Morphological and Molecular Sexing of the Monochromatic Barbados Bullfinch, *Loxigilla barbadensis*

Author(s): Jean-Nicolas Audet, Simon Ducatez, and Louis Lefebvre


Published By: Zoological Society of Japan

DOI: [http://dx.doi.org/10.2108/zs140025](http://dx.doi.org/10.2108/zs140025)

The bullfinch *Loxigilla barbadensis* is an endemic passerine on the Caribbean island of Barbados that has only recently been taxonomically split from the Lesser Antillean bullfinch *L. noctis*. The trait that most clearly distinguishes *L. barbadensis* from *L. noctis* is the absence in the male of sexually dimorphic coloration of the body and throat feathers, with *L. barbadensis* males and females sharing the same dull brown plumage. Here we report, in 64 individuals netted throughout the island, the results of a discriminant analysis on two (wing length and tail length) to four morphological traits showing very high (97%) concordance with sexing via PCR using blood samples. Females also show a paler lower mandible, a trait that yields an 80% concordance with PCR sexing. We found one *L. barbadensis* male that had a *nocitis*-like reddish throat patch, supporting the idea that sexual dichromatism is the ancestral condition and that male Barbados bullfinches have evolved cryptic coloration that now makes the species monochromatic.

**Key words:** Barbados bullfinch, *Loxigilla barbadensis*, Lesser Antillean bullfinch, *Loxigilla noctis*, sexing, sex, sexual dimorphism

**INTRODUCTION**

The bullfinch *Loxigilla barbadensis* is an endemic passerine on the Caribbean island of Barbados. Until 2004, it was considered a subspecies of the Lesser Antillean bullfinch *L. noctis*, but it has since been elevated to species status (Buckley and Buckley, 2004; Gill and Donsker, 2014). The trait that most clearly distinguishes *L. barbadensis* from *L. noctis* is the absence in the male of sexually dimorphic coloration of the body (black) and throat (red) feathers, with *L. barbadensis* males and females sharing the same dull brown plumage. This makes sexing of Barbados bullfinches very difficult.

Several field and captive behavioral studies of this species have been conducted, including work on parasites (Fallon et al., 2003; Svensson-Coelho and Ricklefs, 2011) and various aspects of cognition and personality (Webster and Lefebvre, 2000, 2001; Reader et al., 2002; Ducatez et al., 2013). Research on other passerines shows that sex plays a key role in, among many other traits, learning and parasitism. For instance, in great tits, Dunn et al. (2011) showed a strong interaction between sex and the effects of malaria parasites on problem-solving, exploration, and risk-aversion, while Brust et al. (2013) found that male zebra finches are better at reversal learning than females. Sexing the monomorphic Barbados bullfinch is thus important for ongoing field work, but studies to date have overlooked this potential variable. The purpose of this study is to provide an easy tool for sexing Barbados bullfinches in the field.

Buckley and Buckley (2004) have suggested that male Barbados bullfinches display a “dusker” lower mandible than females, although birding guides (e.g., Evans, 1990; Bond, 1993; Raffaele et al., 2010) and articles (e.g., Bond, 1979) qualify the species as sexually monomorphic. In addition, males of passerine species otherwise classified as monomorphic often show slightly longer wings, longer tarsi, and/or a heavier body mass than females (Svensson, 1992). Measurement and subsequent discriminant analysis of these traits is a convenient way of morphologically distinguishing the sexes (Dechaume-Moncharmont et al., 2011). Here, we assess the validity of the beak shade criterion proposed by Buckley and Buckley (2004) and test for size dimorphism by comparing it to DNA sexing. In non-ratite birds, PCR product analysis of DNA taken from blood or feathers also yields distinct CHD gene patterns in males and females (Griffiths et al., 1996; Griffiths et al., 1998; Zagalska-Neubauer and Dubiec, 2006; Ong and Velianan, 2008).

We propose two new methods for sexing the Barbados bullfinch without the need of molecular techniques: i) first, we show that the lower mandible is darker in males, a feature that distinguishes the sexes with an 80% concordance with the PCR method, and ii) we show that a simple mathematical approach derived from discriminant analysis on morphometric traits distinguishes the sexes with a 97% concordance with the PCR method.

**METHODS**

**Fieldwork**

Sixty-four Barbados bullfinches were captured in mist nets between February and April 2013 at eight different sites throughout the island of Barbados. Barbados bullfinches occur in almost all areas of Barbados islands (JNA, SD, and LL, personal observations), from highly urbanized areas to mostly rural habitats. We took

* Corresponding author. Tel. : +1-514-398-6567; Fax : +1-514-398-5069; E-mail: Jean-nicolas.audet@mail.mcgill.ca

doi:10.2108/zs140025
advantage of a field study comparing birds from rural and urban populations to sample individuals in habitats with various urbanization rates (calculated using the percentage of anthropogenic structures in a 1 km² area around the capture point, Table 1). Beak shading (either pale or dark) was visually assessed by the same person (JNA) on all 64 birds at the moment of capture. Photographs were also taken at capture with a Nikon Coolpix P5100 camera for later re-assessment of shading judgments, with a gray scale image included in every picture. Post-hoc quantitative analysis of beak shade was performed by JNA, blind to the identity of the bird, measuring the optical density of the lower mandible divided by a standardized background of the gray scale, using the ImageJ v1.46r software (NIH, USA). Morphological measurements were taken at capture on all 64 birds by the same person (JNA); measurements were taken three times in succession on each bird and the mean value of the three measures was used in the analyses below. Individuals were weighed using a digital pocket scale (precision to 0.1 g). We measured tail length as the length of the longest straightened rectrix using a metal ruler (precision to 0.5 mm). Wing length was taken with a raised-end ruler as the length of the unflattened wing chord (precision to 0.5 mm). Calipers were used to measure the metatarsi, bill, and head (precision to 0.05 mm). Metatarsal length was measured from the intertarsal joint to the last scale before the toes. Bill length was measured from the tip to the anterior edge of the nostril. Head length was measured from the anterior edge of the nostril to the back of the head following the angle of the bill. Measurement errors were calculated with the method proposed by Bailey and Byme (1990): \( \text{ME}_{\text{wing length}} = 4.3\%, \text{ME}_{\text{tail length}} = 6.9\%, \text{ME}_{\text{tarsus length}} = 9.5\%, \text{ME}_{\text{peak length}} = 26.8\%, \text{ME}_{\text{head length}} = 13.0\% \), indicating an overall mean measurement repeatability of 87.9%. Birds were released at their initial site of capture. All manipulations were conducted according to Animal Use Protocol 2013-7140, approved by the McGill University Animal Care Committee and permitted by the CTCC (Animal Care Committee) at the McGill University, the Natural Heritage Department of the Barbados Ministry of Environment and Drainage.

**PCRs sexotyping**

After morphological measurements were taken, approximately 50 μL of blood was sampled by puncturing the brachial vein. Blood was kept at −20°C and shipped to the Jarvis laboratory at the Department of Neurobiology, Duke University Medical Center, Durham NC. DNA was extracted from the 50 μL of blood using a DNeasy tissue and blood extraction kit (Qiagen, USA). DNA quality and quantity was assessed on a Nanodrop (Thermo Scientific, USA). 50 ng of DNA was used for PCR. P8 (5′-CTCCCAAGGAT-GAGRAAYTG-3′) and P2 (5′-TCTGATCAGTAAATTTTT-3′) primers were used, following Griffiths et al. (1998). The PCR program was run as follows: 94°C for 1 min 30 s, 30 cycles of 48°C for 45 s, 72°C for 45 s and 94°C for 30 s, and then 48°C for 1 min and 72°C for 5 min (Griffiths et al., 1998). The PCR products were digested with HAEIII enzyme and then ran on a 1% agarose gel, as per Griffiths et al. (1996). Although P2 and P8 primers produce bands of slightly different molecular weights, we found that digesting with HAEIII facilitates differentiation and clearness of results (see Fig. 2). Female samples display two bands (300 and 400 bp) whereas the male samples show only one band (300 bp). All molecular procedures were done by the same experimenter (JNA).

**Statistical analyses**

The correspondence between PCR sexing and our judgments on beak shade was tested using Fisher’s exact test (Graphpad QuickCalcs, Graphpad software inc.). The correspondence between judgments on beak shade at capture and post-hoc optical density analyses was also assessed with a Fisher’s exact test. For morphological measurements, we first calculated mean differences between males and females identified by the PCR and tested them for significance with t-tests (Graphpad Prism 5, Graphpad software inc.). When data were not normally distributed, a Mann-Whitney test was used instead and when variances differed significantly, Welch’s correction was applied. To validate our morphological model of sex differences, we built a discriminant analysis with traits that were significantly different between males and females (JPM 10.0, SAS Institute). To establish the model, we used a sample of 34 individuals randomly drawn from our 64 bird database and then applied the model to the other 30 birds to see how well it predicted PCR-determined sex.

**RESULTS**

Of the 64 individuals we caught, all but one showed the brown feather coloration that is typical of both male and female Barbados bullfinches (Fig. 1A). This is very different from coloration shown by the sister species of *L. barbadensis*, the Lesser Antillean bullfinch, in which the male is completely black with a red throat patch (Fig. 1C). However, one of the individuals we caught in Barbados did exhibit a reddish throat patch (Fig. 1B). PCR sexing identified 36 of our 64 birds as males (including the bird with the reddish patch) and 28 as females. Our judgments on the shade of the lower mandible (Fig. 1D) yielded a high correspondence (80%) with the results of the PCR sexing: 51 of the 64 birds (Fisher’s exact test \( P < 0.0001, 32 \) males and 19 females) had shadings that fit with the PCR result (Fig. 2). Post-hoc optic density (OD) analysis of the mandible coloration yielded similar results (74% of birds correctly sexed by OD, Fisher’s exact test \( P = 0.0004 \) with significant correspondence between both methods (Fisher’s exact test \( P = 0.0023 \)).

Morphology varied between the sexes on four of the six traits we quantified. Means were significantly different between PCR-sexed females and males for body weight (\( \bar{X}_F = 16.3, \bar{X}_M = 17.3, P = 0.0006 \)), wing length (\( \bar{X}_F = 66.4, \bar{X}_M = 70.6, P < 0.0001 \)), tail length (\( \bar{X}_F = 48.3, \bar{X}_M = 51.5, P < 0.0001 \)) and beak length (\( \bar{X}_F = 11.6, \bar{X}_M = 12.2, P = 0.0003 \)) (Table 2). Tarsus length and head length were not found to significantly vary between sexes.

The discriminant analysis model we built using the four significant morphometric differences was highly concordant with the PCR sexing. Quadratic discriminant analysis correctly predicted PCR sex 94% of the time (32 of 34 birds, \( -2 \log \text{likelihood} = 10.9, F = 11.13, P < 0.0001 \)) on the model sample. When we applied this model to the other half of our database, PCR sex was correctly predicted in 29 out of 30
Sexing of *Loxigilla barbadensis* birds (97%, \(-2\) log likelihood = 8.89, \(F = 8.92, P < 0.0001\)).

Based on the discriminant analysis model, we derived a simplified formula that can be used to estimate sex using wing (W) and tail (T) length measurements: if \((W^*0.318 + T^*0.796) \geq 61.9\), the bird is a male; if <, it is a female. It is as precise as the four-trait model yielded by the discriminant analysis, also predicting 29 of the 30 birds in the sub-sample not used to derive the model.

**DISCUSSION**

Our results yield a fast, non-quantitative criterion for sexing the ‘monomorphic’ Barbados bullfinch in the field, with females showing a paler lower mandible than males. This result supports the suggestion made by Buckley and Buckley (2004) concerning the ‘duskier’ lower mandible of males. While this method is moderately accurate, it can be valuable for researchers wanting, for example, to perform a pre-selection of birds of a particular sex for a field study or manipulation, when no other methods are available. We also show that quantitative measures of two morphological traits, wing length and tail length, are sufficient to distinguish 97% of the males and females identified by the PCR analysis. Conveniently, these two traits are the easiest to measure accurately (our measurement repeatability scores were the highest for these two measurements). When field or captivity conditions preclude DNA sexing, our study thus offers a fast and easy solution to the identification of males and females in this species.

It has recently been argued that the use of morphometric traits to sex passerine birds is inaccurate when extrapolated to larger geographic scales (Ellrich et al., 2010). Here, we sampled sites that vary sharply in urbanization rates, from less than 2% to more than 55%. A large part of Barbados is urbanized or used for agriculture, while limited forested areas remain in central and northern parts of the island. Contrary to previous studies on *L. barbadensis* parasitism and learning, which were all done on birds caught in a limited urbanized coastal area in Saint James Parish, our eight sites were designed to cover both urban and rural habitats (Table 1). Our 64 individuals thus likely represent a rather wide sample for an endemic species restricted to

---

**Fig. 1.** Sexual dimorphism in Barbados bullfinches and Lesser-Antillean bullfinches. (A) Male Barbados bullfinch showing no obvious sexual dimorphism. (B) Male Barbados bullfinch showing a reddish throat patch. (C) Lesser Antillean Bullfinch (*Loxigilla noctis sclateri*) displaying strong sexual dimorphism. (D) Lower mandible shade of a male versus a female Barbados bullfinch.

**Table 2.** Morphometric sex differences in Barbados bullfinch. Means and SEM for morphological measurements of PCR-sexed males and females. \(P\)-values were computed using unpaired bilateral Student’s t-tests. A Welch’s correction was applied for head length to correct for different variances and a non-parametric t-test was used for wing length to account for the non-Gaussian distribution.

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SEM</th>
<th>95% conf. Interval</th>
<th>Cohen’s (d)</th>
<th>t-test</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>16.3</td>
<td>14.0</td>
<td>18.7</td>
<td>0.25</td>
<td>15.75–16.76</td>
<td>0.7</td>
<td>0.0055</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>17.2</td>
<td>14.8</td>
<td>22.2</td>
<td>0.22</td>
<td>16.81–17.68</td>
<td>1.7</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Wing length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>66.4</td>
<td>63.5</td>
<td>69.5</td>
<td>0.32</td>
<td>65.76–67.07</td>
<td>2.2</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>70.6</td>
<td>63.0</td>
<td>81.0</td>
<td>0.56</td>
<td>69.42–71.69</td>
<td>1.0</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Tail length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>48.3</td>
<td>43.5</td>
<td>51.0</td>
<td>0.30</td>
<td>47.68–48.93</td>
<td>0.7</td>
<td>0.0055</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>51.5</td>
<td>48.5</td>
<td>55.0</td>
<td>0.23</td>
<td>51.05–51.97</td>
<td>1.7</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Tarsus length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>23.0</td>
<td>21.1</td>
<td>24.2</td>
<td>0.12</td>
<td>22.73–23.22</td>
<td>0.3</td>
<td>0.1866</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>23.2</td>
<td>21.5</td>
<td>24.4</td>
<td>0.12</td>
<td>22.97–23.44</td>
<td>1.0</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Beak length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>11.6</td>
<td>10.6</td>
<td>12.8</td>
<td>0.10</td>
<td>11.43–11.82</td>
<td>0.5</td>
<td>0.0962</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>12.2</td>
<td>11.0</td>
<td>13.5</td>
<td>0.11</td>
<td>11.99–12.44</td>
<td>0.0</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Head length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>17.6</td>
<td>16.7</td>
<td>19.0</td>
<td>0.10</td>
<td>17.40–17.80</td>
<td>0.0</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>17.9</td>
<td>16.3</td>
<td>19.3</td>
<td>0.13</td>
<td>17.61–18.14</td>
<td>0.5</td>
<td>0.0962</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Sex-typing PCR. Representative samples analyzed for sex determination. After agarose gel migration of the PCR products, there is one band (~400 bp) for males and two bands (~400 and ~500 bp) for females.
Barbados island.

Phylogenetic studies of *Loxigilla noctis* and *L. barbadensis* in the Lesser Antilles (Lovette et al., 1999) suggest that the Barbados bullfinch is a relatively recent immigrant to that island and that a single invasion event is behind the low within-population nucleotide divergence of the species there. The most parsimonious scenario for the lack of color dimorphism in the Barbados bullfinch is thus that the trait has been lost with respect to its common ancestor with *L. noctis*. Our anecdotal finding that a version of the *L. noctis* red throat patch can still be found, albeit in only one of 36 PCR sexed males, on a background of brown body coloration, supports the idea of trait loss in *L. barbadensis*. Although we cannot exclude the possibility that this bird could be a migrant from other islands, it is unlikely since the overall plumage coloration is much closer to *barbadensis* than *noctis* and, to our knowledge, no brown male has been reported in other Caribbean islands. The genetic basis of this loss would be interesting to explore, as would be the effects of female preference on Barbados males painted with the *L. noctis* colors or the reddish throat patch found in our single *L. barbadensis*.

The reason for the male shift to female coloration in Barbados is unknown. Intriguingly, Carib grackles (*Quiscalus lugubris*) have also evolved a monomorphic plumage coloration in Barbados, but in the opposite direction to bullfinches: it is female grackles that have changed their coloration, from the brown seen on other islands of the Lesser Antilles, as well as northern South America, to the black plumage characteristic of all *Q. lugubris* males. Overington (2011) has suggested that the low levels of predation in Barbados may have favored this shift towards monochromatism. Predation rate is known to affect bird coloration, with a higher predation rate being correlated with plumage dullness (Martin and Badyaev, 1996). The Barbados population of Carib grackles is thought to have originated in northern South America (Lovette et al., 1999) and predation there is much higher than it is in Barbados. Venezuela, for instance, has several native Carnivora and 65 species of diurnal raptors (Naveda-Rodriguez, 2013), whereas there are only rareigrant predators in Barbados (e.g., Peregrine falcon, *Falco peregrinus*). Contrary to grackles, however, Barbados bullfinches are thought to have originated from the small neighboring island of Saint-Lucia (Lovette et al., 1999), where predators are also very scarce. Therefore, predation rate is unlikely to have affected sexual dichromatism in Barbados bullfinches.

In birds, species tend to be monochromatic when both sexes participate in parental duties, including nest building (Verner and Willson, 1969; Kear, 1970; Soler et al., 1998). Bird (1983) has shown that male Barbados bullfinches, compared to male Lesser Antillean bullfinches, contribute more to nest building, stay longer in the vicinity of their nest after construction and throughout brood rearing, feed female more often and are more aggressive around their nest (Bird, 1983; Buckley and Buckley, 2004). Breeding system might thus be an important factor in the loss of male dimorphism in this species.

Whatever the reason for the loss of dichromatism, our study validates two rapid methods for the sexing of *L. barbadensis* that can be used in the absence of invasive techniques, and raises fundamental questions with regards to the evolution of sexual dimorphism.

**ACKNOWLEDGMENTS**

We thank Erich D. Jarvis for the logistical support he provided for molecular sexing, Jason T. Howard for technical help and two anonymous reviewers for helpful comments on an earlier version of the manuscript. This work was supported by a FORNT doctoral scholarship to JNA, a post-doctoral fellowship from the Fondation Fyssen to SD and a NSERC Discovery grant to LL.

**REFERENCES**


Naveda-Rodriguez A (2013) Biogeography and conservation status
of diurnal raptors in Venezuela. [MSc. Thesis]. Universidad Internacional Menendez Pelayo, Santander, Spain


(Received February 4, 2014 / Accepted June 16, 2014)