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ABSTRACT

Genetic techniques for sexing birds are potentially valuable tools for refining methods of sexing birds based on plumage. Here we use a female-specific microsatellite locus isolated from Yellow Warblers *(Dendroica petechia)* to evaluate a technique of sexing hatch-year (HY; < 2 mo of age) Yellow Warblers based on both overall brightness of the yellow of body plumage and the ratio of yellow to dull brown in the outer rectrix. All bright plumaged HY birds (n=21) were males and all dull plumaged HY birds (n=24) were females. All birds of intermediate bright plumage were male (n= 11) and all but three (of 14) intermediate pale birds were female. Canonical discriminant analysis based on measured proportions of yellow and dull brown in the outer rectrix correctly classified 85.7% of both females and males; 91.7% of females and 95.3% of males could be correctly classified with these criteria if intermediate plumages were excluded from the analysis. Using Munsell color codes improved the ability to differentiate sexes; Canonical discriminant analysis was able to correctly classify 100% of females and 92.9% of males using these criteria. Similar results were obtained when the discriminant analyses were performed on a jackknifed sample. Scores from 10 observers asked to sex birds based on rectrices improved from 76.3 to 87.3% by using the relative brightness of yellow in the rectrix and the presence of yellow along the narrow edge of this feather. Thus, the use of this sex-specific DNA marker has revealed a plumage characteristic which can be used to sex a significant component of HY birds of this species.

INTRODUCTION

Identification of sex is essential for a wide range of behavioral, ecological, and evolutionary studies of birds (e.g., Gowaty 1991). Unfortunately, use of field observations alone for sexing birds make it a daunting task to test validity of putative sex-specific morphology, and so the number of sex-specific traits identified thus far is undoubtedly less than what exists. This is particularly true of juvenile or hatching-year (HY) birds that can rarely be sexed by plumage characters. The advent of genetical methods for verifying sex of individuals (Griffiths et al. 1992, 1996) are relatively non-invasive, and make possible more thorough evaluations of morphological traits as candidates for distinguishing sexes. Here we use a polymerase chain reaction (PCR)-based protocol which detects a female-specific microsatellite locus in the Yellow Warbler and other related passerine species. We show the usefulness of this marker for verifying sex related plumages in HY Yellow Warblers and show how the sex-specific traits we found improve sexing accuracy over those provided by Pyle et al. (1987) and Pyle (1997).

METHODS

Sex-specific marker in Yellow Warblers: As part of a larger study on genetic structure in Yellow Warblers in North America, a female-specific microsatellite locus (Dpu 11) was isolated from a Yellow Warbler library enriched for microsatellite repeats (Dawson et al. 1997). Based on the clone from which it was isolated, this locus is an imperfect repeat of the structure (CT)5CCC(TC)
5. Primers for this locus:
Forward: 5'-CCTCA CCTATTTCCAATTTC-3'
Reverse: 5'-AATTTCTTT AAAATGCCTCC-3'

amplify a 137 base pair (bp) band in females only, as confirmed by a survey of 20 male and 20 female adult birds. This band is monomorphic in size and is shared by breeding female Yellow Warblers from Alaska to Newfoundland (unpublished data). PCR amplifications of this locus used procedures described in Dawson et al. (1997) including an annealing temperature of 50° C. To control for non-amplification and the potential false-negative diagnosis as male, primers amplifying a 750 bp fragment from the central and right domains of the mitochondrial control region of this species (described in Gibbs et al. 1997) were also included. Reaction products were run on a 2% agarose gel and visualized using ethidium bromide. Success of a reaction was gauged by the presence of the control region fragment. We identified the sex of an individual by scoring for the presence (female) or absence (male) of the Dpu11 product.

Plumage analysis of HY birds: During July 1996 and 1998, HY Yellow Warblers were captured during constant-effort mist-net operations at Delta Marsh Bird Observatory, located on the forested dune ridge separating Delta Marsh from Lake Manitoba (see Sealy 1980). Birds were recognized as HY using the degree of skull ossification (e.g., Pyle et al. 1987) and general plumage characters, and classed as "bright," "intermediate bright," "intermediate pale," or "pale" based on relative brightness of their yellow body plumage. From a random subsample of 21 bright, 11 intermediate bright, 14 intermediate pale, and 24 pale HY Yellow Warblers, we obtained blood for DNA analysis using brachial vein puncture, and we plucked a single outer rectrix for plumage analysis from each bird before release.

The amount of yellow and dull brown coloration in the outer rectrix (see below) was estimated by taping feathers to a digitizing tablet and covering them with a clear acetate cover. We quantified areas of each color (in cm^2) by tracing outlines using a planimeter interfaced with the software program Easydij (Geocomp Ltd., Golden, CO). The proportion of yellow in a feather was defined as the ratio of yellow area to total feather area. Feathers were also assigned color codes based on Pantone (Pantone Color Matching System. Pantone, Inc. Carlstadt, NJ) and Munsell (Anonymous 1976) color charts to define our perception of bright-to-pale categories for feathers. The Pantone color chart was used because of its relatively low cost and wide range of choice in yellow tones; we also provide Munsell color codes due to their widespread use in biology.

After confirming the sex of birds using DNA techniques (see below), we asked 10 people to separate HY males from HY females based on plumage characteristics of rectrices. These people were students or employees of the Canadian Wildlife Service in Saskatoon and varied from individuals having no experience with ageing and sexing passerines to individuals with considerable experience. Feathers were numbered with a random code and observers were initially asked to use criteria outlined in Pyle et al. (1987) and Pyle (1997) to sex HY birds. Pyle et al.'s technique is based on the relative amount of yellow vs dull brown in the outer rectrix. Following the assignment of new random codes to feathers, we later asked the same individuals to repeat the test using additional criteria we supplied. Specifically, we noted that males were more likely to have yellow edging along the outer (i.e. narrow) side of the feather, and that the yellow in their feathers was generally brighter than that in females. All statistical test were performed using SPSS software.

RESULTS

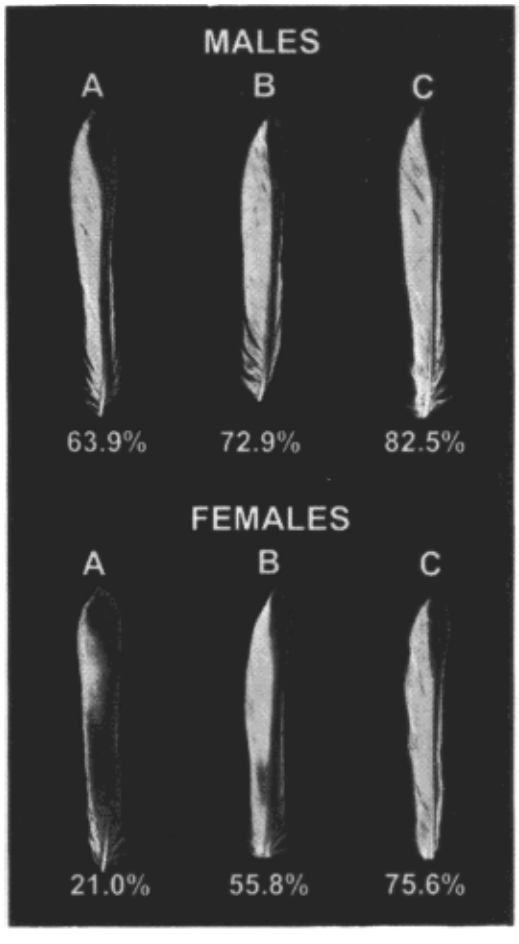
DNA analyses. Based on DNA analyses of blood from HY Yellow Warblers at Delta Marsh Bird Observatory sampled in 1996 and 1998, all 21 brightly plumaged birds were male and all 24 pale plumaged birds were female. All 11 birds of intermediate bright plumages were male, whereas the sample of 14 intermediate pale birds consisted of 3 males and 11 females.

Plumage analyses of HY birds: For all plumages, outer rectrices of male HY Yellow Warblers were larger than females (mean ± SD: male mean 2.1± 0.18cm^2 , range 1.8 - 2.5 cm^2; female mean 1.9 ± 0.13 cm^2, range 1.7 - 2.3 cm^2; two-tailed Student's *t*-test, $t = 6.30$, $P < 0.001$) with a greater proportion of yellow (male mean: 0.71 ± 0.07, range 0.48 - 0.82; female mean: 0.57 ± 0.12, range 0.21 - 0.76;

$t = 5.68$, $P < 0.0001$). Using the area of brown and area of yellow as variables, and defining groups by sex, canonical discriminant analysis correctly classified 85.7% of males and 85.7% of females based on this criteria ($P < 0.0001$). Using Cohen's Kappa test (see Titus et al. 1984), classification results were significantly better than expected by chance alone (Kappa = 0.71; Z= 5.98; $P< 0.0001$). Validation of the classification was done using a jackknifing procedure with the same data (see Phillips and Furness 1997). Eighty-six percent of both sexes were assigned to sex correctly in this validation process.

Considering bright and pale samples only, male HY Yellow Warblers had larger outer rectrices (male mean 2.2 ± 0.02 cm^2 , range 2.0 - 2.4 cm^2; female mean 1.9 ± 0.01 cm^2, range 1.7-2.2 cm^2; two-tailed Student's *t*-test, $t = 8.0$, $P< 0.001$) with a greater proportion of yellow (male mean 0.73 ± 0.002, range 0.64 - 0.83; female mean 0.55 ± 0.019, range 0.21 - 0.76; $t = 6.13$, $P < 0.0001$) than females (Fig. 1). Canonical discriminant analysis correctly classified 91.7% of females and 95.2% of males ($P< 0.0001$). This was significantly better than expected by chance alone (Cohen's Kappa, Kappa = 0.87; Z = 5.80; $P< 0.0001$). The results of jackknifing produced similar classifications, with 92.0% of females and 95.0% of males correctly assigned to sex. With the exception of one intermediate pale feather, all bright and intermediate bright birds had a hue of 7.5Y, while pale and intermediate pale birds had a hue of 5Y (Table 1). Using brightness (as quantified by Munsell color) as an independent factor, Canonical discriminant analysis was able to correctly classify 100% of females and 92.9% of males in a sub-sample of 51 birds ($P< 0.0001$). This was significantly better than expected by chance alone (Cohen's Kappa, Kappa = 0.93; Z = 6.88; $P < 0.0001$). Jackknifing yielded identical results.

Fig. 1. Outer rectrix of HY Yellow Warblers with sex confirmed by the DNA technique described in this paper. Examples of extreme and intermediate proportions of yellow vs brown in the feather as quantified using digital image analysis are shown for both males and females. Numbers refer to percent yellow.



Using criteria outlined by Pyle et al. (1987) and Pyle (1997), observers correctly classified 76.3 ± 12.4% of HY rectrices, with experienced observers tending to score higher (84.4 ± 10 7%, n = 3) than inexperienced observers (72.9 ± 12. 1 %, n = 7; Mann Whitney *U*-test, $U = 4.5$, $P= 0. 17$). Scores increased (to 87.3 ± 6.3%) and variance among observers decreased when they were informed of the two additional criteria of brightness of the yellow and the amount of yellow on the edge of the narrow side of the feather (one-tailed paired *t*-test, $t = 3.0$, $P = 0.007$). Experienced observers did not significantly increase their scores using these criteria (mean score 85.6 ± 7.0%, $n = 3$). However, inexperienced observers showed significantly greater improvement than experienced observers (to 88.1 ± 6.3%, $n = 7$; Mann-Whitney *U*-test, $U = 2.0$, $P = 0. 043$).

DISCUSSION

We used a sex-specific marker to demonstrate that plumage characteristics could be used to sex HY Yellow Warblers. First, we found that the overall brightness of the yellow body plumage of HY Yellow Warblers is a useful sexing criterion. This approach worked consistently well for bright- and pale-plumaged birds. In addition, birds of intermediate plumage coloration that nonetheless

Table 1. Pantone and Munsell color codes for HY Yellow Warblers sampled at Delta Marsh Bird Observatory, Manitoba, 1996-1997.

				Munsell Color		
Color	n	Sex	Pantone Color	Hue	Value	Chroma
Bright	2	M	108u	7.5Y	9	10
	7	M	109c	7.5Y	9	10
	5	M	116c	7.5Y	9	10
Intermediate Bright	3	M	108u	7.5Y	9	10
	8	M	108U	7.5Y	9	8
Intermediate Pale	1	F	129c	7.5Y	9	8
	1	M	107u	5Y	9	8
	1	F	114u	5Y	9	8
	1	F	115c	5Y	9	8
	4*	M,F	122c	5Y	9	8
	6	F	129c	5Y	9	6
Pale	2	F	122c	5Y	9	8
	1	F	128C	5Y	9	8
	1	F	115c	5Y	9	6
	1	F	121c	5Y	9	6
	2	F	127c	5Y	9	6
	4	F	128c	5Y	9	6
	1	F	128u	5Y	9	6
	2	F	129c	5Y	9	6

* 2 M, 2 F

could be classified to "bright" or "pale" were also sexed reasonably successfully using this method. Importantly, overall brightness was more reliable in determining sex than estimating or quantifying the proportion of yellow in the outer rectrix, since there was some overlap in the latter parameter between sexes. Secondly, accuracy of the rectrix approach for sexing HY Yellow Warblers can be improved over the criteria recommended in Pyle (1997) by also estimating brightness of the yellow in the feather and also judging the amount of yellow along the outer edge of the rectrix. Specifically, in the field, we suggest that HY Yellow Warblers with less than about 60% yellow in the outer rectrix be considered female and those with more than 75% be considered male. For intermediate cases, we recommend that those individuals with bright yellow feather coloration and with a yellow edging along the narrow portion of the feather be classed as males whereas those with dull yellow and no yellow edging be classed as female.

We encourage those individuals working with migrant HY Yellow Warblers to record plumage brightness according to the criteria we have provided, initially confirming their classifications with the color chart scores. In this way, it should be possible to record sex ratio information reliably at the population level and to investigate factors influencing this ratio through time. Although we have only conducted our work on fledged HY birds, we suspect that using the same plumage brightness criteria, it should be possible to sex nestling HY Yellow Warblers reliably.

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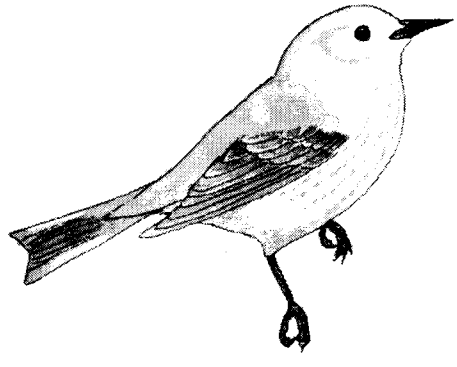

Yellow Warbler by George West