Microsatellite markers for population genetic studies of the rock firefinch, *Lagonosticta sanguinodorsalis*

Jacinta Abalaka[1,2]* & Bengt Hansson[2]

[1]*Department of Genetics, University of Pretoria, Private Bag X20, Hatfield, 0028 South Africa*

[2]*Department of Biology, Lund University, 22362 Lund, Sweden*

Received 27 September 2013. Accepted 20 May 2014

We identified the first set of microsatellites for use in the rock firefinch (*Lagonosticta sanguinodorsalis*). First, we tested existing passerine microsatellite loci for cross-species amplification success in a small sample of rock firefinches and identified 10 loci that were seemingly polymorphic and easy to score. Secondly, we developed and characterized three microsatellite loci *de novo* from the rock firefinch genome. In a larger sample of individuals from three populations, one locus initially interpreted as polymorphic was monomorphic. Among the polymorphic loci, the number of alleles ranged between 2 and 15 and the expected heterozygosity between 0.08 and 0.91 within populations. Two loci had high estimated null allele frequency in at least one population. The microsatellites amplified very well (80% success) in five other African finch species and each locus was confirmed as polymorphic in at least one species. We conclude that these microsatellite markers will be useful for population genetic studies in the rock firefinch and other closely related African finches.

Key words: population genetics, microsatellite, primer, *Lagonosticta sanguinodorsalis*.

Molecular markers have become important tools in understanding the population genetic structure, and population dynamics and colonization history of species. Among the different types of molecular markers, microsatellites (or short-sequence repeats) have been adopted as the marker of choice for detecting intra- and inter-population genetic diversity because of their high variability and abundance throughout the genome (Ellegren 2004).

The rock firefinch, *Lagonosticta sanguinodorsalis*, a seed-eating Estrildidae finch, was described as recently as 1998 (Payne 1998). It has a restricted range and was initially believed to be endemic to Nigeria where it is found in bushy and rocky outcrops on the Jos Plateau and its north and eastward extensions (central Nigeria), on the Mandara Mountains (northeastern Nigeria) and in Rano (northern Nigeria; Payne 1998; Wright & Jones 2005). However, other populations were more recently discovered in Maroua and Mora in northern Cameroon (Mills 2010). The rock firefinch is socially monogamous with a sexually dimorphic plumage where adult males are more brightly coloured than adult females (Fry & Keith 2004). Interestingly, it is the obligate host to a brood parasitic indigobird, the endemic Jos Plateau indigobird (*Vidua maryae*; Brandt & Cresswell 2008).

Although the rock firefinch is listed as species of least concern (http://www.iucnredlist.org/; accessed May 2012), the species' restricted range and specific habitat requirements, and its important role in the life history of another endemic species, make it of conservation interest. Furthermore, it presently occurs in small fragmented populations and the level of movements and dispersal between subpopulations is unknown (Brandt & Cresswell 2008; Abalaka & Jones 2011). As a preparatory step to studying population genetic structure and gene flow in the rock firefinch, we have identified a set of polymorphic microsatellite markers. To our knowledge, no microsatellite markers have previously been reported for *L. sanguinodorsalis*. We also examined the suitability of these markers for use in five other African finches.

Our first approach in identifying usable microsatellite loci was to test 51 existing microsatellite primer pairs designed for other passerine species for cross-species amplification in the rock firefinch. Most of these primers were selected from among those that have proven to amplify successfully across a wide range of species (Simeoni *et al.* 2007; Dawson *et al.* 2010; Olano-Marin *et al.* 2010; Salmona *et al.* 2010). Initially, we tested primers on four individual rock firefinches that had been trapped using mist nets in the Amurum Forest Reserve, Central Nigeria (09°52′44.75″N, 08°58′55.75″E). DNA was extracted from a small amount of blood using a standard phenol-chloroform protocol as described in Bensch *et al.* (1994). The PCR mix contained 4 pmol of each primer

*Author for correspondence. E-mail: jacinta.abalaka@biol.lu.se

(0.4 μM), 1 × NH_4-buffer, 15 nmol (1.5 mM) $MgCl_2$, 2 nmol (0.2 μM) dNTP, 0.5 U AmpliTaq DNA polymerase and 10–25 ng template DNA in 10 μl reaction volume. PCRs were carried out in a GeneAmp PCR System 9700 (Applied Biosystems), and the conditions were as follows: 94°C for 2 min; then 35 cycles with 94°C for 30 s, T_a for 30 s, and 72°C for 30 s; followed by 72°C for 10 min (T_a = locus-specific annealing temperature). The fluorescent-labelled PCR-products were separated and the alleles were detected in an ABI PRISM 3730 capillary sequencer (Applied Biosystems). Ten of the 51 primer pairs tested on the four individual rock firefinces were potentially useful as they had apparently polymorphic products and seemed easy to score. The remaining primers did not amplify (four loci), were polymorphic but weak (three loci), were polymorphic but difficult to score (two loci) or monomorphic (32 loci).

Our second approach was to isolate and characterize microsatellites *de novo* from the rock firefinch genome to increase the number of available markers, as it is more advantageous to invest in the number of highly polymorphic markers than in the number of individuals for many research questions (Landguth *et al*. 2012; Takezaki & Nei 1996).

A microsatellite-enriched library was constructed using protocol provided by Zane *et al*. (2002) with slight modifications following Nordström & Hedren (2007). Genomic DNA (500 ng) of four individuals was digested with 10 U of Bsp1431 (Fermentas) together with 1 × reaction buffer (Fermentas) and 1 μg Bovine Serum Albumin in a total volume of 20 μl. This mix was incubated for 1 hour at 37°C and, to deactivate the enzyme, for 20 min at 80°C. Then, 5 μl ligation reaction mix containing adaptors (5 μM; 5′-GTGGTAGA CTGCGTACC-3′ and 5′-GATCGGTACGCAGTCT AC-3′), ligation buffer (1×; USB) and T4 DNA ligase (0.5 U; USB) was added to the digestion reaction and incubated for three hours at 37°C. The digestion–ligation mix was diluted 1:10, and 10 μl was PCR-amplified using the following conditions: 0.5 μM specific primer (5′-GACTGCGTA CCGATC-3′), 2 mM $MgCl_2$, 0.2 mM dNTPs, 1×polymerase buffer (Applied Biosystems), 0.5 U AmpliTaq DNA polymerase (Applied Biosystems). PCR conditions were as follows: 25 and 30 cycles, respectively, with 94°C for 30 s, 56°C for 1 min, 72°C for 1 min. The samples with the most evenly amplified smear from the 25- and 30-cycle PCRs were pooled and used for the library construction. Pooled PCR products were hybridized with 100 pmol of a mixture of biotinylated oligonucleotides probes ($(AG)_{21}$, $(ATT)_6$, $(AT)_7$, $(AAG)_8$ and $(AAAT)_7$) in a total volume of 100 μl of SSC/SDS buffer. Streptavidin-coated beads (Roche) were then added and incubated at room temperature to capture DNA fragments with microsatellite sequences complementary to the oligonucleotide probes. Beads and attached probes were separated magnetically from the supernatant using a Magnetic Particle Separator (Roche). Following three soft and three hard stringency washes, the bound DNA was recovered by incubating in 1 M Tris-HCL 0.5 M EDTA at 95°C for 5 min.

The TOPO TA Cloning Kit (Invitrogen) was used to clone fragments according to the manufacturer's instructions. Colonies with inserts were identified by white colour and suspended in 150 μl distilled water and heated at 95°C for 3 min. When analyzing the white colonies by PCR, the following conditions were used: 0.4 μM forward primer (5′-TGTAAAACGACGGCCAGT-3′), 0.4 μM reverse primer (5′-CAGGAAACAGCTATGACC-3′), 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 1 × polymerase buffer (Applied Biosystems), 0.5 U AmpliTaq DNA polymerase (Applied Biosystems), 2 μl template in a final volume of 25 μl. The PCR conditions were as follows: 35 cycles with 94°C for 30 s, 50°C for 1 min, 72°C for 1 min. The PCR products were subsequently precipitated and diluted in 10 μl ddH2O. The PCR product (2 μl) was used as template DNA in a sequencing reaction using the BigDye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems) together with the forward primer. The sequencing program was set as follows: 25 cycles with 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. Precipitated products were sequenced on an ABI PRISM 3100 genetic analyser (Applied Biosystems).

Nineteen inserts were sequenced and five unique direpeat microsatellite motifs were detected, in addition to a few monorepeats. Primer pairs were possible to design for four of the five direpeat loci using the program Primer 3 (v. 0.4.0) (Rozen & Skaletsky 2000) and three of them amplified a product of expected length and were interpreted as polymorphic in four test individuals. The repeat motif in the cloned sequence was $(CT)_9$ for Lsa127, $(GT)_6$ for Lsa158 and $(TG)_{18}$ for Lsa165. The position of all three loci in the zebra finch genome was determined by BLAST analyses following Dawson *et al*. (2007) and is given in Table 1.

Table 1. Description of 13 microsatellite loci identified for *Lagonosticta sanguinodorsalis*. Shown are GenBank accession number, reference, primer sequence, position in the zebra finch (ZF) genome, locus specific annealing temperature (T_a) and allele size range in the rock firefinch.

Locus	Accession no. (EMBL/GenBank)	Reference for primer	Primer sequence 5′–3′	ZF chr	ZF start position	T_a (°C)	Allele size range (bp)
TG01-040	DV576233	Dawson *et al.* 2010	F: TGGCAATGGTGAGAAGTTTG R: AGAATTTGTACAGAGGTAATGCACTG	1A	42,620,504	56	280–287
TG05-046	DV957774	Dawson *et al.* 2010	F: AAAACATGGCTTACAAACTGG R: GCTCAGATAAGGGAGAAAACAG	5	50,735,925	56	335–349
TG13-009	DV948691	Dawson *et al.* 2010	F: TGTGGTGGGATAGTGGACTG R: CTGTAAAATGTGCAAGTAACAGAGC	13	3,672,471	56	192–196
TG13-017	CK313422	Dawson *et al.* 2010	F: GCTTTGCATCTTGCCTTAAA R: GGTAACTACAACATTCCAACTCCT	13	18,542	56	296–313
TG22-001	CK317333	Dawson *et al.* 2010	F: TTGGATTTCAGAACATGTAGC R: TCTGATGCAAGCAAACAA	22	1,428,098	56	266–301
Z-002A		Dawson 2007	F: D.A. Dawson unpubl. R: D.A. Dawson unpubl.	Z W	NA	55	209–223
Tgu05	DV946651.1	Slate *et al.* 2007	F:CACAGAAAAGTGAGTGCATTCC R: TGGGAAAACATCTTTACCATCA	1A	64,236,986	58	251–279
Tgu07	DV948303.1	Slate *et al.* 2007	F: CTTCCTGCTATAAGGCACAGG R: AAGTGATCACATTTATTTGAATAT	6	22,807,094	58	91–107
WBSW7	AF130434	McRae & Amos 1999	F: TCTGGAGTTCTGGGACCTG R: CTCACTCAACAGCAGGACC	5	45,275,495	54	141–143
Lswμ14	AF129095	Winker *et al.* 1999	F: GTTATGCTCCAACAAAATAGATA R: AGGTTTTRAAGGATAGATTTATA	2	54,974,454	49	192–202
Lsa127	KF614624	This study	F: TGCAGGCAAATGGTGGTGTG R: TCAAAATACTGTAAATAAATCTTTCCCCC	1A	43,800,199	51–55	231–257
Lsa158	KF614625	This study	F: TGCTTGAGGCAGAGAGAAGGTTATGC R: TCCGGGAGAATGGAGGGTTGC	3	66,032,002	55–58	299
Lsa165	KF614626	This study	F:TGTCAAAGCATCTTCAGTGCTACA R:TGCACACACATGGCAGTTG	1	31,649,863	55–58	161–175

Table 2. Characteristics of 12 microsatellite loci identified for *Lagonosticta sanguinodorsalis* with number of alleles (*A*) , number of successful amplifications (*n*), and observed and expected heterozygosity (H_O, H_E) and *P*-value from Hardy-Weinberg test,

Locus	Amurum					Kagoro					Pankshin				
	A	*n*	H_E	H_O	HWE *P*-value	*A*	*n*	H_E	H_O	HWE *P*-value	*A*	*n*	H_E	H_O	HWE *P*-value
TG01-040	2	18	0.461	0.500	0.7372	3	24	0.405	0.292	0.1308	3	26	0.533	0.577	0.7064
TG05-046	3	19	0.615	0.632	0.6038	3	24	0.442	0.500	0.8410	4	26	0.621	0.731	0.9115
TG13-009	2	19	0.361	0.368	0.7423	2	25	0.077	0.080	1.0000	3	26	0.210	0.231	1.0000
TG13-017	8	17	0.713	0.471	0.0051	7	23	0.713	0.478	0.0051	8	25	0.672	0.480	0.0090
TG22-001	5	18	0.708	0.722	0.5615	5	25	0.735	0.760	0.6308	6	26	0.776	0.885	0.9295
Z-002A*	2	6	0.633	0.632	0.1744	2	11	0.538	0.680	1.0000	3	12	0.652	0.692	0.3410
Tgu05	7	19	0.729	0.789	0.7436	8	25	0.784	0.800	0.5705	7	26	0.744	0.692	0.2179
Tgu07	5	19	0.630	0.579	0.3064	4	25	0.566	0.520	0.3487	7	26	0.686	0.654	0.3436
WBSW7	2	19	0.361	0.474	1.0000	2	25	0.113	0.040	0.0603	2	26	0.233	0.192	0.4077
Lswμ14	2	19	0.432	0.632	1.0000	2	25	0.461	0.480	0.6987	2	26	0.411	0.346	0.3321
Lsa127	12	19	0.792	0.895	0.9577	9	24	0.871	0.792	0.1282	15	25	0.906	0.760	0.0128
Lsa165	5	19	0.636	0.474	0.0359	5	25	0.698	0.720	0.6013	7	25	0.731	0.800	0.8090

*Z-linked marker. Females were excluded from analysis (males: Total = 31; Amurum = 6, Kagoro = 11, Pankshin = 12).

The variability of the 13 successful loci were further tested in 70 individuals from three rock firefinch populations: Amurum (n = 19), Pankshin (n = 26; 09°21′36.23″N, 09°25′52.67″E) and Kagoro (n = 25; 09°34′27.20″N, 08°22′54.39″E). The PCR-mix contained 1 × Q-mix (Qiagen), 1.2 pmol (0.2 μM) each of forward and reverse primer, 10–25 ng DNA template in a 6 μl reaction volume. The PCRs were initiated with a hotstart (95°C for 15 min) followed by seven touchdown cycles with 94°C for 30 s, T_a for 30 s (start T_a depended on the locus; see Table 1) with 0.5 °C reduction per cycle, and 72°C for 30 s. The touchdown was followed by 30 cycles with 94°C for 30 s, T_a minus 3 to 4°C for 90 s and 72°C for 90 s; and a final extension at 72°C for 10 min. PCR products were separated in an ABI PRISM 3730 capillary sequencer (Applied Biosystems).

The results showed that locus Lsa158 which was initially interpreted as polymorphic in the four test individuals was apparently monomorphic in the rock firefinch. Among the 12 polymorphic loci, the number of alleles ranged between 2 and 15 in the different populations (Table 2). Expected heterozygosity ranged between 0.36 and 0.79 in the Amurum population, 0.08 and 0.87 in the Kagoro population, and 0.21 and 0.91 in the Pankshin population (Table 2). Estimation of null allele frequencies implemented in MICRO-CHECKER version 2.2.3 (Oosterhout *et al*. 2004) showed presence of null alleles in TG13-017 for all three populations (ranging from 15–16%) and in Lsa127 for one population (Pankshin; 8%); and these two loci deviated from Hardy-Weinberg equilibrium before (but not after) Bonferroni correction in at least one population (Table 2) using tests implemented in FSTAT version 2.9 (Table 2; Goudet 2001). Z-002A is a Z- and W-linked microsatellite marker that has been used successfully for molecular sexing in several bird species (Dawson 2007). In the rock firefinch, the Z-linked locus was found to be polymorphic with two alleles in 6 and 11 tested males in the Amurum and Kagoro populations respectively, and with three alleles in the 12 tested males in Pankshin population. In addition, genotyping of a few adult male and female rock firefinches – adult birds are easily sexed due to the species' pronounced sexual plumage dimorphism (Fry & Keith 2004) – confirmed amplification of both the Z- and the W-fragments, and since the W-fragment was shorter than all three Z-alleles this primer pair provides a means to sex rock firefinches before

Table 3. Cross-amplification success in five finch species (*n* = number of tested individuals). Number of alleles amplified and no amplification (NA) are indicated.

Locus	*Lagonosticta rara* (*n* = 1)	*Lagonosticta senegala* (*n* = 18)	*Euschistospiza dybowskii* (*n* = 2)	*Vidua maryae* (*n* = 5)	*Vidua chalybeata* (*n* = 2)
TG01-040	1	1	1	5	2
TG05-046	2	6	2	1	1
TG13-009	1	2	1	2	1
TG13-017	1	10	2	5	2
TG22-001	1	5	2	1	1
Z-002A	2	2	3	2	1
TG005	1	9	2	3	3
TG007	1	4	1	1	1
WBSW7	1	1	1	5	1
Lswμ14	1	1	1	2	NA
Lsa127	2	2	3	2	NA
Lsa158	1	4	1	1	1
Lsa165	2	2	2	2	2

they are displaying their sex-specific plumage.

Finally, we tested the set of 13 primer pairs in five closely related African Estrildidae and Viduidae finches: two other *Lagonosticta* species (*L. rara* and *L. senegala*), one other Estrildidae finch (*Euschistospiza dybowskii*) and two Viduidae finches (*Vidua chalybeata* and *V. maryae*; Sorenson *et al.* 2004). The cross-species amplification was highly successful and all loci, except Lsw*μ*14 and Lsa127 in *V. chalybeata*, amplified with varying levels of polymorphism (Table 3). Interestingly, Lsa158, which was monomorphic in the rock firefinch, was polymorphic (four alleles) in *L. senegala*. In three of the species, we tested only one or two individuals (Table 3) and we expect the degree of polymorphism to increase when more individuals and populations are evaluated.

Many species are experiencing fragmentation of their populations as a result of the impact of human activities on their habitat (WCMC 1992). Habitat fragmentation leads to an overall reduction in population size and reduced gene flow among patches (Frankham *et al.* 2002). Genetic diversity can be easily lost in smaller populations, which is unfortunate since it constitutes the raw material for evolutionary change and thus the potential to adapt to changing environments. Loss of genetic diversity can jeopardize the long-term survival prospects of populations due to negative inbreeding effects and even lead to extinction in the event of environmental change (Frankham *et al.* 2002). Thus, understanding and maintaining genetic diversity is of primary concern in conservation biology. The availability of microsatellite markers for the rock firefinch and some related species brings us a step closer to evaluating its genetic status and population genetic structure.

We conclude that our work has detected a set of highly useful microsatellite markers for population genetic research in the rock firefinch and closely related Estrildidae and Viduidae finches.

We would like to thank A.P. Leventis Ornithological Research Institute APLORI, Laminga, Nigeria for logistic support for fieldwork (sample collection). This project was funded by A.P. Leventis Foundation, Swedish Ornithological Society and the Swedish Research Council. Arin Izang was a valuable assistant during the fieldwork. This research was carried out in collaboration with the A.P. Leventis Ornithological Research Institute, Laminga, Nigeria.

REFERENCES

ABALAKA, J.I. & JONES, P.J. 2011. Population densities of the rock firefinch *Lagonosticta sanguinodorsalis* and some other estrildine and viduine finches on the Jos Plateau, Nigeria. In: *Proceedings of the 12th Pan African Ornithological Congress, 2008*, (eds) D.M. Harebottle, A.J.F.K. Craig, M.D. Anderson, H. Rakotomanana & M. Muchai, pp. 131–134. Animal Demography Unit, Cape Town, South Africa.

BENSCH, S., HASSELQUIST, D. & VON SCHANTZ, T. 1994. Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler. *Evolution* **48**: 317–326.

BRANDT, M. & CRESSWELL, W. 2008. Breeding behaviour, home range and habitat selection in rock

firefinches *Lagonosticta sanguinodorsalis* in the wet and dry season in central Nigeria. *Ibis* **150**: 495–507.

DAWSON, D.A. 2007. Genomic analysis of passerine birds using conserved microsatellite loci. Ph.D. thesis, University of Sheffield, Sheffield, U.K.

DAWSON, D.A., ÅKESSON, M., BURKE, T., PEMBERTON, J.M., SLATE, J. & HANSSON, B. 2007. Gene order and recombination in homologous regions of the chicken and a passerine bird. *Molecular Biology and Evolution* **24**: 1537–1552.

DAWSON, D.A., HORSBURGH, G.J., KUPPER, C., STEWART, I.R.K., BALL, A.D., DURRANT, K.L., HANSSON, B., BACON, I., BIRD, S., KLEIN, A., KRUPA, A.P., LEE, J.W., MARTIN-GALVEZ, D., SIMEONI, M., SMITH, G., SPURGIN, L.G. & BURKE, T. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. *Molecular Ecology Resources* **10**: 475–494.

ELLEGREN, H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* **5**: 435–445.

FRANKHAM, R., BRISCOE, D.A. & BALLOU, J.D. 2002. *Introduction to Conservation Genetics*. University of Cambridge Press, Cambridge, U.K.

FRY, C.N. & KEITH, S. (eds) 2004. *Birds of Africa*, Vol. VII. Christopher Helm, London, U.K.

GOUDET, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. Online at: http://www2.unil.ch/popgen/softwares/fstat.htm

LANDGUTH, E.L., FEDY, B.C., OYLER-McCANCE, S. J., GAREY, A.L., EMEL, S.L., MUMMA, M., WAGNER, H.H., FORTIN, M-J. & CUSHMAN, S.A. 2012. Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. *Molecular Ecology Resources* **12**: 276–284.

McRAE, S.B. & AMOS, W. 1999. Characterization of hypervariable microsatellites in the cooperatively breeding white-browed sparrow weaver *Plocepasser mahali*. *Molecular Ecology* **8**: 903–904.

MILLS, M.S.L. 2010. Rock firefinch *Lagonosticta sanguinodorsalis* and its brood parasite, Jos Plateau indigobird *Vidua maryae*, in northern Cameroon. *Bulletin of the African Bird Club* **17**: 86–89.

NORDSTRÖM, S. & HEDREN, M. 2007. Development of polymorphic nuclear microsatellite markers for polyploid and diploid members of the orchid genus *Dactylorhiza*. *Molecular Ecology Resources* **7**: 644–647.

OLANO-MARIN, J., DAWSON D.A., GIRG, A., HANSSON, B., LJUNGQVIST, M., KEMPENAERS, B. & MUELLER, J.C. 2010. A genome-wide set of 106 microsatellite markers for the blue tit (*Cyanistes caeruleus*). *Molecular Ecology Resources* **10**: 516–532.

OOSTERHOUT, C.V., HUTCHINSON, W.F., WILLS, D.P.M. & SHIPLEY, P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535–538.

PAYNE, R.B. 1998. A new species of firefinch *Lagonosticta* from northern Nigeria and its association with the Jos Plateau indigobird *Vidua maryae*. *Ibis* **140**: 368–381.

ROZEN, S. & SKALETSKY, H. 2000. Primer 3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology* **132**: 365–386.

SALMONA, J., DAWSON, D.A., FOUILLOT, D., GHESTEMME, T., THEBAUD, C., CHIKHI, L. & SALAMOLARD, M. 2010. The utility of existing passerine microsatellite markers for genetic studies in endangered species: as demonstrated for a critically endangered forest bird endemic to Réunion Island, the Réunion cuckoo-shrike (*Coracina newtoni*). *Conservation Genetics Resources* **2**: 361–364.

SIMEONI, M., DAWSON, D.A., ROSS, D.J., CHALINE, N., BURKE, T. & HATCHWELL, B.J. 2007. Characterisation of 20 microsatelite loci in the long-tailed tit *Aegithalos caudatus* (Aegithalidae, AVES). *Molecular Ecology Notes* **7**: 1319–1322.

SLATE, J., HALE, M.C. & BIRKHEAD, T.R. 2007. Simple sequence repeats in zebra finch (*Taeniopygia guttata*) expressed sequence tags: a new resource for evolutionary genetic studies of passerines. *BMC Genomics* **8**: 52.

SORENSON, M.D., BALAKRISHNAN, C.N. & PAYNE, R.B. 2004. Clade-limited colonization in brood parasitic finches (*Vidua* spp.). *Systematic Biology* **53**: 140–153.

TAKEZAKI, N. & NEI, M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite data. *Genetics* **144**: 389–399.

WCMC. 1992. *Global Biodiversity: Status of the Earth's Living Resources*. Chapman & Hall, London.

WINKER, K., GLENN, T.C. & GRAVES, G.R. 1999. Dinucleotide microsatellite loci in a migratory wood warbler (Parulidae: *Limnothlypis swainsonii*) and amplification among other songbirds. *Molecular Ecology* **8**: 1553–1556.

WRIGHT, D. & JONES, P. 2005. Population densities and habitat associations of the range-restricted rock firefinch *Lagonosticta sanguinodorsalis* on the Jos Plateau, Nigeria. *Bird Conservation International* **15**: 287–295.

ZANE, L., BARGELLONI, L. & PATARNELLO, T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology* **11**: 1–16.

Responsible Subject Editor: S. Willows-Munro