

## PERMANENT GENETIC RESOURCES

# Isolation and characterization of microsatellite loci from the endangered New Zealand takahe (*Gruiformes*; *Rallidae*; *Porphyrio hochstetteri*)

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## Abstract

Nineteen polymorphic microsatellite loci were characterized from the endangered takahe (*Porphyrio hochstetteri*). Like many of New Zealand's other native avian species, levels of polymorphism were low, with variation detected at only 19 of 110 (17.3%) loci, and most polymorphic loci (78.9%) were diallelic (mean number of alleles = 2.3). Despite these low levels of variation, the microsatellites developed here will be useful for parentage assignment for confirming pedigrees, and investigating relationships between genetic variation, pedigree-based inbreeding and reproductive success in this highly endangered species.

*Keywords:* birds, conservation, inbreeding, microsatellites, takahe, threatened species

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The takahe (*Porphyrio hochstetteri*) is a highly endangered flightless rail endemic to New Zealand. The total census of around 295 birds comprises a remnant population (of around 165 birds) in the Murchison Mountains, Fiordland, along with several island populations themselves descended from 25 Fiordland birds translocated between 1986 and 1991 (Jamieson & Ryan 2000). Because of the small population sizes on the islands, and significant levels of inbreeding (Jamieson *et al.* 2003), takahe will require a high level of management to avoid further inbreeding and loss of genetic diversity [C. Wickes & D. Crouchley Takahe Recovery Plan: 2007–2012 (in review) Department of Conservation, Wellington, New Zealand]. Here, we develop 19 microsatellite loci that will be used to assess the loss of genetic diversity, confirm parentage for pedigree analysis, and assist in the management of this fragile species.

Two *P. hochstetteri* libraries enriched for (GT)<sub>12</sub> and (GA)<sub>12</sub> microsatellite repeats were constructed following the protocol of Perrin & Roy (2000). Overall, 864 GA-enriched and 1056 GT-enriched colonies were screened and 121 (14.0%) and 223 (21.1%) positive clones, respectively, were identified and sequenced using an ABI 3730 Genetic Analyser (Applied Biosystems). SEQUENCHER (Gene Codes

Corporation) was used to ensure no duplicate loci were screened and primers were designed for 119 loci using OLIGO (Molecular Biology Insights Incorporated).

Each locus was screened for variation using 24 randomly selected birds of Fiordland origin, resulting in a 95% probability of detecting polymorphism where  $q > 0.061$  (binomial distribution:  $n = 48$ ,  $x < 1$ , probability = 0.05). Amplifications were carried out in a total volume of 5  $\mu$ L and contained 1  $\mu$ L of extracted DNA, 0.25 U *Taq* polymerase (Bioline Ltd), 200  $\mu$ M each dNTP, 500 nM each primer, 1.5–2.0 mM MgCl<sub>2</sub> and 1 $\times$  *Taq* buffer.

Thermocycling conditions consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 s, locus-specific annealing temperature (Table 1) for 15 s, and extension at 72 °C for 30 s, with a final extension step of 72 °C for 4 min. Amplified products were run for 18 h on 7–10% nondenaturing polyacrylamide gels stained with 50 $\times$  SYBR Green I (Invitrogen) and scored visually. Primer sequences, repeat motifs and their annealing temperatures are listed in Table 1. Of 119 loci screened, 110 (92.4%) produced consistently scoreable results, and of these 19 (17.3%) were polymorphic with 15 (78.9%) of the variable loci diallelic (mean number of alleles = 2.3, Table 1). One locus (Pho06) appeared to be sex-linked, as evidenced by a lack of any heterozygotes for this marker among an additional sample of 66 females, whereas all other loci yielded genotypes consistent with autosomal inheritance. Genotypic data were

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**Table 1** Microsatellite loci isolated from takahe (*Porphyrio hochstetteri*). Locus names are followed by primer sequences. Repeat motif and product length (allele size range) are those of the amplified product. Optimized polymerase chain reaction conditions provided are annealing temperature ( $T_a$  in °C) and  $Mg^{2+}$  concentration. For loci Pho11 through Pho113, numbers of alleles ( $A$ ), and observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities are based on a sample of 25 unrelated Fiordland birds (9 males, 16 females) and significance values ( $P$ ) are provided. Heterozygosity estimates at the sex-linked locus Pho06 are based on males only ( $N = 9$ ). GenBank Accession no. is provided.

Locus	Primer sequences (5'–3')	Repeat motif	Product length (bp)	$T_a$	[ $Mg^{2+}$ ]	$A$	$H_O/H_E$	$P$	Accession no.
Pho06	F-AGGAATCTGCACCAAGAGGA R-TTCCAGTGTGCTTCTGTGT	(GA) <sub>15</sub>	138–142	58	1.5	2	0.667/0.583	0.539	EU185615
Pho11	F-CAGAAGATGCTGTGACGC R-AATCTCAGTCCCTCAGGC	(GA) <sub>2</sub> A(GA) <sub>3</sub> CA(GA) <sub>15</sub> (G) <sub>6</sub>	190–192	55	1.5	2	0.200/0.246	0.384	EU185616
Pho12	F-AGCGAGGGAACCTGCGAG R-AGAAAGCGGTGGGAGGA	(GA) <sub>17</sub>	138–148	59	1.5	2	0.400/0.503	0.420	EU185617
Pho28	F-TCAGTGAACAAAACATC R-AAGTACAATTTGGTATCC	(GA) <sub>2</sub> TA(GA) <sub>7</sub> (GT) <sub>4</sub>	78–80	48	1.5	2	0.400/0.327	0.542	EU185618
Pho38	F-GGTATGAGTTCTGTGTCTCAG R-GCATCAAGAATACATAAAAG	(CAGA) <sub>2</sub> CATACAGA (CA) <sub>4</sub> CG(CA) <sub>3</sub> AA(CA) <sub>8</sub>	92–94	46	1.5	2	0.160/0.372	0.010	EU185619
Pho44	F-AGCTGCCCAGTACCTGAAGG R-CATGAACAGTCAGCCAAAGG	(AC) <sub>13</sub> (AC) <sub>6</sub>	143–145	51	1.5	2	0.040/0.040	>0.999	EU185620
Pho46	F-TGCCATGGTGGAGGTGTG R-TTTGACCACCTGCCCTCTC	(AC) <sub>14</sub>	104–116	51	1.5	4	0.480/0.527	0.400	EU185621
Pho47	F-AGCTAACAAAGGAGTTACCTG R-GAGCATGAGTATCTGAGAAG	(AC) <sub>9</sub>	91–93	48	1.5	2	0.160/0.150	>0.999	EU185622
Pho53	F-GAGGCAACACCTGCTGC R-GCTACCCTGACAATCTCTGG	(TG) <sub>9</sub> TATG(TA) <sub>4</sub> (TGTATA) <sub>2</sub>	204–206	53	1.5	2	0.080/0.077	>0.999	EU185623
Pho60	F-GAAAGCAAGTGTGGCTC R-CACCAGGTATTGCATTAC	(CA) <sub>14</sub> (GA) <sub>6</sub> (CA) <sub>12</sub> (GA) <sub>2</sub> (CA) <sub>13</sub> (GA) <sub>3</sub> (CA) <sub>18</sub> (CT) <sub>12</sub> (CTCA) <sub>2</sub>	198–224	50	1.5	4	0.520/0.608	0.191	EU185624
Pho62	F-CTGTCTTTTTTATACCATAC R-ATGTGATGGGCTGTAG	(CATA) <sub>2</sub> TA(TG) <sub>10</sub>	110–114	47	1.5	2	0.480/0.470	>0.999	EU185625
Pho74	F-CACCCTGTAGGTAAAAGTG R-TAAATGGAACACGGC	(GT) <sub>4</sub> CT(GT) <sub>5</sub> (TGTCTGTGTCTGTGCG) <sub>4</sub>	126–138	46	2.0	3	0.600/0.528	0.537	EU185626
Pho84	F-CACACAGAAGAACTCCCACC R-CCCCAGACAATAAGGTTGC	(CA) <sub>15</sub>	157–161	46	2.0	3	0.600/0.569	>0.999	EU185627
Pho90	F-CGGGAGGTAGGTTTTCTGTC R-TTGTGGAGGAGGTTGTAGG	(GT) <sub>11</sub>	120–122	50	1.5	2	0.440/0.458	>0.999	EU185628
Pho100	F-GCTGCTGCCAATTTTCAGG R-ATTTGTGTCTGTAATTAGC	(GT) <sub>11</sub>	127–129	49	1.5	2	0.160/0.274	0.083	EU185629
Pho104	F-AGATGCTGTCTTACCTGTAGC R-GAGTTTGCCTGCCAAGC	(TG) <sub>10</sub>	138–152	54	1.5	2	0.280/0.246	>0.999	EU185630
Pho107	F-GCTTCCTTCTGCACTGG R-GGAGATGTATGTGTTGGG	(GT) <sub>10</sub>	120–122	54	1.5	2	0.440/0.429	>0.999	EU185631
Pho110	F-CGTGAGGGTAGTGTGTTGG R-AAGGGGAAGCGTGCCTG	(CA) <sub>10</sub>	114–116	51	1.5	2	0.560/0.503	0.691	EU185632
Pho113	F-CCTGGCATGTGGCGGTG R-CATCCCTCCAGAGTGTACCG	(TC) <sub>2</sub> (TG) <sub>9</sub> TC(TG) <sub>7</sub>	132–134	51	1.5	2	0.160/0.274	0.083	EU185633

derived from a sample of 25 unrelated Fiordland birds (9 males and 16 females) and tested for deviations from Hardy–Weinberg equilibrium using GENEPOP (Rousset 2007) (Table 1). One locus (Pho38) showed a significant heterozygote deficit ( $P = 0.010$ ), while the other 18 loci showed no significant deviations from expectations under random mating. GENEPOP was also used to test for linkage disequilibrium and after implementation of sequential Bonferroni adjustment of alpha significance levels (Rice 1989), no significant cases of linkage disequilibrium were observed among pairs of loci. Analysis using MICRO-CHECKER (van Oosterhout *et al.*

2004) revealed no evidence for the existence of null alleles in any of these loci except Pho38, which showed an excess of homozygotes possibly suggestive of null alleles.

The very low microsatellite DNA diversity observed here is consistent with the lack of minisatellite variation previously reported for takahe, and is unusually low even in the context of New Zealand's threatened avian fauna (Lettink *et al.* 2002; Jamieson *et al.* 2006). Nevertheless, the large number of loci developed here should facilitate parentage assignment, and investigations of the relationships between genetic diversity, inbreeding and fitness.

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