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Trinucleotide Microsatellite Loci and Increased Heterozygosity in Cross-Species Applications in the Social Wasp, *Polistes*

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Though microsatellite loci are usually found to be most polymorphic in the species in which they are first identified, we have found significant increases in polymorphisms in some cross-species applications. We present eight new trinucleotide microsatellite loci derived from two species of social wasps, Polistes annularis *and* Polistes bellicosus. *We assessed the primers designed from these species and the degree of polymorphism in two additional species,* P. dorsalis, *which is very closely related to* P. bellicosus, *and* P. dominulus, *which is an Old World congener, thought to have diverged from New World* Polistes *over 80 million years ago. Cross-species applications for these microsatellite loci indicate that the priming sites from* P. bellicosus *loci are conserved in* P. dorsalis *and amplified similarly sized products with higher heterozygosities than the original species in two of three cases. A locus that was monomorphic in* P. annularis *had a heterozygosity of 1.0 in the distantly related* P. dominulus. *Cross-species applications of these loci indicated that alleles were generally of similar lengths in the new and original species when they retained their heterozygosity.*

KEY WORDS: microsatellites; trinucleotide repeats; Vespidae; heterozygosity.

INTRODUCTION

Polistes is a social wasp that builds open nests that are particularly amenable to testing hypotheses about within-group competition because individuals can be

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marked and observed (West Eberhard, 1969; Strassmann, 1981; Turillazzi and Eberhard, 1996). They are especially interesting for such studies because competition for egg laying is not determined by morphological castes (Reeve, 1991). However, tying behavioral observations to exactly who is reproducing is difficult since egg laying is a rare event, and increasing its frequency by devices such as removing eggs from the nest may change the very behaviors under study (Reeve and Nonacs, 1992). Numbers of eggs laid by different females have been estimated from behavioral observations in very few species (e.g., Noonan, 1981; Strassmann, 1981; Queller and Strassmann, 1988). What is needed for accurate assessment of brood parentage is a polymorphic, codominant, mendelian marker that can be genotyped easily from large numbers of individuals. DNA microsatellites provide a solution (Tautz, 1989; Queller *et al.,* 1993). The brood in a nest can be assigned to the appropriate mother with a set of polymorphic microsatellite loci (Queller *et al.,* 1993; Peters *et al.,* 1995).

This paper presents three trinucleotide microsatellite loci from *P. bellicosus* and five from *P. annularis.* These loci amplify repeat regions with AAT, AAC, AAG, CAT, or TAG repeat motifs (Table I). They are the last loci from the same partial genomic libraries described previously (Hughes and Queller, 1993; Strassmann *et al.,* 1997). Not only do they add to our toolbox of clearly scorable, polymorphic loci for *Polistes,* but one of them has the surprising pattern of monomorphy in the original species, low heterozygosity in closely-related species, and 100% heterozygosity in the most distantly related congener we evaluated (Fig. 1). With the loci published previously (Strassmann *et al.,* 1996a, 1997), there are now 57 microsatellite loci for polistine wasps that should prove useful across the Polistinae (Ezenwa *et al.,* 1997).

MATERIALS AND METHODS

Our methods did not differ from those previously described [(Strassmann *et al.,* 1997); see Strassmann *et al.* (1996b) for a description of our approach and specific protocols]. We constructed very large partial genomic libraries of *P. annularis* (Hughes and Queller, 1993) and *P. bellicosus* (Strassmann *et al.,* 1997) in plasmids with inserts (not enriched for microsatellites) averaging 500 base pairs. We separately probed replicate membranes with synthetic oligonucleotides containing 10 or 12 repeats of either AAT, AAC, AAG, CAT, or TAG. After picking up positives, we grew them in LB broth, did plasmid minipreps, cut out the inserts, ran them on an agarose gel, then transferred the DNA to a nylon membrane. We probed this Southern blot to verify that the positives contained the repeat, sequenced positive clones, then designed polymerase chain reaction (PCR) primers for clones containing repeat regions for which we could read the flanking regions (Table I). We did not design PCR primers around all repeats for reasons including the nature of the flanks and the absence of sufficient flanking

Table I. Microsatellite Loci Named for Species (Pan, *P. annularis;* Pbe, *P. bellicosus*), Clone Number, and Repeat Motif[a]

Locus	T_a (°C)	Repeat	PO	Primer sequence 5′–3′
Pan63AAT	50	(AAC)10	F	CTTGTTGCCTTTCTTTCTGTATTC
			R	CCACCTCCTCCTAATTTTTTTCTAC
Pan80AAT	50	(AAT)4TAT(AAT)5	F	ATCAAGGCGAGGAGATCG
			R	GCCGATAACACAACGCTG
Pan82AAT	45.5	(AAT)2T(AAT)4T(AAT)4T(AAT)4	F	ATTTCTGATTCAAATCG
			R	GCGTAATTTATTATACATGCAC
Pan120AAT	50	(TAT)9	F	GAGGATCAAGTGACTGTTAG
			R	TTGTTAGTGTAGCAAAATTG
Pan93AAC	50	(AAC)10	F	CTTGTTGCCTTTCTTTCTGTATTC
			R	ACACACTTTTTTGCATTGTCG
Pbe315CAT	50	(CAT)2AAT(CAT)2CTTCGT(CAT)2AAT (CAT)2CGTCTT(CAT)2CTT(CAT)2	F	ACGTAACTTGATTCCTTC
			R	GATTTTAATCCCTTTCTC
Pbe20TAG	42.5	(TAG)10	F	TTTTCTTATTCTACAATTTC
			R	CTTTGAAGGAAACTAACG
Pbe43AAG	48	(GAA)9GAT(AAG)2AGG(AAG)5	F	ATCGTATTCTTCCGAAAG
			R	CTCCAGCAATCTTCTGAC

[a]Repeat motif, primers, and annealing temperatures are listed. GenBank accession numbers are U79232 to U79239. T_a, annealing temperature; PO, primer orientation; F, forward; R, reverse.

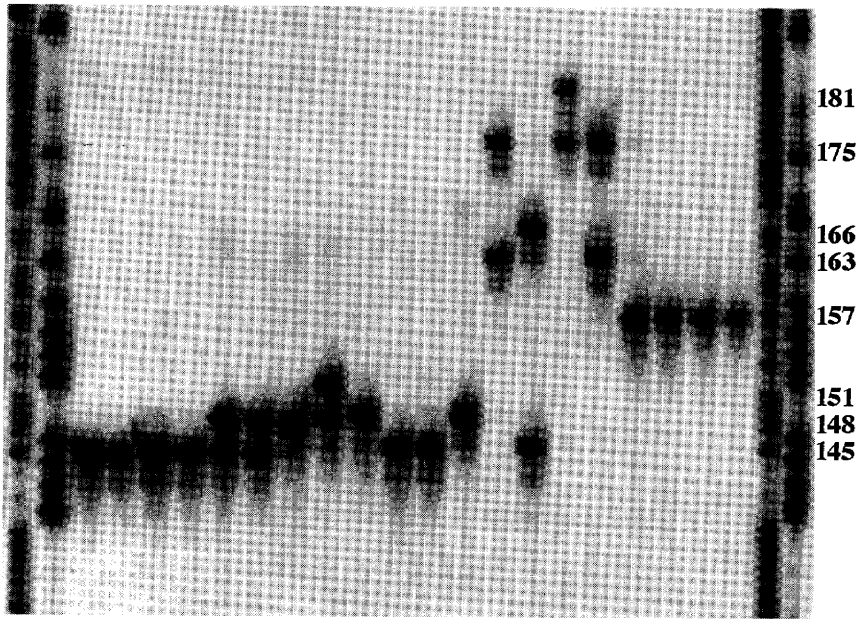

Fig. 1. Length polymorphism at locus Pan80AAT is presented for four individuals each from *P. annularis, P. bellicosus, P. dorsalis, P. dominulus,* and *Parachartergus colobopterus.* Samples are preceded and followed by a ladder consisting of an M13 sequencing reaction terminated with ddCTP only or ddATP, ddGTP, and ddTTP. Allele sizes are given at the right.

sequence or because they were dinucleotide repeats, not the trinucleotides we sought. In three cases (Pan80AAT, Pan82AAT, Pan120AAT) it was necessary to include in the PCR product or in the primers themselves the site (GATC) which provided the target for the restriction enzyme we used, SAU3A. Of course designing primers across a sequence recognized by the restriction enzyme will prove effective only if the site had not been cut by the restriction enzyme, perhaps because of incomplete digestion. In our case designing such primers was a successful gamble since all three primer pairs amplified single products of the expected size and these loci proved to be polymorphic.

We evaluated these primers for heterozygosity on 4 to 10 unrelated individuals of *Polistes annularis, P. bellicosus, P. dorsalis,* and *P. dominulus. P. dorsalis* is very closely related to *P. bellicosus; P. annularis* is a New World congener of these that is in a different subgenus. *P. dominulus* is an Old World (European) species thought to have diverged from the New World species 80 million years ago (Carpenter 1993, 1996).

Genomic DNA was prepared using protocol Strassmann.1 of Strassmann *et al.* (1996b). PCR was carried out under oil in a 10-μl volume made up of 2 μL of diluted genomic DNA (about a nanogram), 2 μL of primer mix (2.5 μ*M*), 0.1 μl of

10 m*M* dNTP mix, 1 μl of 10× buffer (provided with *Taq*), 4.08 μl of dH_2O, 0.62 μl of 25 m*M* $MgCl_2$, 0.05 μl of *Taq* polymerase (5 U/μl; Promega), 0.15 μl of ^{35}S-dATP (12.5 μCi/μl). We did 30 to 35 cycles of 60-sec denaturing at 92°C, 60-sec annealing (at a temperature optimized for the primers used; see Table I), and 45-sec extension at 72°C. PCR products were run on 6% denaturing acrylamide gels (Strassmann *et al.,* 1996b).

RESULTS AND DISCUSSION

All three of the microsatellite loci derived from *P. bellicosus* were polymorphic in that species (Tables I and II). Two of them had repeat motifs of nine or more uninterrupted repeats. The remaining locus had a messy repeat region of 19 triplets, but no more than two uninterrupted repeats. Nonetheless it was polymorphic. Three of the five loci derived from *P. annularis* proved to be polymorphic in that species, and two of the loci were monomorphic (Table II). The loci that were polymorphic in *P. annularis* had from 4 to 10 uninterrupted repeats. A locus with three sets of four uninterrupted repeats separated by a single thymidine nucleotide was monomorphic.

Results of applying these loci to other species varied considerably (Tables II and III). Two of the three loci from *P. bellicosus* were even more polymorphic in *P. dorsalis,* a result in accord with increased heterozygosity observed in other *P. bellicosus* primers when used in *P. dorsalis* (Ezenwa *et al.,* 1997). Locus Pan80AAT was monomorphic in *P. annularis,* yet it is one of the best polymorphic loci we have for *P. dominulus,* which is distantly related (Fig. 1). Pan80AAT was monomorphic in another species, *Parachartergus colobopterus,* which is in a different polistine tribe, the epiponini (Carpenter 1993). Overall, these results are in contrast to the finding of Ellegren *et al.* (1995) of decreased heterozygosity in cross species applications. Clearly, many factors influence microsatellite heterozy-

Table II. Observed Heterozygosity (Number of Individuals Assayed) for These Trinucleotide Microsatellite Loci[a]

Locus	*P. annularis*	*P. bellicosus*	*P. dorsalis*	*P. dominulus*
Pan63AAT	0.60 (10)	x[a]	x	x
Pan80AAT	0.0 (10)	0.5 (6)	0 (6)	1.0 (6)
Pan82AAT	0.0 (10)	x	x	x
Pan120AAT	0.5 (10)	x	x	x
Pan93AAC	0.6 (10)	0.5 (6)	0.0 (6)	0.0 (6)
Pbe315CAT	x	0.5 (10)	0.0 (6)	x
Pbe20TAG	0.0 (4)	0.7 (10)	0.8 (5)	x
Pbe43AAG	0.0 (6)	0.5 (10)	0.83 (6)	0 (4)

[a]An x indicates failure of the primers to amplify product in the cross-species test.

Table III. Allele Sizes (Frequency)[a]

Locus	*P. annularis*	*P. bellicosus*	*P. dorsalis*	*P. dominulus*
Pan63AAT	172 (0.1)			
	181 (0.25)			
	184 (0.55)			
	187 (0.05)			
	190 (0.05)			
Pan80AAT	145 (1)	145 (0.17)	145 (0.67)	145 (0.1)
		148 (0.75)	148 (0.33)	163 (0.3)
		151 (0.08)		166 (0.1)
				175 (0.4)
				181 (0.1)
Pan82AAT	162 (1)			
Pan120AAT	153 (0.15)			
	156 (0.7)			
	159 (0.15)			
Pan93AAC	97 (0.1)	91 (0.58)	91 (1)	91 (1)
	106 (0.25)	94 (0.42)		
	109 (0.55)			
	112 (0.05)			
	115 (0.05)			
Pbe315CAT		110 (0.05)	119 (1)	
		119 (0.15)		
		122 (0.75)		
		152 (0.05)		
Pbe20TAG	118 (1)	96 (0.05)	90 (0.2)	
		99 (0.35)	96 (0.1)	
		102 (0.5)	99 (0.1)	
		105 (0.05)	102 (0.3)	
		108 (0.05)	105 (0.1)	
			108 (0.2)	
Pbe43AAG	114 (1)	132 (0.35)	123 (0.17)	129 (1)
		135 (0.45)	135 (0.17)	
		138 (0.15)	138 (0.33)	
		141 (0.05)	141 (0.17)	
			144 (0.17)	

[a]Sample sizes are the same as those given in Table II.

gosity including population histories which are difficult to trace and may vary among species.

Taken together, studies of these loci extend and confirm results from our other studies (Strassmann *et al.*, 1996a, 1997; Ezenwa *et al.*, 1997). We do not yet know enough about the cross-species distribution of polymorphism in microsatellite loci to predict which loci are likely to be variable in cross-species applications.

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