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A non-invasive technique for obtaining DNA from marine intertidal snails

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DNA was extracted from mucus secreted by snails that had been allowed to crawl over glass microscope slides. The mucus contained many epithelial cells and a few blood cells. Microsatellite DNA regions were amplified using template DNA from the mucus and clear bands obtained showing the same positions as when using template DNA from the foot. Pedal mucus is therefore a reliable source of DNA, which can be extracted by a simple methodology that is readily applied in the field. The technique has considerable potential for conservation- and behavioural ecology.

Effective conservation of threatened species requires knowledge of population–genetic structure (Avise, 1994) and DNA fingerprinting is one of the most effective techniques for achieving this (Beaumont & Bruford, 1999). The importance of DNA fingerprinting is also recognized in many aspects of behavioural ecology (Krebs & Davies, 1993). DNA is generally extracted from blood or organ tissues. However, such invasive techniques may affect the behaviour or quality of life of the target animals and should be avoided wherever possible.

Many marine species are threatened with extinction by human activities (Roberts & Hawkins, 1999). *Nucella freycineti* is a common, predatory, marine snail living on rocky intertidal shores of the north-west Pacific Ocean. Because of tributyltin (TBT) pollution, some populations of *Nucella* are decreasing, although partial recovery from the impact of TBT has been reported for *N. lapillus* (e.g. Harding et al., 1997). Hitherto, molecular research on *Nucella* has required invasive extraction of tissue samples. In this method, snails were killed or damaged in the body to extract DNA. Damaged female *Nucella emarginata* showed different energy trade-off between reproduction and growth from uninjured snails (Geller, 1990). A non-invasive technique for obtaining DNA therefore could be advantageous for studying the conservation- and behavioural ecology of such species. Here, we report a non-invasive technique for obtaining DNA from the pedal mucus secreted onto the substratum by marine snails.

Twelve *N. freycineti* were collected from Usujiri, Hokkaido, Japan (41°57′N 140°58′E) and were briefly washed in filtered seawater. Each snail was placed at one end of a glass microscope slide (76×26×1.5 mm), and allowed to crawl to the opposite side of the slide. After the snail had crawled to the opposite (76 mm distance) or the snail became inactive, the snail was removed and mucus secreted on the glass was wiped by a cotton swab premoistened in a 0.9% NaCl solution containing 1mM $EDTANa_3$ (ethylenediamine tetraacetic acid). The cotton swab was then soaked and rotated in 1 ml of the same solution in a plastic tube to remove the mucus into the solution. This procedure was repeated at least five times. Nine ml of 95% ethanol were then added to the tube, so the final volume of the solution was 10 ml. The solution can be preserved at this stage if necessary. The solution was centrifuged at 2500 rpm for 10 min and the supernatant was discarded. Part of the precipitate was taken by pipette and stained with May–Grünwald–Giemsa stain and the whole was examined using a microscope. To the remaining precipitate was added 500 μl of STE buffer (0.1 M NaCl, 10 mM Tris, and 1mM EDTA, pH 8.0). This solution was transferred into a new 1.5 ml tube by pipette and centrifuged at 2500 rpm for 10 min. After discarding the supernatant, to the precipitate was added 250 μl of STE buffer, 25 μl of 10% sodium dodecylsulphate, and 25 μl of proteinase K (5 mg/ml) at 55°C for 2 hours. The hydrolysates were extracted using 200 μl each of phenol saturated with TE-CIA (chloroform–isoamylalcohol 24:1) and 30 μl of 5 M NaCl, and the DNA was precipitated with a DNA precipitating kit (Ethachin Mate, Nippon Gene). The precipitates were dissolved in 20 μl of TE (10 mM Tris and 1 mM EDTA, pH 8.0). The amount of DNA extracted from the mucus was measured by UV/VIS spectrometer (Perkin Elmer Lambda Bio 20). DNA was also extracted from the foot of each snail.

Mean distance of migration on the slide in the experiment was 37.25 ±2.60 mm (±SE: N=12). Mean amount of DNA extracted from mucus was 32.55 μg/ml ±2.66 (±SE: N=12).

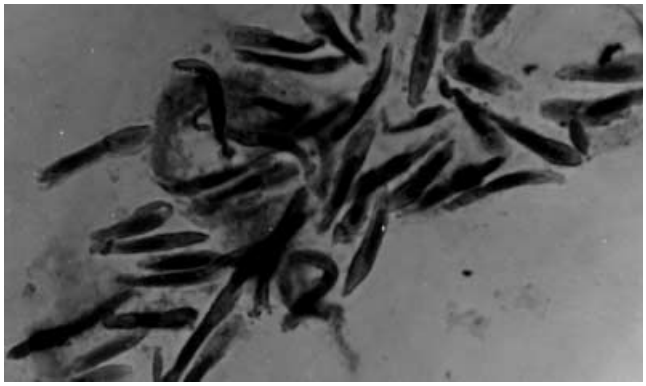

Figure 1. Epithelial cells precipitated from pedal mucus.

Figure 2. Bands characterizing microsatellites that were amplified using template DNA extracted from mucus (M) and the foot (F) in three snails (A, B and C). M: 100bp marker. Target bands are the bands studied.

Polymerase chain reaction (PCR) amplification was carried out in a final volume of 25μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, about 10 ng of above DNA solution, 320 μM of dNTP, 25 pmol of each primer of microsatellite loci Nlw 3, Nlw 5, and Nlw 8 (Kawai et al., 2001), 1.6 units of *Taq* polymerase (Perkin Elmer), 10% DMSO, 0.04% of bovine serum albumin, and 2.5 mM of $MgCl_2$. Thermal cycling (on a Perkin Elmer Gene Amp system 9600) was performed at 6 min at 94°C and 30 thermal cycles with 1 min at 94°C, 1 min at 57°C, and 2 min at 72 °C and an extra extension time of 10 min at 72°C. The PCR products were electrophoresed in 5% polyacrylamide gels (acrylamide–bisacrylamide 29:1) and silver stained.

Many epithelial cells and a few blood cells were observed in precipitates from the mucus (Figure 1). We successfully amplified the microsatellite DNA regions using template DNA from the mucus and obtained clear bands showing the same position and same pattern as template DNA amplified from the foot of the same individual (Figure 2). This result demonstrates the feasibility of amplifying the target DNA while avoiding contamination from the DNA of extraneous micro-organisms. Accordingly, mucus is a reliable source to extract DNA and this non-invasive technique is useful for conservation biology as well as behavioural studies in the intertidal but possibly also land and other marine snails.

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