es were not determined in this study, swine serum contains overall lower total serum Hpt concentrations than does the serum of other species examined.[2,4–6] Dilution of the standards and control facilitates a more accurate measure of swine serum Hpt concentration. According to the manufacturer's protocol, serum samples should be diluted 1:11 when adapted to the older model instrument. With slight adjustments to instrument parameters, an undiluted serum sample may be used, which alleviates an added step of diluting samples. However, a disadvantage to using an undiluted serum sample is loss of linearity at serum Hpt values >50 mg/dl. This is most likely a prozone effect on the assay, and any serum sample identified with a value >50 mg/dl when analyzed on the older model should be diluted into a working range.

This human Hpt immunoturbidimetric assay is valid for the measurement of serum Hpt in swine and may be used routinely in a swine production unit. However, future clinical studies will be necessary to assess the economic impact such assays may have on swine production.

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Sources and manufacturers

a. Incstar Corp., Stillwater, MN.
b. Ciba Corning Diagnostic Corp., Palo Alto, CA.
c. Millipore Corp., Bedford, MA.
d. Kirkgaard & Perry Laboratories, Gaithersburg, MD.
e. Boehringer-Mannheim Corp., Indianapolis, IN.

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Avian sexing: an optimized protocol using polymerase chain reaction–single-strand conformation polymorphism

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Determination of sex is of considerable importance for the understanding of numerous features of behavior, evolutionary ecology, genetics, and evolution[2] and in disease susceptibility.[15] In many cases, sex is difficult or impossible to assess on the basis of phenotype. In birds, where females are heterogametic, the sex determination at the DNA level exploits the use of molecular W-linked markers. A methodology to identify molecular genetic markers for determination of sex using randomly amplified DNA fragments has been described.[8] This technology was followed by the isolation of a highly conserved gene, the chromodomain–helicase–DNA-binding gene (CHD), which exists in 2 copies in the avian genome. One of the genes (CHD-NW), first thought to be autosomal, is probably located on the Z chromosome,[5,7] and the other is W-linked.[9] The amplification of the CHD gene using a pair of polymerase chain reaction (PCR) primers developed previously[9] and digestion with a restriction enzyme, which has to be modified depending on the species studied, is however not always satisfactory.[10] An alternative strategy is to design PCR primer pairs amplifying fragments of appreciably different size from the CHD-W and CHD-NW genes, circumventing the need for restriction digestion

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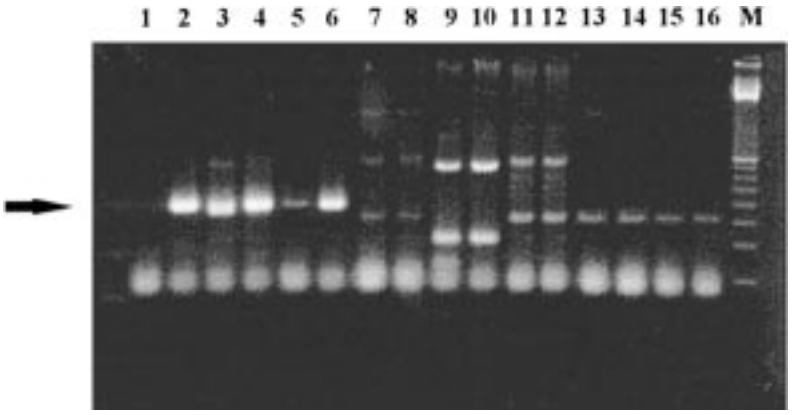

Figure 1. Sex determination in different avian species (from 5 orders) using primers USP1 and USP3 in 2% agarose gels. These primers are unable to discriminate between sexes in the following species (for each species male and female are shown alternatively): partridge (1, 2), stork (3, 4), imperial eagle (5, 6), peregrine falcon (7, 8), eagle owl (9, 10), kestrel (11, 12), lovebird (13, 14), and budgerigar (15, 16). M = molecular marker (100 bp ladder). The arrow points to the 380-bp band.

and restriction fragment length polymorphism analysis.[4] This approach cannot be used to amplify the gene in some birds other than galliforms, passeriforms,[4] goshawks, storks, and barn owls (with a lower annealing temperature, data not shown). Additional studies have described another PCR protocol using a highly conserved region of a 25-kb nonrepetitive sequence from the W chromosome of a chicken.[11] This technique makes use of degenerate primers for gender determination, but the different amplification profiles among species present some interpretation difficulties and the lack of positive control where the amplification of only 1 band is expected in females.

In this study a PCR single-strand conformation polymorphism (SSCP) approach was used to overcome the limitations encountered when using the CHD-W gene. The technique is applicable to different bird orders tested and is likely to be succesful in others with the probable exception of ratites (which do not have different sexual chromosomes[13] and have a primitive temperature-dependant type of sex determination[3]). The SSCP technique is used to detect small mutations (as few as 1 or 2 nucleotides) in short fragments of amplified DNA and is based on the observation that single-stranded DNA forms take on a specific secondary structure, depending on the nucleotide sequence, and thus migrate at different rates.[12] Because both copies situated in chromosome Z and W differ in a few nucleotides (5 in the collared flycatcher[6]), it should be possible to optimize conditions enabling such minor differences to be noted.

For this study, blood samples or feathers were collected to isolate DNA from imperial eagles (*Aquila heliaca, n* = 10), kestrels (*Falco tinnunculus, n* = 6), Gerifalte falcons (*Falco rusticolus, n* = 2), peregrine falcons (*Falco peregrinus, n* = 10), goshawks (*Accipiter gentilis, n* = 6), eagle owls (*Bubo bubo, n* = 6), barn owls (*Tito alba, n* = 2), storks (*Ciconia ciconia, n* = 2), quails (*Coturnix coturnix, n* = 8), red partridges (*Alectoris rufa, n* = 6), blue-fronted Amazons (*Amazona aestiva, n* = 2), lovebirds (*Agapomis roseicolli, n* = 7), and budgerigars (*Melopsittacus undulatus, n* = 2). Five taxonomically distant orders of the class Aves (Falconiformes, Estrigiformes, Ciconiformes, Galliformes, and Psitaciformes) are represented. Sex for all specimens had previously been determined using phenotypic and behavioral criteria.

Previously described primer pairs were used in an attempt to elucidate the most adequate PCR approach for determining sex: P2 (5′-TCTGCATCGCTAAATCCTTT-3′) and P3 (5′-AGATATTCCGGATCTGATAGTGA-3′),[9] and USP1 (5′-CTATGCCTACCAC(A/C)TTCCTATTTGC-3′)and USP3 (5′-AGCTGGA(T/C)TTCAG(A/T)(C/G)CATCTTCT-3′).[11]

When using degenerate primers USP1 and USP3, only the expected 380-bp fragment was observed in both sexes of the goshawk, stork, and imperial eagle and in female galliform (quail, partridge). These primers yielded bands of different sizes in agarose gels for the eagle owl and kestrel and produced very weakly staining amplification products in falcons and in all psitaciforms, even after decreasing the annealing temperature (Fig. 1). In imperial eagles, lower intensity in the male band (lane 5) is diagnostic. Therefore, sex determination with primers USP1 and USP3 is only applicable for those species where sex-specific amplification is achieved (galliforms and some falconiforms).

Primers P2 and P3 yielded a 110-bp fragment (part of an intron of the CHD gene[7]) in all samples analyzed using a reduced annealing temperature of 45 C and 20 pmol of each primer in 25-μl volumes. When this 110-bp fragment was subjected to SSCP analysis following conditions previously proposed,[4] satisfactory results were obtained only for galliforms (data not shown). However, optimal results were obtained when samples were electrophoresed in a 14% nondenaturing polyacrylamide gel[a] in 0.6× Tris–borate–ethylenediametetraacetic acid at 14 C constant temperature (Fig. 2). Bands were visualized by silver staining.[1] To assess the efficiency of the technique, a blind trial was performed by different persons, sexing a total of 54 individuals belonging to different species. No discrepancies were noted, thus confirming the validity of the technique.

Up to now, the determination of sex using direct visualization of the amplified product from a unique primer pair has been impossible to universalize, needing an optimization

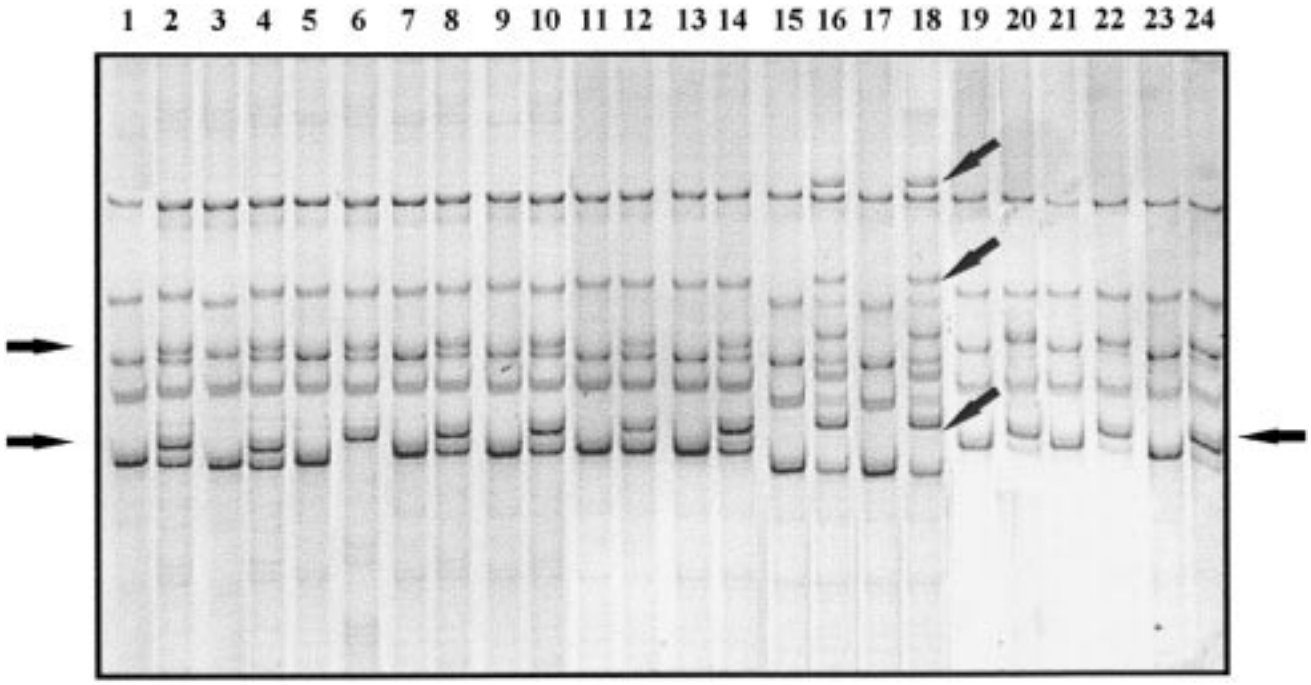

Figure 2. PCR-SSCP profiles of the 110-bp fragment of CHD amplified with primers P2 and P3. Lanes show alternatively male and female samples of imperial eagle (1, 2), kestrel (3, 4), goshawk (5, 6), eagle owl (7, 8), Gerifalte falcon (9, 10), peregrine falcon (11, 12), stork (13, 14), partridge (15, 16), quail (17, 18), blue fronted Amazon (19, 20), budgerigar (21, 22), and lovebird (23, 24). Arrows indicate female-specific bands.

for nearly each species involved. However, the highly conserved CHD-W gene was used successfully as a universal tag for avian sexing that is easily detectable with a unique, simple, and quick SSCP protocol. Reliable and fast determination of the sex of a bird will facilitate the full understanding of the biology[2] and ecology and life history[14] of a species. This test will be an important tool in the design of breeding programs for industrial and conservation purposes and will help aviculturists, avian veterinarians, poultry scientists, ecologists, and diagnosticians to understand sex differences in developmental rates and susceptibility to diseases.[15]

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Sources and manufacturers

a. Penguin™ Dual-Gel Water-Cooled Electrophoresis System, OWL Scientific, MA.

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