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A PROTOCOL FOR NON-DESTRUCTIVE EXTRACTION OF DNA FROM ODONATES

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Genetic methods are often utilised for the ecological study of odon. spp. In many instances, especially from a conservation standpoint, it is desirable to employ a method of extracting DNA that does not affect the subsequent survival of the animal under investigation. Removal of part of an odon. leg has been demonstrated not to affect the subsequent reproductive success of the animal. Thus for odonates, a simple and quick method of extracting DNA from a portion of an odon. leg is presented that provides high yields of DNA suitable for PCR.

INTRODUCTION

Genetic markers are increasingly being used to unravel parts of the ecology of odonates that are difficult to quantify in the field, such the determination of paternity (HADRYS et al., 1993) and study of sperm competition (HOOPER & SIVA-JOTHY, 1996). The efficient extraction of high quality DNA is an essential first step for any of the genetic markers now available. In addition to the many standard protocols available (e.g. SAMBROOK et al., 1989), there are now several methods of extracting DNA specifically from insect samples, including odonates (see CHIPPINDALE et al., 1998).

However, it is frequently desirable to obtain DNA from an animal without killing the organism under study, for example, where the species is rare or endangered, or

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when the genetic identity of individuals needs to be known prior to some subsequent observation or experiment. In this situation, clearly, only a small, non-essential part of the animal can be removed. For non-destructive sampling, recent work on *Megaloprepus coerulatus* has suggested that the tibia may be removed from adult damselflies without having a significant affect upon either the subsequent survival or the ability of the male to hold territory (O.M. Fincke & H. Hadrys, pers.comm.). In addition larvae of the dragonfly *Epitheca cynosura* survived for several months in a field enclosure experiment without any apparent impairment of function following marking by means of tarsus removal (JOHNSON et al., 1995).

Care must be taken when traditional methods of DNA extraction are employed on such valuable, and often small, samples because the washing or precipitation steps can result in a significant loss of available DNA. Less stringent washing, which may increase the yields of DNA, is undesirable for odonate samples because it is likely that there are PCR inhibitants in the cuticle that require thorough washing for their removal. For this reason, the Chelex method, typically used for extraction of DNA from mammalian hair samples, is also unsuitable because it lacks a washing step. Here we describe a non-destructive method of extracting odonate DNA that allows thorough washing and provides high yields of DNA that consistently amplifies during PCR.

METHODS AND RESULTS

PROTOCOL FOR DNA EXTRACTION FROM ODONATE LEGS

This method is based upon that of CHIPPINDALE et al. (1998), who use tissue from the flight-muscles of odonates, and the protocol provided in the Geneclean II DNA purification kit (BIO 101, cat #: 1001-400). Further details on some of the components used in this protocol are provided in the instruction manual provided with the Geneclean II kit.

(1) After a leg has been removed, store the sample in 20 µl of DTAB buffer. For optimal results, samples are usually left for 3-5 days at 4°C. High quality DNA has been obtained from samples left refrigerated for up to 4 weeks, and it is likely that samples can be left for longer periods. (DTAB buffer: 4.0 g dodecyltrimethylammonium bromide, 4.4 g NaCl, 0.84 g EDTA make up to 50 ml with 100 mM Tris-HCl [pH 8.6]).

(2) Immediately prior to extraction freeze the sample in the DTAB buffer at -80°C (or on dry ice). Whilst keeping the sample cold, grind the sample until it is completely homogenised.

(3) Incubate the homogenised sample at 50-55°C for 90 minutes (or preferably overnight), and then increase the temperature to 75-80°C for about 30 minutes. During these incubation stages occasionally agitate the sample; gently spin the sample to the bottom of the tube if necessary.

(4) Add an equal volume (20 µl) of sterile distilled water to the homogenate, and then add 2.5 µl of CTAB buffer. Next, gently mix the sample, and then leave

it at room temperature for between 5 and 20 minutes. (CTAB buffer: 5 % cetyltrimethylammonium bromide in 0.4M NaCl).

(5) Add 4 volumes (*i.e.* 160 μl) of sodium iodide (NaI) buffer and gently mix the sample. (NaI buffer: 6M NaI solution, supplied in Geneclean II kit, Bio101).

(6) Add 5μl of Glassmilk and keep the sample mixing on a mechanical rotor for at least 30 minutes at room temperature. (Glassmilk is composed of a silica matrix that will readily settle out of suspension during storage. It is therefore essential that the Glassmilk is vigorously mixed by vortexing prior to use. Glassmilk is supplied as part of the Geneclean II kit from Bio101).

(7) Spin the samples at 13,000 rpm for 5 seconds and discard the supernatant using a pipette. Repeat this step to ensure that all the NaI solution has been removed.

(8) Add 800 μl of New Wash solution and re-suspend the pellet thoroughly by gentle pipetting. To prevent shearing of DNA it is recommended that wide bore pipette tips are used in this stage. (New Wash is a solution of NaCl, Tris, EDTA and ethanol that should be stored at 22-25°C. It is supplied in the Geneclean II kit).

(9) Spin the sample at 13,000 rpm for 5 seconds and pour off the New Wash solution.

(10) Repeat steps 9 & 10 once or twice more as necessary. After the final washing step use a pipette to remove any residual New Wash solution and then leave the sample to air-dry for about 5-15 minutes to allow the last traces of ethanol to evaporate.

(11) Gently re-suspend the pellet in 25-35 μl of Tris-HCl buffer and incubate at 55°C for 5-30 minutes to elute the DNA. Poor yields of DNA are generally obtained if the sample is eluted in sterile distilled water only. (Tris-HCl buffer: 10 mM Tris-HCl, pH 8.0).

(12) Spin the sample at 13,000 rpm for at least 30 seconds and pipette off the supernatant. Take care not to remove any of the Glassmilk with the eluate as this may inhibit any subsequent PCR. This first elution step should remove about 80 % of the DNA from the Glassmilk. Between 0.5 and 4 μl of the supernatant contains enough DNA for a 10 μl PCR reaction. Optimal PCR results are obtained by diluting the DNA to 20 ngμl^{-1} and using 1 μl of this in a 10 μl PCR reaction.

(13) If more DNA is required a second elution step may be undertaken. However, the expected yield of DNA is only expected to be up to 20% that of the first step.

DISCUSSION

Apart from the benefits to non-destructive sampling in general, there are other advantages to using this method of DNA extraction. First, samples may be stored

Table I
Suggested volumes of buffers, Glassmilk and eluate to be used in this protocol according to different starting samples

Sample	Step (2) DTAB	(4) SDW	(4) CTAB	(5) NaI	(6) Glassmilk	(11) Tris-HCl
Leg	20 µl	20 µl	2.5 µl	160 µl	5 µl	20 µl
Whole Larva	40 µl	40 µl	5.0 µl	320 µl	10 µl	50 µl
Whole Adult	100-200 µl	100-200 µl	12-24 µl	800-1,600 µl	10-15 µl	100-150 µl

in DTAB in the field without degradation of the DNA, and it also prevents some of the problems that may result when samples are stored in ethanol. After the initial storage in DTAB this protocol is rapid and easy to modify if appropriate: for example, this method works equally well on whole animals, although the solution volumes are different. Suggested volumes for DNA extraction from adult and larval odonates are presented in Table I.

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