



Isolation and characterization of 89 SNP markers in the oriental turtle dove, *Streptopelia orientalis*

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Abstract

Streptopelia orientalis is an important commercial species, and natural populations have declined dramatically in recent years because of the application of traditional Chinese medicine. The effective conservation and management of *S. orientalis* have been limited without sufficient molecular markers. In this study, we reported the isolation and characterization of 89 SNP markers in *S. orientalis*. The minor allele frequency ranged from 0.0417 to 0.4792. The observed heterozygosity and expected heterozygosity ranged from 0.0000 to 0.9583 and from 0.0816 to 0.5098, respectively. Polymorphic information content ranged from 0.0767 to 0.3746. The inbreeding coefficient values varied from -0.3149 to 0.8686. Only four loci showed significant deviations from the Hardy–Weinberg equilibrium ($P < 0.05$). The polymorphic SNPs will be helpful for the further population genetic analysis and natural resource conservation of *S. orientalis*.

Keywords *Streptopelia orientalis* · SNP markers · Population genetic diversity · Transcriptome

The oriental turtle dove (*Streptopelia orientalis*) is a widespread polytypic Asian species that breeds from the Ural Mountains to the Pacific coast of the Russian Far East (Brazil 2009; Gibbs et al. 2001; Johnson et al. 2001; Lee et al. 2017). *S. orientalis* has been listed in the International Union for Conservation of Nature (IUCN), *IUCN Red List of Threatened Species* (2016), ranging from north (Heilongjiang Province) to south (Hainan Island), and from west (Xinjiang and Tibet) to east (Hong Kong and Taiwan). Although *S. orientalis* has not yet been classified as an endangered species, natural populations have declined dramatically in recent years because of the application of traditional Chinese medicine and commercial values. Therefore, it is urgent to perform population genetic investigation on *S. orientalis* to conserve and utilize the natural resources. As an important DNA marker, single nucleotide polymorphisms (SNPs) are widely used for genetic studies (Vignal et al. 2002). In this study, SNPs were developed and characterized in *S. orientalis* for the first time with the restriction-site associated DNA tags sequencing (RAD-seq), and will be important genetic markers for the researches on conservation genetics.

Blood was collected from 30 *S. orientalis* individuals from Yantai in Shandong province ($37^{\circ}27'N/121^{\circ}30'E$). Genomic DNA was extracted from blood samples using the DNeasy Blood & Tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. RAD library construction, sample indexing and pooling followed for the natural populations (Baird et al. 2008). To obtain SNP marker resources, pair-end (150-bp) sequencing was performed using Illumina HiSeq4000 (Shanghai BIOZERON Co., Ltd.), and a total of 254,291 putative SNPs in *S. orientalis* was identified.

Primer v3.0 was used to design primers, 89 primer pairs successfully created. PCR reactions were performed in a 25 μ l volume with GenStar PCR Mix according to the manufacturer's instructions (GeneStar, Beijing, China). PCR amplification cycles were as follows: an initial denaturation at 94 $^{\circ}$ C for 5 min; 40 cycles of 94 $^{\circ}$ C for 30 s, annealing for 30 s (for annealing temperatures of each primer pair, see Table 1) for 30 s, and 72 $^{\circ}$ C for 30 s; and a final extension at 72 $^{\circ}$ C for 7 min. Amplified samples were purified by gel extraction and sequenced on ABI 3730 DNA Analyzer (Applied Biosystems). For validated loci, statistics including the minor allele frequency (MAF), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC), inbreeding coefficient (F_{IS}) and P-value representing the deviations from Hardy–Weinberg

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Table 1 Characterization of 89 SNP markers in the oriental turtle dove *Streptopelia orientalis*

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position	H _o	H _E	F _{is}	HWEP	PIC	MAF
33515	F: TGGC AATGTGT CACATCCA R: AGAACCCCTGCTGTGTGATTCA	327	60	T/C	68	0.8750	0.5027	-0.2799	*	0.3711	0.4375
41019	F: GGCATTCAGTGT C ATCCCT R: CGTCATTCAGGCTGGATGGT	281	60	G/A	80	0.9583	0.5098	-0.3149	**	0.3746	0.4792
120812	F: GGAAGATCAGAGACAGTGCT R: CAGAGCAAAGCAGAGGTCGA	304	60	C/T	87	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
121698	F: TGTGACCAAGAAACCAGAGG R: GCTGGGGCAGTCTCCTTAG	293	60	A/G	74	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
128473	F: TAGAGGCTGAGCATTTGCCAG R: AGACAGCAATGAAAAATGGCCT	324	60	G/C	63	0.2500	0.3369	0.1377	*	0.2755	0.2083
128889	F: AGAGTAAAAAGCTTAACCCGAGA R: GTGCTCAGGGCTCTCATG	294	57	T/A	54	0.0417	0.1906	0.6098	NS	0.1692	0.1042
134895	F: GTCCTCTTCAAACTTTCAACTCA R: CTGGTACTACATGACAGAGGC	333	60	T/G	49	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
135047	F: GCAAAAGCTGGGCTGGATTT R: AGCCAGTAGGAAAACCCCTTCT	328	60	T/C	92	0.0417	0.1197	0.4372	NS	0.1103	0.0625
136010	F: TCGAAAACCTCACAAAGCA R: GTGGCTGTAGCGTAGTGTA	292	59	A/G	94	0.0417	0.1197	0.4372	NS	0.1103	0.0625
137106	F: AGGCTGAAGTGCCTACTGAA R: CTCTCAGCATGCTATGGGG	302	59	A/G	76	0.0417	0.1197	0.4372	NS	0.1103	0.0625
138042	F: TCTGTTTGGCAGGGTCTTC R: TGGGAATGGGAAGAATTGGGA	297	60	A/G	83	0.1250	0.1906	0.1975	NS	0.1692	0.1042
140802	F: TAAGCCCTGCTCTGTTGTCC R: ATTGCCCTCCCTGTTCTGTGT	309	60	C/T	58	0.0833	0.0816	-0.0122	NS	0.0767	0.0417
141092	F: ATTCCCTTGTCCAGGAG R: AACTCCTTGTGTAGGCGG	302	60	T/C	75	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
141452	F: GTGGCATAAGCTAAACCAGAGC R: ACAATCAAGGCAGCAAGGATG	270	60	G/A	77	0.0833	0.1560	0.2899	NS	0.1411	0.0833
142319	F: TCAGAGCTGCCACGTATGT R: CTTGTTCAGACTGACACCCCA	352	60	C/G	33	0.0833	0.1560	0.2899	NS	0.1411	0.0833
142716	F: ACACAGCCTGAAATGCAATCAC R: GGTTCAGGAGAGGCAAGCA	330	59	G/T	63	0.0833	0.0816	-0.0122	NS	0.0767	0.0417
144488	F: TCCTCAAACCCTGGAAGCTT R: CAGGAGCTATCAGGTGGCA	264	58	T/C	48	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
144543	F: TGTGAGGCAAGTGCATTTTCT R: CGCCTTCAAGCCAGTTTGT	293	59	C/A	86	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
145456	F: AGATTGCAGCTCTGTTTACTGT R: GTGATCGCCTCTACCCCAAC	258	58	T/G	76	0.1250	0.1197	-0.0234	NS	0.1103	0.0625

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position	H _o	H _E	F _{IS}	HWEP	PIC	MAF
145938	F: TGTCAAGCTAATCCAGACACC R: GGGAGATGCAAGGTCACACA	296	60	C/G	87	0.2500	0.2234	-0.062	NS	0.1948	0.125
145945	F: TGTAAACCCATGAACACAGGTGG R: CACTGGCAGCTGGAAGGTTA	311	60	G/C	67	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
145954	F: AGACAATGTGCAGAGAAGCA R: CCCGAGGAGAACGTCAAAACA	309	57	G/A	86	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
146400	F: GGCTTTTCCCCCACACATCT R: ACTTTTGTGCTTACTCTCT	283	60	A/T	86	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
147303	F: TGCACATTAGCCTGAGCCAA R: GAGAGAGCACCCACAGAGCAG	323	60	T/C	71	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
147628	F: GCCTCACCCAGTGTGAGTAC R: CTGGGGCTCTTAATGGGACC	290	60	A/G	81	0.1667	0.1560	-0.0358	NS	0.1410	0.0833
150367	F: GCCTTTGGCCTCATGGGTAT R: TGTGTGCACATGTGTTTCAGT	256	60	T/G	44	0.3333	0.4220	0.1070	*	0.3278	0.2917
151912	F: CCACCTCCCTAGAGCAGAAGC R: CCAGAACGATGTCATACACGC	329	60	G/A	49	0.3333	0.2837	-0.0884	NS	0.2392	0.1667
212816	F: GGCCATACCTCAAACCTACCA R: TCAAAGCCATAAACCCAGCAAAAAGT	289	59	A/G	57	0.0417	0.1197	0.4372	NS	0.1103	0.0625
213823	F: ACATTTGTGTGATCTAGGCT R: TCTGAAAAATGCAGCCTGAGGT	331	55	T/A	69	0.0000	0.0816	0.6831	NS	0.0767	0.0417
214980	F: TGGCAATCCATTTGTCTCAA R: CCTTCCCCCAAGCAATGTTA	275	56	G/C	90	0.0833	0.0816	-0.0122	NS	0.0767	0.0417
221170	F: TCTTACAGAAAGTTACCAGGAGT R: CCAGGCAGGATGTGAAAACCT	306	58	A/G	47	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
224434	F: TGTGTTTGAAGGCTGGAAAGTG R: CACTGGTACTGCTCAGCAGG	260	60	G/A	98	0.0417	0.1197	0.4372	NS	0.1103	0.0625
225989	F: TGACACATTTCCAGCCCTGT R: CACCTTCAGTAGACCCAGGC	324	60	G/A	56	0.0417	0.1906	0.6098	NS	0.1692	0.1042
227551	F: TGGCAFTTACTGAAGAGCTG R: AGCTGGGGGTTAGGATCT	309	59	G/A	73	0.0000	0.1560	0.8686	NS	0.1411	0.0833
227699	F: ATGGGATGTTCCGACAGCTG R: AGTGTGAGATAAAGGTGACACGA	307	60	C/T	78	0.1250	0.1906	0.1975	NS	0.1692	0.1042
228914	F: TTGAAAGCTGAGCCTGAGGG R: GGCCTGTATCACACCAGTT	289	60	C/G	100	0.0417	0.1906	0.6098	NS	0.1692	0.1042
229943	F: TCCCTATTGTTGGCACCAA R: CTCAGTTCAGCAGGACAGG	298	59	C/T	82	0.0000	0.1560	0.8686	NS	0.1411	0.0833
230950	F: AAGAGGTCCACACTGTCCACC R: AACGAGTGCCAAAGTCTCTCC	260	59	G/C	92	0.0833	0.1560	0.2899	NS	0.1411	0.0833

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position	H_o	H_E	F_{IS}	HWEP	PI_C	MAF
231430	F:ACACTGGCTTTAAATTCCTTTGCA R:GATTTGCAGCCTTCGIGCG	328	60	G/C	78	0.0833	0.1560	0.2899	NS	0.1411	0.0833
231643	F:TCACAGAAACAACATAGCTCC R:TGGCAGCATCACACTGTTCT	280	57	C/G	60	0.0833	0.1560	0.2899	NS	0.1411	0.0833
232294	F:CCTGCTGTTGGCTTCAGTA R:GAGACACTGGCTTCGCTTT	335	60	A/G	104	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
232564	F:TCCATTGATTTGGCATTAAAGC R:CCTGGCTGTTGGCTTCAGTA	278	55	T/C	74	0.0417	0.1197	0.4372	NS	0.1103	0.0625
233731	F:TCTGTGCAACAGCTTATATGCT R:AAAAGTTCAGTGCCACGACAGT	269	59	T/C	61	0.0417	0.1197	0.4372	NS	0.1103	0.0625
233978	F:CTCTTTCGTGGCTGCAGAGA R:ACGAGCAGCTGATGTGCTTT	289	60	C/T	87	0.0833	0.2234	0.4439	NS	0.1948	0.1250
234255	F:AGGATTTGTCAACAGCACACA R:TTGCAGACTCTTTCCACC	249	58	G/T	50	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
234498	F:CAAAACGCCCATGTGACAG R:GACACAGGTGGCTCTCTCAG	301	60	G/C	104	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
234911	F:ACCTCACAGTGCAAAAGAG R:GGGTTAAAAACGAGGGGGTCA	325	60	G/C	91	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
235190	F:ATCGGAGCACTGAGTTTGG R:CAGCTGGATGGCACTTTCAGA	290	60	A/G	91	0.0000	0.0816	0.6831	NS	0.0767	0.0417
235866	F:GGGCTTTTGTCTTCAAACCCC R:CCTGGCCATCCCCATTTCTT	300	60	T/C	73	0.0833	0.2234	0.4439	NS	0.1948	0.1250
236152	F:CATCCACGGGGTAGATGC R:CTCTTGGCCTCACAGGAG	256	60	C/G	58	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
236543	F:CCCCATGAAAACCTGAGTGC R:GCACAGCTCAGTGAAAACCAG	275	59	A/C	58	0.0833	0.1560	0.2899	NS	0.1411	0.0833
236686	F:ATGCTGTCAGGTTCCCTGAGC R:CTGAAGTGACAGGGCAGGTC	262	60	T/G	87	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
236767	F:TCCAAACACTGTCAGGGACG R:GTTACAGGGGAAATGGGTGCT	335	60	T/C	101	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
237156	F:ACCTGACAAGTGCAGGTA AAC R:TTTACACCGTGTCTGAGG	301	58	C/T	76	0.0833	0.2234	0.4439	NS	0.1948	0.1250
237183	F:ACAAGCATCTTGGCAATCA R:ACAATGACATGCAAGTCCAT	288	56	C/A	88	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
237210	F:AAGGTGAGTAGCTGGCATGC R:CCTCCTCCTGCTCCAAAAG	299	60	T/A	85	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
237745	F:CCAAAGGTATGCCACAAATTC R:TGAGCGGCTGTGACTCAAAG	336	59	T/C	49	0.0417	0.1197	0.4372	NS	0.1103	0.0625

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position	H _o	H _E	F _{IS}	HWEP	PIC	MAF
237823	F:GGAATCACTCTGGAACCC R:CCCTTGTATGGCTGTGTA	309	60	C/T	102	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
238083	F:AGGGAAGAATCAGAACTTTGCTCT R:TAGCTCTGCTGGCTGGAATG	274	60	A/G	70	0.1250	0.1906	0.1975	NS	0.1692	0.1042
238127	F:ATTCGGACTGGAGGTGACAG R:GCAGTTTGGTTAGCAGGGC	292	59	A/G	56	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
238315	F:TCAAGCCAACCAAGTTCCCT R:CCCCGTTACAGCTATGCAA	267	59	G/C	50	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
239210	F:TCAACCAAGGAAAAGGCTGA R:CCATGGCTTGTGGGCTTC	311	60	G/A	77	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
239788	F:AGTGGAACTTAAAGTTTTCATTTGCT R:GAAAACGATGACCCGTGTG	295	59	A/G	84	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
240197	F:TCCAGGTCAGAAATGCACAGT R:AATCACGGCTGATCAAGCA	290	60	A/C	103	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
240200	F:TCAAATCTGTGACTCGGTGGT R:ACAAATGGGTCAGAGAGCG	294	60	T/C	105	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
240354	F:TACAATACACTGCTGTGC R:TTTCTGTGGCCGTGGTTTCAT	276	59	A/G	65	0.0833	0.1560	0.2899	NS	0.1411	0.0833
241120	F:CATCCCAGCACACAGTTGA R:TCTCACAAACAGGCAGCACAT	348	60	T/C	55	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
241709	F:TGCAACTAAGTGCATTGCAGA R:AGGGACATCAGCAGCTCAAC	315	59	A/G	73	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
241854	F:TGTCAAACGCAGAACCGATC R:GTGTACCTTCCAGAGGGG	290	59	T/C	51	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
242618	F:TGCCATGGTGAGTAATGCC R:GCTGTGCAAAATCCCCTTCT	321	60	G/T	62	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
243695	F:TAACCTGCAGACAAACGCGG R:AACTCGTCCCAACTTCCC	320	59	G/A	97	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
244077	F:ATGGTGCCAAATGTCCCCTTC R:GGAACCCCTTGGGGAGAAATGT	340	60	T/C	71	0.2083	0.1906	-0.0488	NS	0.1692	0.1042
245338	F:TCTGTACAGCAGCCAGAGC R:ACAAATTCCTCAATCCATAACACA	321	60	C/T	36	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
245390	F:CAGCAGCCAGAGGTGGG R:CGAAATTCAGCACCCAGAGC	351	62	G/C	40	0.2083	0.1906	-0.0488	NS	0.1692	0.1042
245551	F:ACAGGCTAGTTTTATCAGCTCCA R:GCTCACCAAGAAATGCAAGCA	255	59	G/C	72	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
246794	F:ACTGTGCTTGCCTGACATG R:TGGTTTTCTCTGCGATGGG	312	59	A/G	103	0.0833	0.1560	0.2899	NS	0.1411	0.0833

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position	H_o	H_E	F_{IS}	HWE _P	PIC	MAF
246879	F:AGAAAAGATGAGTGGGAAGAGA R:GCTTCAGAGCTGCCCATTTTC	326	56	G/A	69	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
246928	F:AGATGACTCTGAGGTTTTGTAGT R:TTCCCTGTTCCCTCCTCCTCCGT	309	58	G/A	68	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
247124	F:TCACA AATTCAAGGGGCA R:CAAGCAGGGACCAGACTGAG	346	58	C/T	55	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
247894	F:TGCTGTGAAGAAATGATTCA R:CCAGTTTCAGGCTGTGGAGT	326	56	A/C	109	0.0417	0.1197	0.4372	NS	0.1103	0.0625
248120	F:TAAGGGAGGGCTAGAGGACC R:GTTTGGCTGTGCCCTTCCAGG	274	59	G/A	55	0.0000	0.1560	0.8686	NS	0.1411	0.0833
248609	F:ATTCTGAGTGATGCTGCCGC R:GGGGTTCGTTCTGTGTGATCA	270	61	T/C	38	0.2917	0.2544	-0.0752	NS	0.2181	0.1458
249320	F:CTCCTCCTGTTCAAGGCTGG R:TCTGCTCATTTGCTTATGGTTGA	290	60	A/G	72	0.1667	0.1560	-0.0358	NS	0.1411	0.0833
249921	F:CTCGTTGCTGCTCTGAGCT R:GCTACGGATCAGAGGTCAGC	271	60	A/G	103	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
249986	F:CCCCTGGAAAGCGATGACTTT R:TCCCAAATCTTCCCAITCCCA	312	60	A/G	99	0.2917	0.2544	-0.0752	NS	0.2181	0.1458
250879	F:AGACCACATCCCAACCACC R:TGACTCAAGGCAAGACAGAGC	266	60	T/C	85	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
251191	F:ACTGCTTATTTTGACAGATTAGGT R:TGCTTTTCTTCTGGTCAGCA	306	56	T/C	60	0.1250	0.1197	-0.0234	NS	0.1103	0.0625
251324	F:ACAACITCCCTGCATCCCAA R:GGAACATACACCTTTCCCCCA	297	59	T/C	105	0.0417	0.1906	0.6098	NS	0.1692	0.1042
253951	F:TTCTGAGGACAGCTGGCATG R:GCCAACAAAACAGCACACCA	324	60	T/A	62	0.1250	0.1197	-0.0234	NS	0.1103	0.0625

Tm annealing temperature, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, HWE_P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

* $P < 0.05$, ** $P < 0.01$

equilibrium (HWE) were calculated using Cervus 3.0 (Kalinowski et al. 2007).

Eighty-nine primer pairs could be amplified, and 89 SNPs located within these sequences were confirmed by Sanger sequencing. The minor allele frequency ranged from 0.0417 to 0.4792 (Table 1). The observed heterozygosity and expected heterozygosity ranged from 0.0000 to 0.9583 and from 0.0816 to 0.5098, respectively. Polymorphic information content ranged from 0.0767 to 0.3746. The F_{IS} values varied from -0.3149 to 0.8686 . Only four loci showed significant deviations from the HWE after Bonferroni correction ($P < 0.05$). These polymorphic SNP markers will be useful for further population genetic analysis, natural resource conservation and selective breeding of *S. orientalis*.

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