an Amplified Fragment Length Polymorphism (AFLP) analysis protocol which we optimized for the species.

Introduction

Spotted Salamanders and their breeding patterns have been an object of study at Gordon College for over five years. These studies have attempted to correlate egg mass abundance to many ecological factors. However, the driving factors behind salamander breeding success remain unknown, and here we propose a molecular approach to the problem.

AFLP was introduced in 1995 (Vos et al.) and has become a standard genetic analysis technique. It is primarily used to measure genetic diversity, fine-scale population structure, and to test for species hybridization (Bonin et al. 2007).

Methods

Field Work

Egg masses were counted in two vernal pools near Gordon College campus. Each was counted twice, and the higher of the two values was accepted.

At each pool, eggs from four masses were taken and placed in 70% ethanol for storage until DNA could be extracted.

Four toe samples were taken from adult salamanders on 3/22/2010 during mating migration.

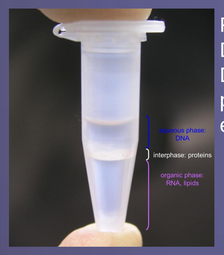


Figure 1: Phenol/Chloroform DNA purification step. Aqueous DNA is decanted and precipitated with absolute ethanol.

http://openwetware.org/wiki/Image:DNA_extraction_w_phenol_chloroform.jpg

DNA Extraction

Prior to egg sample extraction we cut away and discarded outer egg layers. The now-exposed developing embryo was digested with Proteinase K. Toes were placed directly into solution containing the enzyme.

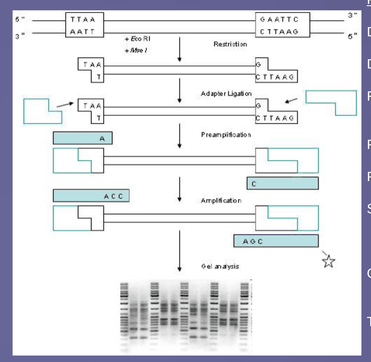
We performed a Phenol/Chloroform purification followed by an ethanol precipitation. This resulted in a white pellet of pure DNA.

Early in our experimentation we used samples resuspended in both 20 µL and 50 µL of TE buffer and found that 50 µL resulted in the clearest electrophoresis bands.

We would like to thank Craig Story for his help with lab protocol details, and the BIO310 class for help in counting egg masses during the spring of 2010.

Amplified Fragment Length Polymorphism

AFLP is a four-part protocol that ends in gel electrophoresis. Figure 2 displays this process visually.



Procedure	Lab Time
DNA Extraction	~2 hours
DNA Restriction	~2 hours
Fragment Ligation	~2 hours
Pre-amplification	~ 2 hours
Primer Pair Addition	~ 30 min
Selective Amplification	~ 3 hours
Gel Electrophoresis	~4 hours
TOTAL:	~16 hours

Figure 2: AFLP protocol from DNA sample to gel analysis

http://www.philsciletters.org/May%202,%202009/Genetic%20fingerprinting.htm

In the final amplification step two primers are attached (see * in Figure 2). These primers vary in effectiveness between species. We experimented with eight different primer pair combinations and determined Mse-CTA and Eco-AGG were the best fit for our sample DNA.

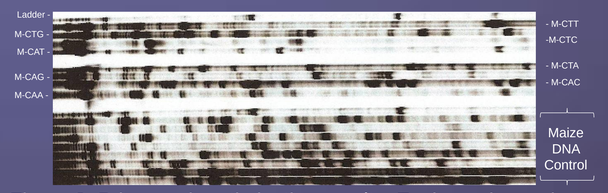


Figure 3: Primer pair optimization results. Each row is a unique Mse-xxx primer paired with Eco-AGG. To determine the best pair we counted the number of bands each pair produced. M-CTA had 21 distinct bands.

After optimizing the primer pair, we ran our full experiment with 22 samples, 11 from Wilson Pond and 11 from Pine Street. Gel electrophoresis was done on a 25 cm 6.5% polyacrylamide gel in a Li-COR 4300 DNA Analyzer.

Results

Our gel electrophoresis results (Figure 4) were converted into binary data groups (0 for band absence and 1 for band presence) and analyzed with software program PopGen. We found that the Wilson pond population had higher genetic diversity in every measure.
Wilson pond also had 3x the number of egg masses. Data is laid out for comparison below:

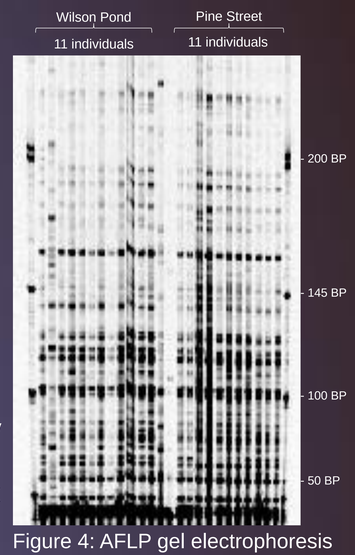


Figure 4: AFLP gel electrophoresis results. Molecular weight ladders flank the sample rows.

	Genetic Diversity			Egg Masses
	H_o	I_s	% P	
Wilson Pond	0.2141	0.3381	**76.18**	**73**
Pine Street	0.1341	0.2048	**40.48**	**23**

Conclusions

-Our preliminary results are highly suggestive of a correlation between breeding success and genetic diversity. Understanding this relationship could help future efforts to curb global decline of amphibians.

-We have extracted genomic DNA from eggs. This is unprecedented in literature and if thoroughly tested could prove to greatly simplify the field work required for genetic analysis of salamander populations.

-We have found an optimum primer pair for use in analysis of salamander populations on the North Shore.

References:

Bonin, A. D. Ehrich and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Molecular Ecology 16:3737-3758.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nuleic Acids Research 23:4407-4414