C and tamara size standard). Mendelian inheritance of alleles was determined by examining the amplified products in two or more full sib families per locus (both parents and 15–20 offspring in each family). To determine allele range and population-level variability, two wild groups of *C. virginica* were examined: one from Virginia Beach, Virginia (latitude

with *C. gigas* ($n = 5$ each from two populations), *C. angulata* ($n = 5$), *Saccostrea glomerata* (formerly *S. commercialis*; $n = 5$) and *Tiostrea chilensis* ($n = 5$). Statistical analyses were performed using GENEPOP version 3.1 (Raymond & Rousset 1995).

Of the 10 primer sets, all amplified from *C. virginica* products of the size expected from insert sequences. Three yielded homologous products and seven loci (Cvi-6, Cvi-7, Cvi-8, Cvi-9, Cvi-11, Cvi-12, and Cvi-13) were polymorphic (Table 1). All seven polymorphic loci exhibited Mendelian segregation. The Virginia *C. virginica* population was surveyed for variation at all seven loci and the Connecticut population was surveyed for Cvi-6, Cvi-8, and Cvi-11. Only three of the seven loci conformed approximately to Hardy–Weinberg equilibrium (Cvi-9, Cvi-11, and Cvi-12). For all loci, observed heterozygosity was lower than expected, suggesting the common occurrence of segregating null alleles. No evidence for linkage was observed among these seven loci. Allelic distribution was significantly different between the two wild *C. virginica* populations (Fisher exact test $P < 0.0001$). When tested with *C. gigas*, *C. angulata*, and *S. glomerata*, four of the 10 primer sets (Cvi6, Cvi9, Cvi12, Cvi13) yielded various homologous products differing substantially in size from the allele sizes observed for *C. virginica*. No amplification was observed for *T. chilensis*.

Acknowledgements

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Microsatellite markers for *Rhytidoponera metallica* and other ponerine ants

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The ant genus *Rhytidoponera* (subfamily Ponerinae) contains 104 described species (Bolton 1995) which are remarkably diverse in their social organization and mating system (Crozier & Pamilo 1996). The greenhead ant *Rhytidoponera metallica* is among the most common ants in Australia, and it possesses an unusual social structure, as the reproductive role is almost invariably taken by multiple mated workers in lieu of queens (Haskins & Whelden 1965). This secondary loss of queens and partitioning of reproduction between morphologically undifferentiated workers offers a good opportunity to study how altruism is maintained in societies with low relatedness (Hamilton 1972). Such studies require detailed genetic data on the social organization and mating system. Recently, microsatellite markers have been described in three species of ponerine ants (Doums 1999; Giraud *et al*. 1999; Tay & Crozier 2000), but only two microsatellites from the most related species proved useful in *R. metallica*. Therefore, we characterized eight new microsatellite markers for *R. metallica*, and tested for cross-species amplification in 10 other species of ponerine ants.

A partial genomic library was constructed from 100 *R. metallica* workers, with gasters removed. DNA was extracted with a CTAB protocol (Hillis *et al*. 1990), digested to completion with *Sau*3A I and *Rsa*I, size-selected for fragments between 300 and 900 bp (Crozier *et al*. 1999), and ligated into a pUC19 vector. The library was screened with an $(AG)_{10}$ oligonucleotide probe end-labelled with ^{33}P, and 62 positive recombinant clones were isolated. Thirty positive clones were sequenced, and primers were designed for 14 of them.

These primers were assayed on a sample of workers collected from the You Yangs Regional Park in Victoria. DNA

Table 1 Characteristics of nine microsatellite loci for *Rhytidoponera metallica*. *n*, number of individuals analysed; *N*, number of nests analysed; H_O, observed heterozygosity; H_E, expected heterozygosity. Deviations from Hardy–Weinberg equilibrium are not significant (exact tests). GenBank accession nos: AF282988–AF282998, AF292086

Locus	Primer sequence (5′–3′)	Core repeat in cloned allele	*n/N*	No. alleles	Size range	H_O	H_E
Rmet3	F: TCTCGGAAAAGAAATAGAGACAG	$(GA)_{40}$	23/13	10	226–248	0.74	0.84
	R: CATGTCTACCTGACCGAGAAC						
Rmet4	F: CATACTATCGCTTATCTCAGC	$(CT)_{26}$	14/14	11	152–178	1.00	0.87
	R: GAACTAACCTCATCGTCCACT						
Rmet7	F: AGACTTCAATCACGAGAAGCG	$(AG)_{30}$	216/27	21	223–269	0.86	0.86
	R: ATTGGCACTTGGTCGATAGG						
Rmet8	F: AAAACACGAGATACCGTCCTC	$(CT)_{50}$	27/13	15	108–144	0.96	0.88
	R: CTGTTGACCCGCCTCCTG						
Rmet10	F: GTCATGGACGGAAATCGC	$(CT)_{37}$	216/27	23	246–296	0.91	0.89
	R: TACCCCCATTCTATCTCGCA						
Rmet12	F: GGAGTTTCTACTCGCCTCTCG	$(GA)_{20}$	216/27	15	275–315	0.85	0.87
	R: CTCATTCGTATCACGCAAGC						
Rmet15	F: CATTCGACCGCATTTTCC	$(AG)_{28}$	216/27	9	154–202	0.44	0.42
	R: CGAGAGAGGGTGCGACAT						
Rmet16	F: TTTAGGGACAAGAGACATGGC	$(CT)_{40}$	18/14	17	117–203	1.00	0.92
	R: ATTGATAGGTCGCGGTCTTG						
Rh12–13525	F: GACATACCGGGAGCGACC	$(CT)_{11}$	216/27	7	178–192	0.72	0.70
	R: CGCCTTCTGACACCTTTGG						

from individual workers was extracted by incubating three crushed legs in 250 µL of 5% Chelex at 95 °C for 20 min (Crozier *et al.* 1999). Amplification was carried out in 10 µL final volume with 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl$_2$, 1.7 µM each dNTP, 0.03–0.05 µM forward primer end-labelled with ^{33}P, 0.4 µM reverse primer, 5 µg of BSA, 0.4 U of *Taq* DNA polymerase (Promega) and 2 µL of template DNA. The polymerase chain reaction (PCR) profile consisted of a 3-min initial denaturation step at 94 °C, followed by 30 cycles of 30 s at 92 °C, 30 s at 50 °C and 30 s at 72 °C. PCR products were separated by electrophoresis through 6% denaturing polyacrylamide gels.

Eight primer pairs yielded suitable amplification products. All eight markers were highly polymorphic, with between nine and 23 alleles detected in the study population (Table 1). Alleles were somewhat difficult to score for Rmet8 and Rmet16, because of stutter bands. Additionally, the previously unpublished marker Rh12–13525, which was developed by W. Tek Tay for *Rhytidoponera* sp. *12*, (Tay & Crozier 2000) had seven alleles in *R. metallica* (Table 1).

The success of cross-species amplification in other genera was low (Table 2). Scorable amplification products were obtained in only 12 out of the 45 tests (27% of the five species assayed for nine markers). Polymorphism among three individuals was detected at a single marker in four species, i.e. in 9% of the 45 tests.

In contrast, the success of cross-species amplification within the genus *Rhytidoponera* was very high (Table 2). Priming sites were well conserved among the *Rhytidoponera* species tested, resulting in strong amplification products in 40 out of the 45 tests (89%). Overall, scorable polymorphism among three individuals was detected in 23 out of the 45 tests (51%). In each species of *Rhytidoponera*, between three and

eight markers were polymorphic, and this figure should increase when more individuals are analysed. Hence, this panel of microsatellites will permit detailed studies of kin structure, breeding system, gene flow and population structure across species of *Rhytidoponera* with variable social structures. Additionally, these markers might help to distinguish between the species yet to be described that are currently lumped into the *metallica* species-complex.

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Table 2 Results of cross-species amplification in 10 other ant species from the subfamily Ponerinae

	Rmet3	Rmet4	Rmet7	Rmet8	Rmet10	Rmet12	Rmet15	Rmet16	Rh12–13525
Tribe ECTATOMMINI									
Rhytidoponera tasmaniensis	+5	+3	—	+5	+2	+3	+2	+2	+4
R. victoriae	+2	+1	+1	+1	+2	+2	+1	—	+2
R. purpurea	+1	+1	+2	—	+1	+3	+2	+1	+4
R. impressa	+1	+1	+5	—	+1	+2	s	+1	+4
R. confusa	+1	+1	+3	—	+2	+1	s	+2	+3
Gnamptogenys menadensis	+1	—	—	—	—	+1	+2	—	—
Tribe PONERINI									
Diacamma cyaneiventre	+3	—	—	—	—	—	+1	—	—
D. ceylonense	+2	—	—	—	—	—	+1	—	—
Harpegnathos saltator	+3	—	—	—	+1	—	+1	—	—
Streblognathus aethiopicus	—	—	—	—	—	+1	+1	—	—

+ n, scorable amplification product with n alleles detected in three individuals.

—, no scorable amplification product.

s, present of supernumerary amplification products.

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Rapid and efficient identification of microsatellite loci from the sea urchin, *Evechinus chloroticus*

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The New Zealand Fiords are characterized by a seawardly flowing surface low salinity layer (LSL), produced by prodigious rainfall. Maintenance of salt balance occurs by a weak oceanic inflow below this LSL. Because the flow of sea water is inwards, planktonic larvae of the fiords are thought to be retained within natal fiords, which could have important consequences on gene flow. This hypothesis was supported by allozyme analyses of *Evechinus chloroticus*, a sea urchin endemic to New Zealand (Mladenov *et al.* 1997). Despite high levels of gene flow found amongst all coastal populations of New Zealand, a population sampled within one fiord was found to be genetically differentiated. Our intention is to address the effects of oceanographic and hydrographic features of all 14 fiords on recruitment and population structuring of *E. chloroticus*. In order to do this we are using highly polymorphic microsatellite markers.

Traditional colony hybridization methods used for microsatellite cloning are time-consuming and relatively inefficient. Several enrichment techniques have previously been published (Gardner *et al.* 1999; Inoue *et al.* 1999). However, these either use radioactivity or include a number of lengthy steps. We report here on an alternative easy, fast, efficient and nonradioactive method of cloning microsatellite markers from the sea urchin *E. chloroticus*.

Size selected fragments (250–800 bp) of *Nde*II-digest genomic DNA from five individuals of *E. chloroticus* were ligated into pUC18 vector (Pharmacia). Inserts were amplified using universal primers (M13) and purified with High Pure PCR product purification Kit (Roche).

In order to hybridize DNA to probes, 100–500 ng of size selected amplified DNA (250–800 bp) was mixed, in separate tubes, with 2 pmol of GA_{12} and GT_{12} 5'-biotinylated repeat probes in 20 µL of extension solution containing: 0.2 mM of each dNTP, 2 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 0.5 U *Taq* DNA polymerase (Roche). This mixture was subjected to one round of polymerase chain reaction (PCR) (5 min at 94 °C, 1 min at 55 °C, 10 min at 72 °C) using a PTC-100 thermal cycler (MJ Research). Purified products were added to Streptavidin MagneSphere Paramagnetic Particles (Promega) and incubated for 15 min at room temperature with 120 µL of 6× SSC/0.1% SDS, mixed continuously. After a series of washes in 150 µL of 6× SSC/0.1% SDS for 15 min: once at 60 °C, 65 °C, 70 °C, 75 °C and twice in 150 µL of 6× SSC at 80 °C, DNA was eluted with 100 µL of 0.1 M NaOH at 80 °C for 10 min. The solution was neutralized with 100 µL TE pH 7.5, purified and amplified as above. A further round of enrichment (hybridization, elution, PCR) was then undertaken.

Size selected fragments (250–800 bp) of *Nde*II-digest from enriched inserts were ligated into pUC18 vector. Ligation