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PRIMER NOTE

Trinucleotide microsatellite loci for a social wasp, *Polistes*

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Polistes is a cosmopolitan genus of bellicose social wasps that build open-faced paper nests in which offspring are reared. The lack of distinct morphological castes and great plasticity in social behaviour among females, combined with the ease of observing individually paint-marked adults, has made this a model genus for behavioural studies (Reeve 1991; Turillazzi & West-Eberhard 1996). All species are eusocial, but at a relatively rudimentary level: colonies are relatively small (Reeve 1991), workers are not consistently different from queens (Haggard & Gamboa 1980), social relations are often characterized by strife (West-Eberhard 1969; Strassmann 1981a; Noonan 1981), and the queen in some species must act as the pacemaker, physically manipulating workers into working (Reeve & Gamboa 1983). *Polistes* has served as a model system for a wide variety of topics including dominance hierarchies (West-Eberhard 1969), advantages of group living (Strassmann *et al.* 1988; Strassmann & Queller 1989; Strassmann 1991), kin selection (Noonan 1981; Strassmann 1981b; Queller & Strassmann 1988), kin recognition (Gamboa *et al.* 1987), usurpation (Klahn 1988), and social control by queens (Reeve & Gamboa 1983). To date, it has been difficult to combine detailed studies of behavioural interactions with studies of genetic relatedness because of the difficulty in obtaining precise estimates of relatedness for individual pairs of interactants with the available genetic markers (e.g. Strassmann *et al.* 1989). Clearly, a more polymorphic set of markers is needed.

Microsatellite loci are highly polymorphic, codominant, and can be genotyped from many sources of DNA including alcohol-preserved tissues and sperm in a female's spermatheca (Evans 1993; Queller *et al.* 1993; Peters *et al.* 1995). Here we present 18 new microsatellite loci derived from *Polistes bellicosus* and eight new loci from *Polistes annularis*. All of these loci contain trinucleotide repeat regions which are much easier to score unambiguously than are dinucleotide repeats.

To obtain these loci we constructed very large partial genomic libraries in plasmids with inserts (not enriched for microsatellites) averaging 500 bp (Hughes & Queller 1993; see Strassmann *et al.* 1996, for the rationale of our approach and specific protocols). We separately probed replicate membranes with synthesized oligonucleotides containing 10 or 12 repeats of either AAT, AAC, AAG, CAT or TAG, five of the 10 possible trinucleotide repeats. We picked up hundreds of potential positives. Reprobing a Southern blot of the inserts indicated that most of these sequences contained repeats. We sequenced part or all of about 80 clones of *P. bellicosus*. Of these, 59 contained at least one repeat region. We designed polymerase chain reaction (PCR) primers for clones containing uninterrupted repeats, five or more repeats long for which we could read the flanking regions (Table 1). We did not design PCR primers around all repeats for reasons including the nature of the flanks, absence of sufficient flanking sequence, or because they were dinucleotide repeats, not the trinucleotides we were after.

We evaluated these primers for heterozygosity on 3–24 unrelated individuals. Genomic DNA was prepared as described in Hughes & Queller (1993) or using protocol Strassmann.1 in Strassmann *et al.* (1996). PCR was carried out under oil in a 10 μL volume made up of 2 μL diluted genomic DNA (about a nanogram), 2 μL of primer mix (2.5 μM), 0.1 μL 10 mM dNTP mix, 1 μL 10× buffer (provided with *Taq*), 4.08 μL dH_2O, 0.62 μl 25 mM $MgCl_2$, 0.05 μL *Taq* polymerase (5 units/μL, Promega), 0.15 μL ^{35}S dATP (12.5 μCi/μL). After an initial denaturing for 5 min at 95 °C, we carried out 30–35 cycles of 60-s denaturing at 92 °C, 60-s annealing (at a temperature optimized for the primers used: see Table 1) and 45-s extension at 72 °C. After that, 5 extra minutes at 72 °C allowed for the completion of the extension. PCR products were run on 6% denaturing acrylamide gels (Strassmann *et al.* 1996).

All of the 18 microsatellite loci proved to be polymorphic in *P. bellicosus* though heterozygosity varied from 0.05 to 1 (Table 1). Seven of the eight loci from *P. annularis* proved to be polymorphic, with heterozygosities ranging from 0.17 to 1 (Table 1).

We have published other microsatellites for *Polistes annularis* and for another social wasp, *Parachartergus*

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Table 1 Microsatellite loci from *Polistes bellicosus* and *Polistes annularis*. Loci are named by the species from which they were identified, clone number, and repeat type. GenBank accession numbers are U64620 to U64645

Locus	T_a	Repeat motif	size (bp)	No. of ind.	No. of alleles	Hetero-zygosity	PO*	Primer sequence 5′ – > 3′
Pbe80AAG	50	(CTT)11…(ACC)6	219	23	6	0.65	F	AGAAAAGACGACCGTCGC
							R	GCGCATCGACGAAGTTT
Pbe128TAG	55	(TAG)12	171	24	6	0.54	F	CCGATATCCGTGCCAGTGATAC
							R	GCTACCGCGACTGCTGTCC
Pbe203AAG	55	(AAG)6 GAG (AAG)6	150	23	13	0.65	F	CGCTTCGCCATTAGTCCCTTCAA
							R	ACGTGCCGACAATCTCCAGAAGG
Pbe216AAG	55	(CTT)7	174	23	2	0.05	F	GCAAGTTCGAGTGTCACGGAAGA
							R	CGACATCCTAGGCGTTGAGAAATG
Pbe269AAG	60	(AAG)10	147	20	8	0.45	F	GAGACGGAAGGTGAGAGTTGTTGGA
							R	CAGTAGAGTTTGTCGAACGGAAA
Pbe411AAT	50	(AAT)11	173	19	7	0.21	F	TCGTTGGGTCCATAGCAGTAACAA
							R	TTAAGAGCATAATACGCAGCCACG
Pbe413AAT	50	(AAT)11	235	20	5	0.4	F	CCAACCCCATCGGATAACATTTTG
							R	CTGGTGTGTTGCGTATATGCTCATTG
Pbe414AAT	55	(AAT)5 ATT (AAT)5 ATT (AAT)10	190	19	6	0.21	F	CGAAACAAAACGGATTTATTGGGA
							R	AGACCACCATTTTTCGCCTGATT
Pbe424AAT	45	(ATT)2 AAT (ATT)19	215	19	7	0.74	F	GGCCAATTATTATCTCCATGCATTA
							R	CGTGCATCCTTCAGAAACAATACTT
Pbe430AAG	55	(TAA)6 (GAA)6 AAA GCA (GAA)5	194	21	4	0.29	F	ACTCTTGTTCCTTGTTATCTCGCC
							R	ACGACGCCAAGTTTTAACTAACG
Pbe433TAG	55	(ACT)8	224	23	2	0.04	F	CAAGATTATCTCTCGGCGAACG
							R	ATCGTGTTATATTAAAAATTTCGCGTAA
Pbe440AAT	50	(AAT)13	224	20	10	0.55	F	AGCTTGCCGATACTAAACAA
							R	GATTACTATTATACCCGCCACT
Pbe442AAT	41	(AAT)16	193	20	9	0.7	F	AAAACTATTCAATTAATTTGTATATGTT
							R	ATTATGAATGTACCGTAAGTAAATTT
Pbe475AAT	55	(AAT)8 ACT (AAT)3	149	22	5	0.5	F	TCGTCTCTTGGTAACCTTCATT
							R	GAACACCATTCGCTAAATCTTT
Pbe484AAT	45	(AAT)10	191	19	2	0.05	F	TCCAAATCAATTTCAACCTACAAACGTATA
							R	TTCACGCATGTTTAGTTAGAAGCCAATA
Pbe492AAT	55	(TTA)4 ATA (TTA)19	179	23	9	0.43	F	ACCGACGAGCGTTAATAATTCATG
							R	CACGTCTGTGCATAAGAAGTACGG
Pbe102TAG	48	(AGT)2 AAT (AGT)2 GGT (AGT)5	108	7	6	0.714	F	TGATTGTTCAAAGTTAGCCG
							R	GGTTCGAGAGAGTTTCCTTG
Pbe205AAG	50	(CTT)2 CCT TTT (CTT)5 CTC (CTT)2 TTT (CTT)2 CGN (CTT)2	234	6	6	1	F	TGTGTTGTTGTCGTCATCGTAC
							R	CTCCGAGTAGTAGACGCAATC
Pan12AAT	50	(AAT)8	171	11	2	0.36	F	AATTATCGAATATAAATCAAAATAACG
							R	GTTGCGAATAAATTACACGTATTAT
Pan27AAT	50	(AAT)8	192	7	4	0.57	F	GTTAGGAATTAAAAGTGGGGATGT
							R	ATTTCACGTAACCCGATAGTTATG
Pan23AAT	55	(AAT)5 AAC (AAT)2	171	9	2	0.22	F	TTAACGGCCATCTTTGTTAC
							R	TATGCGTGTGTATCTGCTTG
Pan36AAT	45	(AAT)8	198	3	2	0.33	F	CGGGAAATAGAAAGAAACTAGC
							R	GCAACTAGCGAAAGCGAT
Pan47AAT	55	(CTT)2 (ATT)5	135	8	1	0	F	TCCCTTTCCCTTACATTCTC
							R	AATTATCCTCGGATTGCG
Pan95TAG	55	TAG CAG (TAG)6 TAA (TAG)9	135	18	4	0.67	F	TTTTACCGGGCACTTTGG
							R	CGGACATCGAACGTTGTG
Pan104TAG	55	(TAG)9 (AAT)7…(TAG)6	224	12	2	0.17	F	AGTCACCGCGAGTAAGATGTT
							R	AAGCGACGAATCAACTTAGCT
Pan109AAT	55	(AAT)12	188	10	7	1	F	GATTTCGATTTGCATGAACGC
							R	AAGCTCGGTTTTCTTCTGCCT

T_a, annealing temperature. *PO, primer orientation *colopopterus* (Choudhary *et al.* 1993; Hughes & Queller 1993; Strassmann *et al.* 1996). Combined, there are now 49 microsatellite loci for social wasps. In this group we investigated the relationship between heterozygosity, repeat type and length (Fig. 1). We assigned each locus to the most common repeat motif between the amplifying primers, though some also had other repeat motifs (Table 1, Strassmann *et al.* 1996). We considered the following repeat types: AAT ($n = 26$ loci), AAG (7), TAG (8), CAT (4), CAC (1), CAG (2), AAGG (1). We detected no pattern between repeat type and heterozygosity for the sample as a whole, or within any of the three species, although we did not have very large samples of most motifs (Kruskal–Wallis tests, Fig. 1). Repeat motifs containing a G or C did not differ in levels of heterozygosity from AAT repeats (Mann–Whitney U $P > 0.6$, $n = 49$).

We analysed the relationship between repeat length and heterozygosity using two measures of repeat length: (i) the longest stretch of perfect, uninterrupted repeats (e.g. AAGAAGAAGAAG – counted as four repeats) and (ii) the longest stretch of repeats including two or fewer repeats with basepair imperfections (e.g. AAGAAGAAGTAGTTGAAGAAG – counted as seven repeats). We had more than two loci per species for four repeat motifs, AAT, TAG, AAG and CAT. We evaluated AAT repeat motifs in *P. annularis*, *P. bellicosus* and *Parachartergus colobopterus* separately. We evaluated TAG repeat motifs in *P. bellicosus* and *Parachartergus colobopterus* separately. We evaluated AAG repeat motifs in *P. bellicosus* and CAT repeat motifs in *Parachartergus colobopterus*. Heterozygosity was significantly correlated with number of perfect AAT repeats in *P. annularis* (Spearman Rho = 0.75, $P < 0.05$, $n= 12$) and was not correlated in *P. bellicosus* (Spearman Rho = 0.61, $P < 0.09$, $n = 9$) or *Parachartergus colobopterus* (Spearman Rho = 0.30, $P < 0.55$). Heterozygosity was uncorrelated with length of total AAT repeat regions in any of the species (Spearman correlations). We found no significant relationships between TAG or CAT repeats (either perfect or total) and heterozygosity in any of the three species (Spearman correlations). It is possible that small sample sizes obscure a relationship between numbers of repeats and heterozygosity for these motifs. However, three of the eight correlations were actually negative (but insignificant), so without further data a relationship between repeat number and heterozygosity must be considered to be absent for TAG, CAT and AAG repeats in these species. For AAT, number of perfect repeats was a better predictor of heterozygosity than was length of total repeat region.

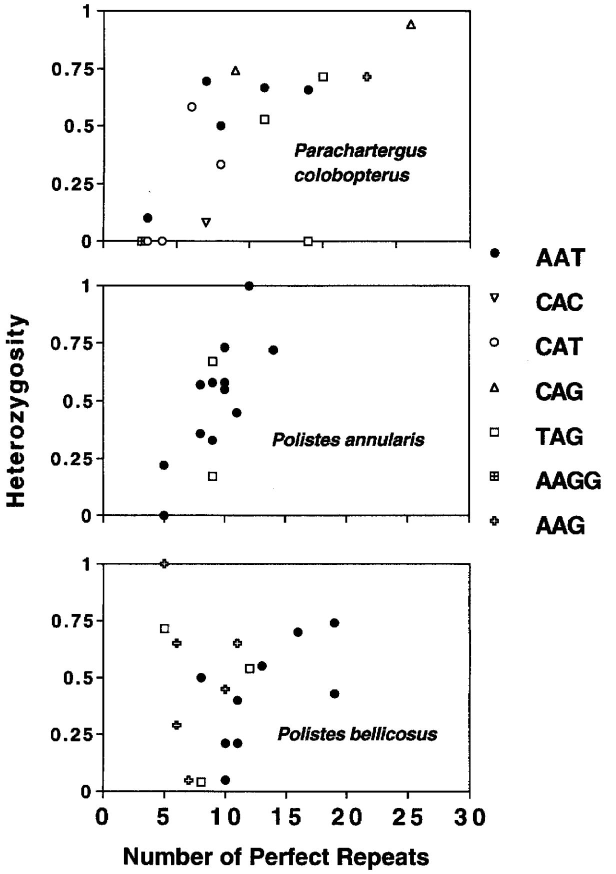

Fig. 1 Relationship between heterozygosity and number of perfect repeats for three species of social wasps. See text for significance levels.

Our 10 most variable loci had heterozygosities over 0.70, came equally from all three species, and had repeat types AAT, AAG, TAG and CAG represented. The 10 most heterozygous loci had from 5 to 26 perfect repeats and 11–31 total repeats. The effort needed to design primers and try them on a population sample is small compared to that needed to get the sequence containing the repeat in the first place, so we feel that it is worth pursuing microsatellites with as few as five perfect repeats. Detailed analyses of parentage or genetic structure in societies often require 5–10 highly polymorphic, unambiguously scorable loci. Cross species studies indicate that these loci are polymorphic in closely related species, and that their utility falls off with increasing phylogenetic distance (V.O. Ezenwa *et al.*, unpubl. data).

When these loci are applied to wasps in conjunction with population studies, we expect great advances in our understanding of social interactions in wasp societies. We will be able to tie individual behaviour to reproductive success (using microsatellites for parentage determination) and to genetic relatedness among interactants, thereby gaining insight on genetic conflicts of interest among related individuals.

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