

## Application of a sex identification technique in juvenile ostriches and its potential application in Botswana

O.M. Mine<sup>#</sup>, M.E. Mochakana, T. Mpapho, D.T.M. Motlhanka and P. Kgwatalala  
Botswana College of Agriculture. Private Bag 0027. Gaborone. Botswana

### Abstract

Sexing ostrich chicks and juveniles before the dimorphous appearance of the plumage that starts at 14 months of age is proving difficult and cumbersome for Botswana farmers. This problem delays early selection of birds for breeding and trading. It also means that birds are often sold as a mixture of males and females. DNA-based sex identification provides a solution and is amenable to large-scale application. The application of the multiplex polymerase chain reaction (PCR) was used to determine the sex of 6-months old juvenile ostriches. The blood from four mature males and four mature females were used to verify the assay. The test group consisted of 19 randomly selected six-month old birds, which turned out to be 10 males and nine females. The PCR technique uses two pairs of primers. The primers SS1 and SS2 amplify a 650 base pair (bp) female-specific fragment, while the primers L014a and L014b amplify a fragment about 280 bp, from both sexes. The females were thus distinguished by amplification of two bands after agarose gel electrophoresis, and the males by one band. Following the successful application of this technique using DNA extracted from blood, further investigations have to be conducted, using DNA extracted from other body tissue samples such as feathers or skin. A scenario is envisaged whereby, during tagging of the chicks, a body tissue sample such as a blood feather could be sent to the laboratory for DNA sexing. This technique would aid farmers in identifying the sex of their birds at a young age.

**Keywords:** Ostriches, sex identification, DNA test

<sup>#</sup> Corresponding author. E-mail: ommine@temo.bca.bw

### Introduction

Commercial ostrich (*Struthio camelus*) farming is a relatively new agricultural enterprise in Botswana, and is expanding rapidly. The products of ostrich farming include meat, hides, eggs and feathers. The ostrich, together with Emu (found in Australia) and Rhea (found in South America) belong to a family of flightless birds, order *Ratitae* (ratites). Although ratites are classified as poultry, the pH of their flesh is similar to that of beef and, therefore, is classified as red meat (Anon, 1996). The special qualities of ostrich meat make it attractive from a health point of view considering that a 100 g serving of raw ostrich meat contains approximately 418 joules, 25 g protein, 2 g fat and 60 mg cholesterol (Anon, 1996). This compares favourably with 100 g of American lean beef, which contains 544 – 711 joules, 20 g protein, 2.5–9 g fat and 65 mg cholesterol. Ostrich skin is considered top quality leather, comparable to those of crocodiles and elephants (Kreibich & Sommer, 1995). Being flexible but tough, ostrich skins are processed into fine quality leather for shoes, purses and handbags. Apart from their importance in breeding, ostrich eggs can be used in the baking industry. The volume of one ostrich egg is on average equivalent to those of 24 chicken eggs. The eggshells may be turned into objects of art by painting or engraving (Kreibich & Sommer, 1995). An advantage of ostrich farming is that every part of the bird can be utilised, with less than 2% being wasted.

The success of ostrich production pivots around the production of healthy chicks in large numbers, which grow to slaughter weight or breeding age as economically as possible (Deeming *et al.*, 1996). One female has the potential to produce 20 or more chicks per season. Problems restricting the development of the industry are the poor survival of chicks after hatching and the lack of knowledge of the possible causes of death of birds (Deeming *et al.*, 1993). In most commercial operations in Botswana, birds are kept together “as hatched”, which means that males and females are not reared separately. This is in contrast to broiler chickens, where the cockerels are normally raised separately because they grow faster, require higher protein diets and convert food more efficiently for a longer period than pullets. However, unlike chickens which display sexual dimorphism relatively young, male and female ostrich chicks and juveniles look identical up to an age of 12–14 months, when the black feathers of males start to appear. In birds from nine months and older, their sex can be determined by watching the birds passing urine or defecating, because the penis emerges during these processes (Hallam, 1992). Alternatively, cloacal sexing which involves the physical examination of the genitalia of the birds can be used. The technique has proven highly successful, but

requires plenty of experience, and even then an accuracy of only 95% is achieved (Deeming *et al.*, 1996). Thus, farmers have to be contented with selecting birds for breeding and sale purposes at a relatively old age, which is costly to both the farmer and customer.

Modern molecular techniques that are non-invasive, accurate, reliable and amenable to large-scale application are required. The ability to sex young birds independent of morphological traits is not only important for breeding and the raising of sexes separately, but also for research purposes such as the testing of theories on the sex ratio evolution (Ellergren & Sheldon, 1997). Traditional methods of sexing birds such as cloacal sexing and karyotype analysis are laborious. The sex chromosomes in birds are designated Z and W, with males being homogametic (ZZ) and females heterogametic (ZW). Genetic sexing relies on the different genotypic composition of males and females, and can be based on counting Z chromosomes. However, the best option is to monitor the presence of W chromosome DNA (Ellegren & Sheldon, 1997). Sex determination at the DNA level is a well-established technique that has been shown to be fast, accurate and inexpensive (Bello & Sanchez, 1999) and has been performed in many different avian species (Griffiths *et al.*, 1998; Bello & Sanchez, 1999). An investigation was conducted to evaluate the application of the multiplex polymerase chain reaction (PCR) technique of Bello & Sanchez (1999) to determine the sex of juvenile ostrich chicks at six months of age.

## Materials and Methods

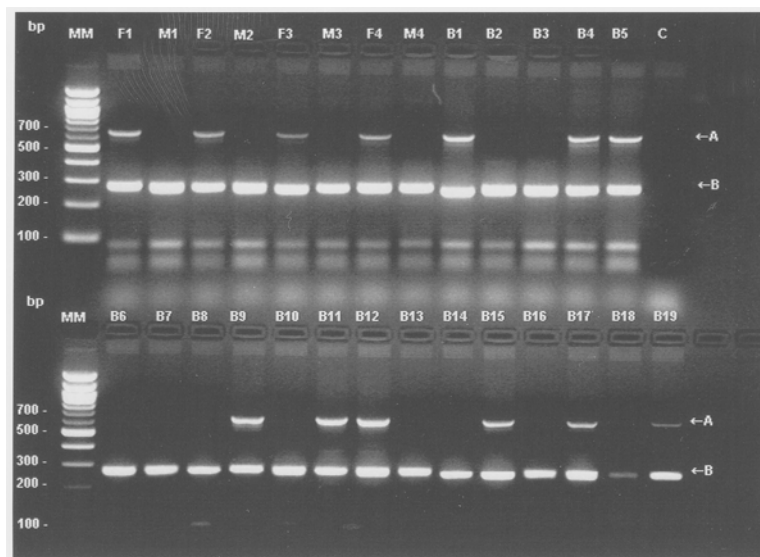
Blood samples were collected from eight mature ostriches (four males and four females) whose gender could be established accurately by the differences in colour patterns of their feathers. Their blood was used to determine the difference in the DNA banding pattern between the two sexes. Blood (200 µl) was drawn from the intertarsal vein under the wing into EDTA tubes using a 23-gauge needle. Twenty additional blood samples were collected from 20 randomly chosen 6-month old juveniles reared for meat production at the Botswana College of Agriculture, located at Sebele, Gaborone, Botswana. Genomic DNA was extracted from the 200 µl of blood using a Genomic Prep Blood DNA Isolation Kit (Amersham Pharmacia Biotech, Johannesburg, South Africa), following the manufacturer's instructions. The DNA was suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA) (pH 8.0).

The DNA samples (50-150 ng) were used as a template in 25 µl reaction containing 1X amplification buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgSO<sub>4</sub>, 0.001% (w/v) gelatin) (Applied Biosystems, Foster City, CA, USA), 200 µM dNTPS, 4 µM of two pairs of primers – SS1 (5'-TCTACACCTAAGGAGCCCATATT-3') and SS2 (5'-GGTCTACACCTGTTGAAAATCATT-3'), L014a (5'-CCAGTCAGGAGCACCTGTTC-3') and L014b (5'-AGAGCAGGGATGACTGTGGC-3') (Bello & Sanchez, 1999) (GENESET OLIGOS, Paris, France) and 0.5 U AmpliTaq DNA polymerase (Applied Biosystems). Amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems) programmed as follows: reactions were heated at 94 °C for 2 min., followed by 30 cycles of 94 °C for 1 min., 64 °C for 1 min. and 72 °C for 1 min. and a final cycle of 72 °C for 15 min. To reduce problems associated with poor amplification, annealing temperature was occasionally reduced or increased by 1–3 °C. A negative control, which contained no DNA, was included with each run. The DNA from the eight ostriches of known sex was used as a positive control. Ten µl of amplification products were electrophoresed in a 2% agarose gel using a TBE (45 mM Tris-borate, 1 mM EDTA) buffer system. The DNA bands were visualised and photographed under ultraviolet light following staining with ethidium bromide.

## Results and Discussion

A characteristic DNA banding pattern for mature females consisted of two bands approximately 280 bp and 650 bp (Figure 1-lanes F1 – F2). In contrast, a characteristic DNA banding pattern displayed by mature males consisted of a single band of approximately 280 bp (Figure 1-lanes M1 – M4). The banding patterns of products of DNA amplification were similar to those of Bello & Sanchez (1999) who first described the technique. Primers SS1 and SS2 amplify a 648 bp female-specific fragment (a RAPD marker converted into a single-locus PCR marker sequence characterised amplified region-SCAR), whereas the additional primers L014a and L014b amplify a 270 – 280 bp fragment in both sexes corresponding to microsatellite L014 (280 bp). Primers L014a and L014b provide an internal positive control for PCR amplification (Bello & Sanchez, 1999). For the 20 DNA samples from the randomly chosen 6-months old juveniles, one sample failed to amplify and the remaining 19 chicks comprised of 10 males (Figure 1 - lanes B2, B3, B6, B7, B8, B10, B13, B14, B16 and B18) showing a single band 280 bp in size and nine females

(Figure 1 -lanes B1, B4, B5, B9, B11, B12, B15, B17 and B19) showing two bands; one at 280 bp and the other at 650 bp. Thus, males and females were distinguishable based on the DNA banding pattern of products of amplification.



**Figure 1** DNA banding patterns on 2% agarose gel of products of PCR amplification of ostrich DNA using two pairs of primers SS1 and SS2, and L014a and L014b. Lane MM represents 100 bp DNA ladder. Lanes F1 – F4 represent DNA from four mature female ostriches, which show 650 bp and 280 bp DNA bands marked A and B respectively, which are characteristic of products of amplification from females. Lanes M1 – M4 represent DNA from four mature males, which show a 280 bp DNA band marked B which is characteristic of products of amplification from males. Lane C represents a negative control that contains no DNA. The juvenile birds comprised of 10 males (Lanes B2, B3, B6, B8, B9, B11, B14, B15, B17 and B20) and nine females (Lanes B1, B4, B5, B10, B13, B16, B18, and B21). One of the samples did not amplify.

## Conclusions

We describe here the use of a multiplex PCR (Bello & Sanchez, 1999) as a molecular technique for sex identification of juvenile ostriches within a developing country set-up. Selection of chicks for breeding and those for sale could be carried out as young as possible, which may ease the problems of transportation. For sexing purposes only, the scenario would be to plug a feather from the chicks during tagging and to send the blood part in a preservative to the laboratory where the blood could be cultured, or DNA could be extracted directly. The use of PCR has an advantage since minute samples are required for analyses. With this technique, farmers exporting birds can provide a certificate of proof and thus a guarantee of the sex of the birds to the buyers. Further research would include the refining of protocols for DNA extraction from samples of body tissues such as feathers or skin that do not require the use of individuals highly trained in phlebotomy. Our laboratory, however, prefers blood, since it can be used also for disease diagnosis such as detecting antibodies against New Castle disease. It is concluded that the sex of the 19 juvenile ostrich chicks could be determined successfully using the multiplex PCR with two pairs of primers, SS1 and SS2, and L014a and L014b and that this technique can be applied under Botswana farming conditions.

## Acknowledgement

The Botswana College of Agriculture provided funding for this project.

## References

- Anonymous, 1996. Focus on: ratites (Emu, Ostrich and Rhea).  
<http://www.fsis.usda.gov/OA/pubs/ratites.html>. Accessed:12 December 2001.

- Bello, N. & Sanchez, A., 1999. The identification of sex-specific DNA marker in ostrich using random amplified polymorphic DNA (RAPD) assay. *Mol. Ecol.* 8, 667–669.
- Deeming, D. C., Dick, A. C. K. & Ayres, L.L., 1996. Ostrich chick rearing. A stockman's guide. A Ratite Conference. c/o Hangland Farm Ostriches Ltd., Upper Wardington, Banbury, Oxfordshire, OX17 1SU, United Kingdom. pp. 24–25.
- Deeming D. C., Ayres, L. & Ayres, F. J., 1993. Observations on the commercial production of ostriches (*Struthio camelus*) in the United Kingdom: rearing chicks. *Vet. Rec.* 132, 627-631.
- Ellegren, H. & Sheldon, B.C., 1997. New tools for sex identification and the study of sex allocation in birds. *Trends Ecol. Evol.* 12, 255–259.
- Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.W., 1998. A DNA test to sex most birds. *Mol. Ecol.* 7, 1071–1075.
- Hallam, M.G., 1992. The Topaz introduction to practical ostrich farming. Savanna Wildlife (PVT) Ltd.
- Kriebich, A. & Sommer, M., 1995. Ostrich farm management. Landwirthschaftsverlag GmbH, Munster-Hiltrup.