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Research article

Seven polymorphic microsatellite loci in honeybees (*Apis mellifera*)

D.J. Rowe[1], T.E. Rinderer[2], J.A. Stelzer[2], B.P. Oldroyd[1,3] and R.H. Crozier[1,*]

[1] *School of Genetics and Human Variation, La Trobe University, Bundoora, Victoria, 3083 Australia, e-mail: DJR: gendjr@lure.latrobe.edu.au, RHC: genrhc@lure.latrobe.edu.au*
[2] *Honey-Bee Breeding, Genetics and Physiology Research, Baton Rouge, LA 70820, USA, e-mail: TER: trindere@asrr.arsusda.gov, JAS: jstelzer@asrr.arsusda.gov*
[3] *Present address: School of Biological Sciences, Sydney University, Sydney, N S W, 2006 Australia, e-mail: boldroyd@abio.usyd.edu.au*

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Summary

Seven microsatellite markers identified by probing clones made from a honeybee population from Victoria, Australia with a GA oligonucleotide are polymorphic in collections of honeybees from Iberia. Five of the microsatellites are GA repeats (three perfect repeats, two imperfect repeats, and one interrupted perfect repeat); of two others found in $(GA)_n$-containing clones, one is a perfect AT repeat and one a mononucleotide T repeat. A further perfect GA repeat is monomorphic in the Iberian bees. Primer sequences are given for each of these microsatellites, and for seven others either not amplifying or giving multiple bands under the conditions used, or which were not tested. In all, eighteen microsatellites were found in twelve clones. One clone contained a minisatellite repeat, not assayed for polymorphism.

Introduction

Populations of Hymenoptera, both eusocial and non-eusocial, are generally believed to have relatively low levels of polymorphism at allozyme loci (Graur, 1985; Pamilo and Crozier, 1981; Pamilo et al., 1978; Shoemaker et al., 1992), although the generality of this tendency for non-eusocial forms needs further investigation (Sheppard and Heydon, 1986). In populations where allozymic variation is relatively low, such as most honey bee populations, population genetic and sociobiological analyses are hindered. Much attention is therefore now being given to DNA markers in the search for polymorphisms adequate for such studies. DNA markers can be divided into substitution differences, such as restriction site variation (Nei

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* Author for correspondence.

and Li, 1979) and RAPDs (Welsh and McClelland, 1990; Williams et al., 1990) and length variations, involving either minisatellites ["DNA-fingerprinting" (Jeffreys et al., 1985)] or microsatellites (Weber and Wong, 1993).

Minisatellites are tandem repeats of length 15 bp or more, generally located in intergenic spaces. Microsatellites have a very short repeat length of 1–6 bp, which can occur in any area of the genome (although microsatellites within protein coding regions are expected to generally be trinucleotide repeats because otherwise the DNA reading frame is disrupted). Microsatellites show a high mutation rate thought to involve replication slippage due to mispairing of the DNA (Li and Graur, 1991); a well-known insect example within a coding region is that in the *Drosophila per* gene (Rosato et al., 1994). Minisatellites are typically studied using appropriate probes and restriction enzyme digests of total DNA, whereas microsatellite variation is revealed as the size of products amplified using the polymerase chain reaction and primers matching DNA sequences flanking the variable region.

Most current information on population structure and relatedness in social insects is based on allozyme data (Crozier and Pamilo, 1996), but DNA markers are being increasingly used following their first use in the study by (Davis et al., 1990) using restriction site variation in the ribosomal RNA genes of *Polistes* wasps. Restriction site variation has also been used to demonstrate genetic polyethism in the dwarf honeybee *Apis florea* (Oldroyd et al., 1994b). RAPDs have been used in the mapping of the honeybee genome (Hunt and Page, 1992; Hunt and Page, 1994; Hunt and Page, 1995), paternity analysis in honeybee colonies (Fondrk et al., 1993), and in a study of a fire-ant hybrid zone (Shoemaker et al., 1994).

DNA fingerprinting has been used to determine relatedness within colonies of honeybees (Blanchetot, 1991; Moritz, 1993; Moritz et al., 1991), halictid bees (Mueller et al., 1994), *Camponotus* ants (Heinze et al., 1994), and solitary bees (Blanchetot, 1992) and wasps (Pfennig and Reeve, 1993).

Although there are methods for estimating relatedness from multilocus DNA fingerprinting (Reeve et al., 1992), such analyses are complicated by the difficulty of assigning bands to alleles. Similar problems exist with the analysis of RAPD data. Single-locus DNA fingerprinting is achievable using sufficiently specific probes (e.g., Moritz, 1993; Moritz et al., 1991), and is usually then amenable to precise genetic analysis.

Because of the more technically difficult molecular biological procedures required for the development of microsatellite markers (Glenn, 1995), these markers have only relatively recently been developed for social insects where, however, they are proving extremely valuable sources of prolific genetic variation. Microsatellite markers have been reported for honeybees (Estoup et al., 1994; Estoup et al., 1993; Estoup et al., 1995b; Hall, 1991; Oldroyd et al., 1994a), bumblebees (Estoup et al., 1995a; Estoup et al., 1993), vespid wasps (Choudhary et al., 1993; Hughes and Queller, 1993; Thorén et al., 1995), and myrmicine (Evans, 1993; Hamaguchi et al., 1993) and formicine ants (Gertsch et al., 1995). Queller et al. (Queller et al., 1993) give an introduction to the use of microsatellites in social insect studies. Indicative of the power of the method is the ability to type males from their sperm in a female's spermatheca (Evans, 1993; Peters et al., 1995). Also encouraging is the fact that some microsatellites developed for *Apis* reveal variation in *Bombus*, and *vice versa*, although the proportion of such portable markers is small (Estoup et al., 1993).

Microsatellite markers, being highly characterised and easily-analysed genetic markers, present the prospect of not only tracing specific chromosome segments in populations but a more powerful means of locating loci important in sociality than anything available before. The demonstration that some genotypes within honeybee colonies escape worker policing (Oldroyd et al., 1994a) illustrates the potential afforded by such highly variable loci. Tanatalizing glimpses of a true genetics of sociality (Ross, 1992; Ross and Shoemaker, 1993) are likely to give way to a clear vision of the genetical evolution of social behavior within a few decades. For this reason it is important to develop further microsatellite markers and to determine their linkage relationships.

In this paper we present primer sequences for 18 honeybee microsatellite markers along with data on their variation in bees from a transect of 30 collections across the Iberian peninsula as part of a larger study.

Materials and methods

DNA was extracted from thoraces of three honeybees from a single colony from Wyperfeld National Park (Victoria, Australia), using an established protocol (Oldroyd et al., 1995), modified for the volumes involved. Approximately 1 µg of DNA was digested to completion with Sau3A and Hae*III*, electrophoresed in a 2% NuSieve (FMC) agarose gel and fragments between 600–800 bp were extracted from the gel using the Prep-A-Gene DNA purification system (BioRad). Phagemid vector pTZ 18R (Pharmacia) was digested with Bam*HI* and Hinc*II*, cleaned with Prep-A-Gene and a filter unit (ultrafree-MC 30,000, Millipore), ligated to the bee DNA and transformed in *Escherichia coli* XL1 Blue (Stratagene) cells.

Colony lifts were taken with nylon membranes (Hybond N+, Amersham) to screen approximately 2,000 white colonies. Prehybridization was carried out for 10 min at 65 °C in 60 ml of Church and Gilbert buffer (Church and Gilbert, 1984) and then 50 pmoles of $(GA)_{10}$ end-labelled with 60 µCi of γ^{33}ATP (activity 1300 to 2000 Ci/mMol) and 25 units of T4 PNK was added to the hybridization solution. After a 20 min hybridization at 65 °C, the membranes were washed in 6 × SSC for 10 min at 65 °C and then in 2 × SSC at 55 °C for another 10 min. The final wash was repeated twice. After overnight exposure of the membranes to Kodak X-AR film, positive colonies were picked, restreaked on selective agar plates, and rescreened to confirm the initial positive signals. Plasmid DNA was prepared from the positive clones using a standard miniprep protocol (Sambrook et al., 1989) and the inserts were sequenced by cycle sequencing using the fmol™ DNA sequencing kit (Promega) and pUC/M13 forward and reverse primers end-labelled with γ^{33}ATP.

Polymerase chain reaction (PCR) primers flanking microsatellite sequences were determined and sent to Baton Rouge where the primers were made and used to assay the Iberian bees.

Template DNA for PCR reactions was extracted using a 10% Chelex solution (Biorad). Degastered bees were ground in aliquots of 400 µl of solution, vortexed for 10 minutes, incubated at 56 °C for 30 minutes, vortexed for 10 seconds, boiled for 5 minutes, vortexed for 10 seconds, then spun for 3 minutes at 12000 rpm. Template DNA was then stored at –20 °C until used. PCR was carried out in 10 µl

volumes with 0.5 µl of template DNA solution, 164 µmol dNTP, 0.8 µmol of one primer (cold), 0.11 µmol of the other primer endlabelled with $\gamma^{33}P$, 200 ng BSA and 0.4 units of Taq Polymerase (Promega) in 1× buffer (Promega). Thermal cycling was standard for all primers (with specific annealing temperatures as shown in Table 1): 3 min initial denaturation at 94 °C, then 30 cycles of 94 °C for 45 sec, 45 sec at the primer-specific annealing temperature and 72 °C for 45 sec, the last cycle being followed by a 10 min extension step at 72 °C. The PCR products were electrophoresed on 5 % polyacrylamide denaturing gels together with sequencing ladders made using pBR322 as template, for sizing the products.

Results

Twelve clones, among approximately 2,000 showing inserts, were identified using the $(GA)_{10}$ probe and sequencing identified eighteen microsatellite sequences and one putative minisatellite sequence (GenBank accession numbers U73917–U73934). In some instances microsatellites with different repeat motifs occurred close together in the same clone. The occurrence of more than one microsatellite sequence in several clones accords with the observation that microsatellite sequences are not evenly distributed in the apid genome (Estoup et al., 1993).

Primer pairs were devised to sixteen potential microsatellite markers (Tables 1, 2); of these eight were assayed for polymorphism at Baton Rouge and several were found to be variable in the Iberian bees, with numbers of alleles varying from two to 18 (Table 2). Table 3 lists primer pairs (and predicted repeat sequence lengths) which have not been assayed for variability but which may be useful to other researchers.

Clone GH contained an apparent minisatellite sequence; the highly repeated sequence immediately adjacent to the minisatellite sequence proved refractory to sequencing, perhaps indicating hairpin loop formation (between GT and AC repeating regions).

Discussion

Our assays of primer sets using the Iberian bees show that seven of these reveal polymorphism, and of these five determine highly polymorphic loci with more than five alleles each. Those primers which show little or no variability in our test bees may well reveal variation in other areas; possibly also in non-apid bees.

The rapid hybridization method described here significantly reduced the time between cloning and sequencing. Selecting large inserts (600 to 800 bp) for cloning detected some microsatellite sequences different from the (GA/CT) probe motif, probably reflecting concentration of dinucleotide microsatellites through their joint occurrence in non-coding regions. With automated methods for sequencing which can reliably generate more than 500 base pairs of sequence from purified plasmid DNA, cloned inserts greater than 500 bp can be sequenced. A marked improvement in the efficient isolation and characterization of microsatellite loci is possible using these methods together.

Table 1. Microsatellite motifs and primer pairs. The third column shows the outcome of PCR amplification; "–" indicates that recorded primers were not synthesised

Micro-satellite	Repeat motif	Amplification	Primers and annealing temp (°C)
B7	perfect GA repeat $(GA)_{12}$	no priming	B7-f B7-r 68 °C
CD	imperfect interrupted GA repeat $(GA)_7AA(GA)_8GTGGGGAGGG$ $(AG)_3GAAGGAAGAAAGACA$ $GAGGAGGGAGAGAGG(GA)_8$	18 alleles 289–321 bp	CD-f CD-r 54 °C
CK	perfect CT repeat interrupted with tetranucleotide TATC repeat $(CT)_{14}(ATCT)_4(CT)_4$	no priming	CK-f CK-r 56 °C
DJ-F	imperfect poly-T mononucleotide repeat $(T)_7(T)_8A(T)_7(T)_7AC(T)_4C(T)_3$	4 alleles 149–152 bp	DJ1-f DJ1-r 58 °C
DJ-R	perfect GA repeat $(GA)_{10}$	monomorphic 270 bp	DJ2-f DJ2-r 58 °C
ED-F	perfect CT repeat $(CT)_8NNNN(CT)_{10}$	17 alleles 174–206 bp	ED1-f ED1-r 62 °C
ED-R	perfect poly-A mononucleotide repeat $(A)_{48}$	no priming	ED2-f ED2-r 56 °C
FE1	interrupted CT repeat $(CT)_5TTT(CT)_{13}$	–	FE1-f FE1-r 60 °C
FE2	perfect GA repeat $(GA)_{23}$	–	FE2-f FE2-r 58 °C
FM-F	perfect GA repeat followed by a perfect TA repeat $(GA)_{23}(N)_{130}(AT)_8$	multiple bands	FM1-f FM1-r 60 °C
FM-R	2 × perfect AT repeats $(AT)_{10}(N)_{149}(AT)_9$	12 alleles 291–303 bp	FM2-f FM2-r 56 °C
GH-F	minisatellite-like repeat ATAAGTACCAGCTAAATTT TTTTTTTTTTTT	no priming	GH1-f GH1-r 60 °C
GH-R	perfect GA repeat $(GA)_{13}$	3 alleles 125–128 bp	GH2-f GH2-r 54 °C
GJ	perfect GA repeat $(GA)_{18}$	multiple bands	GJ-f GJ-r 60 °C
HC	perfect GA repeat $(GA)_{15}$	–	HC-f HC-r 56 °C
IC-F	interrupted perfect GA repeat $(AG)_{13}(N)_{39}(GA)_{13}$	5 alleles 209–215	IC1-f IC1-r 58 °C
IC-R	imperfect CT repeat $(CT)_9GTAT(CT)_9$	2 alleles 197 and 198 bp	IC2-f IC2-r 54 °C
IM	perfect GA repeat $(GA)_{15}$	12 alleles 203–228 bp	IM-f IM-r 56 °C

Table 2. Microsatellite primer sequences and corresponding allele frequencies observed in Iberian honeybees

Micro-satellite	Allele lengths (bp) Numbers seen							Primer sequences (5′ to 3′)-name	
CD	289	290	291	296	297	304		CAGAAATATTTCCATATAC	-CD1
	2	1	1	1	1	5		CTTTATCCACGCTTTGAGC	-CD2
	305	306	307	309	310	311			
	4	7	2	4	4	2			
	312	313	314	315	317	321			
	2	2	2	1	2	1			
							n = 44		
DJ1	149	150	151	152				TTCGAGATTCTTCGATGGGGC	-DJ1-f
	10	29	7	8			*n = 54*	AAGATTATTTCTTATCATTAAGC	-DJ1-r
DJ2	270							GAAAGACGATTGCCAAAAGC	-DJ2-f
	48						*n = 48*	CGATATTTAAAAATCGATTGAAC	-GJ2-r
ED1	174	175	176	177	179	181		CAACAGCCGTGAACGCTATC	-ED1-f
	1	2	1	2	4	8		TCATCGTGTACCAATAACG	-ED1-r
	182	183	184	185	186	187			
	1	2	6	3	3	2			
	188	190	203	204	206				
	1	1	1	1	2		*n = 44*		
FM2	291	292	293	294	295	296		ATTCCCGGTATCATCTCTTG	-FM2-f
	4	14	1	9	1	1		AATTCGTGGTTAAATTCAAAG	-FM2-r
	297	298	300						
	1	1	4				*n = 40*		
GH2	125	127	128					CGGAATATAGTCAAGAAAAT	-GH2-f
	27	11	2				*n = 40*	GATGTATATTTTTTTCTCTTG	-GH2-r
IC1	209	211	212	213	215			GGTTTGATGCTCGTAAGGA	-IC1-f
	3	33	1	1	6		*n = 44*	GGCACCTCTTGCCATCTG	-IC1-r
IC2	197	198						GGATCGATGCGCCTACA	-IC2-f
	22	18					*n = 40*	CTTCCCTTTTCCACGTAG	-IC2-r
IM	203	204	206	207	209	211		ACGCAAATGACAAGTATTAG	-IM1
	3	1	2	7	1	24		GAGTGTATTTCGAAATCGATG	-IM2
	212	213	214	220	227	228			
	1	1	3	15	1	1			
							n = 60		

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Table 3. Microsatellite primer sequences not assayed for variability using the Iberian bees

Microsatellite	Primer name	Primer sequences 5′ to 3′	Expected length (bp)
B7	B7-f	AGTATGAATTACCGATTAACC	170
	B7-r	CCTTTGGATAATTACTTTCC	
ED-R	ED2-f	GCGTTATTATTATTTATCGAG	130
	ED2-r	GAGTTTGAATTGGTAACCG	
FE1*	FE1-f	CATCGCTCGCGATTCTATAG	180
	FE1-r	ATGTCGTCTCGATGGCTACC	
FE2*	FE2-f	CTTGGTCGACCGATATTCG	120
	FE2-r	CCCGCCTTCGTTCCCGG	
FM-F	FM1-f	CCCCTTCCTCGTCCAACAGC	270
	FM1-r	CAATAATCATTCACAATAAAC	
GH-F	GM1-f	TGCTTTGCATCGCGAA	220
	GM1-r	AAATTTCAGAAAGCAATAC	
GJ	GJ-f	GACAATGGACACCTCAAAAGC	130
	GJ-r	CGCAGACTTTCTCCCGAAAG	
HC*	HC-f	TTTTCCATTCGCGGGACG	110
	HC-r	ACGTTTCACCGCAAATATGC	

* Primers not tested.

References

Blanchetot, A., 1991. Genetic relatedness in honeybees as established by DNA fingerprinting. *J. Hered. 82*:391–396.

Blanchetot, A., 1992. DNA fingerprinting analysis in the solitary bee *Megachile rotundata*: variability and nest mate genetic relationships. *Genome 35*:681–688.

Choudhary, M., J.E. Strassmann, C.R. Solís and D.C. Queller, 1993. Microsatellite variation in a social insect. *Bioch. Genet. 31*:87–96.

Church, G.M. and W. Gilbert, 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA 81*:1991–1995.

Crozier, R.H. and P. Pamilo, 1996. *Evolution of social insect colonies. Sex allocation and kin-selection.* Oxford University Press, Oxford, UK.

Davis, S.K., J.E. Strassmann, C. Hughes, L.S. Pletscher and A.R. Templeton, 1990. Population structure and kinship in *Polistes* (Hymenoptera, Vespidae): An analysis using ribosomal DNA and protein electrophoresis. *Evolution 44*:1242–1253.

Estoup, A., P.A. Scholl and M. Solignac, 1995a. Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. *Mol. Ecol. 4*:89–93.

Estoup, A., M. Solignac and J.-M. Cornuet, 1994. Precise assessment of the number of partrilines and of genetic relatedness in honeybee colonies. *Proc. R. Soc. Lond. B 258*:1–7.

Estoup, A., M. Solignac, M. Harry and J.-M. Cornuet, 1993. Characterization of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nucleic Acids Res. 21*:1427–1431.

Estoup, A., C. Tailliez, J.-M. Cornuet and M. Solignac, 1995b. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). *Mol. Biol. Evol. 12*:1074–1084.

Evans, J., 1993. Parentage analysis in ant colonies using simple sequence repeat loci. *Mol. Ecol. 2*:393–397.

Fondrk, M.K., R.E. Page and G.J. Hunt, 1993. Paternity analysis of worker honeybees using randomly amplified polymorphic DNA. *Naturwissenschaften 80*:226–231.

Gertsch, P., P. Pamilo and S.-L. Varvio, 1995. Microsatellites reveal high genetic diversity within colonies of *Camponotus* ants. *Mol. Ecol. 4*:257–260.

Glenn, T.C., 1995. *Microsatellite manual, version 6.* Available electronically via FTP: onxy.si.edu/protocols/msatmanV6.rtf.

Graur, D., 1985. Gene diversity in Hymenoptera. *Evolution 38*:190–199.

Hall, H.G., 1991. Genetic characterization of honey bees through DNA analysis. In: *The "African" honey bee.* (M. Spivak, D.J.C. Fletcher and M..D. Breed, Eds.), Westview Press, Boulder, CO. pp. 45–73.

Hamaguchi, K., Y. Ito and O. Takenaka, 1993. GT dinucleotide repeat polymorphisms in a polygynous ant, *Leptothorax spinosior* and their use for measurement of relatedness. *Naturwissenschaften 80*:179–181.

Heinze, J., J. Gadau, B. Hölldobler, I. Nanda, M. Schmid and K. Scheller, 1994. Genetic variability in the ant *Camponotus floridanus* detected by multilocus DNA fingerprinting. *Naturwissenschaften 81*:34–36.

Hughes, C.R. and D.C. Queller, 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Mol. Ecol. 2*:131–137.

Hunt, G.J. and R.E. Page, 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theor. Appl. Genet. 85*:15–20.

Hunt, G.J. and R.E. Page, 1994. Linkage analysis of sex determination in the honey bee (*Apis mellifera*). *Molec. Gen. Genet. 244*:512–518.

Hunt, G.J. and R.E. Page, 1995. Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. *Genetics 139*:1371–1382.

Jeffreys, A.J., V. Wilson and S.L. Thein, 1985. Individual-specific "fingerprints" of human DNA. *Nature 316*:76–79.

Li, W.-H. and D. Graur, 1991. *Fundamentals of molecular evolution.* Sinauer, Sunderland.

Moritz, R.F.A., 1993. Intracolonial relationship in the honey bee colony (*Apis mellifera*): molecular evidence and behavioural consequences. *Verh. Dtsch. Zool. Ges. 86*:151–158.

Moritz, R.F.A., M.S. Meusel and M. Haberl, 1991. Oligonucleotide DNA fingerprinting discriminates super- and half-sisters in honeybee colonies (*Apis mellifera* L.). *Naturwissenschaften 78*:422–424.

Mueller, U.G., G.C. Eickwort and C.F. Aquadro, 1994. DNA fingerprinting analysis of parent-offspring conflict in a bee. *Proc. Natl. Acad. Sci., USA 91*:5143–5147.

Nei, M. and W.-H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci., USA 76*:5269–5273.

Oldroyd, B.P., D. Rowe, J.-M. Cornuet, T.E. Rinderer and R.H. Crozier, 1995. Racial admixture of *Apis mellifera* in Tasmania, Australia: similarities and differences with natural hybrid zones in Europe. *Heredity 74*:315–325.

Oldroyd, B.P., A.J. Smolenski, J.-M. Cornuet and R.H. Crozier, 1994a. Anarchy in the beehive. *Nature 371*:749.

Oldroyd, B.P., H.A. Sylvester, S. Wongsiri and T.E. Rinderer, 1994b. Task specialization in a wild bee, *Apis florea* (Hymenoptera: Apidae), revealed by RFLP banding. *Behav. Ecol. Sociobiol. 34*:25–30.

Pamilo, P. and R.H. Crozier, 1981. Genic variation in male haploids under deterministic selection. *Genetics 98*:199–214.

Pamilo, P., S.-L. Varvio-Aho and A. Pekkarinen, 1978. Low enzyme gene variability in Hymenoptera as a consequence of haplodiploidy. *Hereditas 88*:93–99.

Peters, J.M., D.C. Queller, J.E. Strassmann and C.R. Solis, 1995. Maternity assignment and queen replacement in a social wasp. *Proc. R. Soc. Lond. [Biol.] 260*:7–12.

Pfennig, D.W. and H.K. Reeve, 1993. Nepotism in a solitary wasp as revealed by DNA fingerprinting. *Evolution 47*:700–704.

Queller, D.C., J.E. Strassmann and C.R. Hughes, 1993. Microsatellites and kinship. *Trends Ecol. Evol. 8*:285–288.

Reeve, H.K., D.F. Westneat and D.C. Queller, 1992. Estimating average within-group relatedness from DNA fingerprints. *Mol. Ecol. 1*:223–232.

Rosato, E., A.A. Peixoto, G. Barbujani, R. Costa and C.P. Kyriacou, 1994. Molecular polymorphism in the period gene of *Drosophila simulans. Genetics 138*:693–707.
Ross, K.G., 1992. Strong selection on a gene that influences reproductive competition in a social insect. *Nature 355*:347–349.
Ross, K.G. and D.D. Shoemaker, 1993. An unusual pattern of gene flow between the two social forms of the fire ant *Solenopsis invicta. Evolution 47*:1595–1605.
Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular cloning. 2nd ed.* Cold Spr Harbor Lab Press, Cold Spr Harbor.
Sheppard, W.S. and S.L. Heydon, 1986. High levels of genetic variability in three male-haploid species (Hymenoptera: Argidae, Tenthredinidae). *Evolution 40*:1350–1353.
Shoemaker, D.D., J.T. Costa and K.G. Ross, 1992. Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. *Heredity 69*:573–582.
Shoemaker, D.D., K.G. Ross and M.L. Arnold, 1994. Development of RAPD markers in two introduced fire ants, *Solenopsis invicta* and *S. richteri*, and their application to the study of a hybrid zone. *Mol. Ecol. 3*:531–539.
Thorén, P.A., R.J. Paxton and A. Estoup, 1995. Unusually high frequency of (CT)n and (GT)n microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). *Ins. Mol. Biol. 4*:141–148.
Weber, J.L. and C. Wong, 1993. Mutation of human short tandem repeats. *Hum. Molec. Genet. 2*:1123–1128.
Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res. 18*:7213–7218.
Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res. 18*:6531–6535.

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