ONLINE RESOURCES

Eighteen polymorphic microsatellites for domestic pigeon *Columba livia* var. *domestica* developed by cross species amplification of chicken markers

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Introduction

Domestic pigeon (*Columba livia* var. *domestica*) belongs to the order Columbiformes of the family Columbidae, and has been used since ages as messengers to carry brief written messages, due to speed, altitude and homing behaviour. The wild rock pigeon was domesticated hundreds of years ago and it was bred into several varieties for their appearance, flying capabilities and for the sport of pigeon racing. 'Racing homer' and 'Birmingham Roller' are the two best known varieties. Pigeons are also reported to be carrier of certain parasites which cause health problems in humans and domestic animals (Trovnicek *et al.* 2002; Haag-Wackernagel and Spiewak 2004). Domestic pigeon is threatened throughout its distribution range by introduced predators, habitat loss and changes in urban landscapes due to rapid development. Around 59 species of pigeon and dove are threatened with extinction today; this is 19% of all species (Walker 2007). No genetic study has been carried out till date for isolation and development of molecular markers in pigeon except by Traxler *et al.* (2000). Despite the relative ease of isolating microsatellites, their development still requires substantial input of time, money and expertise. Therefore, utilization of cross-species markers provides a rapid and cost effective solution for species from which markers have not been cloned or information is limited. Many studies have shown that microsatellite loci are often conserved among closely related species including birds (Moore *et al.* 1991; Primmer *et al.* 1996; Wilson *et al.* 2004; Huang *et al.* 2005; Kupper *et al*. 2007; Zhou *et al.* 2009; Thakur *et al.* 2011). Since we had already transferred chicken microsatellites in other galliformes (Thakur *et al.* 2011) and anseriformes (Mukesh *et al*. 2011). Therefore, we tested whether the sequences flanking the repeats of microsatellites are conserved between species and if chicken primers would amplify the microsatellites in domestic pigeon.

Materials and methods

Sample collection and DNA isolation

Blood samples from 22 individuals of the domestic pigeon were collected on FTA® Classic-Cards (Whatman, Clifton, USA). These pigeons were bred and raised under human control in Dehradun region of Uttarakhand state of India. The genomic DNA was isolated using standard protocol as described by Smith and Burgoyne (2004).

PCR and microsatellite genotyping

Thirty microsatellite primers (AVIANDIV 1998) that were used in 'European Chicken Biodiversity Project' (AVIANDIV 1998) and recommended for the Measurement of Domestic Animals Diversity (MoDAD) by FAO were selected for the present study based on their degree of polymorphism and wide coverage of the genome. The detailed information on the microsatellite markers is available on the website (http://www.dad.fao.org/en/refer/library/guidelin/marker.pdf). Following Qiagen (Mainz, Germany) Multiplex PCR kit, PCR reactions were set up in a 15 μL of reaction volume containing 7.5 μL of 2× Qiagen Multiplex PCR Master mix, 0.50 μL of 10 μM of each primer pair (3.0 μL for six loci), 1 μL of DNA elutant ($\sim$ 20 ng) and 3.5 μL of RNase-free water. The amplification conditions were 15 min initial heat activation of Hot Start (Mainz, Germany) *Taq* DNA polymerase at 95°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at specific temperature (table 1) for 90 s and extension at 72°C for

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60 s with a final extension at 60°C for 30 min. Amplification was checked on 2% agarose gel and fluorescence-based genotyping was performed on ABI 3130 Genetic Analyser (Applied Biosystem, Foster City, USA). Scoring of allele was performed using GeneMapper software (version 3.7, Applied Biosystem).

Statistical analysis

Genetic diversity estimates i.e. observed (N_a) and effective number of alleles (N_e), observed (H_o) and expected heterozygosity (H_e) were performed using POPGENE software (Yeh *et al.* 1999). Polymorphic information content (PIC), a measure of marker's informativeness and predicted null allele frequencies were calculated using Cervus (version 3.0) (Kalinowski *et al*. 2007). For the Hardy–Weinberg equilibrium (HWE) estimation, we followed the probability test approach (Guo and Thompson 1992) using the program GENEPOP (Raymond and Rousset 1995).

Results and discussion

Initially, 30 chicken microsatellites were tested for amplification in pigeons. Of these, seven loci (LEI0166, MCW0020, LEI0094, MCW0284, MCW0014, LEI0192 and MCW0222) did not amplify and five loci showed low success rate i.e. MCW0248 (32%), MCW0034 (37%), MCW0206 (46%), MCW0103 (73%) and MCW0098 (73%). Eighteen loci showed high success rate (>95%) and were therefore subjected for further analysis (table 1). All loci except LEI0234 were polymorphic and the summary of the diversity measures are presented in table 2. Altogether, 139 alleles were found across 18 loci. The number of observed alleles ranged from 2 (MCW0037) to 18 (ADL0278), with overall mean number of alleles per locus of 7.722 (±4.0 s.e.). The observed number of alleles for all loci exceeded the effective number of alleles, which varied from 1.514 (ADL0268) to 12.906 (ADL0278) with mean 4.236 ± 2.77. H_o and H_e ranged from 0.227 (ADL0268) to 0.909 (MCW0016) and

Table 1. Characteristics of chicken microsatellite markers.

Marker	GenBank accession no.	Repeat motif	Chromosome	Primer sequence (5′− > 3′) (forward & reverse)	Allele range	T_a(°C)
ADL0268	G01688	(GT)12	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	102–116	60
MCW0037	L43676	(CA)10	3	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	154–160	64
ADL0112	G01725	(AC)10	10	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	120–134	58
MCW0295	G32052	(AC)10	4	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	88–106	60
MCW0067	G31945	(GT)10	8	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	176–186	60
MCW0104	L43640	(TG)16	13	TAGCACAACTCAAGCTGTGAG AGACTTGCACAGCTGTGTACC	190–234	60
MCW0111	L48909	(AC)8	1	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	96–120	60
MCW0216	AF030586	(GT)9	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	139–149	60
MCW0081	L43636	(GT)7	5	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	112–136	60
MCW0330	G32085	(AC)n	17	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	256–300	60
LEI0234	Z94837	(CTTT)19	2	ATGCATCAGATTGGTATTCAA CGTGGCTGTGAACAAATATG	216–364	60
MCW0069	L43684	(CA)11	E60C04W23	GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA	158–176	60
MCW0016	L40041	(TG)16	3	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	162–206	60
MCW0078	L43686	(TG)8	5	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	135–147	60
MCW0183	G31974	(AC)11	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	296–326	58
MCW0123	L43645	(AC)10	14	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA	76–100	60
MCW0165	L43663	(CA)8	23	CAGACATGCATGCCCAGATGA GATCCAGTCCTGCAGGCTGC	114–118	60
ADL0278	G01698	(GT)6	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	114–126	60

Table 2. Genetic polymorphism of 18 cross-species microsatellite markers in domestic pigeon.

Locus	N_a	N_e	H_o	H_e	PIC	F (null)	P value
ADL0268	3	1.514	0.227	0.347	0.305	0.185	0.027
MCW0037	2	1.995	0.354	0.510	0.374	−0.3133	0.0001
ADL0112	9	6	0.853	0.837	0.814	−0.004	0.120
MCW0295	6	2.205	0.523	0.559	0.512	0.022	0.206
MCW0067	7	2.933	0.674	0.727	0.62	−0.063	0.058
MCW0104	8	4.323	0.666	0.787	0.737	0.004	0.901
MCW0111	11	4.190	0.727	0.779	0.735	−0.0003	0.701
MCW0216	8	2.797	0.818	0.657	0.588	0.148	0.016
MCW0081	11	4.109	0.691	0.753	0.696	−0.008	0.689
MCW0330	4	2.581	0.342	0.626	0.533	−0.258	0.491
LEI0234	1	1	0	0	0	ND	ND
MCW0069	8	4.136	0.775	0.818	0.724	0.002	0.134
MCW0016	7	3.494	0.909	0.730	0.685	−0.148	0.940
MCW0078	12	6.747	0.368	0.874	0.837	0.396	0.001
MCW0183	10	5.822	0.578	0.850	0.809	0.179	0.010
MCW0123	8	6.012	0.535	0.853	0.813	−0.095	0.220
MCW0165	6	3.482	0.729	0.818	0.676	−0.079	0.876
ADL0278	18	12.906	0.636	0.944	0.917	0.179	0.0005
Mean	7.72222	4.236	0.578	0.693	0.63194	ND	ND
SD	4.0118	2.777	0.278	0.228		ND	ND

N_a, number of alleles; N_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity, PIC, polymorphic information content; F (null), frequency of null alleles; ND, not determined; SD, standard deviation.

from 0.347 (ADL0268) and 0.944 (ADL0278), respectively. The H_e values were higher than H_o values for all the 18 loci except for ADL0112, MCW0216 and MCW0016. PIC ranged from 0.305 to 0.917 with an average of 0.631. PIC value for loci ADL0268 and MCW0037 was lower than 0.5. Eleven of the 18 loci confirmed to HWE ($P > 0.05$) while six loci (ADL0268, MCW0037, MCW0216, MCW0078, MCW0183 and ADL0278) deviated significantly from HWE ($P < 0.05$). In the present study, 23 chicken microsatellite loci were transferred to domestic pigeon. Of these, 18 loci showed high amplification success rate (> 95%). Observed heterozygosity was quite high for almost all the loci studied. PIC values higher than 0.5 are considered as informative for population genetic analysis (Botstein *et al.* 1980). Therefore, 16 loci were informative in the present study and could be employed for further genetic studies on pigeon. Departure from HWE for six loci may be due to the presence of null alleles (Pemberton *et al.* 1995) or due to allele scoring errors or combination of both. Most often null alleles occur because of mutations in one or both primer binding sites. This problem is particularly common while transferring microsatellites from one species to other using the same set of primers.

To best of our knowledge, this is probably the first attempt to examine the cross applicability of chicken microsatellites in pigeon while only a few pigeon specific microsatellites have been cloned (Traxler *et al.* 2000). Therefore, this investigation would be of great utility, particularly for researchers working on genetics of wild/domestic pigeon or other columbiformes and the heterologous microsatellites can be used in studies of bird phylogeny, evolution and genetic divergence of different taxa.

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References

AVIANDIV 1998 Development of strategy and application of molecular tools to assess biodiversity in chicken genetic resources. EC contract no. BI04-CT98-0342; http://aviandiv.tzv.fal.de/primer_table.html.

Botstein D., White R. L., Skolnick M. and Davis R. W. 1980 Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32**, 314–331.

Guo S. W. and Thompson E. A. 1992 Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Haag-Wackernagel D. and Spiewak R. 2004 Human infestation by pigeon fleas (*Ceratophyllus columbae*) from feral pigeons. *Ann. Agric. Environ. Med.* **11**, 343–346.

Huang Y., Tu J., Cheng X., Tang B., Hu X., Liu Z. *et al.* 2005 Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds. *Genet. Sel. Evol.* **37**, 455–472.

Kalinowski S. T., Taper M. L. and Marshall T. C. 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106.

Kupper C., Horsburgh G. J., Dawson D. A., Constant R. F., Szekely T. and Burke T. 2007 Characterization of 36 polymorphic microsatellite loci in the Kentish plover (*Charadrius alexandrinus*) including two sex-linked loci and their amplification in four other *Charadrius* species. *Mol. Ecol. Notes* **7**, 35–39.

Moore S. S., Sargeant L. L., King T. J., Mattick J. S., Georges M. and Hetzel D. J. 1991 The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* **10**, 654–660.

Mukesh, Javed R., Gaur U., Jianlin H. and Sathyakumar S. 2011 Cross species applicability of chicken microsatellite markers for investigation of genetic diversity of Indian duck *Anas platyrhynchos* populations. *Afr. J. Biotechnol.* (in press).

Pemberton J. M., Slate J., Bancroft D. R. and Barret J. A. 1995 Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Mol. Ecol.* **4**, 249–252.

Primmer C. R., Moller A. P. and Ellegren H. 1996 A wide-range survey of cross-species microsatellite amplification in birds. *Mol. Ecol.* **5**, 365–378.

Raymond M. and Rousset F. 1995 GENEPOP: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Smith L. M. and Burgoyne L. A. 2004 Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecol.* **4**, 4.

Thakur M., Rai I. D., Mandhan R. P. and Sathyakumar S. 2011 A panel of polymorphic microsatellite markers in Himalayan monal Lophophorus impejanus developed by cross-species amplification and their applicability in other Galliformes. *Eur. J. Wildl. Res.* **57**, 983–989.

Traxler B., Brem G., Muller M. and Achmann R. 2000 Polymorphic DNA microsatellites in the domestic pigeon, *Columba livia* var. *domestica*. *Mol. Ecol.* **9**, 365–378.

Trovnicek M., Cistakova L., Deptula W., Stosik M. and Bhide M. R. 2002 Wild pigeons and pheasants, a source of *Chlamydophila psittaci* for humans and animals. *Ann. Agric. Environ. Med.* **9**, 253–255.

Walker J. 2007 Geographical patterns of threat among pigeons and doves (Columbidae). *Oryx* **41**, 289–299.

Wilson A. C. C., Massonnet B., Simon J. C., Leterme N. P., Dolatti L., Llewellyn K. S. *et al*. 2004 Cross-species amplification of microsatellite loci in aphids: assessment and application. *Mol. Ecol. Notes* **4**, 104–109.

Yeh F. C., Yang R. C., Timothy B. J. B., Ye Z.-H. and Mao J. X. 1999 POPGENE version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada (http://www.ualberta.ca/-fyeh/fyeh).

Zhou X., Xu Y., Ran J., Yue B., Cao L. and Li J. 2009 Polymorphic microsatellites in Buff-throated partridge developed by crossspecies amplification. *Eur. J. Wildl. Res.* **55**, 81–83.

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