Journal of Mahanakorn Veterinary Medicine
Vol. 2 No.2 July - December 2007

Sex Identification of Some Psittacine Birds by Polymerase Chain Reaction

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Abstract

Blood samples from many Psittacine birds including Green-cheeked conure (*Pyrrhura molinae*), Sun conure (*Aratinga solstitialis*), Rosella (*Platycercus spp.*), Amazon parrot (*Amazona spp.*), Congo african gray parrot (*Psittacus erithacus erithacus*) and Blue - and - gold macaw (*Ara ararauna*) were collected for sex identification by PCR – based method. The design of the P2/NP/MP primers was based on differences in sequences between CHD1W and CHD1Z genes using 3'-terminal mismatch primer on point mutation of the female CHD1W gene. We tested this set of primers with DNA samples extracted from a variety of these birds. The PCR amplification using the P2/NP/MP primers showed clearly different patterns of the PCR products between male and female birds of Green-cheeked conure, Sun conure, Rosella, Amazon parrot. For Congo african gray parrot and Blue - and - gold macaw, only male birds were identified. Therefore the results for these two species need to be confirmed by further testing. The results clarify that the PCR method using the P2/NP/MP primers is suitable for sex identification of some Psittacine birds. This method may be applied for sexing of other Psittacine bird species in the future.

Keywords : Psittacine bird, Sex identification, Chromo-helicase-DNA binding protein (CHD) gene, Polymerase chain reaction (PCR)

Introduction

Nowadays, Psittacine bird farming has become a business of interest in Thailand, in particular Psittaciformes such as Parakeets, Lovebirds, Sun conures etc. The imported bird species reach a high price, and the quick coupling of male and female birds could accelerate the return of investment. Sex identification of Psittacine birds especially in juvenile birds could also bring business income. Many species of Psittacine birds are sexually monomorphic. It is difficult to distinguish between male and female based on an external morphological appearance. Many methods have been used to identify bird sex including anatomical analysis, the pelvic bone test, behavior signs, chromosomal karyotyping and hormonal measurement. These conventional techniques present significant problems. The birds may suffer stress, since they are subjected to invasive procedures, time-consuming and potentially harmful.

Various molecular techniques have been developed for sex identification of birds since the 1990s. At the beginning of the development of molecular methods, hybridization-based methods were applied using an oligonucleotide probe or W-chromosome specific probes, but they were laborious and time - consuming. Later, polymerase chain reaction (PCR) based methods became of interest. PCR is precise, sensitive, efficient and less time - intensive. It requires only a drop of blood or a single feather and could be used in juvenile birds.

Based on the chromo-helicase-DNA-binding (*CHD*) gene, in non-ratite birds, the CHD1W gene was found only on the W chromosome of female whereas the CHD1Z gene was found on the Z chromosome of both male and female (Female = ZW, Male= ZZ). However,these genes were not found in ratite or flightless birds such as Emu, Kiwi, Rhea, Moa and Ostrich due to thesimilarity of CHD gene on W and Z chromosomes. The molecular sexing method based on the amplification of the chromo-helicase-DNA-binding 1 (CHD1) gene was first successfully established by Griffith *et al.* (1998). The different PCR methods were designed based on the difference in length between introns in the CHD-Z and CHD-W genes. (Griffiths *et al.*, 1998) Currently, an alternative PCR method named amplification refractory mutation system (ARMS) and based on the size variation of the intron between the CHD1Z and CHD1W, was developed and successfully applied for sex identification in Falconiformes (Ito *et al.*, 2003) and black swans (*Cygnus atratus*). (He *et. al.*, 2005).The efficiencies of P2/P8 primers from Griffiths *et al.* (1998) and of P2/NP/MP primers from Ito *et al.* (2003) for sex identification were compared in some pet birds by Jirajaroenrat and Thammakarn (2007). The results indicated that P2/NP/MP primers can highlight more clearly the difference between female and male of Green-cheeked conure (*Pyrrhura molinae*), Sun conure (*Aratinga solstitialis*), Ring-necked (*Psittacular kramen manillensis*), Finch (*Erythrura gouldiae*) and Lovebird (*Agapornis spp*). This research was established to test the efficiency of P2/NP/MP primers for sex determination in some Psittacine birds.

Materials and methods

Blood samples were collected from the wing vein of male and female Green-cheeked conure (*Pyrrhura molinae*), Sun conure (*Aratinga solstitialis*), Rosella (*Platycercus spp.*), Amazon parrot (*Amazona spp.*), Congo african gray parrot (*Psittacus erithacuserithacus*) and Blue - and - gold macaw (*Ara ararauna*). PCR reactions were carried out using

primers from Griffiths *et al*. (1998), including P2 primer (5'-TCT GCA TCG CTA AAT CCT TT-3') and from Ito *et. al.* (2003), including P2 primer, NP primer (5'-GAG AAA CTG TGC AAA ACA G-3') and MP primer (5'-AGT CAC TAT CAG ATC CGG AA-3') (Figure 1).

The PCR amplifications were performed for 35 cycles: denaturation at 94°C for 45 seconds, annealingat 60°C to 65°C for 45 seconds and extension at 72°C for 45 seconds. PCR products were analysed by 1% agarose gel electrophoresis, comparing with 100-bp DNA ladder (Invitrogen, US).

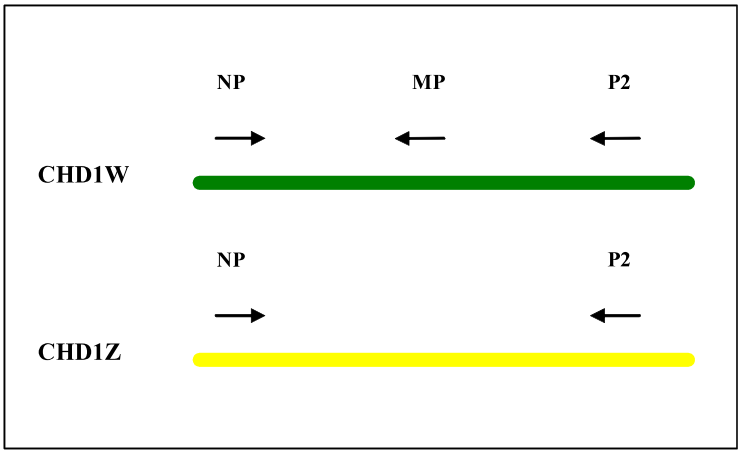

Figure 1 Binding sites of P2/NP/MP primers on the CHD1W and CHD1Z genes.

Results

The P2/NP/MP primers were applied for PCR amplification of CHD1Z and CHD1W genes of Green-cheeked conure, Sun conure, Rosella and Amazon parrot. The female birds showed two bands of PCR products, whereas the male birds showed a single band. The results showed clear differences between female and male birds. (Figure 1). For Congo African gray parrot and Blue – and - gold macaw, only male birds were identified (Table 1).

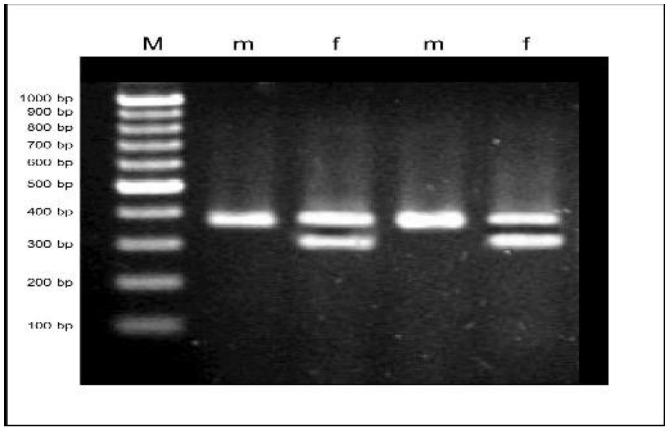

Figure 2 PCR amplification of female (f) and male (m). Lane M is 100-bp DNA ladder.

Table 1 PCR result of Psittacine sexing

Common name	Species	N	Male	Female
Green-cheeked conure	*Pyrrhura molinae*	3	2	1
Sun conure	*Aratinga solstitialis*	21	13	8
Rosella	*Platycercus spp.*	6	2	4
Amazon parrot	*Amazona spp.*	2	1	1
Congo african gray parrot	*Psittacus erithacus erithacus*	4	4	0
Blue – and – gold macaw	*Ara ararauna*	2	2	0

N = total number of samples

Discussion

The results confirmed that the PCR method using the P2/NP/MP primers is able to identify the sex of Green – cheeked conures, Sun conures, Rosella and Amazon parrot. For Congo African gray parrot and Blue – and – gold macaw, only male birds were identified, either because only male birds were collected or because these sets of primers are not suitable. More samples of these two species should be collected and analyzed to confirm the result with these primers.

To be successful in bird sex identification, the primer best suited to each specie should initially be tested.

Acknowledgement

We thank The Subproject Graduate Study and Research in Agricultural Biotechnology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) for material and equipment support.

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Journal of Mahanakorn Veterinary Medicine
Vol. 2 No.2 July - December 2007

การจำแนกเพศนกแก้วปากขอบางชนิดด้วยวิธีปฏิกิริยาลูกโซ่พีซีอาร์

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บทคัดย่อ

การจำแนกเพศนกด้วยวิธีปฏิกิริยาลูกโซ่พีซีอาร์จากตัวอย่างเลือดของนกแก้วปากขอบางชนิดได้แก่ กรีนซีกคอนัวร์ (*Pyrrhura molinae*) ซันคอนัวร์ (*Aratinga solstitialis*) โรเซลล่า (*Platycercus spp.*) นกแก้วอเมซอน (*Amazona spp.*) คองโกอาฟริกันเกรย์ (*Psittacus erithacus erithacus*) และ บลูแอนโกลด์มาคอว์ (*Ara ararauna*) โดยใช้ไพรเมอร์ P2/NP/MP ที่ออกแบบโดยอาศัยความแตกต่างระหว่างยีน CHD1W และ CHD1Z จากการเกิด point mutation ที่บริเวณปลายด้าน 3' ของยีน CHD1W ในนกเพศเมีย ผลการทดสอบพบว่าไพรเมอร์ดังกล่าวให้ผลที่แตกต่างกันอย่างชัดเจนระหว่างนกเพศผู้และเพศเมียในนก กรีนซีกคอนัวร์ ซันคอนัวร์ โรเซลล่า และนกแก้วอเมซอน แต่ไพรเมอร์ดังกล่าวไม่สามารถจำแนกเพศนกชนิด คองโกอาฟริกันเกรย์ และบลูแอนโกลด์มาคอว์ ได้เนื่องจากพบว่าไพรเมอร์ดังกล่าวให้ผลในการทดสอบในนกเพศเมียในรูปแบบเดียวกันกับนกเพศผู้ ทั้งนี้การจำแนกเพศในนกชนิดนี้จำเป็นต้องมีการทดสอบต่อไปในอนาคต จากผลการทดลองดังกล่าวสรุปได้ว่าไพรเมอร์ P2/NP/MP สามารถใช้ในการจำแนกเพศในนกแก้วปากขอได้บางชนิด และอาจสามารถนำไปประยุกต์ใช้ในนกแก้วปากขอชนิดอื่นได้

คำสำคัญ : นกแก้วปากขอ การจำแนกเพศ Chromo-helicase-DNA binding protein (CHD) ปฏิกิริยาลูกโซ่พีซีอาร์