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RESEARCH NOTE

RAPD PROFILING OF SPIDER (ARANEAE) DNA

We present protocols and conditions for specimen storage, DNA extraction and storage, and the subsequent RAPD (Random Amplified Polymorphic DNA) profiling of spiders. Three common UK species, *Lepthyphantes tenuis* (Blackwall 1852), *Enoplognatha ovata* (Clerk 1757) and *Clubiona reclusa* (Cambridge 1863), members of the Linyphiidae, Theridiidae and Clubionidae respectively, were chosen to serve as examples with this highly adaptable technique.

Despite numerous reservations regarding the repeatability, homology, and statistical analysis of the data (see Grossberg et al. 1996 for a comprehensive review), RAPD profiling (Williams 1990) is still the method of choice for many researchers looking to address a wide range of ecological issues in an equally diverse array of organisms. RAPD data have enabled insights into population structure (e.g., Haymer & McInnis 1994; Kambhampati et al. 1992), geographical origins and invasion routes of colonizing species (e.g., Williams et al. 1994), the distinction of new genotypes of parasites (e.g., Majiwa et al. 1993) and conservation genetics (e.g., Rosetto et al. 1995). The RAPD technique can also be a useful initial step in detecting other classes of DNA marker such as microsatellites (Ender et al. 1996).

RAPD profiling is adopted despite the reservations because it possesses many advantages over other molecular marker systems, *viz.*, it is relatively fast and technically undemanding, screens the entire genome for polymorphisms, and can produce a potentially limitless number of markers (simply by screening with more primers). Moreover, due to the amplification process during the PCR thermal cycling, only minute quantities of DNA are required as template, making the analysis of invertebrates unproblematic, e.g., microhymenoptera (Landry et al. 1993).

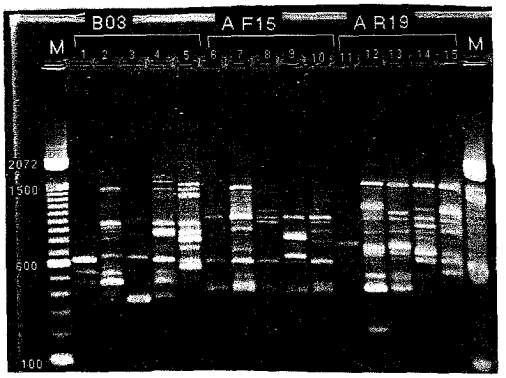
Sample storage prior to DNA extraction was found to be the most crucial stage for this otherwise robust technique, which worked successfully with all the species tested (Fig. 1). Spiders were collected via a D-Vac suction sampler, or by hand, and returned to the lab alive. They were then either stored in ethylene glycol or 70% ethanol at room temperature, or frozen in liquid nitrogen and stored at −80 °C. DNA was extracted after three weeks and examined on a 1% agarose minigel. RAPD reactions were then carried out with DNA stored at 4 °C and −20 °C over a period of one month, to assess the optimal storage for extracted DNA.

Ethylene glycol and 70% ethanol were both found to be poor preservative media for the spider DNA, which had degraded substantially after three weeks storage at room temperature. Storage at −80 °C was found to be the most effective method tested for preserving specimens (at least for one year) prior to DNA extraction if extractions could not be made immediately (Fig. 2). However, it was necessary to identify the spiders prior to storage at −80 °C, as the delicate tissues of the epigyna and palps darkened following freezing, making identification more difficult. Saturated salt solutions have also been used by a number of authors as a means of preserving DNA during field collection of samples, e.g., (Seutin et al. 1991) but these were not investigated in this study.

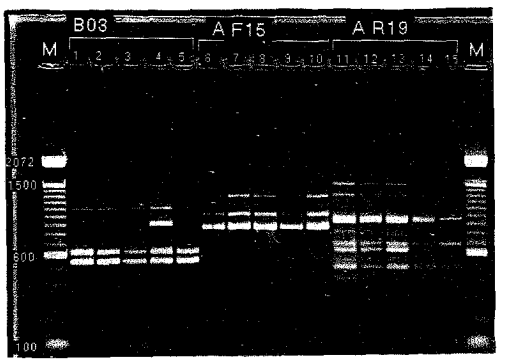
Storage of extracted DNA at −20 °C is recommended if the sample is not to be used directly, as DNA held at 4 °C gave more variable results over time (results not shown). Fresh dilutions of DNA should be prepared from −20 °C stock prior to each RAPD reaction to ensure repeatability of profiles (Fig. 3).

The DNA extraction was carried out as follows. A 1.5 ml Eppendorf tube containing an adult spider was lowered into liquid nitrogen for 10 sec and the spider tipped out onto a Petri dish lid. The abdomen was removed with a sterile scalpel blade, preventing the possible

Lepthyphantes tenuis



Enoplognatha ovata



Clubiona reclusa

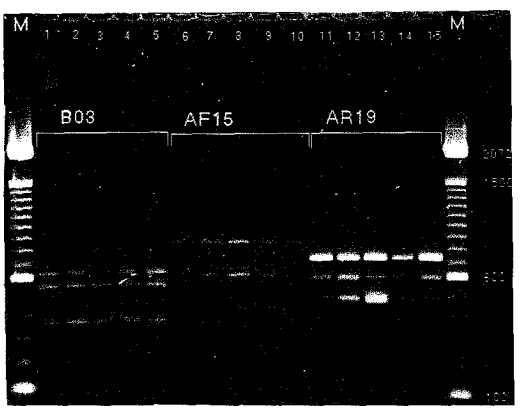


Figure 1.—RAPD profiles produced with three primers (chosen at random from those available in the laboratory) from five individuals from each species. Primer sequences are: OPB–O3 (5′–CATCCCCCAG–3′); OPAF–15 (5′–CACGAACCTC–3′) and OPAR–19 (5′–CTGATCGCGG–3′). M = marker (size in base pairs).

amplification of DNA from prey ingested by the spider or of any parasitic burden. The carapace was then returned to the tube and refrozen. The carapace was homogenized with a sterile plastic Eppendorf pestle (a separate pestle was used for each sample to prevent cross contamination), 500μl chilled DNA extraction buffer (200mM Tris-HCl (pH 8.0), 70mM EDTA, 2M NaCl, 20mM sodium metabisulphite) and 90μl 5% sarcosyl solution added (Cheung et al. 1993), then additional grinding carried out to ensure complete destruction of tissue. The addition of Proteinase K and RNAse was not found necessary to extract DNA which amplified to produce clear repeatable profiles. The tubes were then incubated at 65 °C for 1 h with occasional mixing by inversion. Following incubation, the homogenized tissue was spun in a microfuge at 16,000 × *g* for 3 min to pellet gross debris, and the supernatant, containing the DNA, was transferred to a fresh tube. To precipitate the DNA, 90μl of 10M ammonium acetate and 500μl of chilled isopropanol were added to the supernatant, the tube slowly inverted 50 times to mix, and the sample placed at −20 °C for 2 h.

Total precipitated DNA was pelleted at 16,000 × *g* for 10 min, after which the supernatant was poured off and 400μl 70% ethanol added to wash the pellet. Following a further 4 min spin the 70% ethanol was decanted. Finally, the pellet was air dried for 30–45 min then resuspended in 50μl sterile water (Sigma, UK). Resuspension was aided by heating to 60 °C for 1 h. The quantity of the DNA recovered, as observed on a 1% agarose minigel, was comparable with DNA extracted using the more traditional, solvent extraction method, whilst avoiding the unpleasantness of handling phenol and chloroform.

DNA amplification was carried out on a Perkin Elmer TC-1 thermal cycler, using a step cycle, programmed for 35 cycles of 1 min at 95 °C for DNA denaturation, 1 min at 36 °C for primer annealing, and 2 min at 72 °C for primer extension. This was preceded by an initial denaturation step of 2 min at 95 °C. The cycling was followed by a final primer extension step at 72 °C for 8 min. Following optimization of DNA and magnesium concentrations, in a 50μl reaction volume the following components were employed: 1X Perkin Elmer

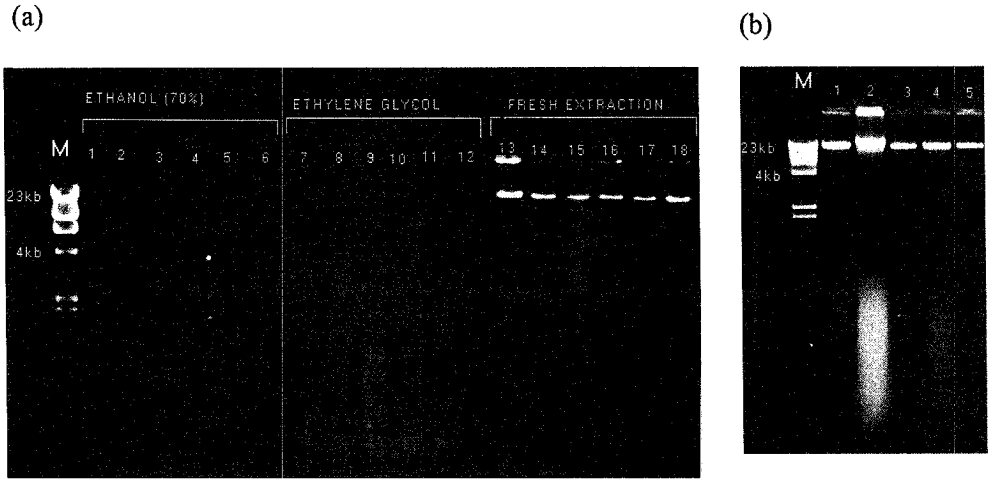


Figure 2.—Effect of specimen preservation on DNA. (a) DNA extractions from 18 *Lepthyphantes tenuis* stored for three weeks at room temperature in 70% ethanol (lanes 1–6), ethylene glycol (lanes 7–12, or recovered from fresh specimens (lanes 13–18). (b) Extraction from 5 *L.tenuis* stored at −80 °C for 12 months. M = marker (size in base pairs).

buffer, 3mM $MgCl_2$, 200μM each of dATP, dTTP, dCTP and dGTP, 0.5 units of Stoffel *Taq* and 0.2μM primer (10-base primers, Operon Technologies Inc., Alameda, California, USA). DNA template was present at a concentration of approximately 40ng per reaction, calculated by comparing by eye the intensity of ethidium bromide stained genomic extracts with dilutions of a DNA marker (λ/HindIII digest) whilst under UV illumination (Sambrook et al. 1989). This allowed dilutions of DNA to be made which were in a good approximation to each other. Finally, prior to PCR, the reaction mix was overlaid with approximately 25μl of mineral oil to prevent evaporation of the sample during cycling.

Amplified RAPD products were visualized on a 1.5% TAE agarose gel following electrophoresis at 80 volts for 2 h. The gel was stained with ethidium bromide (0.5μg/ml) for 20–30 min, rinsed briefly, then examined on a UV illuminator. The results were captured using the IS500 digital image analysis system (Flowgen, UK).

Sample storage in ethanol for future DNA extraction is something of a contentious issue, with reports ranging from vertebrate tissues stored for six years producing good yields of high molecular weight DNA (Smith et al. 1987), to Coleopteran DNA which maintained its integrity for only six weeks in 95% ethanol (Reiss et al. 1995). Laulier et al. (1995) state

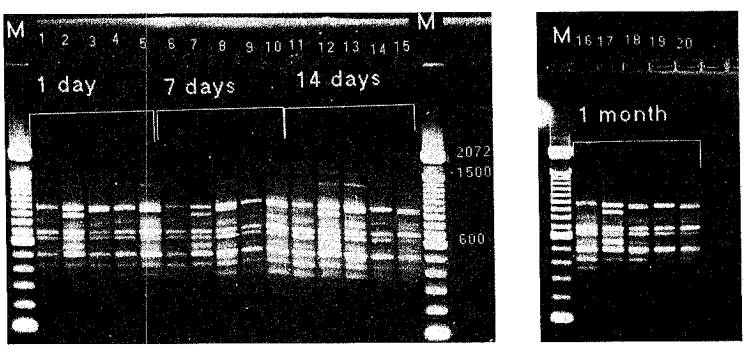


Figure 3.—Reproducibility of RAPD markers over time. Profiles from stock DNA extractions stored at −20 °C with primer, OPAR–19. Five *Enoplognatha ovata* after 1 day (lanes 1–5), 7 days (lanes 5–10), 14 days (lanes 11–15) and one month (lanes 16–20). M = marker (size in base pairs).

that DNA can be recovered from ethanol and methanol preserved samples, but the degree of degradation appears to be species specific, and the yield is generally poor. It can be speculated that any species specificity of degradation may be due to the physical properties of the cuticle of the organism. Ito (1992) reported that unknown contaminants in 100% ethanol can cause degradation of DNA, leading to the simple classification of ethanols as "good" and "bad". Our findings support the difficulty of finding a "good" ethanol and it may be prudent not to take the risk if possible.

In summary, this preliminary study has shown that following optimization, the RAPD technique produces clear and repeatable results and is readily applicable to arachnological studies. Molecular data from such studies should allow new insights into a number of ecological issues if applied appropriately.

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