

# A multiplex microsatellite set for non-invasive genotyping and sexing of the osprey (*Pandion haliaetus*)

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**Abstract** During the 1950s and 1970s the osprey (*Pandion haliaetus*) experienced a dramatic population crash and remains of conservation concern in several parts of the world. We isolated 37 microsatellite loci and assessed these in ospreys sampled in the UK and Norway (using mouth swabs/feathers). From 26 loci variable in four ospreys, we selected 13, combined these into two multiplex-PCR sets and included a sex-typing marker. Additional markers confirmed sexes. In 17 ospreys, feather-sampled in central Norway, we found 3–10 alleles per locus. The 13 loci are autosomal (heterozygotes were present in both sexes) and observed heterozygosities ranged from 0.24 to 0.94. The combined probability of identity for the 13 loci was  $8.0 \times 10^{-12}$ . These microsatellite loci will be useful for genetic monitoring, parentage analysis and population genetic studies of the osprey.

**Keywords** Birds of prey · Feather · Raptor · Sex-typing · Simple Tandem Repeat (STR) · Swabs · Western osprey

## Introduction

The osprey (*Pandion haliaetus*) is a fish-eating raptor with an almost worldwide distribution. It experienced a dramatic decline in population size in the 1950s–1970s primarily due to the use of pesticides and is studied as a sentinel species to detect pollution (Grove et al. 2009). European populations of ospreys are migratory, spending the summer in Europe and winter in Africa, whereas other populations are resident. Although the osprey has recovered to some degree and is no longer threatened globally, it is still of conservation concern in some areas (BirdLife International 2013). To facilitate genetic monitoring through non-invasive sampling of shed feathers, and to enable analyses of genetic diversity, parentage and population structure, we isolated and characterized novel microsatellite loci for the osprey.

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## Methods

Microsatellite sequences were isolated from a male osprey (02/09). This individual hatched at Rutland Water Nature Reserve, near Oakham, UK in 2009 but died of an infection at 6 weeks old. Genomic DNA was extracted from liver tissue, digested with *Mbo*I, enriched for dinucleotide/tetranucleotide sequences, cloned and Sanger-sequenced bidirectionally, identifying 96 unique osprey microsatellites (following Armour et al. 1994). In addition, an Illumina paired-end library was created from the dinucleotide + tetranucleotide-enriched DNA

**Table 1** Assessment of 37 osprey (*Pandion haliaetus*) microsatellite loci in three populations

Locus <sup>a</sup>	Clone name and ENA sequence accession no.	Chromosome location <sup>b</sup>	Primer sequences (5'–3')	Primer T <sub>m</sub> (°C) <sup>c</sup>	Repeat motif	Country where sampled	n	A	Expected allele size (bp) <sup>d</sup>	Observed allele size range (bp)
Pha01 Unreliable	Osp107_A01WZ	Gga and Tgu,	F: [HEX]GTCAAACAGTGTGCCCTAGCAG	60.90	(TG) <sub>10</sub>	NOR	4	0	195	No amp.
	LN829364	Multiple copies	R: TACCCGGGAAGCTTGGAC	61.00		SCOT	6	0	Unreliable	Poor amp.
<b>Pha02</b>	Osp107_A08	NCBI: GgaW & Z	F: Set 1 = [PET]TTATCTGCAAGGCCCTGGTGG	63.37	(CA) <sub>15</sub>	ENG	48	0	No amp.	No amp.
	LN829365	Gga6, 26441864 Tgu6, 24709071	R: ACAGGAGTGGAGGAGGTAGT F: Set 2 (UK) = [6FAM]ATTACTGCAAGGCCCTGGTGG R: CTGCTGCTGGAAATGCTC	55.22 60.10		NOR	4	5	200	196–208
Pha03	Osp107_C03	Gga17, 5182075	F: [6FAM]TCTAGCCCATCTCCAGTGAATC	59.03	(TG) <sub>9</sub>	NOR	4	2	112	105–111
	LN829366	Tgu17, 5717909	R: AATTAGAAAGTTGGTGCAGTCCC	59.17		SCOT	6	1	111	111
Pha04	Osp107_C09	Gga3, 45456042	F: [VIC]ATGACCAGTCTGATGCCTTG	58.67	(CA) <sub>12</sub>	ENG	48	1	111	111
	LN829367	Tgu3, 39010319	R: ACATTTGGAGGGTTTCTTGC F: [HEX] used for UK samples	59.03		NOR	4	4	160	158–168
Pha05	Osp107_C10	Gga3, 21714508	F: [HEX]CATTTAACGGTTTAGAAAGTGAAGG	59.54	(GT) <sub>12</sub>	SCOT	6	5	160	143–167
	LN829368	Tgu3, 11588718	R: TGTAGTGAATGAATAACAAATGAAGC	59.87		ENG	48	6	143–165	143–165
Pha06	Osp107_D06	Gga1, 182509839	F: [HEX]CAAGCTTGTAGCAGTCTTGCAG	60.38	(CA) <sub>19</sub>	NOR	4	0	117	No amp.
	LN829369	Tgu1, 4702446	R: TGCCCTGACAGAAAGCAGCAG	60.35		SCOT	6	3	108–113	108–113
Pha07	Osp107_D07	Gga9, 5205116	F: [6FAM]GATCAACCTCGCTCATCTAG	54.30	(AC) <sub>9</sub>	ENG	48	2	122	108–112
	LN829370	Tgu9, 841456	R: ACGTAACTAAAGAGAGCCTC	54.25		NOR	4	1	125	122
Pha08 Unreliable	Osp107_D08	Gga7, 27710560	F: [HEX]ITACAGGGAGGTCAGCCAATC	60.07	(AC) <sub>12</sub>	SCOT	6	1	121	121
	LN829371	Tgu7, 6096203	R: GGGTTTGCCTACATGGGTATC	60.45		ENG	47	1	121	121
Pha09	Osp107_F09	Gga4, 60184616	F: [6FAM]CTTGTGCCAGTTGCTAGG	59.75	(TG) <sub>11</sub>	NOR	4	0	209	No amp.
	LN829372	Tgu4, 19049909	R: TTAGGGAAGGCAGTTGATGAG	59.32		SCOT	4	3	Unreliable (201–211)	(201–211)
<b>Pha10</b>	Osp107_F12	Gga—no hits	F: Set 1 = [PET]TGGTGAAGCCAGTGAAG	61.78	(GT) <sub>22</sub>	ENG	48	2	248	No amp.
	LN829373	Tgu3, 76487951	R: ACATTAACCTTCACCTTGTCA F: Set 2 (UK) = [6FAM]GAAAGCCCAAGTGAAGTAAAGATAGG	58.49 59.70		NOR	4	4	178	183–211
			R: GTCAGTGAAGGTGGCACAAG	59.31		SCOT	6	5	299	300–332
						ENG	47	6	300–330	300–330

**Table 1** continued

Locus <sup>a</sup>	Clone name and ENA sequence accession no.	Chromosome location <sup>b</sup>	Primer sequences (5'–3')	Primer T <sub>m</sub> (°C) <sup>c</sup>	Repeat motif	Country where sampled	n	A	Expected allele size (bp) <sup>d</sup>	Observed allele size range (bp)
Pha11	Osp107_G04	Gga26, 3808355	F: [HEX]ATCATTGTCTCCGTTGAAATACTC	58.59	(TG) <sub>12</sub>	NOR	4	4	369	362–374
	LN829374	Tgu—no hits	R: TGGCTTAAAGACATGAGCTG	59.02		SCOT	5	3		366–372
Pha12	Osp107_G05Z	Gga—no hits	F: [HEX]JTGCAATCCTAATGAACCTTTGCG	60.09	(CA) <sub>15</sub>	NOR	4	3	299	294–302
	LN829375	TguZ, 23578707	R: AGGCTGGTGGTTAAACATGG	59.85		SCOT	4	3	(females=)	300–304
<b>Pha13</b>	Osp107_G06	Gga12, 12834746	F: [6FAM]AGACAAATTAATTTCTGCCCCTGC	59.49	(AC) <sub>9</sub>	NOR	4	5	193	184–194
	LN829376	Tgu12, 13613680	R: CATAGCTGCACATGACTTCCC	59.05		SCOT	6	5		185–195
Pha14	Osp107_G07	Gga6, 7231355	F: [6FAM]CTGAGCCCTACAGGTCAAGG	59.86	(CA) <sub>14</sub>	NOR	4	3	163	155–163
	LN829377	Tgu6random, 1131071	R: GATCAAAGTATAAGCTTCTGGCACT	59.42		SCOT	6	2		155–163
Pha15	Osp107_H11	Gga—no hits	F: [6FAM]AGGAGAACTGGGCTTGGTC	59.24	(GT) <sub>11</sub>	NOR	4	2	148	149–151
	LN829378	TguLGE11random, 434714	R: TTTGTCACTCTGAACCCCAACTC	59.23		SCOT	6	2		149–151
<b>Pha16</b>	Osp108_C02	Gga4, 60893985	F: [6FAM]JTTAGGACATGAAAGACCATCTAGC	60.04	(GT) <sub>11</sub>	NOR	4	3	300	296–302
	LN829379	Tgu4, 19753992	R: AGGCTCGAATCAAGGAATAGG	59.70		SCOT	6	4		296–302
Pha17	Osp108_D06	Gga3, 6186457	F: [6FAM]GATCAATTTGAGTCAGGGTTGTAGA	59.53	(GT) <sub>12</sub>	NOR	4	2	273	258–261
	LN829380	Tgu3, 23071942	R: CCCAGGCAATGTGTGATAGTAG	59.52		SCOT	6	4		258–263
Pha18	Osp108_D09	Gga14, 7369333	F: [6FAM]JTTGGTCACTTCTGTGGAACC	58.54	(CT) <sub>13</sub>	NOR	4	6	204	205–257
	LN829381	Tgu14, 16292216	R: GGACGCATGGTGTAAACTTC	58.08		SCOT	6	5		205–261
Pha19	Osp108_E06	Gga2, 137582088	F: [6FAM]ATGGTGTCTGGTGGTACTGC	60.62	(GT) <sub>11</sub>	NOR	4	3	94	90–94
	LN829382	Tgu2, 138654459	R: AAGCGATTCACTCCATGCTC	60.37		SCOT	6	2		90–92
Pha20	Osp108_F01	Gga7, 32493856	F: [HEX]CTTTGTGAGCCTGCAAGTACG	59.80	(TG) <sub>9</sub>	NOR	4	2	110	111–113
	LN829383	Tgu7, 35798065	R: CCACCTGAGGACTAAGCCTG	59.46		SCOT	6	3		110–113
Pha21	Osp108_F04	Gga2, 138399255	F: [6FAM]CACAGCCTTAAAGTTCAGCTG	59.77	(AC) <sub>9</sub>	NOR	4	1	146	149
	LN829384	Tgu2, 145579947	R: TTGAGAAGCCTTCCACGACC	59.97		SCOT	6	2		147–149
Pha22	Osp108_F05	Gga8, 19109998	F: [HEX]JCTGCAGGGAGCCGATG	60.02	[GA(CA) <sub>4</sub> ] <sub>5</sub>	NOR	4	8	285	143–149 (266–452)

Table 1 continued

Locus <sup>a</sup>	Clone name and ENA sequence accession no.	Chromosome location <sup>b</sup>	Primer sequences (5'–3')	Primer T <sub>m</sub> (°C) <sup>c</sup>	Repeat motif	Country where sampled	n	A	Expected allele size (bp) <sup>d</sup>	Observed allele size range (bp)
Unreliable	LN829385	Tgu—no hits	R: ATTCGCCTGACCTATGTTGC	60.10		SCOT	6	3	Unreliable	(266–300)
Pha23	Osp108_F09 LN829386	Gga2, 64794670 Tgu—no hits	F: [6FAM]GCTCAGGACAGGAAACAAC	59.76	(CA) <sub>9</sub>	NOR	4	2	180	179, 183
			R: CATGTAGAAGCTGCAGCCTCG	59.34		SCOT	6	2		179, 183
Pha24	Osp108_G03 LN829387	Gga—no hits Tgu1, 38622635	F: [6FAM]GATCTGTTTCTAACCCCTCACAATAC	59.87	(TG) <sub>15</sub>	NOR	4	1	217	(220)
			R: TGTCATTAACAATTCAGAAAAGATTACC	60.07		SCOT	6	3	Unreliable	(214–224)
Pha25	Osp108_H01 LN829388	Gga—no hits Tgu24, 2050527	F: [HEX]CTGGGTTAAAGTCAGTGGGATTG	59.24	(GT) <sub>9</sub>	NOR	4	3	174	177–181
			R: TGTCCATGCACCTATCCATCC	59.58		SCOT	6	1		179
Pha26	Osp108_H08Z LN829389	GgaZ, 55975474 TguZ, 68820524	F: [HEX]TTGAGTTGTTTTAGACTTTTGACA	54.64	(TG) <sub>9</sub>	NOR	4	1	144	(144)
			R: TCCTTATTTTCATCCTCACTGA	54.53		SCOT	6	2	Unreliable	(142–143)
<b>Pha27</b>	Osp34 LN829390	Ggal3, 10093338 Tgu13, 4122045	F: [6FAM]TTTAAACAGCTCCCACTCTGATG	59.38	(GATA) <sub>11</sub>	NOR	4	5	173	164–196
			R: AGCATGCTTGTGGTGAC	59.55		SCOT	6	6		164–192
<b>Pha28</b>	Osp222 LN829391	Gga, no hit Tgu, no hit	F: [6FAM]GGTGGAAAACCTCCCTGAGC	59.65	(CTAA) <sub>11</sub>	NOR	4	5	130	164–196
			R: TGCTTTTGGGGTGAAAAGTC	60.09		SCOT	6	5		117–133
<b>Pha29</b>	Osp354 LN829392	Gga6, 22515994 Tgu9, 22351865 Plus Unknown chr 110289344	F: [NED]AAAGTCCAGGCGCAGTTTGTG	59.19	(TATC) <sub>12</sub>	NOR	4	5	144	116–129
			R: GAACGCTGTTGGACCTTC	59.18		ENG	48	5		117–137
<b>Pha30</b>	Osp428 LN829393	Gga3, 31915082 Tgu3, 35243746	F: [6FAM]CTCAACAACAATTTCTATTGGAACAC	59.03	(TATC) <sub>13</sub>	NOR	4	3	247	135–148
			R: TGGTACTAAGGCTCCATATAGGATAAC	59.35		SCOT	6	3		135–147
Pha31	Osp537 LN829394	Gga, no hit Tgu9, 15738938 And Un 58947724	F: [HEX]AATTATGAGCCATTCTGCAACAG	60.50	(GA) <sub>13</sub>	NOR	4	1	197	247–255
			R: CATCCTGTGTGCCAGTGAG	60.31		ENG	48	5		231–255
Pha32	Osp742 LN829395	Gga, no hit Tgu, no hit	F: [6FAM]CTTGAGCGCCTGCCATAG	60.66	(CA) <sub>22</sub>	NOR	4	0	189	197–220
			R: CACAAGCTAACAGGCCATTCTC	60.18		SCOT	6	4	Unreliable	(183–191)
Unreliable						ENG	48	0	No amp.	No amp.

**Table 1** continued

Locus <sup>a</sup>	Clone name and ENA sequence accession no.	Chromosome location <sup>b</sup>	Primer sequences (5'–3')	Primer T <sub>m</sub> (°C) <sup>c</sup>	Repeat motif	Country where sampled	n	A	Expected allele size (bp) <sup>d</sup>	Observed allele size range (bp)
<b>Pha33</b>	Osp1639 LN829396	Gga, no hit Tgu2, 95818547	F: [VIC]AGGTCAATAGGCTACGTGAACAG R: CACAGGCTACCTTAGACAACACC	59.72 60.10	GATA GATG (GATA) <sub>12</sub>	NOR	4	3	130	129–137
			F: [HEX] used for UK samples			SCOT	5	3		129–137
						ENG	48	5		124–140
Pha34	Osp2311 LN829397	Gga and Tgu, Multiple copies in genome	F: [6FAM]CTGGGCTTGTCATCCAG R: AGGTACGAATATACCCTGAAGCAC	60.20 59.83	(CA) <sub>11</sub>	NOR	4	1	148	145
						SCOT	6	1		145
						ENG	48	2		145–147
<b>Pha35</b>	Osp2323 LN829398	Gga, no hits Tgu, no hit	F: [PET]GAATCCACCCTCAGCAAGTC R: ATAGCAGGATGCTGGAGGAG	59.66 59.41	(G) <sub>7</sub> (GT) <sub>12</sub>	NOR	4	2	110	103–115
			F: [HEX] used for UK samples			SCOT	6	2		109–111
						ENG	46	4		109–115
<b>Pha36</b>	Osp3963 LN829399	Gga, no hits Tgu, no hit	F: [NED]TTTCAGGTGGGCTTCATCTC R: GAATCATCTGAAATGCTTATTTTC	60.20 60.51	(GATA) <sub>13</sub> GATG (GATA) <sub>2</sub>	NOR	4	5	174	166–186
			F: [HEX] used for UK samples			SCOT	6	3		174–182
						ENG	48	5		166–182
<b>Pha37</b>	Osp4029 LN829400	Gga, no hits Tgu, no hit	F: [6FAM]GCTAAGTGATCCCTTCTGC R: GTGCAGCAGCCTTAGCATC	59.98 59.72	(GT) <sub>10</sub>	NOR	4	3	94	86–92
						SCOT	4	2		86–88
						ENG	48	3		86–92
	Summary		Total numbers of loci polymorphic, monomorphic or failing to amplify per region samples were taken				L.	Poly.	Mono.	No amp./ Unreliable
						NOR	37	26	4	7
						SCOT	37	28	3	6
						ENG	37	29	2	6

<sup>a</sup> Loci in bold and underlined were selected for multiplexing; ENA European Nucleotide Archive: <http://www.ebi.ac.uk/ena/data/view/LN829364-LN829400>

<sup>b</sup> Chromosome location in the chicken (Gga) and zebra finch (Tgu) genomes (see Supplementary File)

<sup>c</sup> T<sub>m</sub>, melting temperature, the PCR program used was Norwegian samples: 95 °C for 15 min, 30 cycles of [95 °C for 30 s, 57 °C for 90 s, 72 °C for 60 s] and a final extension step of 60 °C for 30 min. UK samples: 95 °C for 15 min, 35 cycles of [94 °C for 30 s, 56 °C for 90 s, 72 °C for 60 s], and a final extension of 60 °C for 30 min. Six loci were found to be unreliable in all populations, alternative primer sets could be designed if required. *Pha07* was monomorphic in the three populations tested but may be variable in other populations/subspecies. *Pha12* was homozygous in all 21 females genotyped supporting its suggested Z-linked status, *Country* location where individuals were sampled, *NOR* Norway, *SCOT* Scotland, *ENG* England (see text), *A* number of alleles observed, *No amp.* no PCR amplification, *L.* number of loci tested, *Poly.* Polymorphic, *Mono.* monomorphic

<sup>d</sup> The expected allele size was based on the sequence of the male osprey *Pandion haliaetus* individual (02/09; that hatched at the Rutland Water Nature Reserve, Oakham, England, UK) from which the primer sets were designed (see text)

**Table 2** Multiplex microsatellite genotyping and sexing of the osprey (*Pandion haliaetus*)

Locus and primer set	Clone name/reference	Chr.	Fluoro-label	MP set	Final primer concentration ( $\mu\text{M}$ ) <sup>a</sup>	Repeat type	Pop.	<i>n</i>	Allele size range (bp)	A	$H_O$	$H_E$	$P_{HWE}$ (GENEPOP)	$F_{NULL}$ (CERVUS)
Pha04	Osp107_C09	3	VIC	A	0.04	Di	NOR	17	152–168	6	0.71	0.69	0.3845	-0.0350
Pha10 set 1	Osp107_F12	3	PET	A	0.2	Di	NOR	17	165–195	10	0.88	0.87	0.6130	-0.0280
Pha27	Osp0034	13	6FAM	A	0.2	Tetra	NOR	17	164–192	7	0.53	0.63	0.0783	+0.0929
Pha28	Osp0222	Unk.	6FAM	A	0.2	Tetra	NOR	17	117–133	5	0.82	0.76	0.6762	-0.0508
Pha29	Osp0354	6	NED	A	0.04	Tetra	NOR	17	135–151	5	0.76	0.73	0.1537	-0.0427
Pha35	Osp2323	Unk.	PET	A	0.2	Di	NOR	17	115–119	3	0.24	0.36	<b>0.0170</b>	<b>+0.2290</b>
Pha37	Osp4029	Unk.	6FAM	A	0.2	Di	NOR	17	86–92	3	0.59	0.63	0.3211	+0.0294
Pha02 set 1	Osp107_A08	6	PET	B	0.2	Di	NOR	17	188–212	7	0.59	0.50	1.0000	-0.1483
Pha13	Osp107_G06	12	6FAM	B	0.2	Di	NOR	17	182–196	8	0.94	0.88	0.6118	-0.0494
Pha16	Osp108_C02	4	6FAM	B	0.2	Di	NOR	17	296–302	4	0.53	0.57	0.7551	+0.0367
Pha30	Osp0428	3	6FAM	B	0.2	Tetra	NOR	17	235–255	6	0.65	0.78	<b>0.0323</b>	+0.0568
Pha33	Osp1639	2	VIC	B	0.04	Tetra	NOR	17	125–137	4	0.71	0.70	0.9226	-0.0080
Pha36	Osp3963	Unk.	NED	B	0.04	Tetra	NOR	17	166–186	6	0.71	0.77	0.3684	+0.0365
Z-002D <sup>b</sup>	Dawson (2007)	ZW	6FAM	B	0.2	n/a	NOR	5M	127	1	0	0	n/a	
							NOR	12F	118 and 127	2	1	1	n/a	
Z-002D <sup>b</sup>	Dawson (2007)	ZW	6FAM	S-plex	0.2	n/a	UK	28M	127	1	0	0	n/a	
							UK	26F	118 and 127	2	1	1	n/a	
Z-002A	Dawson (2007)	ZW	6FAM	S-plex	0.2	n/a	ENG	27M	210	1	0	0	n/a	
							ENG	21F	210 and 218	2	1	1	n/a	
Z43B	DAD et al. unpublished	ZW	6FAM	S-plex	0.2	n/a	UK	28M	272	1	0	0	n/a	
			Ta <sup>a</sup> =	50 °C			UK	26F	268 and 272	2	1	1	n/a	

<sup>a</sup> The full PCR programs used are provided in the footnotes of Table 1. Chr. chromosome location (see Table 1 and Supplementary Figure), Unk. unknown, MP multiplex set, S-plex marker amplified separately in a single-plex, Pop population genotyped: NOR Norway, ENG England, UK individuals sampled in England and Scotland combined, *n* number of unrelated individuals genotyped, M Male, F Female, A number of different alleles observed,  $H_O$  observed heterozygosity,  $H_E$  expected heterozygosity,  $H_E$  expected heterozygosity,  $P_{HWE}$  probability of deviation from Hardy–Weinberg equilibrium (data in bold indicates  $p > 0.05$ ),  $F_{NULL}$  estimated frequency of null alleles (data in bold indicates  $F_{NULL} > 0.2$ )

<sup>b</sup> The Z-002A and Z-002D (Dawson 2007) and Z43B (DAD et al. unpublished data) were used for identifying the sex of the individuals. Ta, PCR annealing temperature (50 °C for Z43B and 56/57 °C for all other markers, see Table 1 footnotes); M Male, F Female, n/a not applicable

( $\sim 1 + 1 \mu\text{g}$ ) and MiSeq-sequenced. This allowed more (tetranucleotide) marker choices for multiplexing. Primer sets were designed from 37 sequences (26 Sanger and 11 MiSeqs) using PRIMER3 v0.4.0.

Samples were collected from wild ospreys including: (1) 17 feathers from nine nests in central Norway (two plucked from unrelated nestlings and 15 shed from adults); (2) six feathers from two nests in Scotland; and (3) mouth-swabs from 48 osprey chicks at Rutland Water Nature Reserve, England. For genotyping, DNA was extracted from feather calamus ('Norwegian' ospreys) using the Maxwell<sup>®</sup>16 Research System (Promega), and from feathers ('Scottish' ospreys) or mouth swabs ('English' chicks) using ammonium acetate. We sexed the chick and feather samples using the *Z-002A*, *Z-002D* (Dawson 2007) and *Z43B* markers (DAD et al. unpublished data). Initially, each locus was amplified in ospreys sampled in Norway ( $n = 4$ ), Scotland ( $n = 6$ ) and England ( $n = 48$ ; Table 1). PCR was performed with fluorescently-labeled forward primers using QIAGEN's Multiplex PCR kit and protocol [annealing temperature = 56/57 °C (Table 1); 2/10- $\mu\text{l}$  reactions]. Multiplex-PCR was used to genotype/sex-type the 17 presumably unrelated ospreys, sampled in Nord-Trøndelag county (64°06'N, 12°50'E), central Norway (Table 2). PCR products were separated on an ABI Genetic Analyzer and allele sizes assigned using GENEMAPPER software.

## Results

Genotyping revealed that all feathers were from different individuals. The genetic sexing revealed that  $\sim 10\%$  of osprey chicks were incorrectly sexed in the field (5/52 errors when based only on size/morphology). Microsatellite sequences were submitted to the EMBL-EBI European Nucleotide Archive (LN829364–LN829400; Table 1; S1). Of the 37 loci tested, 31 could be assigned a location in the chicken (*Gallus gallus*) and/or zebra finch (*Taeniopygia guttata*) genome based on sequence similarity (following Dawson et al. 2006) and 2–3 were Z-linked (Table 1, Supplementary Figure). From the 26 loci polymorphic in four individuals sampled in Norway, we selected 13 for multiplex-PCR that were placed into two sets based on fragment size, genetic variation and peak interpretation in the Norwegian samples. Multiplex genotyping of 17 ospreys sampled in Norway revealed a mean of 5.7 alleles per polymorphic locus (range 3–10; genotyping was performed in duplicate; Table 2). Heterozygotes were present in both sexes for these 13 loci indicating they are autosomal. Observed heterozygosity ranged from 0.24 to 0.94 per locus

(Table 2). Two loci deviated from Hardy–Weinberg equilibrium in the Norwegian population ( $p < 0.05$ , GENEPOP v4.2; Table 2); possibly due to a Wahlund effect (*Pha30*) and/or allelic dropout/null alleles (*Pha35*, estimated null allele frequency  $>0.2$ , CERVUS v3.0). Despite the source of DNA being feathers there was no evidence of dropout at any other loci (CERVUS). No pairwise locus combinations displayed significant linkage disequilibrium ( $p < 0.01$ , GENEPOP). The combined probability of identity for the 13 loci was  $8.0 \times 10^{-12}$  (GENALEX v6.501).

In conclusion, this multiplex set of novel microsatellite loci combined with the sex markers will be useful for genetic analyses of osprey, including typing non-invasive samples, such as shed feathers.

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### Compliance with ethical standards

**Sampling** Visiting and observing osprey nests at Rutland Water Nature Reserve was performed under an English Schedule 1 Licence, issued by the British Trust for Ornithology on behalf of Natural England. Sampling permission was provided by Tim Mackrill, Senior Reserve Officer at Rutland Water Nature Reserve.

**Conflict of interest** The authors declare that they have no conflict of interest.

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