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TECHNICAL NOTE

Isolation, characterisation and predicted genome locations of Light-bellied Brent goose (*Branta bernicla hrota*) microsatellite loci (Anatidae, AVES)

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Abstract We isolated 137 unique microsatellite loci from an enriched Light-bellied Brent goose (*Branta bernicla hrota*) genomic library. Thirty-seven polymorphic loci were characterised in 24 unrelated individuals sampled from a Light-bellied Brent goose population located at Ringneill quay in Strangford Lough, Northern Ireland. The 37 polymorphic loci displayed between 2 and 38 alleles. Sequence homology was used to assign a predicted chromosome location for 31 polymorphic loci (31 in the chicken (*Gallus gallus)* and 30 in the zebra finch (*Taeniopygia guttata*) assembled genome). Two polymorphic microsatellite loci were Z-linked based on the typing of known sex individuals (24 females and 25 females) and sequence homology.

Keywords Aves · *Branta bernicla hrota* · Brent goose · Microsatellite · Predicted genome locations · Z-linked loci

The East Canadian High Arctic (ECHA) population of the Light-bellied Brent goose (*Branta bernicla hrota*) breeds in the Canadian eastern Queen Elizabeth Islands, stages in western Iceland and winters around the coast of Ireland (Robinson et al. 2004). This species is amber listed in the Birds of Conservation Concern in the UK because 50% or more of the non-breeding population can be found at 10 or fewer sites (Eaton et al. 2009). We have isolated and characterised new microsatellite loci in the Light-bellied Brent goose in order to investigate kin structure and relatedness in the migratory flyway.

Genomic DNA was extracted using an ammonium acetate precipitation method (Nicholls et al. 2000). A microsatellite-enriched library was constructed from a single female ECHA Light-bellied Brent goose (ring combination NZRY) sampled at Alftanes, Iceland in 2008. The library was constructed using the method of Armour et al. (1994) and enriched for the following di- and tetranucleotide microsatellite motifs: $(GT)_n$, $(CT)_n$, $(GTAA)_n$, $(CTAA)_n$, $(TTTC)_n$ and $(GATA)_n$ and their complements, which had been denatured and bound to magnetic beads following Glenn and Schable (2005). Transformant colonies were not screened for the presence of a repeat region but were directly sequenced by the NERC Biomolecular Analysis Facility at the University of Edinburgh.

A total of 137 unique Light-bellied Brent goose microsatellite sequences were isolated (EMBL accession numbers: FN691780–FN691904 and FN812687–FN812698). Primer sets were designed for all 137 unique microsatellite sequences using PRIMER3 (Rozen and Skaletsky 2000). Fifty-two primer sets were initially tested for amplification and polymorphism in two unrelated Light-bellied Brent goose individuals from the ECHA population. The two individuals were amplified using a gradient of 12 different annealing temperatures (56–64°C) using a DNA Engine Tetrad 2 thermal cycler (MJ Research, Bio-Rad, Hemel Hempstead, Herts., UK). Polymorphic loci were typed in 24 additional individuals using the temperature that produced the cleanest and strongest PCR product when observed on a 1.5% agarose gel stained with SYBRSafe. Each 2-µl PCR contained approximately 10 ng of lyophilised genomic DNA, 0.2 µM of each primer and 1 µl QIAGEN multiplex

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Table 1 Characterisation of 37 polymorphic microsatellite loci in the Light-bellied Brent goose, *Branta bernicla hrota* (Anatidae, Aves)

Locus	EMBL	Repeat motif	Primer sequence (5′–3′)	T_m	T_a	Multiplex/ Fluoro Label (F)	Expected/ Observed size (bp)	n	A	H_0	H_E	P_{HWE}	Est. null allele Freq.	Predicted genome location	BLAST E value
Bbh011	FN691790	$(GT)_{10}$	F 6FAM-CAAATCTTGCCCACCCTAAC	59.43	60	1/NED	210/	24	4	0.70	0.62	0.61	−0.07	Gga13, 9487155	1.20E−42
			R CCAGGGCTACCTCAGCAG	59.52			204–212							Tgu13, 2779170	4.50E−20
Bbh018	FN691797	$(AC)_9$	F HEX-ATTGCTCTGGCAAGCAGATAC	59.49	60	–	250/	24	5	0.45	0.57	0.16	0.12	Gga1, 98546576	2.00E−23
		$(AT)_7$	R AGTGTTGGGACAGGATGGAC	59.82			235–257							Tgu1, 105882799	0*
Bbh021	FN691800	$(ATAG)_7$	F 6FAM-TGAGGAAGACAAATGTAATGGGATG	63.31	58	1/PET	207/	24	6	0.71	0.70	0.86	−0.02	Gga1, 96062895	3.90E−13
		$(GTAG)_1$	R AAATATGAAATCTTTCCAAGTCTCTCTGC	63.36			194–214							Tgu1, 66914171	4.00E−22
		$(ATAG)_3$													
Bbh027	FN691806	$(ATCT)_{13}$	F 6FAM-TCCAGACAGACATTGAAAGAGTGAGTAAG	64.25	62	2/PET	374/	24	6	0.75	0.77	0.92	−0.01	Gga2, 150488838	0*
		$(AT)_2$	R GCATGAAGCCCAGTCCATTG	63.87			362–386							Tgu2, 151721189	6.00E−46
		$(TTAT)_2$													
Bbh029	FN691808	$(GGAGA)_5$	F HEX-GAGAAAGGCAAAGTATCAGGTAAATGG	63.38	58	1/6FAM	398/	24	27	1.00	0.98	0.69	−0.02	Gga5, 50901191	3.00E−08
		$(GAGAA)_5$	R GCAGCTTCACATCCCTCTGC	63.42			370–478							Tgu5, 49727935	0*
		$(A)_2$													
		$(GAGAA)_{16}$													
Bbh031	FN691810	$(CTATT)_9$	F 6FAM-TTGAAGGTTCCTTCCAACTGAG	60.63	62	–	201/	24	17	0.96	0.94	0.77	−0.02	Gga2, 44586108	4E−107
		$(CTATC)_9$	R TTGAAAGTCAGGCATCAAAGG	60.23			198–303							Tgu2, 29927904	8E−30
Bbh043	FN691822	$(AG)_{28}$	F HEX-CATTTGGCCCCATGGACTTG	65.66	62	2/6FAM	179/	24	4	0.33	0.30	1	−0.08	Gga NSH	–
		$(GT)_9$	R GGCAGACCACTTATCCTTGAGGTG	65.29			176–184							Tgu NSH	–
Bbh047	FN691826	$(AC)_3$	F 6FAM-CACGGGCTCTGTTCCCATTC	65.12	58	–	300/	24	3	0.29	0.47	0.05	0.23	Gga2, 3007903	7.40E−80
		$(AT)_1$	R CGAAGGAATTAAACCACATGTCCTG	64.78			298–302							Tgu2, 1406605	4E−45[U]
		$(AC)_{12}$													
Bbh056	FN691835	$(A)_7$	F HEX-TTACTGAAATGCCATGGAGAGAA	60.96	60	–	152/	24	4	0.46	0.63	0.02	0.14	Gga1, 162768361	2.70E−23
		$(TC)_1$	R CTATGACAACCACAGCATTCCA	60.94			150–156							Tgu1, 42763148	5.50E−16
		$(AT)_4$													
		$(AC)_9$													
Bbh062	FN691841	$(CA)_{15}$	F 6FAM-TCTGTTCAGCACTGGTGAGG	60.02	60	–	276/	24	10	0.79	0.77	0.58	−0.01	Gga NSH	–
			R CCAAGAAGGAGATTCCACAAAC	59.98			272–292							Tgu NSH	–
Bbh064	FN691843	$(TC)_4$	F HEX-CCTGCCACATGTCCAGTTC	60.1	60	1/NED	209/	24	10	0.75	0.80	0.05	0.04	Gga NSH	–
		$(AC)_4$	R AACAGCTGTGTGCCAGAGG	60.04			180–210							Tgu NSH	–
		$(GC)_2$													
		$(AC)_7$													
		$(GC)_5$													
		$(AC)_{12}$													

Table 1 continued

Locus	EMBL	Repeat motif	Primer sequence (5′–3′)	T_m	T_a	Multiplex/ Fluoro Label (F)	Expected/ Observed size (bp)	n	A	H_0	H_E	P_{HWE}	Est. null allele Freq.	Predicted genome location	BLAST E value
Bbh068	FN691847	$(AC)_8$	F 6FAM-TATCACGAGCGAGTGTACCG	59.89	60	–	244/	24	8	0.60	0.80	0.35	0.12	Gga1, 85096045	2.00E−93
		$(GC)_1$	R GGCCAGGATTTCATTCTTTG	59.5			180–210							Tgu1, 94338347	3.00E−85
		$(AC)_6$													
Bbh070	FN691849	$(AC)_3$	F 6FAM-TGAATCAATTCAAGCTGAGTCC	59.32	60	2/VIC	151/	24	5	0.82	0.82	0.82	−0.01	Gga14, 5688707	3.00E−30
		$(AT)_1$	R GTTTCCCAACAGTTCCCAAC	59.28			147–159							Tgu14, 9912941	1.30E−11
		$(GT)_1$													
		$(AC)_{16}$													
Bbh075	FN691854	$(AAGAGAGG)_8$	F 6FAM-GTGGGTTTCATTGTTCGTTCAGG	64.83	58	–	392/	24	22	0.88	0.93	0.63	0.02	Gga1, 27868755	2.00E−20
		$(GGAA)_5$	R TGTCCTTGGTCATCTTTCAAGCAG	64.84			268–424							Tgu1A, 25498321	0*
Bbh080	FN691859	$(GA)_{16}$	F 6FAM-TTTCAGTGTGGTCCTGGAATG	60.94	60	1/VIC	184/	24	13	0.79	0.88	0.22	0.05	Gga NSH	–
			R TTTATCTAGACTACCCAACAATAGCTCTG	60.71			164–208							Tgu NSH	–
Bbh083	FN691862	$(AAAT)_5$	F 6FAM-TCAGGAAAAGTCTGGTGTCTGAA	61.19	58	–	243/	24	6	0.44	0.44	0.23	0.02	Gga2, 7051280	4E−168*
			R GAAACGTTGCTGTTTTCAAATTCT	60.85			209–255							Tgu2, 7767759	1E−19U
Bbh086	FN691865	$(GATA)_{14}$	F HEX-TTTGCCATGAACTTCTCTTTGG	61.48	62	–	205/	24	13	0.96	0.90	0.86	−0.05	Gga1, 154050787	4E−32*
			R ATGAGCAACAGATTTCATACAGCTC	61			198–262							Tgu1, 44967106	1.00E−22
Bbh087	FN691866	$(GAAA)_8$	F 6FAM-GCTGGTTGGATACAGACCATTCC	63.98	60	–	340/	24	28	0.96	0.99	0.03	0.003	Gga4, 27974814	4.00E−79
			R TGCAAATCTGCGTGTGTTTGTC	64.38			267–395							Tgu4, 1119997	2.30E−27
Bbh089	FN691868	$(GAAAA)_6$	F 6FAM-GGAGAAGGCAGGAAGAGAAGCAC	65	60	2/NED	384/	24	15	1.00	0.98	0.33	−0.02	Gga1, 11879764	2.00E−51
			R GGCTCGTGTCCTGCAGTCAG	64.56			362–422							Tgu1A, 9983440	1.90E−21
Bbh091	FN691870	$(TTCTT)_6$	F HEX-GCAGACTGCAGACTTCCCTTCG	65.82	62	–	389/	24	36	0.96	0.99	0.54	0.003	Gga1, 82614294	4.00E−48
			R GTGGTGGAAGGCCCACCTG	66.19			320–500							Tgu1, 91648919	2E−133*
Bbh094	FN691873	$(TTCT)_7$	F HEX-CCCTGCAACTCATTCCATGC	63.87	58	–	329	24	38	0.96	0.99	0.51	0.006	Gga2, 28256118	1.00E−29
			R CTGTTGTCCCATGGTGTGATAATG	63.47			222–390							Tgu2, 47903235	1.30E−10
Bbh112	FN691891	$(TTCT)_{36}$	F HEX-GCATGCCTGCAAAAGTCAAGC	65.33	58	2/VIC	298/	24	20	1.00	0.95	0.72	−0.04	Gga1, 78980000	8.60E−58
			R TCATGCACCTGGGGAGAAAGA	65.22			245–356							Tgu1, 88244272	3.00E−56
Bbh113	FN691892	$(GATA)_{12}$	F 6FAM-ACCACACATGCAGCAAGTATAAATCTAGG	65.33	58	1/VIC	294/	24	8	0.92	0.81	0.63	−0.07	Gga1, 250153	2.60E−19
			R GGGTCTCTTCCTGCCTTGCTG	65.71			237–299							Tgu NSH	–
Bbh115	FN691894	$(GAAA)_6$	F HEX-AAATTGCCCTGCATCAGCAC	63.41	58	–	462/	24	29	0.78	0.98	0.001	0.01	Gga NSH	–
			R TTCCATAAATAATTCCATTTCCTTTAATTAACTC	64.5			334–522							Tgu NSH	–
Bbh120	FN691899	$(GAAA)_{10}$	F HEX-TCATTTCTTCTGACCTGCACTCTG	63.04	58	2/NED	237/	24	17	0.96	0.95	0.52	−0.02	Gga2, 121996868	0*
			R ACTTGAAGGGCATTGAAACATACG	63.44			167–243							Tgu2, 123539975	1.3E−10U

Table 1 continued

Locus	EMBL	Repeat motif	Primer sequence (5′–3′)	T_m	T_a	Multiplex/ Fluoro Label (F)	Expected/ Observed size (bp)	n	A	H_0	H_E	P_{HWE}	Est. null allele Freq.	Predicted genome location	BLAST E value
Bbh123	FN691902	$(GAAA)_{22}$	F HEX-TGCAGCAGAGACACGGTAAA	60.6	58	2/6FAM	277/	24	21	0.92	0.97	0.35	0.02	Gga2, 89995309	2.00E−33
			R GCTGTTATTTTAGTCCTGAATTCACTTT	60.6			224–308							Tgu2, 92831665	3.30E−44
Bbh126	FN812687	$(AT)_4$	F 6FAM-TCCTTTAGCAGGGAACCTCAC	60.62	60	–	231/	24	3	0.38	0.46	0.3	0.1	Gga1, 154076317	1.00E−81
		$(GA)_{11}$	R CAGGAGGCTAAGGCCATAAAG	60.22			225–229							Tgu1, 44995874	3.80E−45
Bbh128	FN812689	$(GA)_{10}$	F HEX-TTCCCTGTAACCCACCTCTG	59.96	60	–	199/	24	3	0.56	0.51	0.8	−0.06	Gga2, 36636601	1.50E−48
			R GCTTTACATCTTGGCTGTTGG	59.76			198–202							Tgu2, 56804450	9.10E−18
Bbh129	FN812690	$(AT)_5$	F 6FAM-GGGCAAAGACAGTTGTCAGC	60.84	62	–	163/	23	5	0.66	0.65	0.41	−0.01	Gga2, 63188208	2.80E−21
		$(AC)_{11}$	R GCAAGAGTCCCTTTGGACAAC	61.04			151–167							Tgu2, 69661449	1.60E−18
Bbh130	FN812691	$(A)_{17}$	F HEX-TGTTCTTCAGCATTTGATTTGC	60.25	60	–	160/	24	2	0.12	0.11	1	−0.02	Gga1, 73312401	8.50E−49
		$(GA)_{11}$	R TTTCCTTAAGTAACCATGCAATCC	60.56			151–157							Tgu1A, 61655965	1.50E−54
Bbh131	FN812692	$(GT)_9$	F HEX-TTTCCTCCCTTCCATCCAG	59.99	60	–	120/	24	6	0.47	0.84	0	0.26	Gga1, 139510365	2.00E−54
		$(A)_9$	R GTACCTCTCCGCCGTGTG	60.27			112–124							Tgu1, 28358130	8.20E−47
		$(AT)_6$													
Bbh133	FN812694	$(TC)_{10}$	F 6FAM-TGCCTGAGATTATTGGGACTC	59.14	60	–	199/	24	6	0.54	0.79	0.004	0.17	Gga11, 4542779	2.00E−16
			R ATCGCGACGTCAGTAAACAG	58.95			197–207							Tgu11, 8394313	4.30E−17
Bbh135	FN812696	$(TATC)_4$	F HEX-GGAGGTGCAAAGAGATGAGC	59.96	60	1/PET	257/	24	5	0.62	0.75	0.67	0.08	Gga5, 15904566	6.50E−63
		$(TAATC)_1$	R TGCTATCTGGTTTCCCGTATG	59.97			251–267							Tgu5, 14758685	1.40E−28
		$(TATC)_7$													
Bbh136	FN812697	$(AG)_{18}$	F HEX-TCTCTCTTGGAGCTCTGCTG	58.57	60	1/6FAM	147/	24	7	0.75	0.71	0.32	−0.03	Gga2, 133226667	1E−14
			R GCCATGAAGAGGTATTGTGC	58.17			133–153							Tgu2, 134802087	0*
Bbh137	FN812698	$(GT)_8$	F HEX-GGGTTCCAGATGCACATACC	60.2	60	–	248/	24	7	0.79	0.65	0.32	−0.12	Gga NSH	–
		$(ATCCCTCTG)_1$	R GAAATGGCAATTCCAAGCAC	60.46			246–258							Tgu NSH	–
		$(A)_9$													
		$(AT)_6$													
Bbh008	FN691787	$(AAAC)_3$	F HEX-GCATTGTATGGGGAGGACAG	60.34	60	–	189/	25	3	0.72	0.63	0.24	−0.08	GgaZ, 18652977	3.00E−17
(Z-linked)		$(AAAAC)_4$	R CCAAACTTTTCTCCGTGCAG	60.8			185–192 (M)	24	3	0.00				TguZ, 49706592	4.40E−07
		$(AAACC)_2$					185–192 (F)								
		$(AAAC)_3$													

Table 1 continued

Locus	EMBL	Repeat motif	Primer sequence (5′–3′)	T_m	T_a	Multiplex/ Fluoro Label (F)	Expected/ Observed size (bp)	n	A	H_O	H_E	P_{HWE}	Est. null allele Freq.	Predicted genome location	BLAST E value
Bbh067	FN691846	$(AG)_1$	F HEX-GCATGTTCACAGCAGGAATG	60.27	60	–	247/	25	4	0.68	0.69	0.24	0.01	GgaZ, 39611374	0*
(Z-linked)		$(AAG)_1$	R TGGCAGGAAATATGAGGTCTG	60.08			242–248 (M)	24	4	0.00				TguZ, 52672100	5.90E−11
		$(AG)_4$					242–248 (F)								
		$(AA)_1$													
		$(AG)_{14}$													

T_m, Melting temperature; T_a, annealing temperature; n, number of unrelated light-bellied Brent goose, *Branta bernicla hrota* individuals genotyped; A, number of alleles; M, males; F, females; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HWE}, Hardy–Weinberg equilibrium test P-value as identified by GENEPOP v3.4 (Rousset 2008)

Chromosome Location: [U]Hits to Unknown Chromosome in zebra finch also detected; * Assigned by indirect BLAST (methods as in Dawson et al. 2007); NSH—No significant hits

PCR mix (QIAGEN Inc.; Kenta et al. 2008). PCR amplification was performed using a DNA Engine Tetrad 2 thermal cycler (MJ Research, Bio-Rad, Hemel Hempstead, Herts., UK) with the following program: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, annealing temperature (Table 1) for 90 s, 72°C for 1 min, and finally 60°C for 30 min. Amplified products were loaded an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA) and allele sizes were assigned using GENEMAPPER v3.7 (Applied Biosystems, California, USA). Individuals were sex-typed with the Z002A primer set (Dawson 2007) to enable the identification of sex-linked loci.

Of the 52 loci tested in two individuals, 9 loci did not did not amplify or produced non-specific product, 6 were monomorphic and 37 were polymorphic (Table 1). These 37 polymorphic loci were then typed in 24 unrelated individuals (12 male/12 female) belonging to the ECHA population and sampled at Ringneil Quay in Strangford Lough, Northern Ireland (Co-ordinates: 54.51584 N, 5.64585 W). The 37 polymorphic loci displayed between 2 and 38 alleles when genotyped in the 24 individuals. Two loci (Bbh008, Bbh067) displayed a genotype pattern consistent with linkage to the Z chromosome with both loci being homozygous in all 24 female individuals amplified but were heterozygous or homozygous in 25 males. A Fisher's Exact test comparing numbers of male and female homozoygotes confirmed that both loci were Z-linked (both P-values <0.001). Observed and expected heterozygosities, and predicted null allele frequencies were calculated using CERVUS v3.0.3 (Kalinowski et al. 2007). Tests for departures from Hardy–Weinberg equilibrium and assessment of genotypic disequilibrium were conducted in GENEPOP v3.4 (Rousset 2008). Three loci deviated from Hardy–Weinberg equilibrium after correction for multiple tests (Bbh115, Bbh131, Bbh133) (Rice 1989). Seven loci displayed a high predicted null allele frequency (Table 1). Nine pairs of loci showed evidence of linkage disequilibrium (Bbh018–Bbh056, Bbh021–Bbh129, Bbh027–Bbh115, Bbh043–Bbh070, Bbh083–Bbh135, Bbh 083–Bbh136, Bbh112–Bbh062, Bbh113–Bbh086, Bbh126–Bbh056) however following a sequential Bonferroni correction, no pairs of loci showed evidence of linkage disequilibrium. Fifteen markers were selected for the creation of a multiplex marker set (Table 1).

Predicted chromosome locations were assigned by comparing the sequence of the Light-bellied Brent goose with the location of its sequence homolog on the chicken (*Gallus gallus*) genome and zebra finch (*Taeniopygia guttata*) genome assemblies (methods as in Dawson et al. 2006, 2007). A genome map was prepared using MapChart software (Voorrips 2002) (Fig. 1). Two loci were less than 1 Mb apart in the chicken genome (Bbh086 and Bbh126) and therefore may be physically linked in the Light-bellied Brent goose.

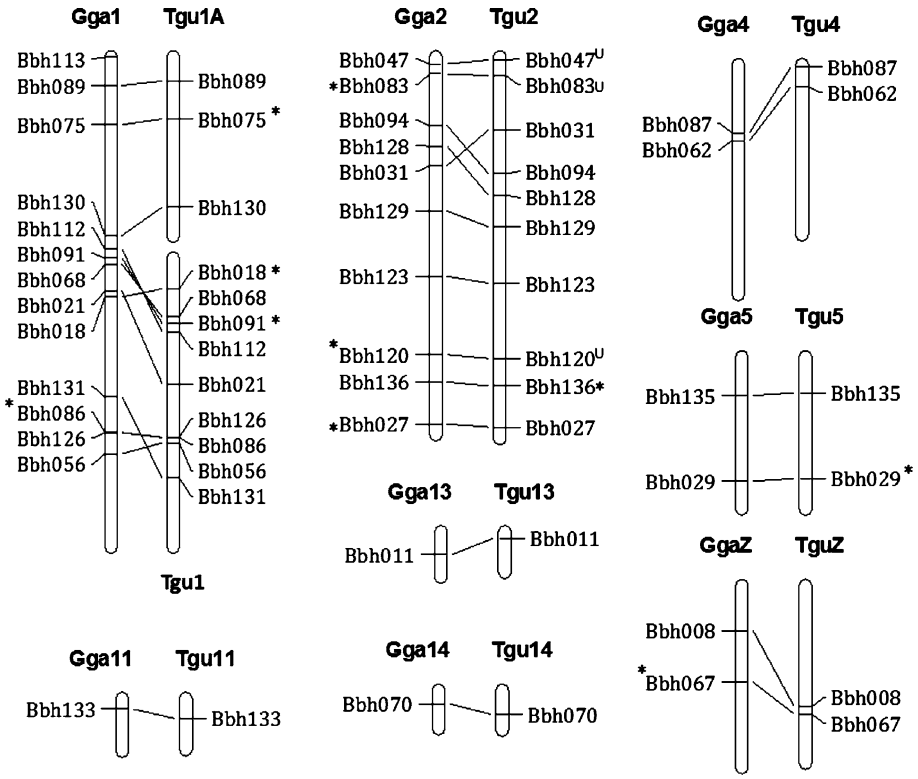

Fig. 1 Chromosomal locations on the chicken (*Gallus gallus*) and zebra finch (*Taeniopygia guttata*) assembled genomes of thirty-one microsatellite loci which are polymorphic in the Light-bellied Brent goose (*Branta bernicla hrota*)—location assignments were based on sequence homology and BLAST comparisons made against the zebra finch genome assembly (using the assembled zebra finch genome as released on 5/3/2009 version *Taeniopygia guttata*-1.1 http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=59729) and chicken genome assembly (using the assembled chicken genome as released on 29/11/2006 version *Gallus gallus*-2.1 http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9031). Genome locations in the chicken and zebra finch genomes were checked by performing a WU-BLAST 2.0 implemented on the Washington University webpage http://genomeold.wustl.edu/tools/blast/ (using a DUST/SEG filter and RepeatMasker). Sequence is also homologous to a region on the "Unknown" chromosome which may be due to an assembly error where the sequence has not been removed from the Unknown chromosome when it was assigned to a named chromosome. * Sequences with no significant hits in either the chicken or zebra finch were subsequently assigned using an indirect BLAST (methods as in Dawson et al. 2007). Gga: chicken (*Gallus gallus*) chromosome name; Tgu: zebra finch (*Taeniopygia guttata*) chromosome name

These polymorphic microsatellites will be useful for population genetics studies of *Branta bernicla* and may also be of utility in studies of endangered congeners such as the Hawaiian goose (*Branta sandvicensis*) and the red-breasted goose (*Branta ruficollis*).

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