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RESEARCH

Development and Characterization of Seven Polymorphic Microsatellite Loci in *Bembidion atrocaeruleum* (Coleoptera: Carabidae)

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ABSTRACT. We isolated seven polymorphic microsatellite loci from a ground beetle (*Bembidion atrocaeruleum*, Coleoptera, Carabidae (Stephens, 1826)) associated with naturally and regularly disturbed floodplain habitat in northwest Europe. Loci were tested on 157 individuals collected from five distinct habitat patches across two adjacent drainage basins in Wales, United Kingdom, to assess their potential for revealing population structure across a relatively short spatial extent. Alleles per locus ranged from 4 to 12. For a central representative population, expected heterozygosity ranged from 0.23 to 0.78 (mean: 0.63), and observed heterozygosity ranged from 0.16 to 0.94 (mean: 0.56). Analysis of molecular variance indicated significant structure among populations, even when one locus potentially containing null alleles was removed. These loci have the potential to aid the study of dispersal mechanisms of this important riparian species along and between river corridors, a recurring question in floodplain conservation studies. In addition, given the diversity of the *Bembidion* genus, they may have utility in the study of sister species.

Key Words: Coleoptera, *Bembidion*, microsatellite, floodplain, disturbance

Bembidion atrocaeruleum (Coleoptera, Carabidae) (Stephens 1828) is a widespread but highly specialized ground beetle exclusively associated with coarse riparian sediments, having a largely montane distribution across Europe (Luff 1998). It is associated with patchy habitat, subject to frequent flood disturbance. This habitat type supports a high number of specialist species, many of which are rare, and in some cases endemic (Eyre et al. 2000, Anderson and Hanssen 2005, Sadler and Bates 2008). The habitat is vulnerable to anthropogenic degradation, through alteration of hydrology (Petts and Gurnell 2005) or river channel (Florsheim et al. 2008), and the ability of specialist species to colonize alternative habitat patches between rivers and catchments is poorly understood. *B. atrocaeruleum* uses environmental cues to instigate short flights (maximum 200 m) between habitat patches (Bates et al. 2006), but data on dispersal potential across greater distances are lacking. Microsatellite loci have been identified in other carabids (e.g., Keller and Largiader 2003, Contreras-Diaz et al. 2006), but this represents the first attempt to identify them within the *Bembidion* genus that we are aware of.

Materials and Methods

Tissue samples from 10 individuals collected from a single centrally located site on the River Severn, United Kingdom, were used by Genetic Identification Services (Chatsworth, CA) to construct four microsatellite-enriched libraries with the magnetic bead-based enrichment procedure described by Jones et al. (2002). Libraries were prepared in parallel using Biotin-CA (15), Biotin-GA(15), Biotin-AAC(12), and Biotin-ATG(12) as capture oligonucleotides. Sequencing of randomly selected recombinant clones and primer design followed Jones et al. (2002). Primers for 24 microsatellite loci of high quality and with suitable flanking regions were tested and screened for polymorphism on respective corresponding clones using an amplification reaction mixture consisting of 1× Biolase Buffer (from 10× stock solution supplied by the manufacturer), 2 mM $MgC1_2$, 0.2 mM dNTPs, 6 µM each primer, 0.025 U/µl Biolase DNA Polymerase (Bioline US, Taunton, MA), and 0.2 ng/µl clonal template DNA in 50 µl of final reaction volume. Polymerase chain reaction (PCR) consisted of an initial 3-min denaturation at 94°C, followed by 35 cycles of denaturation (94°C, 40s), annealing (55°C, 40s), and extension (72°C, 30 s), with final extension time of 4 min at 72°C. As a simple screening procedure, PCR products were run on a 3.5% agarose gel stained with ethidium bromide. This approach revealed clear polymorphism in 13 of the 24 screened loci.

To assess the potential for detecting population structure both among local habitats along the River Severn and between the River Severn and adjacent River Wye, we initially attempted to amplify all 13 polymorphic loci across five populations (central Severn, three Severn tributaries, and one Wye population; $n = 29$–32 each). Forward primers were tagged with dyes obtained from Applied Biosystems (Foster City, CA; Table 1), PCR mixtures and program were as above, products were run on a 3,730 automated sequencer (Applied Biosystems), and genotyping was performed on GENEMAPPER v 4.0 (Applied Biosystems). Only 7 of the 13 loci amplified product in the natural populations, and we report on these 7 polymorphic loci here (but see Table 2 for sequences of the six failed primer pairs).

Results

Number of alleles per locus ranged from 4 to 12 (Table 1), and overall $F_{ST} = 0.025$ ($P < 0.01$), as calculated via Analysis of Molecular Variance (AMOVA) with an infinite allele model in Arlequin version 3.5.1.2 (Excoffier and Lischer 2010). Six of 10 pairwise F_{ST} values were significant ($P < 0.05$, Table 2). Arlequin analysis revealed no evidence for linkage disequilibrium between any loci, either across the full sample extent or within individual populations. Arlequin was also used to perform exact tests of Hardy–Weinberg equilibrium for each locus in each population. Observed and expected heterozygosities did not differ significantly at any locus except D101, which showed a significant homozygosity excess in all five populations (Table 1 for central Severn population), probably due to null alleles. Therefore, AMOVA was rerun including only the six loci that consistently demonstrated Hardy–Weinberg equilibrium. Excluding D101, overall $F_{ST} = 0.005$

Table 1. Details of seven microsatellite loci isolated from *B. atrocaeruleum*

Locus	Primer sequence 5′–3′	Dye	Repeat motif	NA	Size range	H_e[a]	H_o[a]	*P*
Ba-A108	F: AACGCACTTTCGACTTCGATA R: ATCGGCCCATTACCATAAATC	NED	Di	9	110–141	0.72	0.56	0.19
Ba-C1	F: ACCGCCCTCAATGATGAC R: TTCCTCTGCCTCGTCCAC	6FAM	Tri	~	93–138	0.78	0.81	0.57
Ba-C2	F: ATATGCAGTCCAAACCAAGAC R: GCTGAGGATAATGTTGAGAATG	HEX	Tri	12	129–165	0.78	0.94	0.64
Ba-C102	F: AGCCCAACACGATAAAACG R: CAACCATCATCCAGTTCGA	HEX	Tri	4	186–195	0.48	0.44	0.72
Ba-C103	F: CCTGCTGCATGATATTTGG R: AGCCAGTGTACGTGCAAAC	6FAM	Tri	7	262–282	0.23	0.25	0.26
Ba-C104	F: TCCGTTTCTTTCACTGACC R: CATCATCCGTTACACCAC	NED	Tri	9	198–220	0.78	0.74	1
Ba-D101	F: ACCAATACGTGCTTCTCGTGT	NED	Tri	10	244–263	0.66	0.16	<0.001**

All anneal temperatures were 55°C. N_A, number of alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; *P*, exact test *P* value.
[a]Heterozygosities calculated for the central population on the River Severn, main channel ($n = 32$) ("Severn" in Table 3).
**Statistically significant.

Table 2. Sequences for primer pairs designed from clones that failed to amplify product in natural populations

Locus	Primer sequence 5′–3′
Ba-A6	F: TAACGCCACCCTTGCTTA R: TACGCCGCTAACCTATGTG
Ba-A110	F: CACACACGCAAACACACATA R: TAGGTGCCTGTGGTTGTTC
Ba-B1a	F: AGAGAGAGTTGCGGCAGATA R: GACCAATGTTCAGGCTATTCC
Ba-B3	F: GTTTCGGAACAAGATAGGTTT R: GGTCCCTCCATACAAATACC
Ba-B103b	F: AGCGACAACATCATTTTAGTG R: TGCGTTTACACAATAGACCC
Ba-B111	F: GCAGTGGGGTATGGTG R: TCGCAGGCAGAGATTTGT

Table 3. Pairwise F_{ST} (above diagonal, *$P < 0.05$), first value including and second value excluding locus D101; and Euclidean distance (km, below diagonal) between five populations of *B. atrocaeruleum* (from two catchments, Severn [S] and Wye [W])

River	Banwy (S)	Severn (S)	Tanat (S)	Vyrnwy (S)	Wye (W)
Banwy		0.01*/0.009	<0.001/<0.001	0.03*/0.003	0.01/0.001
Severn	21.5		0.03*/0.003	0.05*/0.01*	0.01/0.01*
Tanat	14.8	36.3		0.02*/0.01*	0.04*/<0.001
Vyrnwy	13.2	24.8	17		0.06*/0.004
Wye	33.5	15.8	48.7	39.7	

($P = 0.04$), and 3/10 pairwise F_{ST} values were significant (Table 3), two within the Severn region, and one between the Severn and Wye regions.

The population dynamics of patchily distributed riparian invertebrates, their responses to disturbance events, and the potential for recolonization routes after these events are important questions in floodplain conservation biology, particularly given the globally threatened status of natural floodplain systems (Tockner and Stanford 2002). The ability of these microsatellite loci to detect significant structure among *B. atroceruleum* populations across a minimal spatial extent suggests their strong potential for their use in addressing these questions, in addition, the diversity of the *Bembidion* genus may give these primers some utility in sister species.

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