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PERMANENT GENETIC RESOURCES

# Characterization of new microsatellite markers derived from sequence databases for the emu (*Dromaius novaehollandiae*)

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## Abstract

**The emu (*Dromaius novaehollandiae*), a member of ratite family, is native to Australia and has been introduced to other countries worldwide. In this work, 10 polymorphic microsatellite loci were isolated and characterized for emu from public sequences. Polymorphism was surveyed in 22 individuals from two different populations kept in captivity. Between two and 11 alleles were found per locus, and the observed heterozygosity ranged from 0.05 to 0.85, in accordance with expectations. These markers will be useful as tools for detecting levels of genetic variation, reconstructing pedigrees (for quantitative genetic analysis) and identifying markers associated to fitness traits in emu populations.**

*Keywords*: database search, *Dromaius novaehollandiae*, emu, microsatellite, ratite, SSRs

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The emu (*Dromaius novaehollandiae*) is a member of ratite family, a group of large flightless bird species with keelless breastbones. Emus are native to Australia and they are found across the continent in almost all habitat types. Despite the fact that the actual census number of this species is relatively high, care is needed when the aim is to introduce this species in a new habitat, and microsatellite markers have proved to be effective in estimating relatedness and to maximize the genetic variability in the founder population. To date, there is only one study that has aimed to isolate and characterized microsatellite loci in the emu. Taylor *et al*. (1999) have identified six polymorphic microsatellite loci. In addition, using only five microsatellite loci, they have found relatively high genetic diversity and little evidence of inbreeding within populations (Hammond *et al*. 2002). Despite this, a larger number of markers are needed for an accurate reconstruction of pedigrees and for identifying markers associated to desirable traits in natural populations.

We developed 10 novel polymorphic microsatellite markers by screening through genomic DNA sequences from *D. novaehollandiae* deposited in GenBank by other researchers. A total of 2.4 Mb from 19 different bacterial artificial chromosome (BAC) clones were analysed. We found 35 simple sequence repeats (SSRs) in these sequences. Two of them showed a gap in the sequence submitted; therefore, these loci were excluded from the primer design. Of the 33 primer pairs designed, 11 were randomly selected for amplification (Table 1). Ten out of these SSRs showed variation and one primer pair did not yield product.

The sequences were downloaded from the GenBank database using the Entrez nucleotide query webpage (www.ncbi.nlm.nih.gov/Entrez), converted to FASTA format and the analysis was carried out using the program Tandem Repeats Finder (Benson 1999). This software identifies repeated patterns of nucleotides in DNA sequences, allowing insertions, mismatches and deletions. Default parameters were used in the search. Primers flanking each microsatellite were designed using the online primer design software Primer 3.0 (Rozen & Skaletsky 2000).

The allelic variation was assessed in a total of 22 individuals. Genomic DNA was isolated from muscle tissue of 20 *D. Novaehollandiae* from farms in the fourth region in Chile and from feathers of two individuals from the National Zoo located in Santiago, Chile, using DNeasy Blood and Tissue Kit (QIAGEN). Polymerase chain reactions (PCR) were performed in a total volume of 25 μL containing 1×

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**Table 1** PCR primer sequences, repeat motif, optimal annealing temperature ($T_{an}$) and preliminary population estimates ($n$ = 22)

| Locus | Clone (GenBank Accession no.) | Primer sequences | $T_{an}$ | Repeat motif | $N_A$ | $H_E$ | $H_O$ | $P$ |
|---|---|---|---|---|---|---|---|---|
| Dn01 | VMRC16-71P5 (AC157880) | F: CGATGGTGCTGATGAATAAT<br>R: TGAGGTAAAAGCCACTGTATGT | 59 | $(TG)_{22}$ | 3 | 0.09 | 0.09 | 0.02 |
| Dn02 | VMRC16-71P5 (AC157880) | F: CCTTTCTTTCCCCCTCCT<br>R: GTCTTCTCTTCATTTTCCTGCTT | 59 | $(ACAC)_{12}$ | 2 | 0.64 | 0.51 | 0.78 |
| Dn03 | VMRC16-127E6 (AC158282) | F: TGTCAGTTTGTTCGCAGGT<br>R: TGGAAAGAAAGAAAGGGAAT | 52 | $(TC)_{19}$ | 4 | 0.50 | 0.50 | 0.62 |
| Dn06 | VMRC16-248F6 (AC159173) | F: CAAGCCAGCCCCAAAGA<br>R: ATAATCCCACTCACTGCGGTA | 59 | $(TG)_{17}$ | 5 | 0.82 | 0.75 | 0.75 |
| Dn08 | VMRC16-243G7 (AC159950) | F: GATTCATCGTTTTGTTTCTTGG<br>R: TTGGAGCAGGAGGAGGAG | ND | $(CCCTT)_{13}$ | ND | ND | ND | ND |
| Dn10 | VMRC16-2M13 (AC160232) | F: TGTTGAGTTTTGGGTTGG<br>R: TTGTTGTGCTCTGTATTCCTT | 52 | $(TTTA)_7$ | 4 | 0.82 | 0.75 | 0.76 |
| Dn13 | VMRC16-278A18 (AC162760) | F: TGACTTCTTCAGCTTACCATCC<br>R: TTTGCTCCGTTTCTCATTTT | 52 | $(TG)_{30}$ | 10 | 0.82 | 0.80 | 0.64 |
| Dn15 | VMRC16-150I9 (AC158284) | F: GGAGGCAGCCCTGTTTT<br>R: CCGCCATTTCTAGGTGTGT | 59 | $(AT)_{15}$ | 5 | 0.59 | 0.72 | 0.04 |
| Dn22 | VMRC16-201J9 (AC154080) | F: TGCGTGTCTGTAAGGGATG<br>R: CCCCCTGCACTACGATTT | 59 | $(TG)_{31}$ | 11 | 0.82 | 0.85 | 0.42 |
| Dn28 | VMRC16-186M19 (AC154079) | F: CGGCACAGACGATCAAGAG<br>R: GACAGGGGCACGAAGGA | 59 | $(CAT)_8$ | 2 | 0.05 | 0.05 | 0.00* |
| Dn35 | VMRC16-130G15 (AC155794) | F: ATTCCAGCCCTTCACATCT<br>R: TGCCTTTCCTCATACACCTC | 62 | $(TA)_{17}$ | 7 | 0.86 | 0.65 | 0.99 |

*Significant heterozygote deficiency ($P < 0.005$, after Bonferroni correction) and ND, not detected. $N_A$, number of alleles; $H_E$, expected heterozygosity; *H*o, observed heterozygosity; *P*, exact *P* values for heterozygote deficiency (after Kalinowski & Taper 2006), for 10 microsatellites in the emu (*Dromaius novaehollandiae*).

buffer, 2.5 mM of $MgCl_2$, 200 μM of each dNTPs, 0.5 μM of each primer, 1 U of *Taq* DNA polymerase (Bioron) and 100 ng of DNA template. Samples were amplified in a Techne TC-412 thermal cycler with an initial 5-min denaturation at 94 °C, and 30 amplification cycles of 30 s at 94 °C, 1 min at the locus-specific annealing temperature (see Table 1), 1 min at 72 °C, and a final extension of 5 min at 72 °C. The PCR products were separated on 10% polyacrylamide gels (29:1 acrylamide: bis-acrylamide, 1× TBE, 120 V for 12–14 h) and displayed with silver nitrate staining (Allen & Budowle 1989). The pUC19-*Msp*I digest DNA marker used in electrophoresis was purchased from MBI Fermentas. The length of alleles was identified with QuantityOne 4.5.1 software (Bio-Rad).

A total of 53 alleles, ranging from two (Dn02 and Dn28) to 11 (Dn22), were found in the 10 loci. Expected and observed heterozygosities, Hardy–Weinberg equilibrium test and linkage disequilibrium were analysed with GenePop 3.4 (Raymond & Rousset 1995) and the Hardy–Weinberg test for heterozygote deficiency using maximum likelihood with ML-null for the presence of null alleles (Kalinowski & Taper 2006). The expected heterozygosity ranged from 0.86 (Dn35) to 0.05 (Dn28), with a mean of 0.60 (see Table 1). This value was not significantly different from the mean observed heterozygosity (0.57; $P > 0.01$). One (Dn28) out of 10 loci showed evidence of heterozygote deficiency (Table 1). Concomitantly, this one was the least polymorphic; therefore, it would be difficult to assess properly the presence of null alleles or heterozygote deficiency in this locus due to inbreeding in the sample. The other loci did not depart significantly from Hardy–Weinberg equilibrium ($P > 0.01$). Alleles from pairs of loci did not show significant linkage disequilibrium ($P > 0.01$). The probability of excluding both parents (Jamieson & Taylor 1997) using information from loci Dn06, Dn10, Dn13 and Dn22 jointly is 0.997.

These markers will provide the required and powerful molecular tools for management and conservation studies in wild and farmed emu populations and related species. In addition, we leave a set of primers for further testing of polymorphism in this species (see Table S1, Supplementary material).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Primer set designed for testing polymorphism in emu (*Dromaius novaehollandiae*) populations

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