MOLECULAR ECOLOGY RESOURCES

Molecular Ecology Resources (2011) **11**, 877–889 doi: 10.1111/j.1755-0998.2011.03015.x

Description of microsatellite markers and genotyping performances using feathers and buccal swabs for the Ivory gull (*Pagophila eburnea*)

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Abstract

We report 22 new polymorphic microsatellites for the Ivory gull (*Pagophila eburnea*), and we describe how they can be efficiently co-amplified using multiplexed polymerase chain reactions. In addition, we report DNA concentration, amplification success, rates of genotyping errors and the number of genotyping repetitions required to obtain reliable data with three types of noninvasive or nondestructive samples: shed feathers collected in colonies, feathers plucked from living individuals and buccal swabs. In two populations from Greenland (n = 21) and Russia (Severnaya Zemlya Archipelago, n = 21), the number of alleles per locus varied between 2 and 17, and expected heterozygosity per population ranged from 0.18 to 0.92. Twenty of the markers conformed to Hardy–Weinberg and linkage equilibrium expectations. Most markers were easily amplified and highly reliable when analysed from buccal swabs and plucked feathers, showing that buccal swabbing is a very efficient approach allowing good quality DNA retrieval. Although DNA amplification success using single shed feathers was generally high, the genotypes obtained from this type of samples were prone to error and thus need to be amplified several times. The set of microsatellite markers described here together with multiplex amplification conditions and genotyping error rates will be useful for population genetic studies of the Ivory gull.

Keywords: allelic dropout, amplification success, Arctic, buccal swab, feather, genotyping errors, Ivory gull, microsatellites, multiplex PCR, *Pagophila eburnea*

Received 6 September 2010; revision received 1 March 2011; accepted 7 March 2011

Introduction

The ‘most Arctic’ of all birds, the Ivory gull (*Pagophila eburnea*) has been the focus of much recent attention (e.g. Krajick 2003). Over its entire breeding range (Canadian Arctic, Greenland, Svalbard and Western Russian Arctic islands), it breeds either on inland cliffs and nunataks, i.e., rocky outcrops emerging from icecaps, or on high-Arctic barren islands or flatlands (Gilg *et al.* 2009). The precise number and distribution of breeding colonies is uncertain (see Gilchrist *et al.* 2008), and the wintering habits of the species are even less well documented (but see Gilg *et al.* 2010). The International Union for Conservation of Nature and Natural Resources (IUCN) classified the Ivory gull as near threatened on the red list of threatened species, with major threats including pollution and climate change (IUCN 2010). In Canada, where the status of the species was recently updated to ‘Endangered’, recent studies claim that 80% of the breeding population was lost during the past 20 years (Gilchrist & Mallory 2005). Although its status is still under evaluation in all other countries holding breeding populations, a recent international circumpolar ‘Conservation Strategy

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and Action Plan' was presented by leading seabird experts from Arctic countries (Gilchrist *et al*. 2008), which among other issues pointed out that more information on genetic diversity was needed. Such information would be valuable *per se* because genetic diversity is directly linked with the ability of populations to cope with environmental change. In addition, genetic tools would help us resolve population structure (especially regarding the connectivity of remnant populations), past population demography (e.g. by identifying genetic signatures of population decline), and individual relationships (e.g. parentage). The primary object of this paper is the description of a set of microsatellite markers that will allow genetic studies of the Ivory gull. We detail also the conditions for successful multiplex polymerase chain reaction (PCR) amplification, which consists of the co-amplification of several microsatellites in a single reaction, thereby reducing cost, increasing speed and reducing consumption of DNA (Butler 2005; Luikart *et al*. 2008; Beja-Pereira *et al*. 2009). Reducing manipulation and handling (fewer PCR per individual) also minimizes the possibility of contamination and error during laboratory process (Beja-Pereira *et al*. 2009).

The secondary object of this paper is the estimation of microsatellite genotyping performances (amplification success, error rates and number of genotyping repetitions required) using shed feathers, plucked feathers and buccal swabs from the Ivory gull. With endangered species, sampling should be as nonintrusive as possible. Feathers provide a readily available source of DNA that can be noninvasively collected for genetic studies of birds (Horváth *et al*. 2005; Rudnick *et al*. 2007; Hogan *et al*. 2008). However, considerable variation may occur in the quantity and the quality of the recovered DNA, depending on whether the samples are fresh plucked feathers (Harvey *et al*. 2006) or shed feathers (Hogan *et al*. 2008). Buccal cells collected using cotton swabs provide another source of DNA that is now regularly used in a number of species: amphibians (Pidancier *et al*. 2003; Broquet *et al*. 2007a), fish (Smalley & Campanella 2005), mammals (Mitrecic *et al*. 2008) and more rarely birds (Bush *et al*. 2005; Handel *et al*. 2006; Brubaker *et al*. 2011). Nondestructive and noninvasive samples such as feathers and buccal swabs may contain small quantities of DNA and/or degraded DNA. Multilocus microsatellite genotypes may thus be difficult to obtain, and they may be imperfectly reliable because of the occurrence of genotyping errors that cannot be easily avoided: allelic dropouts (ADO: one allele of a heterozygous individual is not amplified during a positive PCR) and false alleles (FA: PCR-generated allele as a result of a slippage artefact during the first cycles of the reaction). The number of times that each sample must be independently genotyped to ensure the reliability of the data depends upon the frequency of such errors (Taberlet *et al*. 1996; Pompanon *et al*. 2005). Quantifying the rates of ADO and FA requires a comparison of the genotypes obtained using noninvasive sampling with a reference genotype of the same individuals (typing of noninvasive *vs* invasive samples), or a repetition experiment (repeated typing of noninvasive samples) (Taberlet *et al*. 1996). While Handel *et al*. (2006) showed that buccal swabs can be used for sexing, genotyping, and sequencing mitochondrial DNA from chickadees (*Poecile* spp.), the reliability of multilocus microsatellite genotypes obtained from bird buccal swabs has yet to be quantified.

Material and methods

Population sampling and study area

The Ivory gull, the only species of the genus *Pagophila*, breeds in the Canadian Arctic Archipelago, in Greenland, in Svalbard and in the western Russian Arctic Islands (Mallory *et al*. 2008). Samples were collected on breeding colonies from geographically distinct populations within the northeast Atlantic range of the species, that is, Svalbard (Norway), northeastern Greenland, Franz Josef Land and Severnaya Zemlya Archipelago, Russia (Tables 1 and 2). Two nondestructive DNA sampling methods (collection of mouth swabs and plucked feathers) and a noninvasive sampling method (collection of shed feathers) were used. In Svalbard and Greenland, birds were trapped using a noose on a pole or flap nets, respectively, and buccal cells were collected using synthetic cotton swabs individually packaged in sterile polypropylene tubes (Milian). Each sample required only 5–10 s to be collected, and birds were immediately released after sampling. Buccal swabs were air-dried for 10–15 min before be placed back in individual plastic collection tubes. Then, buccal swabs were placed in plastic bags containing silica gel beads and kept at ambient temperature (usually around 0–10 °C) in the field for up to 1 month and finally stored at −20 °C back in the laboratory. In Svalbard, plucked feathers (from birds' breasts) and blood samples were taken from each individual in addition to buccal swabs (Table 2). In Greenland, muscle and buccal swabs were occasionally sampled on fresh carcasses found in the colonies. In Russia, we collected shed feathers found in the breeding colony (single feathers were placed dry in individual paper envelopes).

To assess the variability of the isolated markers in natural populations, we genotyped 42 individuals, that is, 21 from Station Nord, Greenland and 21 from Domashny Island, Severnaya Zemlya Archipelago, Russia (Table 1). To estimate the amplification success and genotyping error rates obtained from different sampling methods, we randomly picked a subset of plucked feathers (n = 8),

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Table 1 Samples used for evaluating microsatellite genetic variability

Country	Region	Site	Latitude	Longitude	N	Status	Type of samples
Greenland	Northeast Greenland National Park	Station Nord	81°35′49.20″N	16°39′24.18″W	21	Adults	Buccal swab
Russia	Severnaya Zemlya Archipelago	Domashny	79°30′34.34″N	91°3′11.04″E	21	Adults	Shed feather

Table 2 Samples used for measuring genotyping performances

Country	Region	Site	Latitude	Longitude	Shed feather	Plucked feather	Buccal Swab
Russia	Severnaya Zemlya Archipelago	Domashny	79°30′34.34″N	91°3′11.04″E	2		
Russia	Kara Sea Islands	Heiberg Islands	77°36′50.99″N	101°30′31.08″E	1		
Russia	Franz Josef Land	Komsomalets	80°45′58.79″N	94°50′21.61″E	4		
Russia	Franz Josef Land	Rudolfa	81°45′8.20″N	58°23′30.76″E	1		
Russia	Franz Josef Land	Eva-Liv	81°38′7.75″N	63°13′2.65″E	2		
Russia	Franz Josef Land	Nagurskoje	80°42′54.46″N	48°13′8.36″E	2		
Norway	Svalbard	Svenskoya	78°43′1.60″N	26°37′46.69″E		8	8*
Greenland	Northeast Greenland National Park	Station Nord	81°35′49.20″N	16°39′24.18″W			1†
Greenland	Northeast Greenland National Park	Amdrup Land	80°50′46.55″N	14°37′39.63″W			3†
Total					12	8	12

*The genotype was confirmed from blood extract.
†The genotype was confirmed from muscle extract.

shed feathers (n = 12) and buccal swabs (n = 12) from populations across Svalbard, Greenland and Russia (Table 2). Genotypes obtained from blood or muscle were used as reference genotypes (see Table 2).

Microsatellite development

Specific markers were developed from genomic libraries enriched for microsatellite motifs constructed by Genetic identification services (http://www.genetic-id-services.com). Libraries were built using a sample containing 100 μg of genomic DNA extracted from a dead Ivory gull chick collected on Domashny Island, Severnaya Zemlya Archipelago, Russia (79°30′N, 91°01′E). Libraries were enriched for GT, CAG, CATC and TAGA following Jones *et al.* (2002). Out of 116 different microsatellite-containing clones, 81 had flanking sequences of sufficient length for designing primers, which was done using DESIGNER-PCR version 1.03 (Research Genetics, Inc.). Out of 49 pairs of primers tested, 22 pairs amplified microsatellites that were both easy to score and polymorphic enough to be potentially useful for population genetic analyses.

Microsatellite genotyping and genetic diversity

Genomic DNA was extracted from a single feather or buccal swab using the DNeasy Tissue kit or the BioSprint 96 DNA Blood Kit used with the BioSprint 96 Workstation (QIAGEN), following manufacturer's instructions. DNA was eluted in a final volume of 150–200 μl of buffer AE (QIAGEN). The yield of DNA was quantified using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc). Individuals were genotyped independently in 10-μl multiplex reactions containing 3 μl of DNA extract (0.3–60 ng/μl per reaction depending on the DNA sources, see Results), 0.6 × Multiplex PCR Master-Mix (QIAGEN), providing a final concentration of 1.8 μM $MgCl_2$ and multiplex primer sets (concentrations ranged from 0.05 to 0.35 μM, see Fig. 1). Either four or six loci were co-amplified in the same reaction (details of multiplex conditions are shown in Fig. 1). For each locus, the 5′ end of the forward primer was fluorolabelled with a FAM, HEX or NED dye. A negative control containing no tissue was included in each set of DNA extractions, and a no-template negative control was included in each PCR. PCR was performed on GeneAmp PCR Systems 2700 or 9700 (Applied Biosystems), according to the following thermal profiles: initial denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, annealing at 57 °C for 90 s, elongation at 72 °C for 1 min, and a final elongation step at 60 °C for 30 min. Amplification products were run on an ABI PRISM 3100 (Applied Biosystems) automated DNA sequencer. Alleles were scored with GENEMAPPER 4.1 (Applied Biosystems). Note that in the case of the Domashny Island population, where samples were obtained from shed feathers, we performed three PCR replicates of each locus to obtain reliable information on marker polymorphism (see Results). All

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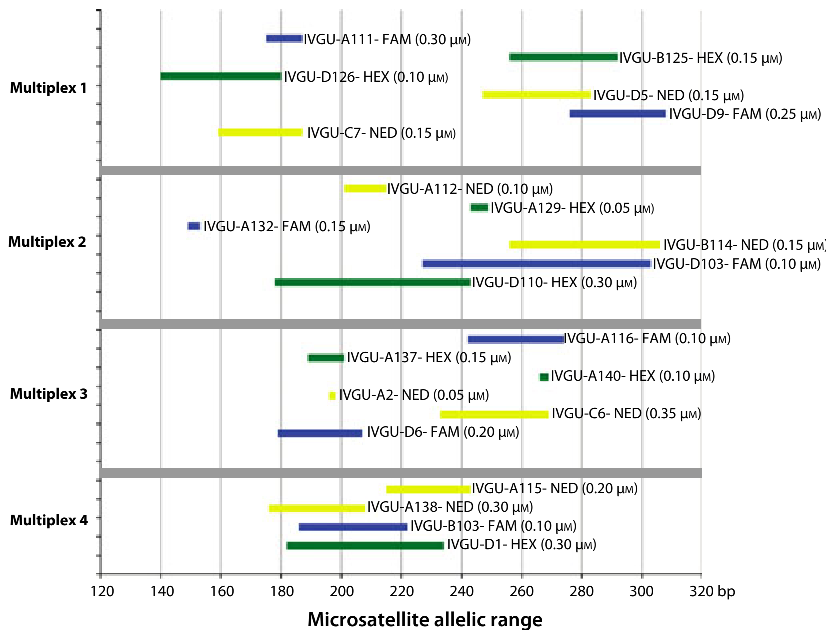

Fig. 1 The allelic range and colour assignment of primers for multiplex polymerase chain reactions, where FAM, HEX and NED are fluorable dyes used. The primer concentration is also given for each locus.

PCR repetitions were performed totally independently: that is, starting from a given DNA extract obtained from one sample, independent DNA templates are pipetted and used in independent PCR.

The software GENEPOP 4.0 (Rousset 2007) was used to estimate allele number, observed heterozygosities (H_o) and expected heterozygosities (H_e) per population, and Hardy–Weinberg (HW) equilibrium within populations. We used the software FSTAT 2.9.4 (Goudet 1995, 2001) to test for linkage equilibrium in each pair of loci using a likelihood-ratio statistic. Bonferroni corrections were applied to account for multiple comparisons. As departure from Hardy–Weinberg equilibrium may be explained by the presence of null alleles, we used MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to detect potential null alleles and to calculate their frequency using Brookfield's method (Brookfield 1996, equation 2).

Evaluation of genotyping performances

The genotyping performances obtained using different types of samples were evaluated using the multitubes approach described in Taberlet *et al.* (1996): we amplified and genotyped eight times each subset of buccal swabs (n = 12), plucked feathers (n = 8) and shed feathers (n = 12), independently at each locus (i.e. 5632 amplifications). Following Taberlet *et al.* (1996), this repetition procedure allowed us to determine the consensus genotype of each individual, which in 12 cases could also be confirmed by independent genotyping obtained using other sources of DNA (i.e. blood and muscle tissue). Once reliable consensus genotypes were obtained, we quantified genotyping performances using five parameters. First, amplification success was estimated for each locus as the proportion of PCR leading to a readable genotype (following e.g. Pompanon *et al.* 2005). Second, locus-specific rates of ADO and FA were calculated following equations (1) and (3) in Broquet & Petit (2004), respectively. These estimators provide unbiased estimates of error rates (in particular by taking into account the fact that ADO should be estimated considering heterozygous genotypes only). Third, global genotyping performances using buccal swabs, plucked feathers and shed feathers were evaluated using the quality index (QI) described by Miquel *et al.* (2006). This statistics integrates amplification failures and genotyping errors to provide a standardized index of performance (ranging 0–1) that can be easily used to compare samples, loci and field/laboratory protocols. Following Miquel *et al.* (2006), we calculated the quality index (QI) of each locus and each sample used in our genotyping repetition experiment, and we combined these values into a global quality index for each sampling method. Fourth, we used the software GEMINI 1.3.0 (Valière *et al.* 2002) to determine the number of repetitions required to achieve an acceptable level of genotyping reliability with buccal swabs and plucked or shed feathers. To this end, GEMINI simulates genotypes based upon given allelic frequencies and genotyping error rates (ADO and FA) and calculates the proportion of accurate multilocus genotypes obtained when genotyping is repeated a number of times (Valière *et al.* 2002). We performed 100 replicates of such simulations for 200 theoretical individuals created from the average allelic frequencies observed in Greenland and Russia (that is,

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Table 3 Characterization of 22 polymorphic microsatellite loci for the Ivory gull, *Pagophila eburnea*

Locus	Repeat in clone	Primer sequence (5′–3′)	Ta (C°)	Size range (bp)	Domashny Island, Russia						Station Nord, Groenland							
					n	No. Alleles	*Ho*	*He*	*HWE*		*n*	No. Alleles	*Ho*	*He*	*HWE*		$P_{(ID)Sib}$	GenBank Accession no.
IVGU-A111	$(GT)_{12}$	F: FAM-TGTCCAGTCCAGTCCTCAC R: TTCCTACAAAACGCAGTTGA	56.8	175–187	21	6	0.48	0.54	0.07	NS	21	6	0.67	0.65	0.92	NS	5.06E-01	HM171614
IVGU-A112	$(CA)_{14}$	F: NED-CATGGAAGGACAAGTGATTG R: TGGAATACCTGCTCAGAGC	57	201–215	20	5	0.75	0.63	0.31	NS	21	6	0.67	0.77	0.22	NS	4.37E-01	HM171620
IVGU-A115	$(GT)_{19}$	F: NED-CCCAAGACTACAATGATTGC R: TGTTAAACGGCTCTTCTGTC	56.9	215–243	21	8	0.81	0.73	0.92	NS	21	6	0.71	0.73	0.10	NS	4.12E-01	HM171633
IVGU-A116	$(GT)_{22}$	F: FAM-GGCAGCAAAGGTATGTGG R: AATCGGAACTATCGCCAAC	57.8	242–274	21	9	0.95	0.85	0.00	*	20	12	0.75	0.89	0.07	NS	3.15E-01	HM171629
IVGU-A129	$(GT)_{12}$	F: HEX-GACCCCTCGGACAAACTG R: CCCAGGACCAGCCATTAG	58.1	243–249	21	3	0.67	0.58	0.72	NS	21	4	0.48	0.59	0.52	NS	5.23E-01	HM171622
IVGU-A132	$(AC)_{12}$	F: FAM-GGAAGGGAGGAAACAACTC R: GGTTCAAGGTGGATTTTACC	56	149–153	21	3	0.57	0.54	1.00	NS	21	3	0.48	0.53	0.02	NS	5.59E-01	HM171618
IVGU-A137	$(CA)_{12}$	F: HEX-AGTGGGAAGCAAAAGTGG R: TTGGGGAAGAAACTCTGC	56.8	189–201	21	4	0.33	0.30	1.00	NS	21	2	0.10	0.09	0.10	NS	8.15E-01	HM171626
IVGU-A138	$(CA)_{14}$	F: NED-GAGCCTCCAGCACCTTTC R: CCCTGCCCTTCCTAAGAA	57.1	176–208	20	10	0.85	0.90	0.11	NS	21	12	0.95	0.87	0.49	NS	3.15E-01	HM171630
IVGU-A140	$(GT)_{9}$	F: HEX-CAGTGGGGTTCAGTCCTG R: AAGGTTAGCCAGATGTGCTG	57.8	266–269	21	2	0.52	0.47	0.66	NS	21	2	0.29	0.42	0.27	NS	6.35E-01	HM171627
IVGU-A2	$(GT)_{12}$	F: NED-AGCCGACTCTTTAGGACGC R: AATCCCCCAGACTCAGTGG	57.8	196–198	21	2	0.29	0.32	1.00	NS	21	2	0.05	0.05	NA	NA	8.23E-01	HM171624
IVGU-B103	$(TGGA)_{13}$	F: FAM-TTCACGCAGAGAAGTGTCC R: GGCAAAGGGAAGGATCAT	57.3	186–222	20	6	0.75	0.80	0.88	NS	21	6	0.71	0.72	0.34	NS	3.93E-01	HM171631
IVGU-B114	$(CCAT)_{14}$ CCCAT	F: NED-GTGAGAGGTGCTGACACATAC R: CGGCTGAACAAATACTGATAA	57.3	256–306	20	10	0.90	0.87	0.06	NS	21	10	0.95	0.88	0.95	NS	3.32E-01	HM171623
IVGU-B125	$(CCAT)_{16}$ CCCT	F: HEX-GCCGCTGTGTCTCTCTTC R: TTCCAAGGGTGTCTGAGG	57.8	256–292	21	9	0.90	0.87	0.80	NS	21	9	0.90	0.83	0.93	NS	3.38E-01	HM171616
IVGU-C6	$(CCAT)_{16}$	F: NED-ACAAGGCACTCAGTCCAGT R: GCCTAGTAAAGTTGAAGAATGC	57	233–269	21	8	0.86	0.85	0.22	NS	21	7	0.86	0.81	0.70	NS	3.47E-01	HM171628
IVGU-C7	AGGA $(TGGA)_{14}$	F: NED-TCCAGGTCTTACATCCCAG R: AAGCCCAATCCAGTATGAA	56.3	159–187	21	6	0.81	0.76	0.82	NS	21	8	0.86	0.80	0.39	NS	3.90E-01	HM171613
IVGU-D1	TTAT $(CTAT)_{16}$ CTAA	F: HEX-ATGCCACAACTGTGAAGACTC R: TCTGCACATCATAGGTGGAATA	57.5	182–234	18	11	0.78	0.82	0.04	NS	20	12	0.85	0.90	0.70	NS	3.32E-01	HM171632
IVGU-D103	GAGA $(TAGA)_{6}$	F: FAM-GGGGACCACTTGATGACC R: TTCAGGCAGCAGAGATGC	57.9	227–303	20	13	0.90	0.92	0.11	NS	21	14	0.76	0.91	0.04	NS	2.99E-01	HM171621
IVGU-D110	$(CTAT)_{10}$	F: HEX-TTTCCAAAGAGCAAGATG R: ATGAGAGGTGACCATACAAG	55.3	178–243	17	8	0.35	0.80	0.00	*	20	13	0.60	0.91	0.00	*	3.19E-01	HM171619

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Table 3 Continued

Locus	Repeat in clone	Primer sequence (5′–3′)	Ta (C°)	Size range (bp)	Domashny Island, Russia					Station Nord, Groenland						
					n	No. Alleles	Ho	He	HWE	n	No. Alleles	Ho	He	HWE	$P_{(ID)sib}$	GenBank Accession no.
IVGU-D126	$(TAGA)_{15}$ TAGG	F: HEX-AGGAGTGCTGGGAGATG R: CGGAGAGTGTTGGGTTCC	57	140–180	20	9	0.95	0.81	0.00 NS	21	10	0.81	0.84	0.40 NS	3.59E-01	HM171612
IVGU-D5	$(CTAT)_{16}$	F: NED-CAGGATTGCTGATACGAGTC R: ACTTTGACCCAGGCTGTAG	57.1	247–283	20	9	0.75	0.88	0.06 NS	21	7	0.90	0.82	0.75 NS	3.40E-01	HM171617
IVGU-D6	$(GACA)_6$	F: FAM-TCTCCCAGGATCTACTTATCAC R: GAAACACCTGAGGAAAAGATTA	56.4	179–207	21	7	0.29	0.85	0.00 *	21	6	0.52	0.83	0.01 *	3.39E-01	HM171625
IVGU-D9	$(GATA)_9$	F: FAM-GTGCAACATAGCACAAAATAGC R: CTGCTCTTAGCTGAACAGTCAG	57.7	276–308	21	7	0.57	0.78	0.02 NS	21	6	0.71	0.75	0.04 NS	4.00E-01	HM171615

Reported are: locus name; repeat motif, sequences for forward (F) and reverse (R) primers; optimal annealing temperature (Ta) for simplex amplification; allele size range; number of individuals successfully genotyped per population (n); number of observed alleles; observed (H_O) and expected (H_E) heterozygosity; as well as the significance of departure from Hardy–Weinberg equilibrium (HWE) test. NS, Non-significant; *, significant, after multitest adjustment, based on a sequential goodness of fit metatest (SGoF; Carvajal-Rodriguez *et al.* 2009); probability of identity ($P_{(ID)sib}$); and GenBank Accession no. NA, data not avalaible.

the two populations used for describing the polymorphism of the newly developed microsatellites). Based upon the error rates measured in our repetition experiment (see above), GEMINI determines the reliability of the genotypes that would be obtained for these 200 theoretical individuals if they would be sampled using buccal swabs, plucked feathers and shed feathers, and independently genotyped 1–8 times. Because downstream applications may differ in the number of microsatellites required, the genotyping accuracy that must be attained, or the quantity of DNA available, we ran this analysis for two extreme cases, first using the complete panel of 22 markers, then using only the 10 markers showing the highest quality index. Most potential applications using these markers should fall in between these two scenarios.

Finally, we calculated the probability that two individuals drawn at random from a population will have the same multilocus genotype (P_{ID}) when using either one of these two microsatellite panels (i.e. 22 or 10 loci). This statistic is especially useful for analyses requiring reliable fingerprinting (e.g. individual identification, estimation of population size or parentage analyses). The software GEMINI was used to calculate $P_{(ID)sib}$, a conservative estimator corresponding to the probability of genotype identity among sibs (Waits *et al.* 2001, equation 3).

Results and discussion

All loci were polymorphic, with 2–17 alleles detected per locus across populations and between 2 and 14 alleles per locus per population. Observed heterozygosities (H_O) ranged from 0.05 to 0.95 and expected heterozygosities (H_E) ranged from 0.05 to 0.92 (Table 3). No significant difference in the number of alleles, allelic richness and gene diversity was observed between the two genotyped populations. Two loci (IVGU-D110 and IVGU-D6) departed significantly from H–W equilibrium in the two populations and a third one (IVGU-A116) in Domashny (Russia) only. This departure may be linked to null alleles (frequency of null alleles as calculated in MICRO-CHECKER v2.2.3 (Van Oosterhout *et al.* 2004): IVGU-D110, $r = 0.53$ and IVGU-D6, $r = 0.30$). Null alleles were also detected in locus IVGU-D9 ($r = 0.11$). No linkage disequilibrium between loci was detected after Bonferroni corrections (Goudet *et al.* 1996).

DNA concentration extracted from a single plucked feather ranged from 0.12 to 0.85 ng/μl (mean ± SE = 0.41 ± 0.27) and that from a single shed feather ranged from 0.89 to 1.79 ng/μl (1.35 ± 0.39). The DNA concentration obtained from a buccal swab ranged from 0.90 to 65.4 ng/μl (11.76 ± 18.10). We report in Table 4 published examples of DNA concentrations obtained from similar DNA sources in birds, *i.e.*, plucked feather, shed feather and buccal swab as well as from blood for com-

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Table 4 Examples of biological samples, taxa and purposes for which noninvasive or nondestructive sampling have been used in birds; When avalaible, DNA concentrations are given as well as methods of extraction. Examples of blood sampling are also given for comparison

DNA Source	Bird Species	Weight (g)*	Invasiveness category	DNA concentration (ng/µl)†	DNA extraction methodology	Purpose of the study	Reference
Plucked feather	Ivory Gull (*Pagophila eburnea*)	520–700	Nondestructive	0.41 ± 0.27	DNeasy Tissue kit‡	Methodology	Present study
Plucked rectrix feather	Black-capped Chickadee (*Poecile atricapilla*)	9.2–14	Nondestructive	1.16 ± 0.72	DNeasy Tissue kit‡	Sex determination	Harvey *et al.* 2006
Plucked feather	Greater Flamingo (*Phoenicopterus roseus*)	2100–4100	Nondestructive	NA	Alkaline	Sex determination	Balkiz *et al.* 2007
Plucked feather	Capercaillie (*Tetrao urogallus*)	1700–4300	Nondestructive	NA	DNeasy Tissue kit‡	Methodology	Segelbacher 2002
Plucked feather	47 species (39 genera, 21 families & 10 orders)	/	Nondestructive	NA	Chelex 100 resin§	Sex determination	Jensen *et al.* 2003
Shed feather	Capercaillie (*Tetrao urogallus*)	1700–4300	Noninvasive	NA	DNeasy Tissue kit†	Methodology	Segelbacher 2002
Shed feather	Ivory Gull (*Pagophila eburnea*)	520–700	Noninvasive	1.35 ± 0.39	DNeasy Tissue kit†	Methodology	Present study
Molted contour and body feather	Greater Sage-Grouse (*Centrocercus urophasianus*)	1350–3200	Noninvasive	13	DNeasy Tissue kit†	Methodology	Bush *et al.* 2005
Molted wing and tail feather	Greater Sage-Grouse (*Centrocercus urophasianus*)	1350–3200	Noninvasive	0	DNeasy Tissue kit†	Methodology	Bush *et al.* 2005
Clot-shed Feather	Spanish-Imperial Eagle (*Aquila heliaca*)	2500–3500	Noninvasive	92.19 ± 76.8	DNeasy Tissue kit†	Methodology	Horváth *et al.* 2005
Tip-shed Feather	Spanish-Imperial Eagle (*Aquila adalberti*)	2500–3500	Noninvasive	–	DNeasy Tissue kit†	Methodology	Horváth *et al.* 2005
Molted remige feather	Roseate Spoonbill (*Platalea ajaja*) Jabiru Stork (*Jabiru mycteria*)	1400 8000	Noninvasive	115.5 ± 13	Prot K + phenol–chloroform	Methodology	Miño & Del Lama 2009
Shed feather	Powerful Owl (*Ninox strenua*)	1250–1500	Noninvasive	NA	Saline Solution	Methodology	Hogan *et al.* 2008
Molted feather	Macaws (*Ara* spp)	900–1700	Noninvasive	0–40	DNeasy Tissue kit†	Methodology	Gebhardt *et al.* 2009
Shed feather	Lesser Spotted Eagle (*Aquila pomarina*)	1100–2000	Nondestructive	NA	NucleoSpin Tissue¶	Social organization, sex determination and individual assignment	Meyburg *et al.* 2007
Shed feather	Eastern Imperial Eagle (*Aquila heliaca*)	2450–4530	Nondestructive	NA	Prot K + ammonium acetate	Individual identification, genetic parentage analyses, and population monitoring	Rudnick *et al.* 2005
Shed feather	Eastern Imperial Eagle (*Aquila heliaca*)	2450–4530	Nondestructive	NA	Prot K + ammonium acetate	Population genetics	Rudnick *et al.* 2008
Buccal swab	Ivory Gull (*Pagophila eburnea*)	520–700	Nondestructive	11.76 ± 18.10	BioSprint 96 DNA Blood kit‡	Methodology	Present study

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Table 4 Continued

DNA Source	Bird Species	Weight (g)*	Invasiveness category	DNA concentration (ng/μl)†	DNA extraction methodology	Purpose of the study	Reference
Mouth swab	Greater Sage-Grouse (*Centrocercus urophasianus*)	1350–3200	Nondestructive	41	DNeasy Tissue kit‡	Methodology	Bush *et al.* 2005
Buccal swab	Black-capped Chickadee (*Poecile atricapilla*) Boreal Chickadee (*P. hudsonica*)	9.2–14 7–12.4	Nondestructive	4.3 ± 4.9	QuickExtract**	Methodology, individual and Sex identification	Handel *et al.* 2006
Buccal swab	Black-capped Chickadee (*Poecile atricapilla*) Boreal Chickadee (*P. hudsonica*)	9.2–14 7–12.4	Nondestructive	2.7 ± 3.9	Salt-extraction protocol	Methodology, individual and Sex determination	Handel *et al.* 2006
Buccal swab	Japonese Quail (*Coturnix japonica*) Eastern Screech-Owl (*Megascops asio*)	90 166–194	Nondestructive	NA	DNARelease Additive††	Sex determination	Brubaker *et al.* 2011
Blood	Black-capped Chickadee (*Poecile atricapilla*) Boreal Chickadee (*P. hudsonica*)	9.2–14 7–12.4	Nondestructive	257 ± 202	Salt-extraction protocol	Methodology, individual and Sex determination	Handel *et al.* 2006
Blood	Roseate Spoonbill (*Platalea ajaja*) Jabiru Stork (*Jabiru mycteria*)	1400	Invasive	~130	Prot K + phenol–chloroform modified protocol	Methodology	Miño & Del Lama 2009
Blood	Greater Sage-Grouse (*Centrocercus urophasianus*)	1350–3200	Invasive	119	DNeasy Tissue kit‡	Methodology	Bush *et al.* 2005
Blood	Ivory Gull (*Pagophila eburnea*)	520–700	Invasive	22.05 ± 7.59	BioSprint 96 DNA Blood kit‡	Methodology	Present study
Blood	Black-capped Chickadee (*Poecile atricapilla*)	9.2–14	Invasive	30.95 ± 18.79	DNA Blood mini kit‡‡	Sex determination	Harvey *et al.* 2006

*Source: del Hoyo *et al.* (1992–2011).
†Note that raw material used for extraction and elution volume may vary among studies.
‡QIAGEN Inc., Valencia, California.
§Sigma-Aldrich Co, St Louis, MO.
¶Macherey-Nagel GmbH & Co. KG, Düren, Germany.
**EPICENTRE, Madison, Wisconsin.
††Eppendorf, Westbury, New York.
‡‡Finnzymes, Woburn, MA, USA.
NA, data not available.
(Harvey *et al.* 2006) (Balkiz *et al.* 2007) (Segelbacher 2002) (Jensen *et al.* 2003) (Bush *et al.* 2005) (Horváth *et al.* 2005) (Miño & Del Lama 2009) (Hogan *et al.* 2008) (Gebhardt *et al.* 2009) (Meyburg *et al.* 2007) (Rudnick *et al.* 2005) (Rudnick *et al.* 2008) (Handel *et al.* 2006) (Handel *et al.* 2006) (Miño & Del Lama 2009) (Brubaker *et al.* 2011).

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Table 5 Amplification success, frequency of false alleles (FA) and rate of allelic dropout (ADO) for microsatellite genotypes of *Pagophila eburnea* using feathers and buccal swabs. Variables *n* and *n(Hz)* are the number of PCR trials and of heterozygote genotypes used for estimating error rates following equations 1 and 3 in Broquet & Petit (2004)

	IVGU-A111	IVGU-A112	IVGU-A115	IVGU-A116	IVGU-A129	IVGU-A132	IVGU-A137	IVGU-A138	IVGU-A140	IVGU-A2	IVGU-B103
Plucked feathers											
n	62*	64	64	62*	64	64	62*	64	62*	62*	64
Amp suc (%)	100	100	96.88	100	100	100	100	98.44	100	100	98.44
FA (%)	0	0	0	0	0	0	0	0	0	0	0
n (Hz)	48	48	63	47	32	48	8	55	24	15	55
ADO rate (%)	0.00	2.08	0.00	0.00	3.13	0.00	0.00	0.00	0.00	13.33	0.00
Shed feathers											
n	96	96	96	96	96	96	96	96	96	96	96
Amp suc (%)	83.33	93.80	93.80	85.42	97.92	98.96	93.80	95.83	89.58	93.75	91.67
FA (%)	2.53	2.22	1.15	2.47	1.07	0	1.11	0	0	0	0
n (Hz)	31	75	79	65	38	63	16	79	31	7	70
ADO rate (%)	6.45	2.67	13.92	16.92	23.68	6.35	0.00	10.13	12.90	14.29	7.14
Buccal swabs											
n	95*	94*	96	96	94*	94*	96	96	96	96	96
Amp suc (%)	100	100	100	100	100	100	100	100	100	100	100
FA (%)	0	0	0	0	0	0	0	0	0	0	0
n (Hz)	63	63	72	72	47	63	32	80	48	24	72
ADO rate (%)	0.00	0.00	0.00	0.00	2.13	0.00	0.00	0.00	2.08	4.17	0.00

	IVGU-B125	IVGU-B114	IVGU-C6	IVGU-C7	IVGU-D1	IVGU-D103	IVGU-D110	IVGU-D126	IVGU-D5	IVGU-D6	IVGU-D9
Plucked feathers											
n	62*	64	62*	62*	64	64	64	62*	62*	62*	62*
Amp suc (%)	100	100	100	100	98.44	100	100	100	100	98.39	100
FA (%)	0	0	0	0	0	0	1.56	3.23	0	0	0
n (Hz)	62	64	55	46	47	56	32	62	46	30	56
ADO rate (%)	0.00	0.00	0.00	0.00	2.13	0.00	0.00	0.00	0.00	0.00	0.00
Shed feathers											
n	96	96	96	96	96	96	96	96	96	96	96
Amp suc (%)	96.88	96.90	92.71	96.88	77.10	97.92	93.80	97.92	96.88	85.42	96.90
FA (%)	1.08	1.15	0	1.08	2.94	0	3.66	0	0	0	1.08
n (Hz)	77	79	55	85	58	79	42	63	93	48	61
ADO rate (%)	6.49	2.53	1.82	1.18	8.62	7.59	7.14	0.00	2.15	10.42	4.92
Buccal swabs											
n	95*	94*	96	95*	96	94*	94*	95*	95*	96	95*
Amp suc (%)	100	100	100	100	100	100	100	100	100	100	100
FA (%)	0	0	0	0	0	1.06	0	0	0	0	0
n (Hz)	95	86	88	47	27	78	46	95	72	48	79
ADO rate (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

These estimates are based on 24 individuals sampled (see Material and methods) either using plucked feathers ($N = 8$), shed feathers ($N = 12$) or buccal swabs ($N = 12$), and repeatedly genotyped eight times at each locus (the number of amplifications for each locus is $n = 64$ for shed feathers and $n = 96$ for plucked feathers and buccal swabs).
*In this case, a problem with size standard during electrophoresis did not allow scoring all the alleles. Only the available amplifications were therefore used to estimate genotyping success at this locus.

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parison. The DNA concentrations achieved in this study are slightly lower than values reported for plucked feathers from smaller size birds (1.16 ± 0.7 ng/µl; Harvey *et al.* 2006), but are in the order of magnitude or higher for buccal swabs (e.g. 4.3 ± 4.9 ng/µl; Handel *et al.* 2006). However, it is worth noting that Nanodrops have quite limited accuracy at low DNA concentration (i.e. ≤2 ng/µl), which is where all of the feather extractions were estimated to be. Moreover, concentration estimates can be influenced by extraction reagents. The smallest values reported here must thus be interpreted with caution. Amplification success, recorded as the proportion of PCR that lead to a readable genotype, ranged between 97 and 100% for plucked feathers, 77 to 99% for shed feathers and was 100% for buccal swabs extracts for all loci (Table 5). No significant relationship could be drawn between DNA concentrations and amplification success, neither between allele size range or microsatellite repeat motif and error rate (data not shown, see e.g. Broquet *et al.* 2007b for a comparative analysis of these issues using larger data sets). In 12 cases, the genotypes obtained from feathers or swabs could be compared with that obtained from blood or muscle of the same individual. In all these cases, the consensus genotype obtained in the repetition experiment was confirmed. The reliability of the genotypes was very good overall, but shed feathers produced more genotyping errors than swabs and plucked feathers (Table 5). Buccal swabs produced particularly reliable genotypes (one false allele was observed at one locus, while three loci were affected by ADO with locus-specific rates ranging between 2.08–4.17%). Using plucked feathers, two and four loci were affected by FA (rates 1.56–3.23%) and ADO (2.08–13.33%), respectively. Finally, with shed feathers FA occurred in 12 loci (1.07–3.66%), while ADO were observed for 20 loci (1.18–23.68%). The rates of genotyping errors obtained with shed feathers are similar to those obtained with hair or faeces in mammals (Broquet *et al.* 2007b). In agreement with the rates of amplification success and genotyping errors presented above, the quality index per sample (Fig. 2) was very good (QI > 0.97) for all buccal swabs and plucked feathers, while it was more variable for shed feathers (range 0.36–1). As expected, the quality index per locus (Fig. 3) was also very variable for shed feathers (range 0.63–0.98), while it was consistently high for plucked feathers (0.97–1) and buccal swabs (0.99–1). Overall, the global quality index for shed feathers reached 0.858, while it was equal to 0.990 and 0.998 for plucked feathers and buccal swabs, respectively.

In Fig. 4, we present the genotyping accuracy expected from buccal swabs, plucked feathers, and shed feathers, would each sample be genotyped 1–8 times independently. For shed feathers, and using the complete set of markers that we described here (22 loci), at least three repetitions are required to achieve a reasonable level of genotyping accuracy (that is, >93% of error-free genotypes, Fig. 4a). One or two additional repetitions will be needed for analyses requiring very high accuracy levels (e.g. parentage or mark-recapture based upon genetic fingerprinting). Buccal swabs and plucked feathers appeared much more reliable, even when using all 22 loci, but 100% accuracy nonetheless requires more than one genotyping. These guidelines are conservative because one could lower the risks of error by removing a few error-prone genetic markers. This is illustrated by the results obtained for a reduced panel composed of the

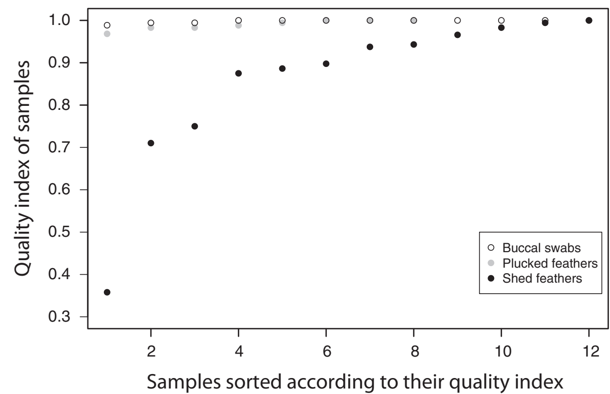

Fig. 2 Quality indexes obtained from the 22 loci for the three different DNA sources: buccal swabs (n = 12 individuals), plucked feathers (n = 8 individuals) and shed feathers (n = 12 individuals). Indexes are obtained according to the method by Miquel *et al.* (2006). Overall, the global quality indexes are QI = 0.858 for shed feathers, 0.990 for plucked feathers and 0.998 for buccal swabs.

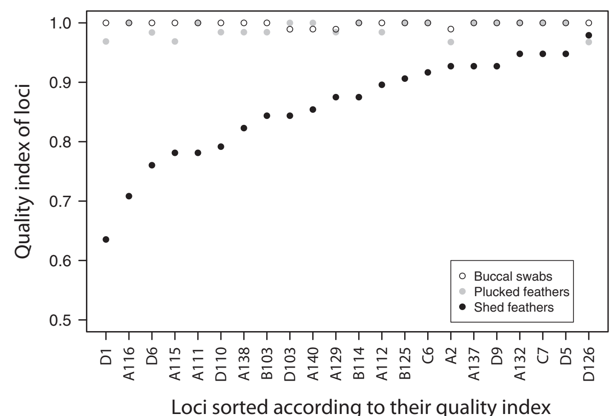

Fig. 3 Quality indexes obtained for the 22 different loci from the three different DNA sources (buccal swabs, plucked feathers and shed feathers). Indexes are obtained according to the method by Miquel *et al.* (2006).

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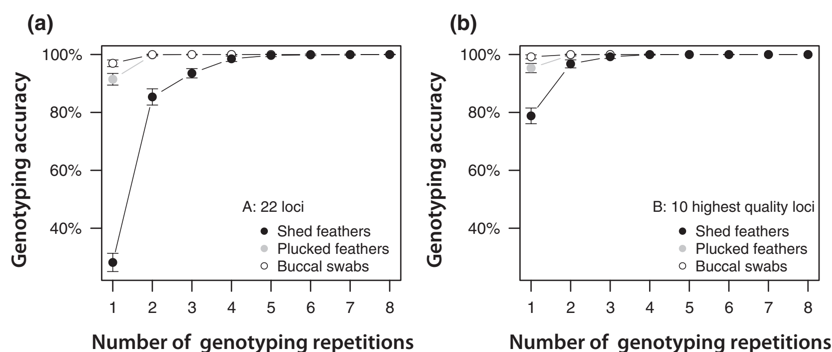

Fig. 4 Genotyping accuracy obtained for the 22 loci (a) and a subset of 10 loci with the highest quality index (b) after 1 to eight repetitions using buccal swabs, plucked feathers and shed feathers.

ten loci showing the highest quality index (i.e. the loci that will be most easily amplified and that will generate fewest errors). Using such a limited panel allows reduction in the number of repetitions required (Fig. 4b). While a number of additional factors (e.g. locus polymorphism, suspicion about null alleles and multiplex combinations) will drive the final choice of markers for a particular application, it will most likely fall in between the two extreme examples provided here in regard to typing reliability. Finally, it is interesting to note that even the reduced panel of 10 loci provides reasonable power to distinguish among individuals (10 loci: $P_{\text{(ID)sib}} = 3.7{\cdot}10^{-4}$; 22 loci: $P_{\text{(ID)sib}} = 3.5{\cdot}10^{-9}$), with probabilities of identity well below the minimum recommended by Waits *et al.* (2001) for individual discrimination, considering that the size of the entire circumpolar Ivory gull population is believed to range between 8000 and 11 500 breeding pairs (Gilchrist *et al.* 2008).

These results show that nondestructive or noninvasive sampling methods based upon the collection of plucked feathers, buccal swabs or shed feathers are very efficient and less invasive methods than blood sampling for obtaining DNA. However, one should be aware that when employing noninvasive sampling strategies, such as the use of shed feathers, considerable variation can occur in the quality of the recovered DNA (Hogan *et al.* 2008). In most cases, the history of shed feathers is unknown (see also, Segelbacher 2002; Rodriguez-Munoz *et al.* 2007; Rudnick *et al.* 2007). Shed feathers could be in the field for several months prior to analysis. Stochastic environmental events, such as heavy rainstorms, can compromise DNA quality even in relatively fresh material (Waits & Paetkau 2005). Consequently, the degree of DNA degradation (*i.e* fragmentation into fragments <100 bp and to single strand breaks) in shed feathers is largely unknown (Hogan *et al.* 2008), leading to variable amplification success and unpredictable error rate compared to plucked feathers or buccal swabs. The lower amplification success obtained with shed feathers in this work is in accordance with other studies that reported DNA degradation, presence of PCR inhibitors and limited success with nuclear marker amplification using moulted feathers (e.g. Segelbacher 2002; Bush *et al.* 2005; Gebhardt & Waits 2008). The two other DNA sources (plucked feathers and buccal swabs) need also to be stored in a safe manner and kept perfectly dry to avoid moisture development that could irreversibly degrade DNA (Taberlet *et al.* 1999). Our results will add valuable insights for estimation of the number of genotyping repetitions required given the sampling methods, the choice of microsatellites and the genotyping precision that must be attained.

Acknowledgements

This work was supported by a foundation Agassiz (Switzerland) grant to TB, and by grants from foundation Ellis Elliot (Switzerland), Société vaudoise des Sciences naturelles (Switzerland), and Nos Oiseaux (Switzerland) to GY and EP. This work has also been supported by Nicolas Perrin's research group (University of Lausanne). Field work in Greenland was supported by the National Geographic Society, Prix Gore-Tex initiative, Fondation Avenir Finance, the Arctic Ocean Diversity Census of Marine Life Project, CNES, CLS and F. Paulsen. The field work in Norway and Russia was funded by the Norwegian Ministry of Environment, the Norwegian Polar Institute and Arctic and Antarctic Research Institute. The project was part of the work plan of the Joint Norwegian-Russian Commission on Environmental Protection. The work in Russia was part of the Russian IPY program and we thank here leader of the *Arktika-2008 IPY Expedition* Vladimir Sokolov for providing opportunity to visit Domashny Island colony and collect Ivory gull samples. Subject editor G. Travis and three anonymous reviewers contributed constructive insights to this work.

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Data accessibility

Sequence data deposited with GenBank (see Table 3).

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