Inferring Genetic Variability between Two Crayfish Species And between Two Fish Species Using RAPD-PCR

Christopher L. Romano
Department of Biological Sciences, Fordham University, Bronx, NY.

Abstract:

Random amplified polymorphic DNA PCR (RAPD-PCR) is an effective technique for studies of genetic variability, conservation and population genetics, taxonomy and conservation biology. RAPD-PCR was employed here to assess genetic variability between two species of crayfish (*Cambarus maculatus* and *Astacus astacus*) and two species of fish (*Carassius auratus* and *Poecilia latipinna*). A high level of genetic variability was observed through the use of five primers. To achieve greater resolution for future studies, the use of more primers is necessary and would likely decrease the degree of genetic variability observed. Through the incorporation of additional species into the analysis, degrees of relatedness can also be determined.

Introduction:

Random amplified polymorphic DNA PCR (RAPD-PCR) has been shown to be an effective technique for use in wide ranging issues such as population and conservation genetics (Haig et al. 1994), taxonomy (Chapco et al. 1994), assessment of genetic variability (Sofia et al. 2005, Zhang et al. 2005) and insights into geographic origins and invasion routes of colonizing species (Williams et al. 1994). The principle behind RAPD-PCR is the detection of polymorphisms, either intraspecific or interspecific,

through the PCR amplification of DNA fragments with primers of arbitrary nucleotide sequence (Williams et al. 1990). Primers used for RAPD-PCR are usually 10 nucleotides in length and between 50-70% G + C content. Each RAPD-PCR reaction volume contains a single primer which functions as both the forward and reverse primer for amplification. DNA amplification products are generated from any region where primers bind in the proper orientation and within 5 kbp of each other (Perez and Dominguez 1998). Following the PCR, amplification products are visualized on sizing gels for analysis. Most bands resulting from RAPD-PCR originate from repetitive DNA sequences (Nkongolo et al. 2002). Polymorphisms are detected as either the presence or absence of bands, caused by nucleotide sequence differences between individuals (Perez and Dominguez 1998). Differences in nucleotide sequence may be attributable to the deletion of sites, insertions which increase the size of the PCR products or insertions which cause primer binding sites to be too distant for amplification (Williams et al. 1990).

RAPD-PCR has significant advantages over other techniques used for the analysis of populations, such as microsatellites and restriction fragment length polymorphisms (RFLPs). For example, RAPD-PCR is relatively fast, simple and inexpensive to conduct. In addition, it requires only small quantities of template DNA and potentially provides a large number of polymorphic loci; both of which are especially valuable when working with rare or endangered species where populations sizes and genetic variability are low (Palacios and Gonzalez-Candelas 1997). An additional advantage is that no prior knowledge of the target DNA sequence is needed to employ this technique (Martinez et al. 2006).

In spite of the many advantages of RAPD-PCR, limitations do exist. Arguably, the biggest limitation of RAPD-PCR is the dominant nature of bands generated by this technique. As a result, it is impossible to distinguish a DNA segment amplified from a homozygous locus from that of a heterozygous locus with a dominant RAPD marker (Williams et al. 1990, Zhang et al. 2005). Despite this shortcoming, statistical power can be gained through the use of appropriate statistical analysis. Common methods of analysis include the Unweigthed Pair Group Method with Arithmetic Averages (UPGMA) (Sneath and Sokal 1973) and the Shannon-Weaver index (Chakraborty and Rao 1991) modified for RAPD analysis.

The other main drawback of RAPD-PCR is its sensitivity to reaction conditions allegedly making reproducibility difficult. For example, changes in conditions such as template DNA concentration, magnesium concentration, the type of polymerase used or changes in thermal cycler conditions may lead to an alteration of the banding profiles achieved (Ellsworth et al. 1993, Palacios and Gonzalez-Candelas 1997, Perez et al. 1998, Williams et al. 1990,). Stringent laboratory protocol should circumvent this limitation.

The aim of this study was to employ RAPD-PCR in order to estimate genetic variability between two species of crayfish (Freckled Crayfish: *Cambarus maculatus* and Noble Crayfish: *Astacus astacus*) and two species of fish (Goldfish: *Carassius auratus* and Molly: *Poecilia latipinna*). High degrees of polymorphism are expected due to the distant relatedness of these species. An additional goal of this study was to determine the reproducibility of RAPD-PCR results, in light of claims that this is a weakness.

Materials and Methods:

Genomic DNA

Genomic DNA was extracted from both Goldfish and Molly using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's protocol with the exception that final elution was carried out using 50ul of water in place of 200ul of Buffer AE. This was done to increase the final DNA concentration. 25mg of tissue were used for DNA extraction and removed from the posterior region of the fish. Lysis with proteinase K at 55°C was allowed to proceed overnight. Following DNA extraction, DNA concentrations were determined and the DNAs were stored at -20°C.

Table 1

Primers used in RAPD analysis

Primer	Sequence 5'-3'
RAPD 1	GAATGCGAGG
RAPD 2	TCTAAGCTCG
RAPD 3	AGGAGTGAGA
RAPD 4	GAGCCAGAAG
RAPD 5	AACGCGTAGA

Crayfish genomic DNA was kindly provided by Dr. James Fetzner at the Carnegie Museum of Natural History in Pittsburgh. Upon receipt, DNA concentrations were determined and the DNAs were stored at -20°C.

Preliminary Screening of Primers

Ten decamer oligonucleotide primers were arbitrarily designed with 50 and 60% G + C content and supplied by Invitrogen. These primers were tested in PCR amplification on *C. auratus* and *A. astacus* DNA for their capability of producing clear bands (data not shown). Five primers were subsequently chosen from these ten for use with all four species (see Table 1 for sequences).

RAPD-PCR

The PCR was performed using the Amplicycle Sequencing Kit (Applied Biosystems). For each reaction, 1μl of DNA (40ng/μl) was mixed with 1μl of 10X cycling buffer, 0.25μl of 10mM dNTPs, 0.4μl of primer, 0.25μl of [α-33P]ATP and dH2O to a final volume of 10μl. 12ul of mineral oil were added to each reaction mix to prevent evaporation during the PCR. The PCR conditions were as follows: (step 1) 94°C for 3 min (step 2) 94°C for 30 sec, 36°C for 30 sec, 72°C for 1 min and 30 sec (50 cycles), (step 3) 72°C for 10 min and hold at 4°C. Following the PCR, 3ul of stop solution were added and samples were denatured by heating at 94°C for 3 min. The RAPD-PCR products were separated on a denaturing polyacrylamide gel and visualized by autoradiography (Figure 1).

Results

The genomic DNAs from crayfish, *C. maculaues* and *A. astacus*, and fish, *C. auratus* and *P. latipinna* were used to generate RAPD patterns using five oligonucleotide primers. As expected, a high degree of polymorphism was observed at the interspecific level. The five primers generated 90 bands when used with both crayfish DNAs, 100% of which were polymorphic. The five primers generated 131 bands when used with both fish DNAs, 94.7% of which were polymorphic. Out of the 221 bands observed, 7 bands appear to be common to both species of fish, while no pattern similarities were observed between the two crayfish species. The banding profiles of all four study species, can be seen in Figure 2.

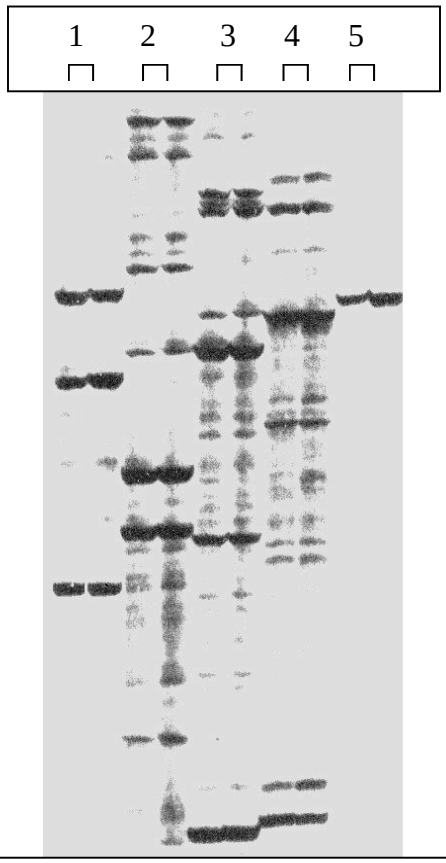

Figure 1. Reproducibility of RAPD-PCR markers. From left to right, duplicate RAPD patterns obtained with primers RAPD 1, RAPD 2, RAPD 3, RAPD 4 and RAPD 5 with *P. latipinna* genomic DNA.

Reproducibility of RAPD-PCR results was easily achieved in this study. As seen in Figure 1, patterns are nearly identical in duplicate PCR reactions, which supports the reliability of this technique when variables known to create divergent results (DNA concentration, annealing temperature) are held constant. For an in depth evaluation of the reproducibility of RAPD-PCR results see Albornoz and Dominguez (1998).

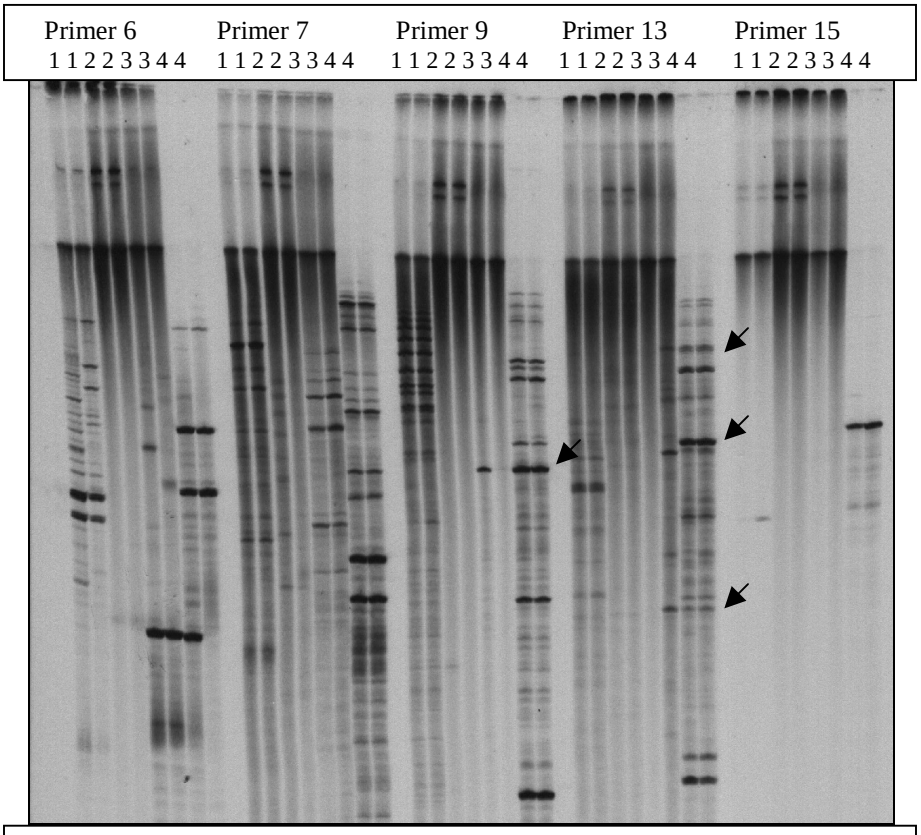

Figure 2. RAPD banding profiles of primers RAPD 1, RAPD 2, RAPD 3, RAPD 4 and RAPD 5. From left to right, duplicates of noble crayfish *Astacus astacus* **(C1)**, freckled crayfish *Cambarus maculates* **(C2)**, goldfish *Carassius auratus* **(F1)** and molly *Poecilia latipinna* **(F2)**. Arrows indicate bands shared by both *C. auratus* and *P. latipinna*.

Discussion

RAPD-PCR was used here to make preliminary inferences concerning genetic variability between two species of crayfish (*Cambarus maculatus* and *Astacus astacus*) and two species of fish (*Carassius auratus* and *Poecilia latipinna*). This technique is based on the detection of polymorphisms, bands observed in some individuals but absent in others, which are amplified by random primers during the PCR. In this study, a high degree of polymorphism was observed, suggesting a high degree of genetic variability between the pairs of species compared. Despite these findings, it is probable that the

high level of genetic variability observed is the result of using too few primers in this analysis. For future studies, the use of a greater number of primers would likely decrease the level of polymorphism observed and thus reveal a greater degree of similarity between the two crayfish species and two fish species evaluated. In addition, by the incorporation of additional species into the analysis, degrees of relatedness can also be determined.

Acknowledgments

I would like to thank Jinsong Qiu and Lisa Sarran for their continuous help and enduring patience. I would also like to thank Dr. Berish Rubin for his helpful assistance and for the use of his laboratory. In addition, I thank Dr. James Fetzner at the Carnegie Museum of Natural History in Pittsburgh for his generous contribution of crayfish genomic DNA. Last but not least, I thank my classmates for providing good cheer and encouragement.

References

A'Hara, S., R. Harling, R. McKinlay and C. Topping. 1998. RAPD Profiling of Spider (Araneae) DNA. The Journal of Arachnology 26: 397-400.

Chapco, W., N.W. Ashton, R.K. Martel, N. Antonishishyn and W.L. Crosby. 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome 35: 569-574.

Ellsworth, D.L., D. Rittenhouse and R.L. Honeycutt. 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. Biotechniques 14: 214-217.

Haig, S.M., J.M. Rhimer, and D.G. Heckel. 1994. Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. Molecular Ecology 3: 581-595.

Martinez, R., A. Carolina, and F. Sonsoles. 2006. Genetic variability among *Alexandrium tamarense* and *Alexandrium minutum* strains studied by RAPD banding pattern analysis. Harmful Algae 225: 1-9.

Nkongolo, K.K., P. Michael, and W.S. Gratton. 2002. Identification and characterization of RAPD markers inferring genetic relationships among Pine species. Genome 45: 51-58.

Palacios, C., F. Gonzalez-Candelas. 1997. Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Molecular Ecology 6: 1107-1121.

Perez, T., J. Albornoz and A. Dominguez. 1998. An evaluation of RAPD fragment reproducibility and nature. Molecular Ecology 7: 1347-1357.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman, San Francisco.

Sofia, S.H., F. M., de Paula, A.M. dos Santos and F.S. Almeida. 2005. Genetic structure analysis of *Eufriesea violacea* populations from southern Brazilian Atlantic rainforest remnants. Genetics and Molecular Biology 28: 479-484.

Williams, J.G.K., A.R. Kubelia, K.J. Livak, J.A. RAfalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Williams C.L., S.L. Goldson, D.B. Baird and D.W. Bullock. 1994. Geographical origin of an introduced insect pest, *Listronotis bonariensis* (Kuschel), determined by RAPD analysis. Heredity 72: 412-419.

Zhang, Y., J. Yang and G.Y. Rao. 2005. Genetic diversity of an amphicarpic species, *Amphicarpaea edgeworthii* Benth. (Leguminosae) based on RAPD markers. Biochemical Systematics and Ecology 33: 1246-1257.