Development and characterization of 13 new, and cross amplification of 3, polymorphic nuclear microsatellite loci in the common myna (*Acridotheres tristis*)

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Abstract We used a next generation sequencing (NGS) approach to screen for genome-wide nuclear microsatellite loci in the common (Indian) myna, *Acridotheres tristis*. In addition, markers previously developed for other Sturnidae species were tested for cross-amplification in *A. tristis*. In total, we identified 20 loci from NGS data and tested 26 loci for cross-amplification. Out of all loci (NGS developed and cross-amplified), 16 unlinked loci showed polymorphism, ranging from 2 to 9 alleles per locus. Test individuals were obtained from the invasive ranges of common myna in South Africa. Overall, expected and observed heterozygosities ranged from 0.089 to 0.802 and from 0.094 to 0.906, respectively. These markers will be used to shed light on invasion genetics and landscape-scale dynamics of invasive *A. tristis* in South Africa.

Keywords *Acridotheres tristis* · Common myna · Invasive species · Microsatellite

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The common (Indian) myna, *Acridotheres tristis*, is one of the most successful invasive bird species in the world (Lowe et al. 2000). It is native to India and South-East Asia (del Hoyo et al. 2009) and has established and invaded many Near East countries and all Indian Ocean coasts (Global Invasive Species Database, www.issg.org/database/). In South Africa, the common myna has been introduced at least a century ago (Peacock et al. 2007) and now is a problematic invading species throughout the eastern and north-eastern parts of the country (van Rensburg et al. 2011). The recent arrival of common mynas in many important conservation areas is especially concerning (Peacock et al. 2007) as, at least by the mid-1980 s, no undisturbed natural habitat in South Africa has been invaded by alien birds (Brooke et al. 1986).

The relatively long residence time of common myna in South Africa, coupled with extensive spread throughout many bioregions in the country, have led to clines in morphological features linked to foraging and dispersal behaviour (Berthouly-Salazar et al., unpublished data). This emphasizes the need for a thorough population genetic study to better understand the influence of contemporary evolution and phenotypic plasticity during the invasion process.

Microsatellites are currently one of the most widely used molecular markers in population genetic studies because of their high polymorphism, co-dominance and their genome wide abundance (Le Roux and Wieczorek 2009).

Table 1 Summary of microsatellites loci tested for cross-amplication in *Acridotheres tristis*

Locus name	Genbank	Size range	Na	Reference
Ase18	AJ276375	–	–	Richardson et al. (2000)
Ase19	AJ276376	172-174	2	Richardson et al. (2000)
MIG-1	U82673	–	–	Li et al. (1997)
Pcaμ3[a]	AJ279805.1	163-185	3	Dawson et al. (2000)
SS1-12	AY906871	–	–	Rubenstein (2005)
SS1-6	AY906875	–	1	Rubenstein (2005)
SS2-106	AY906877	–	1	Rubenstein (2005)
SS2-114	AY906878	–	1	Rubenstein (2005)
SS2-119	AY906879	274-280	2	Rubenstein (2005)
SS2-130	AY906884	260-262	2	Rubenstein (2005)
SS2-16	AY906886	–	1	Rubenstein (2005)
SS2-32	AY906889	–	1	Rubenstein (2005)
SS2-40	AY906892	–	1	Rubenstein (2005)
SS2-46	AY906896	–	1	Rubenstein (2005)
SS2-52	AY906898	–	1	Rubenstein (2005)
SS2-56	AY906901	–	1	Rubenstein (2005)
SS2-68	AY906905	135-139	2	Rubenstein (2005)
SS2-71B[a]	AY906906	320-340	6	Rubenstein (2005)
SS2-80	AY906910	–	1	Rubenstein (2005)
SS2-83	AY906912	–	1	Rubenstein (2005)
SS3-42C[a]	AY906917	122-147	5	Rubenstein (2005)
Sta213	DQ860242	–	1	Celis et al. (2007)
Sta269	DQ860238	–	1	Celis et al. (2007)
Sta294	DQ860240	–	1	Celis et al. (2007)
Sta308	DQ860237	131-135	2	Celis et al. (2007)
Sta317	DQ860244	–	–	Celis et al. (2007)

Na number of alleles

[a] Markers used in multiplexing

Traditionally the development of microsatellites for single species is prohibitively expensive and extremely time consuming. However, the rapid and growing application of next generation sequencing (NGS) is now creating opportunities for fast and relatively cost effective means to identify and characterize DNA markers for population genetics studies (Allentoft et al. 2009, Heather and Fleischer 2010). In addition, the transfer of orthologous microsatellite loci across closely related species represents another valued approach to overcome the limitations of de novo development (Dawson et al. 2010). The aim of this study was to (1) cross-amplify and characterize polymorphic nuclear microsatellite loci in *A. tristis* that were previously tested in other Sturnidae species (*Sturnus vulgaris*, Dawson et al. 2010, Rollins et al. 2009, Rubenstein 2005) and (2) develop new microsatellite loci using a NGS approach for *A. tristis*.

A total of 32 common myna individuals that originated from throughout the KwaZulu-Natal Province in South Africa were used to test for polymorphism. Whole genomic DNA was extracted at Stellenbosch University's Central Analytical Facility.

Twenty six microsatellite loci previously characterized in Sturnidae (Dawson et al. 2010, Rollins et al. 2009, Rubenstein 2005) were tested for successful amplification first in only eight individuals (Table 1).

In addition, novel microsatellite loci were developed following the partial genome NGS methodology of Gardner et al. (2011). Briefly, we sequenced 12.5% of a plate using the GS-FLX 454 platform (Roche, Germany), providing 86 493 sequenced reads with an average read length of 331 bp. Sequence data were scanned for the presence of microsatellite repeat motifs using QDD v 1.3 (Meglécz et al. 2010) and subsequent primers developed using Primer 3 (Rozen and Skaletsky 1999). Twenty one new primer pairs were developed and tested for amplification and subsequent polymorphism. PCR was conducted using a Multigene cycler (Labnet International, Inc.) using the QIAGEN multiplex kit (Qiagen, supplied by Whitehead Scientific, Cape Town) with a thermocycle of: initial denaturation of 94°C for 30 min; 35 cycles at 94°C for 30 s, 60°C for 90 s, 72°C for 90 s; followed by a final extension at 72° C for 10 min. Optimized loci were amplified in all 32 individuals (Table 2).

Table 2 Characteristics of 13 newly developed and 3 cross-amplified microsatellite loci for *Acridotheres tristis*

Locus	Multiplex	Dye	Primer sequences (5′–3′)	Repeat	Na	Size range	PIC (%)	H_E	H_O
Acri1	1	VIC	GAAGCCATAGGAATTGGGAA CAGTTGCAAAGCAGCAGTTC	$(TG)_9$	3	143–147	42.6	0.493	0.375
Acri141	2	NED	CATTGGTTGGGAGCAGATAGA AAGCCATCACTGTGGTCTCC	$(AGAT)_{11}$	9	280–329	75.4	0.782	0.719
Acri142	2	6FAM	CAATGACTGTGCTCTCCAGTG GGAGGTCTGTGGTTAAGGCA	$(TATC)_7$	3	194–203	54.6	0.616	0.594
Acri191	1	NED	GAAGGAGTGGGTGGAAACAA CAGTTCAGAGGGCAGGAAAC	$(AG)_9$	3	96–100	58.3	0.657	0.719
Acri192	2	PET	AGAGGAGTGCGTGTTGGTG GATGTACGGAACTCCCTTTCTG	$(CA)_{10}$	2	93–95	8.5	0.089	0.094
Acri1	2	NED	CATCTTTGCATGACAGTCTGG CAGCCTTCAAGATCTGGTGA	$(AT)_{10}$	3	125–129	18.8	0.203	0.161
Acri22	1	VIC	TCCCACACCAATCCTACTCC AGGTGTATTGGGGCAAGATG	$(ATCC)_{13}$	8	232–260	77.2	0.798	0.903
Acri5	1	PET	TGTAGTGTGAGTATTCCAGACAATCTC TCTGAAAAAGAAGCCATAAAGGA	$(AG)_8$	5	208–214	73.7	0.774	0.781
Acri81	1	6FAM	CAGGCTGGAGAGGAGAATTG TGGCCAGGACAGGGTTAATA	$(GGTGT)_8$	7	330–380	62.5	0.679	0.250*
Acri82	1	NED	TCACTGATGATGCACAATGC GATGCATGTCTGAATAAAGACCC	$(AC)_8$	6	132–144	77.3	0.802	0.906
Acri83	2	VIC	GTGACAGGGTGGCTCCAGT CGACTCTGAACTCCCAACCT	$(TCCA)_{10}$	3	170–176	56.9	0.642	0.563
Acri9	1	NED	GGTCTCCTATGGCAAAACCA TGACTTTTGTTCTCCCAGCA	$(TGGA)_{14}$	4	96–100	64.3	0.696	0.531
AcriNa	2	VIC	GCAGCGCCGCTTCTGCTG GGAGACGGAGGAAGAGAACG	$(TTCCTC)_9$	8	72–120	65.9	0.686	0.625
Pcaμ33[a]	2	PET	Dawson et al. (2000)		7	163–185	73.9	0.771	0.781
SS2-71b[a]	2	VIC	Rubenstein (2005)		7	320–340	69.1	0.720	0.813
SS3-42c[a]	2	VIC	Rubenstein (2005)		6	122–147	73.3	0.771	0.813

Size range, number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (H_E) are based on 32 individuals sampled from throughout South Africa's KwaZulu Natal Province

* Significant deviation from HWE

[a] Primer and other details obtainable from reference

Polymorphism at each locus was screened using an ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, CA, USA), and PCR products were sized relative to a molecular size marker (LIZ500, PE Applied Biosystems, CA, USA). DNA fragments were analysed using GeneMapper (PE Applied Biosystems, CA, USA). Polymorphic Information Content (PIC) values were estimated using MOLKIN 3.0 (Guttiérrez et al. 2005). We calculated expected and observed heterozygosities, significant deviation from Hardy–Weinberg equilibrium (HWE), and the existence of genotypic linkage disequilibrium (LD) using GENEPOP (Rousset & Raymond 1995) for all loci. We also tested for the presence of null alleles using the programme Micro-Checker (van Oosterhout et al. 2004). Among the 26 markers tested for cross-amplification, eight were found with more than two alleles however only three of them were selected for the following analyses according to their range size, annealing temperature and polymorphism.

Among the twenty-one new markers developed from NGS data, 13 were found to be polymorphic, i.e. having at least two alleles. In total, 16 markers (13 new and 3 from cross-amplification) were organized in two multiplexes and tested on 32 individuals. The expected and observed heterozygosities ranged from 0.089 to 0.798 and from 0.094 to 0.906 respectively (Table 2). Fifteen loci had heterozygosities conforming to those expected under HWE. One locus, Acri82b, was the only marker with a significant deficiency in heterozygotes, due to the presence of null

alleles. No significant LD was found for any of the tested loci.

South African populations of common myna are thought to have originated from a few cage bird escapees from the pet trade (Peacock et al. 2007, van Rensburg et al. 2011). The variable loci identified here will be useful to study the genetic structure, dispersal patterns and evolutionary dynamics of common myna invasions in South Africa and could be utilized to do similar studies across the common myna's invasive ranges globally.

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References

Allentoft ME, Schuster SC, Holdaway RN, Hale ML, McLay E, Oskam C, Gilbert MTP, Spencer P, Willerslev E, Bunce M (2009) Identification of microsatellites from an extinct moa species using high-throughput (454) sequence data. Biotechniques 46:195–200

Brooke RK, Lloyd PH, de Villiers AL (1986) Alien and translocated terrestrial vertebrates in South Africa. In: Macdonald IAW, Kruger FJ, Ferrar AA (eds) The ecology and management of biological invasions in southern Africa. Oxford University Press, Cape Town, pp 63–74

Celis P, Gil D, Graves JA (2007) Isolation and characterization of polymorphic microsatellites isolated from sportless starling (*Sturnus unicolor*) and cross-species amplification in the European starling (*Sturnus vulgaris*). Mol Ecol Notes 7:251–253

Dawson DA, Hanotte O, Greig C, Stewart IRK, Burke T (2000) Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Mol Ecol Notes 9:1941–1944

Dawson DA, Horsburgh GJ, Küpper C, Stewart IRK, Ball AD, Durrant KL, Hansson B, Bacon I, Bird S, Klein A et al (2010) New methods to identify conserved microsatellites loci and develop primer sets of high cross-species utility- as demonstrated for birds. Mol Ecol Res 10:475–794

del Hoyo J, Elliott A, David C (2009) Handbook of the birds of the world. Bush-shrikes to old world sparrows, vol 14. Barcelona: Lynx Edicions, p 735

Gardner MG, Fitch AJ, Bertozzi T, Lowe AJ (2011) Rise of the machines—recommendations for ecologists when using next generation sequencing for microsatellite development. Mol Ecol Resour. doi:10.1111/j.1755-0998.2011.03037.x

Guttiérrez JP, Royo LJ, Alvaréz I, Goyache F (2005) MOLKIN v2.0: a computer program for genetic analysis of population using molecular coancestry information. J Hered 96:718–721

Heather L, Fleischer RC (2010) Prospects for the use of next-generation sequencing methods in ornithology. Auk 127:4–15

Le Roux JJ, Wieczorek AM (2009) Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. Ann Appl Biol 154:1–17

Li S-H, Huang Y-J, Brown JL (1997) Isolation of tetranucleotide microsatellites from the Mexican jay *Aphelocoma ultramarina*. Mol Ecol 6:499–501

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species. Aliens 12:S1–S12

Meglécz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin JF (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404

Peacock DS, Rensburg BJV, Robertson MP (2007) The distribution and spread of the invasive alien common myna, *Acridotheres tristis* L. Aves: Sturnidae, in southern Africa. S Afr J Sci 103:465–473

Richardson DS, Jury FL, Dawson DA, Salguiero P, Komdeur J, Burke T (2000) Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. Mol Ecol 9:2226–2231

Rollins LA, Woolnough AP, Wilton AN, Sinclair R, Sherwin WB (2009) Invasive species can't cover their tracks: using microsatellites to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. Mol Ecol 18:1560–1573

Rousset F, Raymond M (1995) GENEPOP (version 3–4): population genetics software for exact test and ecumenism. J Hered 86: 248–259

Rozen S, Skaletsky HJ (1999) Primer3 on the www for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Rubenstein DR (2005) Isolation and characterization of polymorphic microsatellites loci in the plural cooperatively breeding superb starling, *Lamprotornis superbus*. Mol Ecol Notes 5:739–744

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellites data. Mol Ecol Notes 4: 535–538

Van Rensburg BJ, Weyl OLF, Davies SJ, van Wilgen LJ, Peacock DS, Spear D, Chimimba CT (2011) In: Pimentel D (ed) Biological invasions: economic and environmental costs of alien plant, animal, and microbe species, 2nd edn. CRC Press, Boca Raton, pp 325–378