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Label free molecular sexing of monomorphic birds using infrared spectroscopic imaging

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ABSTRACT

The absence of sexual dimorphism in many birds often makes sex determination difficult. In particular immature birds and adults of monomorphic species show no external sex characteristics. Molecular techniques based on DNA hybridization or polymerase chain reaction (PCR) are standard methods for sex identification. However, these methods are expensive and time consuming procedures and require special sample preparation. Noninvasive methods for a rapid determination of bird's gender are of increasing importance for ornithologists, breeders as well as for successful captive-breeding programs. Fourier transform infrared (FT-IR) spectroscopy is one such technique that can provide gender specific information. In this study, using the example of domestic pigeons (*Columba livia* f. dom.) we demonstrate that only a small amount of the feather pulp is needed to determine the gender. FT-IR spectroscopic images of feather pulp suspensions were recorded in transmission mode. Principal component analysis (PCA) and linear discriminant analysis (LDA) were performed to identify the sex. The gender related information are described by 2nd and 4th principal component principle component (PC). The 2nd PC represents different amounts of proteins while the 4th PC shows variations within the amide I and amide II bands as well as in the region of phosphate vibrations of nucleic acids. Blood cells of male pigeons exhibit a significantly higher amount of proteins and nucleic acids than those of female pigeons. Feather pulp samples of male species were assigned with 100% accuracy. Seven from eight female samples were assigned correctly while one sample could not be classified. This study demonstrates that the sex of domestic pigeons can be accurately and and rapidly identified by infrared spectroscopic imaging.

1. Introduction

A large number of avian species show no sexual dimorphism. The lack of sex-specific external characteristics in nestlings, juveniles and immatures or even adult birds makes it difficult or impossible to perform gender determination based on phenotypic features. Sex determination in birds is of particular importance for breeders and ornithologist when considering production, exploitation, population ecology studies or even conservation programs [1,2]. The standard molecular-based method for bird sex determination is polymerase chain reaction (PCR) [3]. The most universal tag for PCR sexing is the chromo-helicase-DNA binding (CHD) gene which is located on the Z and W chromosomes [4]. The most universal tag for PCR sexing is the CHD gene which is located on the Z and W chromosomes [4]. Although sexing by PCR provides a highly sensitive identification, the method has some limitations due to expensive and time consuming processing steps. Recently, vibrational spectroscopy was used to determine the gender of chicken and turkey poults by analyzing feather pulp samples [5,6]. The spectroscopic-based sex determination is based on difference in the biochemical composition of male and female cells as well as on the different size of male and female genomes. Male birds are homogametic with two Z sex chromosomes whereas female birds have one Z- and one W sex chromosome [7,8]. Z- and W-chromosomes have a species-specific different number of base pairs. These slight variations can be observed in Raman- and infrared spectra. Molecular markers from feather pulp

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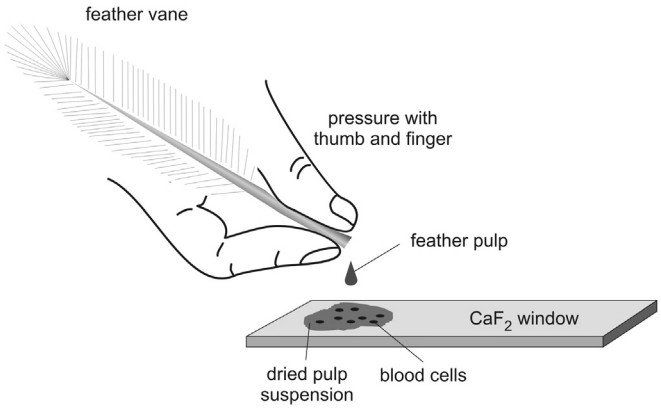

Fig. 1. Illustration of the procedure for extraction of a very small amount of feather pulp suspension from the feather and transferring onto an IR-transparent CaF_2 window.

minimizes the stress during sampling compared to other invasive techniques (e.g. laparoscopy), and provides a rapid and safe procedure for sex determination. Only proliferating cells of the feather pulp or nucleated blood cells from blood vessels inside the feather pulp provide the molecular information on the sex. Therefore spectroscopic-based sexing is difficult if the concentration of cells is low or the total difference of the number of base pairs between the male and female genome is less than 1%.

Hyperspectral imaging is a powerful tool for examining the spatial distribution of biochemical species [9]. In particular Fourier transform infrared (FT-IR) spectroscopic imaging has attracted considerable interest in the characterization and differentiation of cells and tissue [10]. For example, the spatial resolution allows the detection of single cells from a germinal disk that carry the spectral signature of the gender [11].

Herein, we present a novel application of FT-IR spectroscopic imaging to determine the sex of pigeon species. Domestic pigeons (see Fig. S1 in the Supplementary material section) were used as example for a large group of birds that show no sexual dimorphism and that exhibits only small differences in size between males and females.

With the exception of so-called 'autosexing breeds', the absence of conspicuous sexual dimorphism often makes it difficult to determine the gender of domestic pigeons on the basis of external characteristics [12]. The approach that we report in this paper continues the efforts to use FT-IR spectroscopy as a new tool for sexing birds, now for birds that exhibit only small differences in size of the genomes between male and female species.

2. Experimental

2.1. Sample preparation

Growing contour feathers of pigeons of known sex were plucked. Feather pulp suspensions were extracted as illustrated in Fig. 1 from the feather shaft by exerting a slight pressure with the thumb and the finger. A small amount of few microliters of the suspension was transferred onto an infrared transparent CaF_2 window and air dried. The pulp suspension contains also blood cells from capillaries vascularizing the pulp of the growing feather ('blood feather'). Samples from eight female and nine male pigeons were collected and prepared. Prepared suspension samples were dried overnight at room temperature. The sex of the pigeons was determined previously by courtship displays, egg-laying or position during copulations. PCR analysis of the gender was performed for one animal which did not show the typical gender specific behavior. The PCR was performed according to a pigeon-specific protocol as described by Wu et al. [13].

2.2. FT-IR spectroscopic imaging

FT-IR spectroscopic images were collected in transmission mode using a FT-IR spectrometer Vertex 70 coupled with an infrared microscope Hyperion 3000 (both from Bruker Optik GmbH,

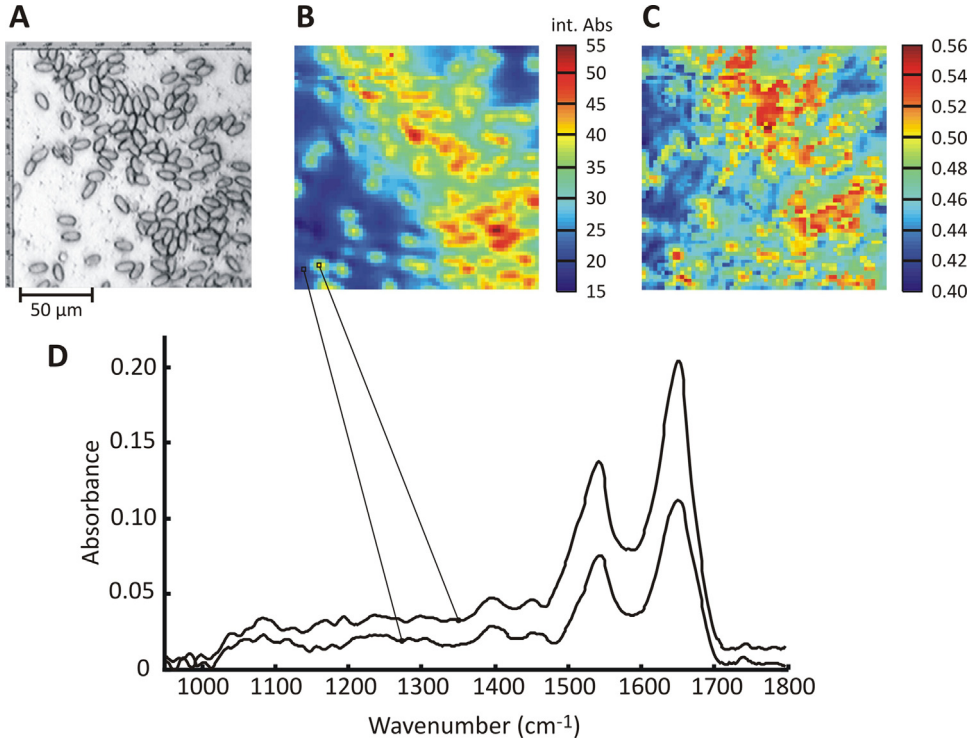

Fig. 2. Selection of spectra for classification. (A) microscopic image, (B) infrared spectroscopic bright field, (C) ratio intensity amide I band to the integrated intensity, (D) example for spectra from cells and pulp suspension.