Conserv Genet (2009) 10:1177–1179
DOI 10.1007/s10592-008-9738-3

TECHNICAL NOTE

Variable nuclear markers for a Sonoran Desert bark beetle, *Araptus attenuatus* Wood (Curculionidae: Scolytinae), with applications to related genera

R. C. Garrick · C. A. Meadows · J. D. Nason · A. I. Cognato · R. J. Dyer

Received: 4 October 2008 / Accepted: 9 October 2008 / Published online: 25 October 2008

Abstract We report eight new co-dominant nuclear markers for population genetics of the bark beetle *Araptus attenuatus* Wood. Several loci include introns from low-copy genes, and four cross-amplify in one or more related genera. The markers show moderate levels of polymorphism (2–19 alleles per locus), and no loci showed significant deviations from Hardy–Weinberg or linkage equilibrium across both of the two populations examined, consistent with Mendelian inheritance patterns.

Keywords Baja Peninsula · Co-dominant loci · Coleoptera · Introns · Population structure

Bark beetles (Curculionidae: Scolytinae) are ecologically and economically important agents of tree damage and mortality in forests worldwide. Their interactions with host plants have also served as models for understanding speciation processes, including in bark beetles associated with plants of the family Euphorbiaceae (e.g., Jordal and Hewitt 2004, 2006). As part of a research program investigating the genetic structure of plant–insect species pairs from the Sonoran Desert (Nason et al. 2002; Dyer and Nason 2004), we report the first set of population-genetic markers for *Araptus attenuatus* Wood. This beetle occurs in Baja California and Sonora, Mexico, and is known only from senescing stems of *Euphorbia lomelii* V. W. Steinm. (synon. *Pedilanthus macrocarpus* Benth.). *Araptus attenuatus* may comprise a species complex based on mitochondrial DNA sequence data (R. C. Garrick et al. unpublished) and morphology (A. Cognato personal observation). The following gene regions were assayed for intraspecific variation: *enolase* (*ENO*), *elongation factor-1α* (*EF-1α*), *wingless* (*WNT*), *muscle protein 20* (*MP20*), *kuzbanian* (*KUZ*), *ATP synthetase* subunit α (*ATPSα*), *lysidyl aminoacyl transfer RNA synthetase* (*LTRS*), and an anonymous microsatellite locus (*AML*).

Selected low-copy nuclear genes were initially amplified using degenerate exon-priming intron-crossing (EPIC) oligonucleotides. We surveyed GenBank for accessions from diverse beetle genera, and used resulting sequence alignments to design primers in conserved regions flanking highly variable non-coding regions. We also trialed a suite of published EPICs (Jarman et al. 2002) and other arthropod primer sets (Brower and DeSalle 1998).

Genomic DNA was extracted using a DNeasy® animal tissue kit (Qiagen). Polymerase chain reactions (PCRs) were performed using a PTC-200 thermocycler (MJ Research) in 10 µl volumes containing 5 µl JumpStart REDTaq Ready-Mix (Sigma), 3 µl dH_2O, 0.5 µM each primer and 1 µl template DNA. Each primer pair was tested on a panel of five individuals, using two annealing temperatures with the following profile: 95°C 2 min (1 cycle), 95°C 30 s, 44 or 48°C 30 s, 72°C 1 min (35 cycles), 72°C 2 min (1 cycle). PCR products were separated via electrophoresis (2% agarose gels stained with ethidium bromide, 80 V 55 mA for 2–3 h), and viewed on a UV transilluminator. Discrete, similarly-sized bands (±50 bp) that amplified in multiple individuals

R. C. Garrick (✉) · C. A. Meadows · R. J. Dyer
Department of Biology, Virginia Commonwealth University, Richmond, VA 23284, USA
e-mail: rcgarrick@vcu.edu

J. D. Nason
Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011, USA

A. I. Cognato
Department of Entomology, Michigan State University, East Lansing, MI 48824, USA

Table 1 Characterization of nuclear markers developed for *Araptus attenuatus*

Locus name	Primer sequence (5′–3′)[a]	*T* (°C)	Amplicon sizes (bp)	Assay (restriction enzyme)	N_A	Population 1 ($N = 37$)			Population 2 ($N = 53$)			GenBank accessions
						H_O	H_E	*P*-value[b]	H_O	H_E	*P*-value[b]	
Aa*ENO*	F: ATGATTCTTCCAACTGGWGCTGC R: ATCGAAGTAGTGAYGRCGAATC	48	167–168	RFLP (*Bcc*I, *Tsp*5091)	5	0.189	0.367	<0.001	0.019	0.038	1.000	FJ347566–FJ347571
Aa*WNT*	F: TTAAGGATCGATACGATGGAGC R: ACTGTATTTGTGYTTACG	50	229	RFLP (*Dra*I, *Hha*I)	5	0.378	0.394	1.000	0.377	0.425	0.511	FJ347584–FJ347586
Aa*LTRS*	F: ACAACTTTTTCAAACTCGTGC R: ATCAAAGCCACCTACTACC	46	217	RFLP (*Hae*II)	2	0.189	0.198	1.000	0.245	0.288	0.605	FJ347577–FJ347578
Aa*EF1α*	F: GCACCTGGACACAGAGATTTC R: CGTTCAATAGCCCATCCCTTG	48	380	RFLP (*Dde*I)	2	0.027	0.054	1.000	0.358	0.487	0.082	FJ347561–FJ347565
Aa*KUZ*	F: ATCTCCATTTAGCATGATTATCCGC R: TGCCGCAAAATGCRCCTGAAATG	46	266	RFLP (*Nci*I)	2	0.108	0.220	0.039	0.170	0.234	0.153	FJ347572–FJ347576
Aa*MP20*	F: **FAM-**ATTTGCTCCAGGATCTGTTC R: ATCTGAGAATGKTTTACG	46	303–323	indel	19	0.703	0.584	0.249	0.660	0.708	0.349	FJ347579–FJ347583
Aa*ATPSα*	F: CGTGAATTGATTATTGGAGATCGTC R: **TET**-ATACCTAATGCACGTTTCAAGTAG	44	196–210	indel	10	0.378	0.395	0.800	0.019	0.038	1.000	FJ347558–FJ347560
Aa*AML*	F: **HEX**-TGTSATARRCATTTGCAATGC R: ACGCTACATCAGGATCTTGCAC	50	135–166	indel	13	0.541	0.680	0.504	0.509	0.697	0.025	FJ347550–FJ347557

T annealing temperature, RFLP restriction-fragment-length polymorphism, N_A number of alleles resolved, *N* number of individuals, H_O observed heterozygosity, H_E expected heterozygosity

[a] 5′ fluorescent labels used for assaying insertion/deletion (indel) mutation with a MegaBACE are shown in bold

[b] For multiple tests of Hardy–Weinberg equilibrium, critical *P*-value = 0.00625

Table 2 Cross-taxon amplification of nuclear markers developed for *Araptus attenuatus*

Taxon	*N*	Locus (*T*°C)								GenBank accessions
		Aa*ENO*	Aa*WNT*	Aa*LTRS*	Aa*EF1α*	Aa*KUZ*	Aa*MP20*	Aa*ATPSα*	Aa*AML*	
Pseudopityophthorus pubipennis	5	(50)[a]	–	(50)[a]	(50)[b]	–	–	–	–	FJ347587–FJ347590
Pseudopityophthorus pruinosus	5	(50)[a]	–	(50)[a]	(50)[a]	–	–	–	–	FJ347591–FJ347593
Dendroterus striatus	3	(50)[a]	–	(50)[a]	(50)[a]	–	–	–	–	FJ347594–FJ347596
Conophthorus coniperda	2	(50)[a]	–	(50)[a]	(50)[a]	(50)[a]	–	–	–	FJ347597–FJ347600
Ips confusus	6	–	–	(50)[b]	(44)[b]	–	–	–	–	FJ347601–FJ347604

N Number of individuals assayed via double-stranded conformation polymorphism (6% non-denaturing acrylamide gels run at 50 V, 10 mA for 4 h at 4°C)

T Annealing temperature

[a] Successful amplification

[b] Intraspecific polymorphism confirmed by sequencing

– No amplification

were excised, eluted in 15 µl dH_2O overnight, then used as template for reamplification (as above, but with 55°C annealing). Diploid PCR products were sequenced using a MegaBACE 1000 (Amersham Biosciences). Multi-allele alignments were used to redesign primers, and annealing temperatures were optimized empirically (Table 1). Cross-amplification was tested for five related scolytine taxa, with intraspecific polymorphism assessed via double-stranded conformation polymorphism followed by sequencing of selected amplicons (Table 2).

For loci with alleles that showed little or no size variation in *Araptus attenuatus*, restriction-fragment-length polymorphism (RFLP) assays were designed and implemented following Garrick et al. (2008). Loci with insertion-deletion (indel) polymorphisms were genotyped on a MegaBACE. Briefly, each locus was amplified with a 5′ fluorescent label added to the forward or reverse primer (Table 1). PCR products were diluted with dH_2O, desalted using 0.1% Tween 20, and run with an ET550-R size standard (GE Healthcare). Allele sizes were estimated in FRAGMENT PROFILER V1.2 (Amersham Biosciences), and all sample plates were run three times to resolve any ambiguous genotype assignments.

Tests of Hardy–Weinberg and linkage equilibrium were performed on samples from each of two populations using ARLEQUIN v3.0 (Excoffier et al. 2005). No significant deviations were detected following Bonferroni correction, with the exception of locus Aa*ENO* which showed an excess of homozygotes in Population 1 (Table 1). This anomaly may be driven by within-population substructure or the presence of kin groups in that sample. Four primer pairs amplified homologous gene regions in other species representing up to four different genera. Despite small sample sizes, population-level polymorphism at two of these cross-amplifying loci was confirmed for some taxa (Table 2), a result indicating that some of the new markers are of potentially broad taxonomic utility. We are using this new marker set to examine evidence for cryptic species in *Araptus attenuatus*, and to gain further insights into the biogeographic history of Sonoran Desert biota.

Acknowledgments This work was funded by National Science Foundation grant DEB-0543102 to RJD and JDN and, in part, by the National Research Initiative of the USDA Cooperative State Research, Education, and Extension Service Grant 2003-35302-13381 to AIC.

References

Brower AVZ, DeSalle R (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. Insect Mol Biol 7:73–82. doi:10.1046/j.1365-2583.1998.71052.x

Dyer RJ, Nason JD (2004) Population graphs: the graph theoretic shape of genetic structure. Mol Ecol 13:1713–1727. doi:10.1111/j.1365-294X.2004.02177.x

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Garrick RC, Meadows CA, Nicolas AN, Nason JD, Dyer RJ (2008) A set of polymorphic nuclear intron markers for conservation genetics and phylogeography of *Euphorbia* species (*Pedilanthus* clade). Conserv Genet. doi:10.1007/s10592-008-9521-5

Jarman SN, Ward RD, Elliott NG (2002) Oligonucleotide primers for PCR amplification of coelomate introns. Mar Biotechnol 4:347–355. doi:10.1007/s10126-002-0029-6

Jordal BH, Hewitt GM (2004) The origin and radiation of Macronesian beetles breeding in *Euphorbia*: the relative importance of multiple data partitions and population sampling. Syst Biol 53:711–734. doi:10.1080/10635150490468710

Jordal BH, Emerson BC, Hewitt GM (2006) Apparent 'sympatric' speciation in ecologically similar herbivorous beetles facilitated by multiple colonizations of an island. Mol Ecol 15:2935–2947. doi:10.1111/j.1365-294X.2006.02993.x

Nason JD, Hamrick JL, Fleming TH (2002) Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert columnar cactus. Evolution 56:2214–2226. doi:10.1111/j.0014-3820.2002.tb00146.x