Conserv Genet
DOI 10.1007/s10592-008-9798-4

TECHNICAL NOTE

Twenty-three polymorphic microsatellite markers for the Caribbean endemic Zenaida dove, *Zenaida aurita*, and its conservation in related *Zenaida* species

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Received: 10 December 2008 / Accepted: 16 December 2008
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Abstract Twenty-three polymorphic microsatellite loci, six dinucleotidic loci and 17 tetranucleotidic loci, were developed for the Zenaida dove (*Zenaida aurita*), a bird species endemic to the Caribbean Islands. From a set of 30 individuals captured at one single location in Barbados, we obtained 20 loci that did not deviate from Hardy–Weinberg Equilibrium. Number of alleles per locus ranged between 2 and 11 (average 7.05) and the expected heterozygosity per locus, *He* ranged between 0.321 and 0.881 (average 0.712). This gives an exclusionary power for parental analysis of 0.9999 and 1.0000, knowing the genotype of one social parent, or both, respectively. Such results indicate that these 20 loci will be useful for both studying population genetics and mate choice patterns in *Z. aurita*. All 20 loci amplified in four other *Zenaida* species, the Galápagos dove, *Z. galapagoensis*, the eared dove, *Z. auriculata*, the mourning dove, *Z. macroura*, the Pacific dove, *Z. meloda*, with 30–96% being polymorphic.

Keywords Endemic species · Extra-pair paternity · Gene flow · Mate choice · Microsatellite · Zenaida dove

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The Zenaida dove (*Zenaida aurita*) is a columbid species endemic to the Caribbean Islands (Wiley 1991). This species is of patrimonial interest, and is legally hunted in Puerto Rico, Guadeloupe and Martinique. It suffers in some islands from competition with the invasive Eurasian collared dove (*Streptopelia decaocto*), habitat destruction, and illegal hunting (Barré et al. 1997; Rivera-Milan 1997), very little is known, of movements of individuals between islands. In addition, knowledge of the mating system of the Zenaida dove remains limited. Although the species is territorial and socially monogamous (Wiley 1991), the occurrence of extra-pair paternity has not been investigated so far. However, data on mate fidelity and extra-pair paternity in tropical species that maintain year-round pair bonds is particularly valuable, yet rare (Stutchbury and Morton 2001).

Molecular markers are needed to both assess the conservation genetics of this species (i.e. allelic diversity related to island size, level of gene flow, and differentiation) and study mate choice and mating patterns (i.e. female choice related to male heterozygosity and genetic similarity, and rates of extra-pair paternity).

Four *Zenaida aurita* partial genomic DNA libraries enriched for microsatellite sequences containing CA, GA, CATC and TAGA repeat motifs were constructed following the methods described in Jones et al. (2002). High molecular weight DNA was extracted from blood using chloroform/phenol/isopropanol extraction (Sambrook et al. 1989) from 10 individuals from one location in Barbados. DNA was partially digested with a cocktail of seven blunt-end restriction enzymes (*Rsa*I, *Hae*III, *Bsr* BI, *Pvu*II, *Stu*I, *Sca*I and *Eco* RV). Fragments 350–700 bp long were adapted with 20 bp oligonucleotides which contained a *Hind*III site at the 5′ end, and subjected to magnetic bead capture using 5′-biotinylated microsatellite oligonucleotides as capture

Published online: 04 January 2009

Table 1 Characterization of 23 polymorphic microsatellite loci for Zenaida dove (*Zenaida aurita*)

Locus	Repeat motif	Primer sequence (5′–3′)	Genbank Accession #	Size Range (bp)	N	N_A	*Ho*	*He*	PE-1	PE-2
ZaA4	$(GT)_{19}$	F: AGCACGCTCATTAACACATTAG	GF091744	243–273	29	6	0.897	0.796	0.396	0.575
		R: ACCTGTAGTTCAGGGCGTATAG								
ZaA5	$(GT)_{21}$	F: GGCACACAAATACTTTAAGAGG	GF091766	251–285	29	11	0.724	0.848	0.501	0.670
		R: GACCTTTGCTCACTCACTATTC								
ZaA7	$(AC)_{24}$	F: TGGCACTGAAGAACAAGC	GF091745	101–147	26	9	0.769	0.759	0.367	0.551
		R: CTGGGACTCGGGATTTAC								
ZaA112	$(AC)_{19}$	F: CTCCCTGGTTTCATTTCAGAC	GF091746	194–204	29	6	0.862	0.736	0.310	0.485
		R: ATGGTGCATTTGTGTTGTAGG								
ZaA113	$(AC)_{21}$	F: AGCTTTTTGATAAGCCTGAAC	GF091747	210–248	29	9	0.828	0.872	0.549	0.712
		R: CTTGCTGTATGTCCACAATG								
ZaA127	$(CA)_{17}$	F: GCATTTGTCTCTCCAGTAGATT	GF091748	245–247	30	2	0.333	0.427	0.088	0.166
		R: AAGCCTGACAGTATTCAAGAAG								
ZaC1	$(TCCA)_9$	F: TCCTGAGCTTCTGGGACAC	GF091749	174–194	29	4	0.586	0.607	0.187	0.344
		R: GGGACAAGGAGCATCCAC								
ZaC3	$(TCCA)_{10}$	F: TTCTTCTGCCAGCACTGTG	GF091750	252–272	28	4	0.429	0.459	0.103	0.238
		R: TTTTCCCTACCTGCCTTTTC								
ZaC11	$(TGGA)_4GGGA(TGGA)_4$	F: GCTTTCATCTGTGTGAGGAC	GF091751	123–151	29	5	0.828	0.791	0.386	0.565
		R: AGGACATCCATAGCCTGATAC								
ZaC12	$(TCCA)_{12}$	F: AGCGTCCGAACTCCTAACTAC	GF091752	279–323	30	9	0.700	0.788	0.400	0.579
		R: GATAGGCTCTCTCTGCTGATG								
ZaC117	$(GGAT)_{11}$	F: AAGGCAAAAAGGAAGGAAAG	GF091753	128–156	27	7	0.852	0.665	0.246	0.412
		R: TCCGTGATTCTATGCTGTAGC								
ZaD1	$(TAGA)_{11}$	F: TAGCGTGGACAGACTTGTTG	GF091754	121–165	29	8	0.759	0.846	0.492	0.664
		R: GGGTGATTTGATGTTCCTTC								
ZaD5	$(TATC)_{13}$	F: ACCACCCTCATTGTAAAAGAT	GF091755	162–282	29	7	0.207*	0.797	–	–
		R: ACACCACAGCAGTAAGTTGAC								
ZaD6	$(TAGA)_8$	F: TCTTGACTTCACTCCTGACAC	GF091756	241–269	30	6	0.500	0.548	0.163	0.330
		R: CCCTACTCCACATCTTCTGTT								
ZaD7	$(TCTA)_4TCCTAA(TCTA)_9$	F: CCTTTTGAAGGTGTTCATC	GF091757	304–332	30	8	0.833	0.833	0.477	0.651
		R: GCATCCAAATGAGTATGC								
ZaD10	$(TTCTA)_8(TTCTG)_4N_{21}(TCCTA)_{16}$	F: CGAGCCTGTCAGTGTTTC	GF091758	368–416	30	11	0.400*	0.894	–	–
		R: TCATGGGTTACTGTTACAACTC								
ZaD11	$(TCTA)_{15}$	F: TGAACCATTGCTCCAGAG	GF091759	230–266	28	7	0.893	0.803	0.418	0.596
		R: TGCCATAACTTGTAGAACTGC								
ZaD104	$(GATA)_{10}$	F: ACCGAAATCCTAACCTGAAC	GF091760	245–273	28	7	0.929	0.841	0.477	0.651
		R: ATGCCAGTCTGTGAAACAAG								

Table 1 continued

Locus	Repeat motif	Primer sequence (5′–3′)	Genbank Accession #	Size Range (bp)	N	N_A	*Ho*	*He*	PE-1	PE-2
ZaD105	$(GATA)_{11}$	F: GCAGGAATCACTTTCAGAGTAC R: TTGAAGATGATACAGCAGAGTG	GF091761	264–284	30	6	0.733	0.647	0.226	0.388
ZaD108	$(TCTA)_{10}$	F: CCTTCCTTTCAATGACTTACC R: GCATCTGTGTCCATCCAG	GF091762	141–173	30	9	0.833	0.881	0.571	0.729
ZaD116	$(CTAT)_{13}$	F: CAGGGGGAAAAAAAAATCT R: TTCTGAGTGAAGATGGGTAAAT	GF091763	123–143	28	6	0.321*	0.761	–	–
ZaD119	$(GATA)_{12}$	F: CATTAACGCAATGTTTTCTGAG R: TGCCCAAAGGTTTGTATTTTAC	GF091764	162–186	29	7	0.897	0.758	0.353	0.531
ZaD121	$(GATA)_{13}$	F: CTGAGGTTCCAACAAACTTG R: TTAGAGGACTGTCCGTTCTGTG	GF091765	203–247	29	10	0.897	0.909	0.641	0.783
Overall									0.999	1.000

N = Number of individual typed, all coming from the same location in Barbados. N_A = Number of alleles per locus. *Ho* and *He* = Observed and expected heterozygoties, respectively. PE-1 and PE-2 are exclusion probabilities of knowing the genotype of one or both parents, respectively

* Significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction for multiple tests ($P < 0.00212$)

molecules. Fragments enriched for microsatellites were subsequently PCR amplified using primer complementary to the adaptator, digested with *Hind*III to remove the adaptor sequences, and ligated into the *Hind*III site of pUC19. The plasmids were then electroporated into *Escherichia coli* DH5α. Recombinant clones, identified by white–blue screening, were chosen at random for sequencing on an ABI373 automated sequencer, using ABI Prism Taq dye terminator cycle sequencing methodology. A total of 81 out of 94 positive clones sequenced contained microsatellites (86.17%). Primers were designed for 50 microsatellite-bearing clones using Designer PCR, versus 1.03 (Research Genetics, Inc.). Enriched library construction, sequencing of positive clones and primer design were performed by Genetic Identification Services (www.genetic-id-services.com). Primers pairs were synthesized (GENCUST, France) for 33 potential loci, each forward primer being 5′ tailed with a M13 sequence (5′-CACGACGTTGTAAAACGAC-3′). PCRs were carried out in 10 µl volume including 5 ng DNA template, 200 µM each dNTP, 200 nM each primer (Table 1), 0.025 µM of 5′ labelled M13 primer with 700 and 800 fluorochromes (LICOR), 1× reaction buffer and 0.15 U HotMaster DNA polymerase (5-PRIME). A T3 thermocycler (Biometra) was used beginning with an initial denaturation at 94°C for 1.5 min, followed by 35 cycles consisting of 20 s at 94°C, 30 s at 46°C and 30 s at 65°C, and a final extension step at 65°C for 3 min. All 33 loci successfully amplified as tested on 6 cm long 2% agarose gels (TBE 1×) for 30 min at 100 V in a RunOne electrophoresis chambers (EmbiTec). Product size variations were visualized using a LICOR 4000L automated sequencer, 6.5% acrylamide, 41 cm long gels. Image analysis and allele scoring were carried out by eye by two independent investigators and then compared. Ten primer pairs either failed to reveal any polymorphism or produced unreadable patterns on a set of eight individuals from one location in Barbados. Polymorphism of the 23 remaining loci was assessed on an extra set of up to 22 Zenaida doves from the same location. Estimates of observed (*Ho*) and expected (*He*) heterozygosities, deviations from Hardy–Weinberg equilibrium (HWE), and genotypic linkage disequilibrium (LD) were estimated using the software FSTAT version 2.9.3.2 (Goudet 1995). Exclusion probabilities knowing the genotype of one or both social parents, PE-1 and PE-2, respectively, were estimated for loci not deviating significantly from HWE using the software CERVUS 2.0 (Marshall et al. 1998).

No linkage disequilibrium was detected after Bonferroni correction for multiple tests. Three loci ZaD5, ZaD10 and ZaD116 showed a heterozygote deficiency, significantly deviating (after Bonferroni correction) from Hardy–Weinberg Equilibrium (Table 1) probably due to null alleles associated with absence of conservation of flanking

Table 2 Trans-specific test of amplification and polymorphism of 23 microsatellite loci initially developed for Zenaida dove (*Zenaida aurita*) for four other *Zenaida* species and one *Colombina* species

Locus	*Z. galapagoensis* ($n = 3$)	*Z. auriculata* ($n = 3$)	*Z. macroura* ($n = 1$)	*Z. meloda* ($n = 3$)	*Colombina passerina* ($n = 2$)
ZaA4	1	3	1	2	1
ZaA5	2	2	1	1	–
ZaA7	1	2	2	1	–
ZaA112	1	2	1	1	1
ZaA113	4	3	1	1	–
ZaA127	2	3	2	2	1
ZaC1	2	2	1	4	2
ZaC3	4	3	1	2	1
ZaC11	2	4	1	1	3
ZaC12	4	4	1	–	1
ZaC117	4	3	2	1	–
ZaD1	2	3	2	4	–
ZaD5	1	2	2	2	–
ZaD6	4	3	2	2	1
ZaD7	4	3	1	3	1
ZaD10	2	2	1	–	1
ZaD11	6	4	1	4	–
ZaD104	1	4	1	1	1
ZaD105	3	2	1	1	1
ZaD108	1	1	2	1	1
ZaD116	2	3	1	–	–
ZaD119	2	2	1	2	1
ZaD121	5	4	1	1	1
AS (%)	100	100	100	87	65
P (%)	74	96	30	50	13
Na	3.18	2.86	2.00	2.70	2.50

N = Number of individuals tested. – Denotes an absence of amplification.
AS = Amplification success, percentage of amplifying loci. P = Percentage of polymorphic loci. Na = Mean number of alleles for polymorphic loci

sequences (Wattier et al. 1998). Therefore, we recommend not using these loci. The remaining 20 loci consist of 2–11 alleles (average 7.05), an expected heterozygosity (*He*) ranging from 0.427 to 0.909 (average 0.712) and an exclusionary power of 0.9999 and 1.0000, knowing the genotype of one social parent, or both, respectively (Table 1). This exclusionary power remains extremely high if only the eight loci with the highest individual PE-1 are taken into account, combined PE-1 = 0.9972 and PE-2 = 0.9999. These results indicate that the 20 polymorphic loci not deviating from HWE will be useful for both studying population genetics and mate choice patterns in *Z. aurita*.

Amplification success and polymorphism were assessed for four other *Zenaida* species (Table 2): the Galápagos dove, *Z. galapagoensis* (three from Galápagos), the eared dove, *Z. auriculata* (two from Uruguay and one from Venezuela), the mourning dove, *Z. macroura* (one individual from the USA), the Pacific dove, *Z. meloda* (three from Peru) and one *Colombina* species, the common ground-dove, *Columbina passerina*. (one from Mexico and one from Venezuela). PCRs and genotyping conditions were identical to those used for *Z. aurita*. The overall success of amplification (Table 2) for the four *Zenaida* species is extremely high (87–100%), being 100% for the three most related to *Z. aurita*, that is *Z. galapagoensis*, *Z. auriculata* and *Z. macroura* (Johnson and Clayton 2000). Many loci are polymorphic (30–96%) with 2.00–3.18 alleles per loci on average (Table 2). These results are very encouraging about the usefulness of the loci developed in *Z. aurita* in other *Zenaida* species, especially taking into account the limited sampling size used ($n = 3$ for three species and only one for *Z. macroura*). Until now, only five loci were available for one species, the Galápagos dove (Santiago-Alarcon et al. 2006). From the 65% of the loci amplified in *C. passerina*, 13% were polymorphic with 2.5 alleles on average. These results also show that the loci developed for *Z. aurita* are likely to be useful for other Columbidae.

Acknowledgements This work was supported by grants to F. C. and R. W. by "Institut Buffon" Université de Bourgogne, to K. M. from the French Ministère de l'Education Nationale (grant # 01121) and to D. S.-A. and P. G. P. from the Whitney R. Harris World Ecology Centre, Idea Wild, The Frank M. Chapman Memorial Fund of the

American Museum of Natural History, The Field Research for Conservation program of the Saint Louis Zoo (FRC 05-2 and FRC 08-2), the Organization for Tropical Studies, and by the Des Lee collaborative vision in zoological studies. Logistical support and permits were provided by the Galápagos National Park, the Darwin Research Foundation and the Nature Heritage Department of Barbados. Authors thank Kenneth Jones and Mark Todd from Genetic Identification Services for their help and patience in developing the microsatellite loci, and Jérôme Moreau for helping collecting biological material.

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