Microsatellite Analysis of Genetic Diversity in Wild and Farmed Emus (*Dromaius novaehollandiae*)

E. L. Hammond, A. J. Lymbery, G. B. Martin, D. Groth, and J. D. Wetherall

The emu (*Dromaius novaehollandiae*) occupies most regions of the Australian continent and in recent times has been farmed for meat, oil, and leather. Very little is known about the genetic structure of natural or farmed populations of these birds. We report a preliminary study of genetic variation in emus undertaken by typing birds from five farms and two natural populations at five polymorphic microsatellite loci. Genetic diversity was high for all populations and there was little evidence of inbreeding, with most populations conforming to Hardy–Weinberg equilibrium for most loci. Significant heterozygote deficiencies at one locus in a number of populations were detected and may indicate the presence of null alleles. Comparisons of allele frequencies showed little evidence of genetic differentiation either among farmed populations or between farmed and natural populations.

The emu (*Dromaius novaehollandiae*) is a well-known member of the ratite family...
of flightless birds and is widely dispersed across the Australian continent. There is very little morphological variation among emu populations across Australia and there are no data on their genetic variability. Several studies have used mitochondrial sequence analysis to examine the phylogenetic relationships among ratite birds (Baker et al. 1995; Freitag and Robinson 1993; Haddrath and Baker 2001), but no studies to date have examined ratite population structure using microsatellite analysis.

The emergence of an emu industry in the 1980s, aimed at commercial production of meat, oil, and leather, has heightened interest in characterizing emu pedigrees and improving their genetic management. Within Western Australia, many farms have birds that are descendants of emus from the founding stock of the first emu farm established in Wiluna in 1976. However, poor written records of male:female ratios, overall stock numbers, and the source of breeding stock make it difficult to estimate the level of relatedness among domesticated stock. The number of emus at each farm appears variable over time, and culls and severe reductions in stock size can follow large breeding seasons. Estimation of expected levels of genetic variation among farmed stock is further confounded by a lack of knowledge of existing genetic variation in wild populations. More information would be very helpful in defining pedigrees, assessing the levels of inbreeding, and (eventually) in defining markers for desirable heritable traits.

We have previously published the first panel of five microsatellite markers isolated in this species (Taylor et al. 1999) and here we report a preliminary assessment of genetic variation in emus sampled from farmed farms and the wild in Western Australia. Data from wild emus were used to assess whether isolating mechanisms are operating in natural populations of these birds. Any factor that limits gene flow among populations can act as an isolating mechanism, including the long stretches of vermin-proof fences that have been erected in an effort to control emu movements. Data from farmed emus were used to determine if farmed populations were less genetically diverse than wild populations and to assess the genetic relationships among different farmed populations. The results described here represent the first molecular genetic characterization of emu populations.

Materials and Methods

Sampling

Blood was sampled from populations of wild and farmed emus from various locations in Western Australia and from one farmed population in Thailand (Table 1). When sampling birds from commercial farms, the farmer was usually able to advise which birds were unrelated, and samples were not knowingly taken from related birds. Farmed emus were restrained and calmed, usually by holding the bird under the wings, while 200 μl of blood was taken by syringe from the jugular vein. The blood was immediately mixed with 1 ml 70% ethanol and stored at 4°C pending DNA extraction by standard phenol-chloroform procedures (Maniatis et al. 1982). Wild emus panic when restrained, and previous attempts to capture them have stressed the birds to such an extent that they have seriously injured themselves. The use of drugs, either in water sources or as a dart, has also been largely unsuccessful. We therefore obtained a scientific research permit to kill a number of wild birds; we employed the services of professional marksmen to ensure a clean shot. Groups of emus—consisting of a number of smaller birds of approximately the same height and one full-sized bird—were considered a family unit, and only one member was sampled.

From seven distinct populations, 107 individual animals were sampled. These were identified as either wild or farmed, together with the location from which they were obtained (Table 1). Most of the emus on Western Australian farms sampled in this study originated, directly or indirectly, from the Wiluna emu farm that was established in 1976 with 100 breeding pairs caught from the surrounding area. The Mount Gibson and Toodyay farms supplemented their stock with more wild birds. The Toodyay farm contained some birds that were descendants of emus taken from the Ben Covens and Sand Plains areas around Toodyay. The Thailand farm was established in the 1990s with emus imported from the United States, but the origin of these emus is unknown. Wild emus were sampled from two areas in Western Australia, to the west and to the east of a vermin-proof fence that was erected in 1900 and subsequently extended and maintained to control emu movements into agricultural areas.

Microsatellite Typing

Loci were amplified by polymerase chain reaction (PCR) in 10 μl reaction volumes, using 50 ng DNA, 4 pmol forward primer, end-labeled with an IRD 800 infrared dye (Li-Cor Inc., Lincoln, NE), 1 pmol unlabeled forward primer, and 5 pmol unlabeled reverse primer, 200 μM dNTPs, 0.1 U AmpliTaq Gold (Perkin-Elmer, USA), 0.1 M Tris-HCl pH 8.3, 0.5 M KCl, and 1.5 mM MgCl₂ (Taylor et al. 1999). A summary of the primer pairs and properties of the final panel of five microsatellite loci is given in Table 1. Gel electrophoresis was used for genotyping, as described previously (Taylor et al. 1999). All loci generated light stutter bands typical of dinucleotide microsatellite loci, but these did not preclude successful identification of the main DNA fragments used to assign genotypes.

Analysis

Genotypic and allelic frequencies were calculated for all populations at all microsatellite loci. Genetic diversity within populations was described by the mean number of alleles per locus and the mean expected unbiased heterozygosity or total gene diversity (H; Nei 1978). Genotypic frequencies expected under Hardy–Weinberg equilibrium were calculated from allelic frequencies using Levene’s (1949)

### Table 1. Mean number of alleles per locus and total gene diversity (H) in samples of emus from seven populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Source</th>
<th>Sample size</th>
<th>Mean alleles/locus</th>
<th>Total gene diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. Gibson</td>
<td>Farmed</td>
<td>20</td>
<td>12</td>
<td>0.89</td>
</tr>
<tr>
<td>Harvey</td>
<td>Farmed</td>
<td>19</td>
<td>11</td>
<td>0.87</td>
</tr>
<tr>
<td>York</td>
<td>Farmed</td>
<td>15</td>
<td>10</td>
<td>0.82</td>
</tr>
<tr>
<td>Toodyay</td>
<td>Farmed</td>
<td>19</td>
<td>11</td>
<td>0.87</td>
</tr>
<tr>
<td>Thailand</td>
<td>Farmed</td>
<td>9</td>
<td>6</td>
<td>0.79</td>
</tr>
<tr>
<td>Wild A</td>
<td>Wild—west of fence</td>
<td>11</td>
<td>8</td>
<td>0.80</td>
</tr>
<tr>
<td>Wild B</td>
<td>Wild—east of fence</td>
<td>14</td>
<td>11</td>
<td>0.87</td>
</tr>
</tbody>
</table>

All farmed populations, except Thailand, were in Western Australia. The two natural populations were also from Western Australia and separated by a vermin-proof fence.
mean, $F_{IS}$. Burrow’s composite measure of linkage disequilibrium ($\Delta_{AB}$) was estimated for all pairwise combinations of loci in all populations and tested for significance as outlined by Weir (1990).

Genetic differentiation among populations was assessed by an analysis of molecular variance (AMOVA; Excoffier et al. 1992). Distances among all multilocus genotypes were estimated by counting the number of different alleles (Michalakis and Excoffier 1996) and the total variation among genotypes partitioned into that due to differences within populations, that due to differences among populations in Australia, and that due to differences among populations in Australia and Thailand. A nonparametric correction for small sample size. Deviations of observed from expected frequencies were tested by the exact test of Guo and Thompson (1992). The extent of deviation from Hardy–Weinberg equilibrium within populations was expressed for each locus by Wright’s fixation index ($F_{IS}$), and across all loci for each population, $F$-values were summarized by the weighted
permutation approach (Excoffier et al. 1992) was used to test the significance of the variance component estimates.

**Results and Discussion**

A total of 96 alleles were detected in the 107 individual emus typed. As shown in Table 2, the allele frequency distributions for the five microsatellite loci were similar between the groups of emus, and indicate that the groups sampled are not differentiated. The distributions of the alleles are similar to those seen with microsatellite loci in humans and many other species, displaying a large number of alleles and high levels of heterozygosity. Locus *emu63* was exceptionally polymorphic and is clearly the most informative for population characterization and individual identification, including parentage assignment (Taylor et al. 2000). Over all populations, the mean number of alleles detected per locus was 19, although the actual number of observable alleles at each locus ranged from 13 at locus *emu18* to 38 at locus *emu63*. Some alleles were detected in just one population. There was a direct relationship between the number of samples taken from a source and the number of alleles detected, indicating that increased sample size is required to provide more accurate knowledge of alleles and their frequency distribution. There was no evidence of associations between alleles at different microsatellite loci, and none of the 10 pairs of loci showed significant linkage disequilibrium as measured by $(\Delta_{AB})$ (data not shown).

Values for total genetic diversity at all loci are compared among populations in Table 1. Given the sample size limitations of the study, these values should be viewed as minimum estimates, as further sampling may reveal even higher levels of genetic diversity. In general, the farmed Thai and natural Wild A populations were less genetically diverse than the other farmed or natural populations, but again further sampling is required to determine whether these data accurately reflect the total genetic diversity of the source.

For most loci, in most populations, the genotypic frequencies did not deviate significantly from Hardy–Weinberg equilibrium (Table 3). The exception was locus *emu5*, where there were significant heterozygote deficiencies in five of the seven populations. Such locus-specific effects cannot be accounted for by genome-wide phenomena such as inbreeding or a Wahlund effect. Null alleles (alleles that fail to amplify because of large increases in the size of the product or mutations at flanking primer sites) could explain the heterozygote deficiencies, because heterozygotes for the null allele will appear as homozygotes for the amplified allele.

Although farmed populations of emus tended to have higher fixation indices than natural populations, the general concordance between the expected and observed heterozygosity values suggests that, for the most part, the populations are not inbred (Table 3). The mean $F_s$ value over loci was significantly greater than zero for the Thai population, but this was principally due to the relatively greater value at locus *emu5*, and does not seem to indicate a general heterozygote deficiency in this population. There is therefore little evidence for extensive inbreeding in farmed emus. This suggests, first, that initial selection of emus for breeding stock sampled a large proportion of the genetic diversity present in wild populations, and second, that there has been little restriction of the gene pool in farmed populations since they were founded. The pair bonding of males and females (Blache et al. 2000) has prevented emu farms from implementing systematic programs of genetic improvement that would tend to increase inbreeding and reduce genetic diversity (MacHugh et al. 1997; Norris et al. 1999).

When total genetic variation was partitioned within and among populations by AMOVA, most genetic variation (94%) was found within populations, with only 3% found between emus in different countries (Thailand and Australia) and 3% between different populations from within Australia. The general lack of genetic differentiation among different farmed populations, or between farmed and natural populations, may be attributable to the relatively short time the populations have been separated and
the absence of selective breeding in captivity. However, given the large number of alleles detected at each locus, relatively small changes in genetic structuring may not be detectable with the limited number of samples analyzed in this study.

We did not detect any genetic differences between emus sampled from wild populations that are separated by vermin-proof fences. The fences may be effective in preventing emu movement, but they were erected only 100 years ago, and this may be insufficient time for genetic differences to develop. The data suggest that no isolating mechanisms exist, so the morphological homogeneity seen in the emu (O’Brien 1990) appears to be a true indicator of genetic uniformity in the species. However, most of the wild emus in this study originated from regions within 1000 km of Perth, a limitation in sampling that must be taken into account. Samples from populations located in Eastern Australia or from the far north of Western Australia were not available for this study and are needed to permit better estimates of genetic diversity in this widely disseminated, apparently homogeneous species.

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Received September 11, 2001

Accepted August 8, 2002

Corresponding Editor: Susan J. Lamont