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PERMANENT GENETIC RESOURCES

Characterization of microsatellite loci in the subsocial spider *Stegodyphus lineatus* (Araneae: Eresidae)

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Abstract

***Stegodyphus lineatus* spiders live in groups consisting of closely related individuals. There appears to be no discrimination against related individuals as mates but females mate multiply, despite the fact that matings are shown to carry a cost. We have developed eight polymorphic dinucleotide microsatellite markers that allow us to assess levels of heterozygosity and relatedness among individuals of this species. These molecular markers are likely to prove highly effective tools for estimating levels of inbreeding and thus allow us to test hypotheses about the relationships between social structure, mating strategies and inbreeding avoidance.**

Keywords: inbreeding, mating system, microsatellites, population structure, spider, *Stegodyphus*

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The evolution of female multiple mating (polyandry) such as is found in the subsocial spider *Stegodyphus lineatus* is intriguing, because a single mating by a female is sufficient to secure the fertilization of eggs while repeated mating is associated with considerable costs (Bilde *et al*. 2005; Maklakov *et al*. 2005). Recent empirical evidence suggests that polyandrous females in some species may bias paternity to maximize the heterozygosity of their offspring, which may translate into survival benefits such as increased resistance against diseases (Hansson & Westerberg 2002; Foerster *et al*. 2003). We have developed molecular markers to allow us to investigate, for the first time, whether such a relationship between mating strategy and genetic variability exists in *S. lineatus.*

A microsatellite dinucleotide-enriched library was obtained from a single *S. lineatus* using the fast isolation by amplified fragment length polymorphism (AFLP) of sequences containing repeats protocol (FIASCO; Zane *et al*. 2002) which is based upon an AFLP approach (Vos *et al*. 1995). Briefly, total genomic DNA was extracted from 25 to 50 mg of tissue according to a standard phenol–chloroform protocol (Sambrook *et al*. 1989) then simultaneously digested with *Mse*I and ligated to an *Mse*I-adaptor (5′-TACTCAGGACTCAT3′/5′-GACGATGAGTCCTGAG-3′). Restricted, ligated fragments were amplified with *Mse*I adaptor-specific primers and hybridized with a biotinylated $(AC)_{17}$ probe for 15 min at room temperature. Molecules hybridized with the probes were selectively captured by streptavidin-coated beads (Roche), separated by a magnetic field and DNA eluted from the bead probes using TE 1× buffer at 95 °C for 5 min. DNA was precipitated using sodium acetate and ethanol, re-amplified with *Mse*I adaptor-specific primers and cloned using the TOPO TA cloning kit (Invitrogen). Clones were screened by polymerase chain reaction (PCR) for microsatellite repeats using M13 forward–reverse primers and sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems). Sequences were resolved on an ABI PRISM 310 Genetic Analyser (Applied Biosystems).

Primer 3 software (Rozen & Skaletsky 1998) was used to design primers to amplify these repeats. Oligonucleotide primers were optimized for PCR amplification testing over a range of annealing temperatures and $MgCl_2$ concentrations using the following PCR mixtures: 20-μL volume containing ~20 ng of genomic DNA, 0.5–2.5 mM $MgCl_2$ (Table 1), 0.5 mM of each primer, 200 μM dNTPs, 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100 and 1 U of *Taq*

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Table 1 *Stegodyphus lineatus* microsatellite loci.

Locus and Accession nos.	Primer sequences (5′–3′)	Repeat in sequenced clone	T_a (°C)	$MgCl_2$ (mM)	No. of alleles (size range)	H_O/H_E population 1 (P)	H_O/H_E population 2 (P)
R4 EU789520	F: HGAGGTGGAATTAAGAAGCTG R: TTGCCGATTAACAACCTAAG	$(CA)_{10}$	55	1.5	4 (187–209)	0.15/0.14 ($P > 0.99$)	0.53/0.77 ($P = 0.08$)
R5N EU789521	F: FCGCTTTCCTGTAACCCATTA R: TTTGCGAGAAAAGCTGACTT	$(CA)_{16}$	55–50*	2.5	7 (252–266)	0.29/0.44 ($P = 0.44$)	0.31/0.76 ($P < 0.01$)†
R7 EU789522	F: FTTCACGTTTCTTCAGTACCAGTT R: GGCCCTGGGTCTATTAGCTT	$(GT)_{16}$	55	1.5	3 (122–132)	0/0.10 ($P = 0.03$)	0.40/0.63 ($P = 0.01$)
R7N EU789523	F: HCGGAAAACCAGAAGTCAAAA R: AGGCATCACTCCTCTTAGGAA	$(GT)_{20}$	55–50*	2.5	2 (140–144)	0.56/0.46 ($P = 0.60$)	0 (1 allele present)
R16 EU789524	F: TCTTACACACAGCACGAACGA R: CCTGAGTAAGGCCGGATAAT	$(CA)_{15}$	55	1.5	5 (210–218)	0.18/0.42 ($P < 0.01$)	0.60/0.76 ($P = 0.26$)
R20 EU789525	F: HCCTGCAATGACATAAAATGA R: TAATGCTAAAGCACCGATGT	$(GT)_{11}$	55	2	2 (217–219)	0 (1 allele present)	0/0.31 ($P < 0.01$)
RP4 EU789526	F: FCGCTTCCCTATAGCCCATT R: CCTACTCCACCCGATTAATTT	$(CA)_{12}$	55	2	2 (279–285)	0/0.44 ($P < 0.01$)	0/0.36 ($P = 0.11$)
V14 EU789527	F: HCATAAACAGATACATACACACA R: CAGTAAGATAAAAGATCAAAGA	$(AC)_{18}$	55–50*	2	3 (134–138)	0.47/0.58 ($P = 0.61$)	0.70/0.60 ($P = 0.87$)

Observed and expected heterozygosities (H_O/H_E) for the two populations tested are given together with the P values for deviation from Hardy Weinberg equilibrium (†indicates significance after Bonferroni correction). Forward primers were fluorescently labelled using the ABI system (H, HEX; F, 6-FAM; T, TAMRA).

Polymerase (Promega) with PCR amplification parameters: 3 min at 94 °C for 1 cycle, followed by 94 °C for 30 s, annealing temperature (see Table 1) for 30 s and 72 °C for 30 s for 35 cycles, followed by 1 cycle of 5 min at 72 °C. Genotyping was performed by allele sizing on an ABI PRISM 310 sequencer, using the ROX HD500 size standard.

Approximately 200 recombinant clones were obtained, 64 were screened for the presence of simple sequence repeats and primers were designed to amplify potential microsatellite loci in 20 of these loci. Primers that reliably amplified a single locus were tested for allelic polymorphism on a total of 40 *S. lineatus* individuals; 20 from each of two geographically distinct populations, Israel and Greece. Eight of these primers were polymorphic (Table 1) and one locus, R2N, amplified successfully but was monomorphic in the samples tested.

Observed and expected heterozygosities for polymorphic loci and tests for linkage disequilibria among loci were calculated using GenePop version 3.4. (Raymond & Rousset 2003). Linkage disequilibrium among loci and deviations from Hardy–Weinberg were calculated using Fisher's exact test with the *P* value estimated using the Markov chain method of Guo & Thompson 1992 (http://genepop.curtin.edu.au/). Mean heterozygosity over all eight loci was 0.14 (Greek population) and 0.39 (Israeli population 2). No evidence was found for linkage among loci. The significance of deviations from frequencies expected under Hardy–Weinberg equilibrium was estimated by permutation (1.000 batches of 10 000 replicates).

Only heterozygote deficit for one locus remained significant after Bonferroni correction for multiple tests. This may reflect the level of inbreeding within this species, which is predicted to be high (Johannesen & Lubin 1999; Bilde *et al*. 2005), suggesting deviation from the expectations of Hardy–Weinberg equilibrium. F_{ST} was calculated between Israeli and Greek populations with a significance test at 10 000 permutations implemented in Genetix 4.03 (www.genetix.univ-montp2.fr/genetix/genetix.htm) indicating genetic differentiation of the two populations ($F_{ST} = 0.29$, $P < 0.001$). Indeed, clustering analyses implemented in Structure 2.2 (http://pritch.bsd.uchicago.edu/structure.html) showed a clear genetic distinction. Values of Q = 93.47 SE = 0.83 for Israel, and Q = 80.58 SE = 2.27 for Greece based on five replications using 1 000 000 permutations (likelihood = 0.99 for $K = 2$) clearly separated the two populations. Hence, the discriminatory power of these markers is highly significant even when the level of genetic variation is low (due to inbreeding), revealing the potential to address evolutionary questions of wide interest.

Tests for linkage disequilibrium between pairs of loci within each population showed only three comparisons to have probabilities of less than 0.05 (population 1: R16 with R5N $P = 0.039$, R5N with R20 $P = 0.046$ and R16 with R20 $P = 0.018$). None of these values was significant after Bonferroni correction ($P > 0.05$). It was noted that some specimens failed to amplify (typically failing for more than one locus). We calculated the expected failure rate if due to null alleles according to Brookfield 1996: $[r_2 = (H_t - H_o)/(1 + H_t]$, and found that the actual failure rate was lower than this value for each locus and population. Failed amplifications are thus most likely due to insufficient good quality template DNA. All microsatellite-containing clone sequences are deposited in GenBank (accession nos EU789520–EU789527). This information has also been deposited in the MEN primer database.

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