*Development of 13 microsatellites for Gunnison Sage-grouse (*Centrocercus minimus*) using next-generation shotgun sequencing and their utility in Greater Sage-grouse (*Centrocercus urophasianus*)*

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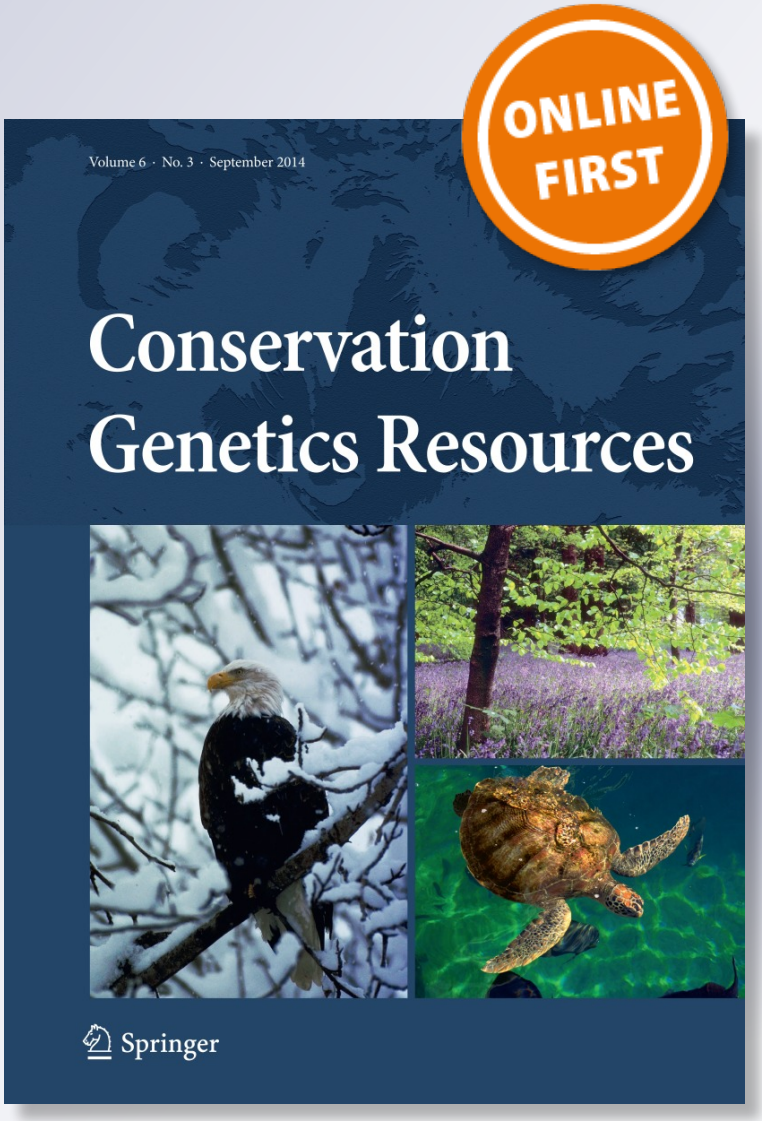

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MICROSATELLITE LETTERS

Development of 13 microsatellites for Gunnison Sage-grouse (*Centrocercus minimus*) using next-generation shotgun sequencing and their utility in Greater Sage-grouse (*Centrocercus urophasianus*)

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Abstract Gunnison Sage-grouse are an obligate sagebrush species that has experienced significant population declines and has been proposed for listing under the U.S. Endangered Species Act. In order to examine levels of connectivity among Gunnison Sage-grouse leks, we identified 13 novel microsatellite loci though next-generation shotgun sequencing, and tested them on the closely related Greater Sage-grouse. The number of alleles per locus ranged from 2 to 12. No loci were found to be linked, although 2 loci revealed significant departures from Hardy–Weinberg equilibrium or evidence of null alleles. While these microsatellites were designed for Gunnison Sage-grouse, they also work well for Greater Sage-grouse and could be used for numerous genetic questions including landscape and population genetics.

Keywords Gunnison Sage-grouse · Greater Sage-grouse · Microsatellites · *Centrocercus minimus* · *Centrocercus urophasianus*

Gunnison Sage-grouse (*Centrocercus minimus*) have experienced significant range reductions and population declines, and as a result, has been proposed for listing under the U.S. Endangered Species Act. The range of the Gunnison Sage-grouse consists of one moderately sized population in the Gunnison Basin surrounded by six small, isolated, satellite populations (Oyler-McCance et al. 2005). Within the Gunnison Basin and throughout the species' range, there is a need to examine levels of connectivity among Gunnison Sage-grouse leks and to analyze those delineations in relation to habitat and anthropogenic variables, allowing managers to better understand functional connectivity among areas. Previous research revealed that Gunnison Sage-grouse have much less genetic diversity than Greater Sage-grouse (Oyler-McCance et al. 1999) and thus require a large suite of highly polymorphic microsatellite loci to adequately assess fine scale landscape genetic variation. For this reason, we identified and designed primers for 13 microsatellite markers in Gunnison Sage-grouse.

Genomic DNA from the blood of a Gunnison Sage-grouse was isolated using a standard phenol–chloroform protocol. Microsatellites for Gunnison Sage-grouse were identified from a next-generation shotgun sequencing run that provided massive numbers of candidate microsatellite loci. The next generation sequencing protocol is described elsewhere (Castoe et al. 2012). We chose 20 potential microsatellites (including di-, tri-, and tetra-nucleotide repeats), of which, 13 amplified consistently and were used for initial screening.

For polymorphism screening we isolated DNA from the blood of 31 Gunnison Sage-grouse originating from a population in Gunnison Basin, CO using an ammonium acetate protocol (modified from the PUREGENE kit; Gentra Systems) and from 14 Greater Sage-grouse sampled from a population in North Evanston, WY using a standard phenol–chloroform protocol. Screening PCRs were performed in 10 μL reactions containing 0.2 mM of each

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Table 1 Characterization of 13 polymorphic microsatellite loci developed for Gunnison Sage-grouse

Locus	GenBank accession no.	Primer sequence	T_A (°C)	Motif	Gunnison					Greater				
					A	N	Size range	H_E	H_O	A	N	Size range	H_E	H_O
SG21	KJ486474	F: M13-AGGCAAAACAGTCACACATGC R: ATCACAAGCAGAGTGCAGGC	60	$TC_{(27)}$	8	31	195–231	0.739	0.710	9	14	205–241	0.706	0.786
SG23	KJ486475	F: M13-CCAGTCACAGCCCAGAAGC R: GCAATCGTTTATCACCTGCG	55	$ATGG_{(13)}$	7	30	329–361	0.616	0.533[a]	4	13	325–349	0.532	0.462[b]
SG26	KJ486476	F: M13-TGGCCAGAAATTTAGGTGTGG R: TTAAGCAATCTGAAACCCCTTACC	60	$ATT_{(12)}$	2	31	136–139	0.252	0.226	5	14	121–142	0.598	0.643
SG27	KJ486477	F: M13-TGAACTCTTCACTGTCTAAAGGGG R: TCACTCCCTAGGAACCTCCG	60	$TC_{(15)}$	2	29	149–153	0.313	0.379	12	13	121–169	0.920	0.769[ab]
SG28	KJ486478	F: M13-ACAGGGGAAGGACAGACTGG R: ACCTCTGCTTTTCCATTGCC	60	$AC_{(25)}$	8	31	144–172	0.821	0.871	12	14	128–172	0.894	0.857
SG29	KJ486479	F: M13-AAGGGGCTTAGGGTTTTAATGG R: AGTTAACTAAGTTGGGCAGGGG	60	$AC_{(25)}$	12	30	149–191	0.860	0.800	8	14	137–155	0.889	1.000
SG30	KJ486480	F: M13-TTATTAAGTGCCTTGGTGTGGC R: GAATTGCTAACTGTCATGAGCCC	60	$AAAT_{(10)}$	3	31	181–189	0.476	0.452	3	14	181–193	0.685	0.786
SG31	KJ486481	F: M13-GAACCGTTGTTTCTTCTGCC R: AAACCTGTTCAGTTGTCATGTCC	60	$AATG_{(11)}$	5	31	150–166	0.554	0.581	6	13	150–170	0.772	0.538[ab]
SG33	KJ486482	F: M13-AGCTTCCCAGTGAATGAGCG R: GGTGGAGACTGAGGTGTAACCC	60	$AAAT_{(10)}$	4	31	147–159	0.750	0.645[b]	5	14	143–163	0.720	0.571[b]
SG34	KJ486483	F: M13-TGAGATCAAGAAGATAACAGGAGG R: AGTTGTAGAAGACTTTATAGAGAGAAATCC	60	$ATT_{(12)}$	2	31	165–168	0.178	0.194	5	14	162–174	0.553	0.571
SG36	KJ486484	F: M13-TTCCAGACATTTTGGGAGCC R: CACATGTCCATCCAACCACC	60	$ATGG_{(13)}$	4	31	242–254	0.628	0.677	5	13	222–258	0.803	0.615[b]
SG38	KJ486485	F: M13-CAGCAATGGTAGGTGATGGC R: AAATGTTGCTGAGCCTCTTGG	60	$AC_{(20)}$	11	31	180–216	0.868	0.903	13	14	174–232	0.937	0.929
SG39	KJ486486	F: M13-GAAAGTCTGAATGCTGGAGAACC R: AAGCGTACTGTTTGCTCCCC	60	$ATC_{(15)}$	2	31	188–191	0.094	0.097	7	14	176–197	0.751	0.714

Annealing temperature (T_A), number of alleles (A), number of individuals screened (N), expected (H_E) and observed (H_O) heterozygosities are reported for Gunnison and Greater Sage-grouse

[a] Significant deviation from Hardy–Weinberg equilibrium ($\alpha = 0.05$)

[b] Frequency of null alleles >0.05

dNTP, 1X GoTaq Flexi Buffer (Promega), 1.5 mM $MgCl_2$, 0.03 μM M13-tailed forward primer, 0.5 μM non-tailed reverse primer, 0.5 μM M13 dye-labeled primer with either a 6FAM, VIC, NED, or PET label (Applied Biosystems) and 0.5 U of Taq DNA polymerase (Promega). Amplification conditions were as follows: 94 °C for 2 min, 40 cycles of 94 °C for 1 min, annealing temperature (Table 1) for 1 min and 72 °C for 1 min, then 60 °C for 45 min and a final extension at 72 °C for 10 min. The PCR products were run on an AB3500 Genetic Analyzer (Applied Biosystems). All loci were run with 600LIZ size standard (Applied Biosystems) and analyzed using GeneMapper v4.1 (Applied Biosystems).

For each polymorphic locus, we calculated observed heterozygosity (H_O), expected heterozygosity (H_E) and null allele frequencies using CERVUS 1.0 (Marshall et al. 1998). GENEPOP version 3.4 (Raymond and Rousset 2000) was used to test for evidence of linkage disequilibrium and deviations from Hardy–Weinberg equilibrium. The number of alleles per locus ranged from 2 to 12 for Gunnison Sage-grouse and from 3 to 13 for Greater Sage-grouse, and single locus heterozygosities ranged from 0.097 to 0.903 for Gunnison Sage-grouse and from 0.462 to 1.000 for Greater Sage-grouse (Table 1). Significant deviations from Hardy–Weinberg equilibrium were observed at one locus for Gunnison Sage-grouse and at 2 loci for Greater Sage-grouse (Table 1). High null allele frequencies were detected at one locus for Gunnison Sage-grouse and at 5 loci for Greater Sage-grouse (Table 1). We found no evidence of linkage disequilibrium for Gunnison or Greater Sage-grouse after a Bonferroni correction was applied ($P < 0.000128$). While these microsatellites were designed for use in describing fine scale genetic data for Gunnison Sage-grouse, they also work well for Greater Sage-grouse and could be used for landscape genetic, population genetic, or unique identification of individuals for both species.

Acknowledgments The use of any trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix: Microsatellite sequences for Gunnison Sage-grouse (*Centrocercus minimus*)

SG21

TAAAAAGCAAAAGGCAAAACAGTCACACATGC
AAAAAGAACCACAACAAAAGAGAGAGAGAGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAAAGTATA
GGAAGGCTAAGTGCCTTGTCCTACCTCCTGCAGGA
GATGGACAGGAAGGAGCGCAGAGCAAATACCGA
GATCTTCTTCACCCCAGCCTGCACTCTGCTTGTGA
TGTTCCCTTTGCCCTTGCAGTACTCTGTAACCTGCC
TTTTGCTTTTTA

SG23

CCCAGTCACAGCCCAGAAGCAGTTAAAGCAAG
GAGGTGTGATGACTGCAATGAATGGATGGATGGA
TGGATGGATGGATGGATGGATGGATGATTTTAAA
AGGCACCTCATGTCACCAACTTTGCAGCTCTTTGG
TTCCAGGTTTCATCATGAAACCCTTCAGAGACATT
GAGCTGCCTGTAGCATCCTCCCTTTCTCATCTGCC
TGTCCTGGGGGCAGCTGAAGGACACACTTGGAGA
AGGGAAGCTCCCAGCCCCACGGCTACCCCAGGTA
CCTTGGAAGAGGGCAGGCTGTGCGCAGGTGATAA
ACGATTGCAAAGCAGTCCATTAATTCAGGAGCAA
AA

SG26

TTTCTTTTACAATGAAGTGAAATTGGCCAGAAA
TTTAGGTGTGGTTTTGTTTGTTTATTATTATTATTA
TTATTATTATTATTATTATTATTTTAAATATGAAAA
GTAACAGTATTGCTGTGGTAAGGGGTTTCAGATTG
CTTAAAGAACTGTTA

SG27

TGTTTTTGTTTGTGTTGAACTCTTCACTGTCTAA
AGGGGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGAGAGAAAGAGAAAGATGAGCTGAAATCC
TCGGAGGTTCCTAGGGAGTGAGAGAAGAAGCACC
AAGGCTATGTTTCTTCACAAACAAAAACA

SG28

TATCCCTACCTCAGCTTAAGGACTTATTAACTT
AGTAACTTAAGGACACTTATACCACAGGGGAAGG
ACAGACTGGCGTCGTACAACAACACAGGTCATTA
GCACACACACACACACACACACACACACACACAG
AGGATACATCTTGGCCCTGTGTGTTTCCTCAGGGC
AATGGAAAAGCAGAGGTAGGGATA

SG29

TAGAAGAAACAAGACAGTTGTATTCAGAGTTA
AGGGGCTTAGGGTTTTAATGGAGAAAAATATATA
CACACACACACACACACACACACACACACTTTAG
ACATTGCTAGCTTCAGCCACTAAATAAAATAAGTT
GTGTCCCCTGCCCAACTTAGTTAACTCTGAATACA
ACTGTCTTGTTTCTTCT

SG30

TGTTTTATTAAGTGCCTTGGTGTGGCCTTGACTC
AGCTTTACTGAAACAAATTCTTGGCCTGTGAGAGA
GCAAGTGAAAAGTCAGGCAGAATAAGAAAAGTA
AATAAATAAATAAATAAATAAATAAATAAATAAA
TAAAAGGTTAGTGGGCTCGTGGGCTCATGACAGT
TAGCAATTCAAGAGAAAAAATACTGAACAAAAAG
GCACTTAATAAAACA

SG31

TTGTCACAGAAAATCATTAAATTGATGCAAGAA
CCGTTGTTTCTTCTGCCTTTTGAGAAAAGAAACAC
AAATTCAGATGAGATTTTACCACTGAATGAAGAA

TGAATGAATGAATGAATGAATGAATGAATGAATG
AATGAATGGATGAAAGGACATGACAACTGAACAG
GTTTATTATTCAGGCAGTTTATTTCCAGAAA

SG33

TATTTATTTAAGTAATAGAGTGGAGTAACATTC
TCTAGCTTCCCAGTGAATGAGCGTCTTTTCTACTG
TGGGTCTTTATTTATTTATTTATTTATTTATTTATTT
ATTTATTTATTTATTTATTACATAGCTTCATATGGG
CAATAAGAAGGGTTACACCTCAGTCTCCACCCTAT
TACTTAAATAAATA

SG34

TAAATGCATATAATATCTCTGAGATCAAGAAGA
TAACAGGAGGAATCCTCATCCCAGGAGAAGTCTT
CAATTCTGGCCATTGTGTTTTTATTATTATTATTAT
TATTATTATTATTATTATTATACTGCAGATTAGATT
TGGATTTCTCTCTATAAAGTCTTCTACAACTTTCA
GAGATATTATATGCATTTA

SG36

TCCCACCTATTCCAGACATTTTGGGAGCCAGCT
GAATTGGTGAAGCATTTTGTTTTTTATTTGGCATA
TGACATCCATCCCTTTGTATTTGCCCACAGTTTGT
GTGGGGACTTTCATTTCTCCAAGGCAAAGCAGCTC
TGGCAGAAGGATGGATGGATGGATGGATGGATGG
ATGGATGGATGGATGGATGGATGGATGGATGGAT
GGATGGATGGAAGAACTGGGTGGTTGGATGGACA
TGTGGATTGATGGATGGGTGGATAGGTGGGA

SG38

TGTTCCAATCAGTTCAGCAATGGTAGGTGATGG
CCTTGTAGTAGGAAAGTAAGAGTCCTGTCCAAAA
ACAGAAGTTAAGCATCAGAAAGAGCTAAACACAC
ACACACACACACACACACACACACACACACACACAC
ACACTGTAAAAGCTATTCTCAAACCTCAGTCCCAA
GAGGCTCAGCAACATTTCTATCCAGAACTGATTGG
AACA

SG39

TATATTAAGGTAAAGCGTACTGTTTCTCAACTG
CAATTCTTCAGTTTCTTGAGTAGCAGAAAGTCTGA
ATGCTGGAGAACCTGACGGCAGGATGCATCAAAT
AAATCGTATTACTCATGATGATGATGATGATGATG
ATGATGATGATGATGATGATGATGATGATGATGA
TGATGATGATGATGATGATGATGAAGCAGTGGGG
GGGTGGTGTGGGGGAGCAAACAGTACGCTTTACC
TTAATATA

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