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PERMANENT GENETIC RESOURCES NOTE

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Abstract

This article documents the addition of 229 microsatellite marker loci to the Molecular Ecology Resources Database. Loci were developed for the following species: *Acacia auriculiformis* × *Acacia mangium* hybrid, *Alabama argillacea, Anoplopoma fimbria, Aplochiton zebra, Brevicoryne brassicae, Bruguiera gymnorhiza, Bucorvus leadbeateri, Delphacodes detecta, Tumidagena minuta, Dictyostelium giganteum, Echinogammarus berilloni, Epimedium sagittatum, Fraxinus excelsior, Labeo chrysophekadion, Oncorhynchus clarki lewisi, Paratrechina longicornis, Phaeocystis antarctica, Pinus roxburghii* and *Potamilus capax*. These loci were cross-tested on the following species: *Acacia peregrinalis, Acacia crassicarpa, Bruguiera cylindrica, Delphacodes detecta, Tumidagena minuta, Dictyostelium macrocephalum, Dictyostelium discoideum, Dictyostelium purpureum, Dictyostelium mucoroides, Dictyostelium rosarium, Polysphondylium pallidum, Epimedium brevicornum, Epimedium koreanum, Epimedium pubescens, Epimedium wushanese* and *Fraxinus angustifolia*.

This article documents the addition of 229 microsatellite marker loci to the Molecular Ecology Resources Database. Table 1 contains information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column. A full description of the development protocol for the loci presented here can be found in the Molecular Ecology Resources Database (http://tomato.biol.trinity.edu/).

Table 1 Information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Acacia auriculiformis × *Acacia mangium* hybrid	20	*Acacia peregrinalis*, *Acacia crassicarpa*	44734–44741, 44812–44823	HQ110862–HQ110881	Sukganah, A; Liew, W.Y.; Wickneswari, R.
Alabama argillacea	10	n/a	44502–44508, 44510–44512	GF102184–GF102193	Pavinato, V.A.C.; Bajay, M.M.; Martinelli, S.; Monteiro, M.; Pinheiro, J.B.; Zucchi, M.I.; Omoto, C.
Anoplopoma fimbria	13	n/a	44824–44836	GO616605.1, GO616986.1, GO617191.1, GO618107.1, GO618227.1, GO618807.1, GO618865.1, GO619216.1, GO620444.1, GO620529.1, GO629344.1, GO638529.1, GO646855.1	Messmer, Amber M.; Sanderson, Dan; Nelson, R. John; Koop, Ben F.
Aplochiton zebra	13	n/a	44587–44599	HM997136–HM997140, HM997142–HM997148, HQ003931	Vanhaecke, Delphine; Croxford, Adam; Allainguillaume, Joel; Garcia de Leaniz, Carlos; Consuegra, Sofia

Table 1 Continued

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Brevicoryne brassicae	9	n/a	44548–44556	FN820283–FN820291	Esselink, GD; den Belder, E; Elderson, J; Smulders, MJM
Bruguiera gymnorhiza	14	B. cylindrica	44644–44654, 44656–44658	AB571659–AB571669, AB571671–AB571673	Takayama, Koji; Tamura, Mariko; Ono, Junya; Tateishi, Yoichi; Kajita, Tadashi
Bucorvus leadbeateri	12	n/a	44565–44567, 44569–44577	HM590197–HM590203, HM590206–HM590210	Dalton, Desiré L; Kotzé, Antoinette
Delphacodes detecta, *Tumidagena minuta*	10, 7	*Delphacodes detecta*, *Tumidagena minuta*	44673–44693	HM626384–HM626400	Sheridan, C. K.; Douglas, M. R.; Power, L. D.; Wimp, G. M.; Hamilton, M. B.
Dictyostelium giganteum	12	*Dictyostelium macrocephalum*, *Dictyostelium discoideum*, *Dictyostelium purpureum*, *Dictyostelium mucoroides*, *Dictyostelium rosarium*, *Polysphondylium pallidum*	44709, 44710, 44712–44721	GU904555, GU904556, GU904559, GU904560, GU904562–GU904565, GU904567–GU904569, GU904573	Sathe, Santosh; Lalremruata, Albert; Aggarwal, Ramesh K.
Echinogammarus berilloni	11	n/a	44600–44610	HQ185684–HQ185694	Drees, Michael; Reusch, Thorsten B. H.; Meyer, Elisabeth I.
Epimedium sagittatum	8	*Epimedium brevicornum*, *Epimedium koreanum*, *Epimedium pubescens*, *Epimedium wushanese*	44557–44564	HM623765–HM623772	Li, Chunhong; Guo, Baoling; Hong, Yan
Fraxinus excelsior	15	*Fraxinus angustifolia*	44694–44708	FR635387, FR636736, FR637753, FR638723, FR639294, FR639485, FR639792, FR640915, FR642190, FR644535, FR644953, FR645030, FR645771, FR645842, FR646655	Sannier, J.; Bertolino, P.; Frascaria-Lacoste, N.; Fernández-Manjarrés, J. F.
Labeo chrysophekadion	9	n/a	44578–44586	HM641012–HM641020, AJ291680, AJ507524	Nguyen, Thuy T. T.
Oncorhynchus clarki lewisi	12	n/a	44536–44547	HM153812–HM153823	Vu, Ninh V.; Kalinowski, Steven T.
Paratrechina longicornis	15	n/a	44611–44625	HM210893–HM210895, HM210900, HM210909, HM210910, HM210912, HM210913, HM210915–HM210917, HM210919–HM210921, HM210929, HM210934, HM210935, HM210937, HM357722	Matthews, Emily A.; Pearcy, Morgan; Witte, Volker; Keller, Laurent; Goodisman, Michael A. D.
Phaeocystis antarctica	8	n/a	44636–44643	HQ132752–HQ135759	Gäbler-Schwarz, Steffi; Leese, Florian; Hayes, Paul K.; Medlin, Linda K.

Table 1 Continued

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Pinus roxburghii	19	n/a	44793–44811	See text for details.	Chauhan, Priti; Ginwal, H.S.; Rawat, Anita; Barthwal, Santan
Potamilus capax	12	n/a	44661–44672	HM991151, HM991153–HM991163	Díaz-Ferguson, E.; Williams, A.S.; Moyer, G.R.

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New microsatellite markers in the longhorn crazy ant, Paratrechina longicornis

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New microsatellite markers in the longhorn crazy ant, *Paratrechina longicornis*

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Abstract

We report the development of 15 microsatellite markers in the longhorn crazy ant, *Paratrechina longicornis*. The loci displayed modest levels of variation (mean of 2.16 alleles per locus) in workers sampled from 14 invasive populations. In addition, almost all loci displayed levels of observed heterozygosity greatly exceeding Hardy-Weinberg proportions, suggesting that *P. longicornis* possesses a nonstandard breeding system. Finally, populations displayed significant genotypic differences. Consequently, the microsatellite markers should prove useful in charting the invasion history and breeding biology of this introduced pest.

Main Text

The longhorn crazy ant, *Paratrechina longicornis*, is one of the most broadly distributed invasive social insects (Wetterer 2008). However, little is known about its breeding biology or patterns of invasion. Molecular genetic markers can provide considerable insight into these basic issues (Avise 2004). Here we report the development of microsatellite markers for *P. longicornis* and document intriguing results arising from analyses of genetic variation.

We constructed a *P. longicornis* genomic DNA library by extracting genomic DNA from ~50 fresh, adult workers using the DNeasy™ kit (Qiagen). DNA was partially digested with the enzyme Tsp509I (New England Biolabs). Fragments 500-1500 bps in size were selected using agarose gel electrophoresis and then purified using the Prep-A-Gene® (Bio-Rad) system. The fragments were then ligated into pBluescript™ II plasmid (Stratagene). Plasmids were then transformed into XL10 Gold™ (Stratagene) competent cells producing a library of ~571,000 cfus.

We screened 960 clones from the library for of DNA microsatellites using the method of Gaublomme *et al.* (2003). Briefly, PCRs conducted in Eppendorf Mastercycler® PCR machines were carried out on individual clones using either the T3 or T7 primer in conjunction with all of the following primers: $(CT)_{10}$, $(TG)_{10}$, $(GAA)_8$, $(TAA)_8$, and $(TGTA)_6(TG)$. The presence of a smeared PCR product visualized on an agarose gel suggested that a microsatellite was present in the clone. PCRs contained a final concentration of 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Fisher, HotMaster™), and 0.5 μM of each primer, in addition to 1 μL of template DNA obtained from a bacterial colony resuspended in 1000 μL of water, in a final volume of 10 μL. PCRs consisted of 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min. In total, 80 clones appeared to contain a microsatellite. These clones were sequenced. The program PRIMER 3 (Koressaar& Remm 2007) was then used to design PCR primers for 29 high-quality loci.

We conducted initial assays of these primers using PCRs that contained a final concentration of 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase, 0.5 μM of each forward and reverse primer, and 1 μL of genomic DNA extracted from ethanol-preserved workers using the Chelex® protocol (Crozier *et al.* 1999) in a final volume of 15 μL. PCR cycling was as described above except that the annealing temperature was locus-specific (Table 2).

Eighteen loci produced consistent bands on agarose gels and were thus further analyzed for variability by labeling the forward primer for each locus with 6FAM and HEX dyes (Sigma-Genosys) and sizing the amplicons on an ABI Prism 3100 DNA Analyzer (Applied Biosystems). The variability of markers was assessed on workers obtained from several populations (Table 1). We used the program GENEPOP (Raymond& Rousset 1995) to determine observed and expected heterozygosities, inbreeding coefficients (F_{IS}), deviations of genotype frequencies from Hardy-Weinberg proportions (probability test), levels of gametic disequilibrium, and significance of differentiation among populations.

Three of the loci were monomorphic. The other 15 loci showed unusual patterns of genetic diversity. Almost all of the variable loci displayed significantly negative F_{IS} values, indicating an *excess of heterozygotes* in populations (Table 2). This pattern was associated with highly significant ($P < 0.001$) gametic disequilibrium among most loci.

To determine if these results arose as an artifact associated with primer development, we genotyped ants from the Oracle population (Table 1) with primers developed from two other species, *Camponotus festinatus* and *C. consubrinus*. The loci Cfes1, Cfes3 (Goodisman& Hahn 2005), Ccon12, Ccon20, Ccon42, and Ccon70 (Crozier *et al.* 1999) successfully amplified in *P. longicornis*. More importantly, *P. longicornis* workers displayed the same fixed heterozygous genotypes at all polymorphic loci (Cfes1, Cfes3, Ccon12, and Ccon70) consistent with findings from our Plon loci. Thus, the unusual patterns of heterozygosity were not an artifact of our development method. Detailed analyses indicate that the high observed heterozygosities in *P. longicornis* result from an unusual mode of reproduction. These results will be described elsewhere.

In addition to the unusual patterns of heterozygosity, most loci displayed relatively low levels of genetic diversity. This may be related to the fact that the ant is introduced in all sampled populations and thus may possess limited genetic diversity due to population bottlenecks. Regardless, significant ($P < 0.05$) genotypic differentiation was present among populations for all loci. Thus the markers will be informative for examining patterns of gene flow among invasive populations.

Acknowledgments

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Table 1. Number (*N*) of *Paratrechina longicornis* workers sampled.

Population	*N*
Scotts Head, Dominica	8
Forres Park, Trinidad and Tobago	8
Port of Spain, Trinidad and Tobago	7
Jupiter, Florida, United States	8
Hamilton, Bermuda	9
Funchal, Madeira	8
Nananu-i-ra Island, Fiji	8
Bonaire, Netherlands Antilles	9
Dry Tortugas, United States	8
Mapusaga, American Samoa	9
Efate, Vanuatu	9
Arnos Vale, St. Vincent and the Grenadines	9
Oracle, Arizona, United States	82
Bangkok, Thailand	245

Table 2. Characteristics of 15 polymorphic microsatellite loci in *Paratechina longicornis*.

Locus	Genbank ID(s)	Primers	T_a [1]	Core repeat	$\overline{A}$ [2]	A_T [3]	Size Range	$\overline{H}_O$ [4]	$\overline{H}_E$ [5]	$\overline{F}_{IS}$ [6]
Plon-1.B9[7]	HM210895	F: AACGGGAAGGAAGGTGAGAC	66	$(GA)_3AC(GA)_{10}$	2.000	2	202 - 206	0.996	0.501	-0.991***
		R: AGGGAGAAACCACACACGAG								
Plon-1.H3	HM210893	F: TCAGTGCGATTCACAACCAT	61	$(CT)_{16}$	3.308	14	195 - 225	0.898	0.64	-0.474***
	HM210894	R: TGTAAGTCCGACCCTCAACC								
Plon-3.C4	HM210900	F: CGTACATGCACTCATACAT	55	$(CT)_{14}$	2.071	7	163 - 181	0.455	0.379	-0.190***
		R: GCGCTTCGCACTAGTTTC								
Plon-4.E6	HM210909	F: ACAACGTGCATAAATATCTC	61	$(AC)_7(GC)_6AC(GC)_4$	2.357	6	116 - 129	0.934	0.575	-0.782***
		R: TAGAATTTTATGCGGAAAG								
Plon-5.C3	HM210912	F: GTAGGTCAAATCTCAGTGAA	61	$(GA)_{43}$	1.857	8	157 - 193	0.216	0.312	0.333***
	HM210913	R: GTCATTTTAAGCGATACATT								

Plon-5.F7[7]	HM210910	F: TAAGAGGGGTCACAGCAT R: CTTTTTCATCATCCCTTC	59	$(GA)_{10}$	2.000	2	160 - 172	0.996	0.501	-0.992***
Plon-7.H4	HM210915	F: TGTCGACTACAGTTCACTATC R: GAACTTTAATTCGGTCATC	61	$(CT)_5TT(CT)_6$	1.500	4	152 - 160	0.431	0.250	-0.771**
Plon-8.A8	HM210916	F: TTCATCTTTCCGATAGTTC R: TCCTCTACACTCAGAGATTG	55	$(CT)_{11}$	2.000	7	162 - 190	1.000	0.556	-0.963***
Plon-8.F2	HM210921	F: AGCGACGATTCGCTTTTA R: CCTCTCTTTTCGATCACAAC	61	$(AC)_9$	2.357	8	159 - 182	0.968	0.592	-0.769***
Plon-8.G5[7]	HM210919 HM210920	F: TAAGCTCTCGTCTTCATTAC R: GCTTAAACGAAACTCACAC	61	$(CT)_{13}$	2.000	2	340 - 368	0.996	0.501	-0.992***
Plon-8.G7	HM210917	F: TATATAGCGATTCTGCTTTT R: CGTTAAGTTAAATGAAGCTC	55	$(CT)_{10}$	1.077	2	145 - 147	0.009	0.023	0.636

Plon-9.A4[7]	HM357722	F: AAGCGCAAAAGAGAGACTGC	65	$(CT)_{12}$	3.000	3	159 - 175	1.000	0.627	-0.597***
		R: GCGGGCGAGAAGTGCATC								
Plon-10.B7[7]	HM210934	F: ATCGTTAGTAAGGAAGGAAC	61	$(CT)_{38}$	3.000	3	210 - 223	1.000	0.627	-0.598***
	HM210935	R: GAGCAAAGATAGATGGATAG								
Plon-10.E7[7]	HM210937	F: TCGTTCCATGTAACACATA	59	$(TA)_7$	2.000	2	183 - 195	0.996	0.501	-0.991***
		R: GCATCAACCGTAATTTAGTA								
Plon-10.F11	HM210929	F: AGTAACTTAAGATCCTGACG	61	$(AC)_3(AG)_7A(AG)_7$	2.000	2	226 - 228	0.970	0.532	-0.948***
		R: AGAGAGTGTCAAAGGAGAGT								

[1] Annealing temperature.
[2] Mean numbers of alleles per population.
[3] Total numbers of alleles in all populations combined.
[4] Mean observed heterozygosity across all populations.
[5] Mean expected heterozygosity across all populations.
[6] Mean F_{IS} across all populations. Significance of deviation from Hardy-Weinberg proportions: ** P < 0.01, *** P < 0.001.
[7] Measures of genetic diversity obtained from Bangkok population only.