Characterization of six polymorphic microsatellites for the polychaete tubeworm *Hydroides elegans* and cross-species amplification in the congener *Hydroides hexagonus*

J. B. PETTENGILL,* M. G. HADFIELD,† M. D. SCHUG* and D. E. WENDT*‡

*Department of Biology, The University of North Carolina, Greensboro, NC 27403, USA, †Kewalo Marine Laboratory, University of Hawaii, 41 Ahui Street, Honolulu, HI 96813, USA, ‡Biological Sciences Department, Cal Poly State University, San Luis Obispo, CA 93407, USA

Abstract

We isolated and characterized six polymorphic microsatellite loci for the polychaete tubeworm, *Hydroides elegans*. Two additional loci were not reliably scorable and estimates of heterozygosity were obtained for the other six. In addition, cross-species amplification was successful for two loci using the congener *H. hexagonus*. Given that few microsatellite loci are available for polychaetes, these markers will be useful in assessing dispersal and gene flow in *H. elegans* and probably also other polychaetes.

Keywords: gene flow, larval dispersal, microsatellites, polychaete

Obtaining direct evidence of dispersal for marine invertebrates is often extremely difficult. For species with sessile adult stages, the free-swimming larval phase is probably a primary mechanism of migration between populations. The advent of genetic markers has increased the ability of population ecologists to indirectly assess larval dispersal between populations (e.g. Palumbi 1995) and the impact of anthropogenic-mediated dispersal (Gollasch 2002). Microsatellites are good markers for such studies as they have relatively high mutation rates (e.g. Dallas 1992) and are thought to be selectively neutral (Jarne & Lagoda 1996). We report here on the development of six polymorphic microsatellite loci for the serpulid polychaete tubeworm *Hydroides elegans*.

We performed a pooled extraction on 25 live animals to obtain a significant amount (606.8 ng/mL) of high quality ($OD_{260/280}$ 1.82) genomic DNA, which was quantified using spectrophotometry (biophotometer; Eppendorf). Several extraction 'kits' and a standard digestion buffer (100 mM Tris at pH 7.5–8.0, 100 mM EDTA at pH 8.0 and 0.5% sodium dodecyl sulphate) followed by a phenol/chloroform/isoamyl precipitation were attempted. Although these methods yielded sufficient high molecular weight DNA, the stability of the DNA decreased quickly during enzymatic reactions. A cetyltrimethyl ammonium bromide phenol/chloroform/isoamyl combination was used to more adequately remove polysaccharides and proteins, as described by Winnenpenninckx *et al.* 1993). A similar protocol for the polychaete *Pectinaria koreni* was utilized by Weinmayr *et al.* (2000).

The microsatellite library was generated following the methods of Dr Travis Glenn at the University of Georgia Savannah River Ecology Laboratory. Briefly, ~500-bp fragments were created by digesting genomic DNA with the restriction enzyme *Rsa*I. SNX linkers were then ligated onto the fragments, which ultimately allows for the enrichment of products through polymerase chain reaction (PCR). These fragments were probed with biotin-labelled oligonucleotide repeats (di-, tri- and tetranucleotide) and exposed to straptavidin Dynabeads® (Dynal Biotech). Straptavidin Dynabeads® bind to those fragments containing a sufficient number of hybridized biotin oligonucleotides, which are collected with a magnet. After enrichment through PCR, the products were cloned with a PCR Cloning^Plus Kit (QIAGEN) according to the manufacturer's protocol. Minipreps of positive colonies were performed using the Wizard® Plus SV Miniprep DNA Purification System (Promega) with centrifugation.

Table 1 Characteristics of six polymorphic microsatellite loci and two additional loci for *Hydroides elegans*

Locus	Primers*	Core repeat sequence	Size of PCR product (bp)‡	No. of alleles†	H_O	H_E	GenBank Accession no.
Hel 3	F: 5′-TGGCAAATATGACTGCAGATG-3′ R: 5′-TGTAAATAACGCCGCCAAAT-3′	$(ATC)_{11}$	256	15	0.763	0.723	ss 5607065
Hel 8	F: 5′-GCACACGCAATGGAACATAC-3′ R: 5′-GCAGCATCGCAATCAATAAT-3′	$(GAT)_{10}AAT(GAT)_5$	200	19	0.781	0.707	ss 5607066
Hel 10	F: 5′-CCGGGATATGAGCCAATTAC-3′ R: 5′-CCTTAGGGATGTCTACTCTAACGA-3′	$(CAA)_5$	135	11	0.625	0.620	ss 5607067
Hel 16	F: 5′-AGATGGATTGCGCTGCTT-3′ R: 5′-CCAGGCCTAGAAGTGGTAGTAG-3′	$(CAA)_{14}$	163	24	0.893	0.896	ss 5607068
Hel 4	F: 5′-GAAACCACAACGTTGCAAATC-3′ R: 5′-GCATAGGTGTAATATGCGCTTT-3′	$(ATC)_8$	204	3	0.465	0.493	ss 5607069
Hel 7	F: 5′-AAATGAGCGAATGAGGGAAA-3′ R: 5′-CACCAAGCAATCTGTCATTCA-3′	$(GAGT)_6GAAT(GAGT)_5$ $GAGC(GAGT)_9$	246	9	0.741	0.668	ss 5607070
Hel 2	F: 5′-TCGGCCCGAAATATTGACTA-3′ R: 5′-CTCACCCGACCCTTCTTTC-3′	$(TG)_7$	175	§			ss 5607071
Hel 11	F: 5′-CACGATCCAAGGCCCTTAC-3′ R: 5′-CGATCATAAGTATCGTCATCGTC-3′	$(GAT)_4GACGATGACGAT$	218	§			ss 5607072

*Minus the M13 forward sequence.
†No. of alleles from 137 individuals.
‡Size of cloned allele.
§Could not be reliably scored due to multiple stutter bands.
PCR, Polymerase chain eaction; H_E, expected heterozygosity; H_O, observed heterozygosity.

To confirm which plasmids had inserts, we performed a 25-μL PCR reaction with 1.0 μL 100 pmol M13 forward and reverse primers, 1.0 μL plasmid DNA, 2.5 μL 10× buffer, 2.0 μL 25 mmol $MgCl_2$, 1 U *Taq* polymerase (Promega) and 17.375 μL ddH_2O (35 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s).

Plasmids containing inserts were sequenced to determine the presence and identity of each microsatellite locus. The PCR reaction mixtures for sequencing were according to the manufacturer's protocol (Epicentre® Sequitherm EXCEL™ II DNA Sequencing Kit-LC for 66-cm gels) adjusted for 4 μL of plasmid DNA per reaction. The cycling parameters were 4.5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 53 °C and 1 min at 72 °C. Sequencing was conducted using a NEN® Global IR² DNA Sequencer System (LiCor) with 41-cm 64-well 5.5% polyacrylamide gels created from the prescribed polyacrylamide solution. The analysis of sequencing gels was done using E-SEQ software (LiCor) and the resultant base sequences were manually analysed for microsatellites.

Fifteen microsatellites had an appreciable flanking region for the program PRIMER3 (Rozen & Skaletsky 1998) to design primers and the forward primer contained the M13 forward sequence. Amplification was successful for 11 of the original 15 loci. Genotyping reactions involved a 10-μL mixture containing 5.175 μL H_2O, 1.8 μL 10× buffer, 0.8 μL 25 mM $MgCl_2$, 0.4 μL 10 mM dNTPs, 0.125 μL of *Taq* DNA polymerase (Promega), 0.5 μL 1 pmol forward and reverse primers and 0.5 μL of 1 pmol IRDye700 or IRDye800 M13 forward primer (LiCor). The thermocycler conditions were the 54 °C low-enhance programme described by LiCor. Reaction products were loaded on a 25-cm gel (8.4 g urea, 4 mL 5× TBE, 2.4 mL LongRanger acrylamide, 200 μL APS and 15 μL Temed) with 0.25-mm spacers. Lanes had either one or two bands and scoring was conducted using GENE IMAGIR® (LiCor) software.

Details of the degree of polymorphisms and scorability are in Table 1. Estimates of observed and expected heterozygosity were conducted using the software program F-STAT (Goudet 1995). Two of the microsatellite loci (*Hel 10* and *Hel 16*) for *H. elegans* were tried on the congener *H. hexagonus* and both successfully amplified. These loci will aid in answering questions of natural and anthropogenic dispersal and gene flow between adult populations of *H. elegans* and possibly other closely related polychaetes.

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