Sex Determination in African Love Birds (*Agapornis Sp.*)
Using P2/P8 set of Primers by PCR

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Abstract

Most of the bird species are not sexually dimorphic. In such cases, molecular methods are more efficient for their sex determination. The objective of this study was to test the feasibility and efficacy of feathers as a molecular method of sex determination in African love birds. From the freshly plucked down feathers, DNA was isolated and the amplification of the CHD gene was performed using P2/P8 set of primers. Sexing was successfully determined in 24 African love birds. P2/P8 primers proved to work well for sexing in African love birds. The males showed single bands and the females showed double bands.

Key words : African love birds – Sexing – P2/P8 primers – PCR

Sex determination of young ones, juveniles and monomorphic birds is very difficult based on morphological analysis (Jensen et al., 2003). Sex can be determined by PCR using feather samples as a source of DNA without posing risk for the life of the birds (Bello et al., 2006; Dubeic and Neubaeur, 2006). Recently, number of studies have been focused on the development of efficient molecular methods for sexing birds (Cerit and Avanus, 2007). However, in India information on sex determination of monomorphic birds is scarce.

Materials and Methods

Freshly plucked down feathers (4 to 6 feathers) from 24 African love birds were collected after proper physical restraint, from pet shops and private aviaries and stored in air tight polythene bags at -20°C. The feather tip was cut into small pieces (5 mm size) and DNA was extracted from as per the protocol given in Qiagen DNeasy blood and tissue kit. DNA extracted was subjected to PCR using primers P2-5′-TCTGCATCGCTAAATCCTTT-3′ and P8 - 5′-CTCCC AAGGA TGAGR AAYTG -3′ (Griffiths et al., 1998). The reaction mixtures were prepared in 10 µl volumes (Initial denaturation step at 94°C for 1 min 30 sec; 35 cycles of 95°C for 30 sec, 50-55°C for 30 sec and 72°C for 30 sec; and final elongation at 72°C for 5 min). The samples were run in 12% Poly Acrylamide Gel Electrophoresis (PAGE) and stained with eithidium bromide (Choryand Pollard, 2001). The gel was analysed by UV transilluminator and photographed with a gel documentation system.

Results and Discussion

Gender was determined by visualization of gel under UV transilluminator (Fig 1) as two bands (375 and 400 bp) in females (Z and W) or one band (375) in males (Z). In this study, 12 males and 12 females were identified. With vent sexing personnel involved, when well trained at chick sexing schools, could easily get 95% accurate results in sexing. This method is based on holding the day old chick upside down in one hand and examining the vent area (cloaca) for the presence or absence of arudimentary male sex organ. Feathers represent the most common sample for DNA isolation in birds (Seki et al., 2006). Moreover, when compared to blood collection, sampling is easy and causes minimum pain, if the feathers are plucked (Leekaew et al., 2008). Feathers can be collected much earlier than blood samples; hence DNA analyses can be performed at a very early stage without any harm (Malago et al., 2002). It is criti-
cally important to reduce the physical restraint induced stress while handling birds (Bosnjak et al., 2013). From the cells at the basal tip of the calamus of the feathers, DNA is extracted (Morin et al., 1994). Hence, in this study we used feathers as source of DNA for gender identification and its reliability was proved in all sampled individuals. Moreover, when compared to the other techniques, the discomfort caused to the individuals by plucking feathers is meagre (Bosnjak et al., loc cit). Considering the above factors, it would be recommended that feathers might be used as DNA source wherever possible while sexing birds. The method for determining sex in African love birds presented in this study is a reliable, economical, fast, simple and less-invasive method.

Summary

African love birds are monomorphic and can be sexed using feathers as a source of DNA extraction and subject the DNA template to PCR using P2 and P8 set of primers.

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