

Sexing of the endangered Floreana mockingbird (*Mimus trifasciatus*) using morphometric measurements

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Abstract: Male and female adult Floreana mockingbird (*Mimus trifasciatus*) have monomorphic plumage features that make them impossible to sex in the field. In this study, we use discriminant function analysis (DFA), a widely used technique, to assess the best measures to determine sex. We measured six morphological characteristics (mass, beak depth, beak width, tarsus length, wing length, and head-beak length) for birds of known sex (determined by molecular techniques) from the two extant populations of *M. trifasciatus* on Champion and Gardner islets, within the Galápagos archipelago. Using a coefficient of sexual dimorphism, we found that males are significantly larger than females in three of the variables. Discriminant functions using wing length and a combination of wing length + mass, and wing length + tarsus length could classify birds with a 98% level of accuracy. Furthermore, we were able to estimate a robust cut-off point to determine the sex of individuals in the field through a decision tree, using only wing length as morphological variable. Fast and accurate sexing of the bird based on one variable will reduce handling times and minimise stress for captured birds.

Reyes, E.M.R.; Smith, A.N.H.; Rueda, D.; Sevilla, C.; Brunton, D.H.; Ortiz-Catedral, L. 2022. Sexing of the endangered Floreana mockingbird (*Mimus trifasciatus*) using morphometric measurements. *Notornis* 69(4): 256–263.

Key words: sex identification, morphometry, discriminant function analysis, Floreana mockingbird, management, Galápagos Islands.

INTRODUCTION

Identifying the sex of individuals is important in ecological research and conservation biology because knowing the sex of birds can tell us about possible biases in population sex ratios and improve our knowledge of the population dynamics of endangered species. For example, in many populations, one sex may have higher mortality rates than the other causing a bias in the operational sex ratio of a population and potentially lowering population growth rates (Brekke *et al.* 2010). An efficient method for sexing monomorphic species can also improve protocols and methodologies for translocations and captive breeding programmes. In addition, the correct interpretation of behavioural and ecological data often relies on knowing the sex of the study individuals. Many avian species can be sexed using visually distinct phenotypes such as size and colour dimorphism; features usually correlated with social mating systems (Owens & Hartley 1998; Dunn *et al.* 2001) or by observing sex-specific behaviours (Lewis *et al.* 2002; Joo *et al.* 2018). However, for monomorphic species or juvenile birds, differences are less obvious and considerable overlap in male and female characteristics can cause uncertainty. When sex-specific behaviours are used for assigning sex, observers may need long periods of observations that are time-consuming and logistically expensive, especially in areas of difficult access.

For sexing monomorphic species, with an absence of sexually dimorphic external factors, a variety of techniques have been proposed. These techniques include both invasive and non-invasive methods such as cloacal inspection, molecular analysis, vocalisations, statistical methods based on morphometrical measurements, and combinations of these (Lessells & Mateman 1998; Bourgeois *et al.* 2007; Volodin *et al.* 2009; Ellrich, *et al.* 2010; Bazzano *et al.* 2012; Morinha *et al.* 2012). Of these methods, one of the most reliable in sexing birds has been the use of discriminant function analysis (DFA) for morphometric measurements. This technique has been used widely in different birds taxa from Procellariiforms (Mischler *et al.* 2015) to passerines including species of Mimidae (Martínez-Gómez & Curry 1998; Fuchs & Montalti 2016), even in juvenile birds (Martín *et al.* 2000; Thorogood *et al.* 2009). This method identifies individuals of known sex by creating a linear function of measurements that best discriminates between males and females (Phillips & Furness 1997) and the coefficient outputs can be used to generate an equation to classify the sex of further sampled individuals (Queen *et al.* 2002). However, despite the wide use of the method, there are some caveats in the robustness of discriminant equations when using

small sample sizes (Dechaume-Moncharmont *et al.* 2011). Moreover, although easy to understand by experts, the equations may be problematic for use by those involved in citizen science or community conservation without an academic background.

The Floreana mockingbird (*Mimus trifasciatus*) is the rarest and most range-restricted mockingbird species in the Galápagos archipelago and is classified as “endangered” by the IUCN (Fig. 1). Historically, *M. trifasciatus* occurred in the lowlands of Floreana Island and its surrounding islets. *Mimus trifasciatus* disappeared in the early 1900s from Floreana Island due to a combination of factors, in particular, the effects of introduced species (Curry 1986; Hoeck *et al.* 2010). Currently, *M. trifasciatus* is restricted to two islets representing less than 1% of its former range. Because of its rarity (<350 individuals) and the inaccessibility of these islets, the ecology of the *M. trifasciatus* is poorly understood, which has prevented the development of management plans directed towards increasing the number of individuals and populations, in particular via reintroduction of birds to the lowlands of Floreana Island. Despite its relevant role in the history of biology (Hoeck *et al.* 2010) and its conservation importance (Ortiz-Catedral 2018), basic aspects of the natural history of this species are still missing. Although there are existing criteria using the wing length for sexing Mimids in the Galápagos, most of these criteria have been developed based in the morphometric characters of a different species, the Galápagos mockingbird (*Mimus parvulus*) (Kinnaird & Grant 1982; Curry 1988; 1989; Curry & Grant; 1989). To date, sex determination for *M. trifasciatus* has been



Figure 1. Adult Floreana mockingbird (*Mimus trifasciatus*) perched on an *Opuntia* cactus. Champion Islet. Photo: Enzo M. R. Reyes.

mentioned in Grant *et al.* (2000) and in Deem *et al.* (2011) but neither of these publications report specific criteria to determine sex of this species using morphometric measurements. Our goal was to determine whether morphological differences could be used to distinguish between the sexes of *M. trifasciatus* using discriminant function analysis. We then used a decision tree analysis to identify the trait(s) that provided the greatest discriminatory power and estimated cut-off points of morphological measurements that could be easily interpreted in the field.

northern part of Floreana Island (Fig. 2). Champion is a 9.4 ha circular islet (~400 m diameter). It is a crater emerged from the seafloor whose maximum elevation is 46 meters above sea level and is located less than one kilometre from the coastline of Floreana Island (Grant *et al.* 2000). The Gardner-by-Floreana islet (76.5 ha) is located 8 km off the coast of Floreana Island. It is a partially sunk volcanic cone, creating an islet covered by cliffs of 50–100 m high and reaching an elevation of 210 meters a.s.l. The islet has a 100 m high plateau of approximately 12 ha located in the eastern part of the islet (Jiménez-Uzcátegui *et al.* 2011) that is the only place accessible and safe for humans.

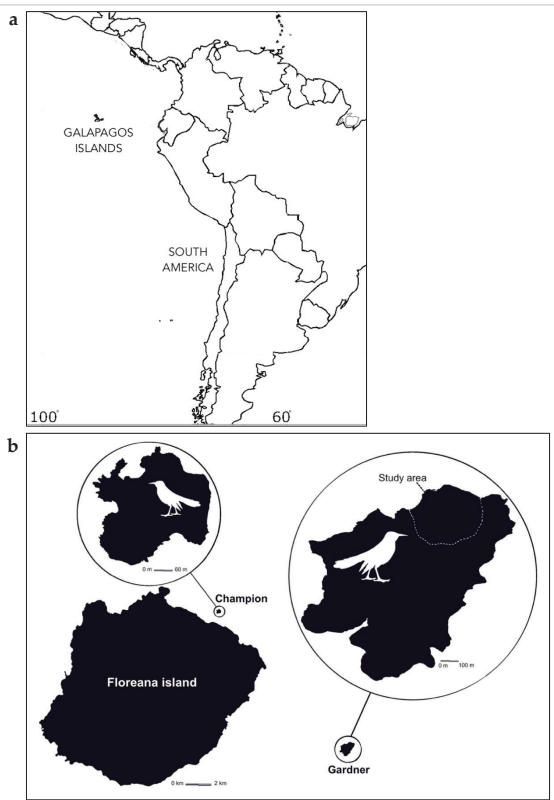


Figure 2. a) Location of the Galapagos Islands in South America. b) Floreana mockingbird (*Mimus trifasciatus*) populations. For the Champion population (90°23'100"W, 01°14'240"S), the study area corresponds to the whole islet. For the Gardner population (90°17'700"W, 01°19'969"S) the dotted area indicates the 12 ha study which is the only area accessible on the island.

MATERIALS & METHODS

Study site

This study was conducted on Champion (90°23'100"W, 01°14'240"S) and Gardner-by-Floreana (90°17'700"W, 01°19'969"S) islets in the

Morphometry and molecular sexing

Birds were captured as part of an annual long-term monitoring study carried out since 2006. During November 2015, a total of 41 birds (24 males, 17 females) were caught using a wire cage trap with a lure; these birds were then banded, measured, and released. We took six morphological body measurements (Fig. 3) following a standardised measurements protocol for the species: (1) mass; (2) beak depth, in vertical plane in the middle of the nares; (3) beak width, in the upper mandible in a horizontal plane in the anterior edge of the nares; (4) tarsus length, from the intertarsal joint to the foot joint; (5) wing length, with the wing in a natural arc and at 90° angle with the radius/ulna; (6) head-beak length, from the upper beak tip to the nape. All measurements were taken to the nearest 1 and 0.1 mm using a stopped wing ruler and Vernier callipers respectively, the mass was taken to the nearest 0.5 g using a 50 g or 100 g Pesola spring balance. All measurements were taken twice and averaged prior to knowing the sex of each bird, which was later confirmed by molecular analysis of DNA for each bird. For DNA samples, we used a needle to prick the brachial vein of each adult mockingbird shortly after capture. Approximately 5 µL of blood was collected from each individual using a capillary tube (75 µL) and the blood was stored in a CryoTube™ vial with 1.0 ml of Queen's Lysis buffer. Vials were labelled with the respective metal band identity of each sampled bird. Blood samples were sent to the Equine Parentage and Animal Genetic Services Centre, Massey University, New Zealand for molecular analysis of the CHD gene of the avian sex chromosomes using a similar technique described in Norris-Caneda & Elliott (1998).

Analysis

Assumptions of normality were tested using a Shapiro-Wilk Test in R (R Core Team, 2013). All variables met the condition of normality so no

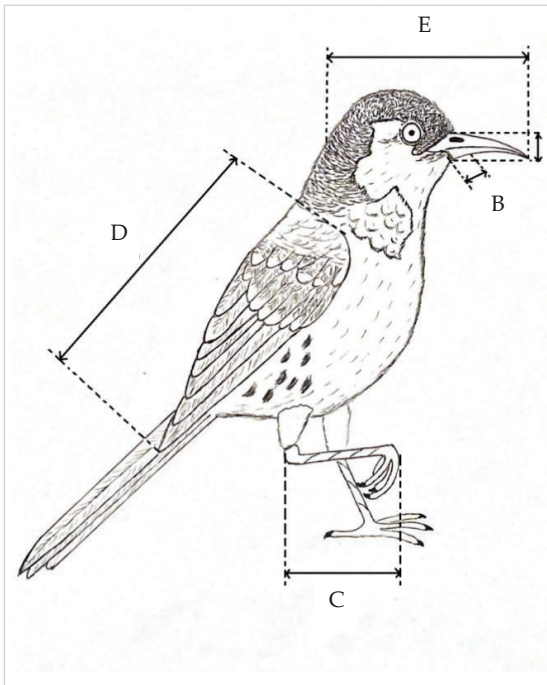


Figure 3. Description of morphological measurements used for the sexing of the Floreana mockingbird (*Mimus trifasciatus*). A) beak depth, b) beak width, c) tarsus length, d) wing length, and e) head-beak length. Drawing by: Enzo M. R. Reyes.

transformation was carried out. To test for intersexual differences among the molecular sexed birds, we used an unpaired t-test for each pair of measurements. Moreover, for each measurement, we calculated the percentage of sexual dimorphism using the formula described in Holmes & Pitelka (1968): $100 \times (m-f)/m$, where m and f are the mean values of the male and female respectively. DFA of the measurements of the known sex birds were then conducted to determine which measurements were most reliable for classifying individuals either as male or female. Wilks' lambda (λ) statistics was used as a variable selection criterion. The accuracy of our discriminant functions were determined by the percentage of known sex individuals correctly classified using all the individuals. In addition, we applied a jack-knifing prediction procedure in which each case was reclassified by repeatedly removing a single individual and then classifying this individual based on the discriminant function generated by the remaining birds. When we obtained the best discriminant functions, we then calculated the threshold of probabilities of being a male or female through a decision tree analysis which predict an outcome based on a set of

predictors classifying particular variables higher than some threshold. The analyses were run on R (R Core Team, 2013) using the packages MASS for the DFA calculations, rrcov for the multivariate lambda values and packages rpart, GGally, and tidyverse for the decision tree analysis. Univariate lambda values were extracted from SPSS software (IBM, 2020).

RESULTS

We sampled 14 birds from Champion (nine males, five females) and 27 birds from Gardner (15 males, 12 females). Morphological measurements from the 41 birds that were molecularly sexed showed that males and females overlapped but generally males were larger than females revealing sexual dimorphism by size (Table 1; Fig. 4). The mean values for five traits: mass, beak depth, tarsus length, head-beak, and wing length, were significantly larger in males than in females. Coefficient of sexual dimorphism shows that mass, beak depth, and wing length are the most dimorph traits. The DFA shows that the wing trait performs the best as a single variable classifying birds correctly 98% of the time (Table 2). Because of this, we created four discriminant functions using a combination of wing length and other variables: wing length + mass, wing length + tarsus length, wing length + all beak, and all measurements combined. From this, wing + mass and wing + tarsus length classified birds correctly 98% of the time in both cases and had a greater discriminatory power based on the Wilks' Lambda values (Table 2). As wing length performed the best as a univariate, the calculated cut-off point by a decision tree analysis was 119.5 mm. The probability of correctly classifying as a female was 94% under the threshold of 119.5 mm, and 100% probability of correctly classifying as a male when the wing length is equal or larger than 119.5 mm. The cut-off point for tarsus length was 39.9 mm. The probability of correctly classifying as a female was 85% under the threshold of 39.9 mm, and 79% probability of correctly classifying as a male when the tarsus length is equal or greater than 119.5 mm. Furthermore, for mass, we obtained two cut-off points: 61.6 g and 56.7 g. Birds under 61.6 g and 56.65 g could be classified as females with 59% and 77% probability, respectively. Meanwhile, birds equal or greater than 56.65 and 61.6 g could be classified as males with 56% and 100% probability, respectively.

DISCUSSION

In this study, we investigated whether *Mimus trifasciatus* can be correctly classified as a male or female by morphological measurements and, if so, which morphological traits are the best to assign

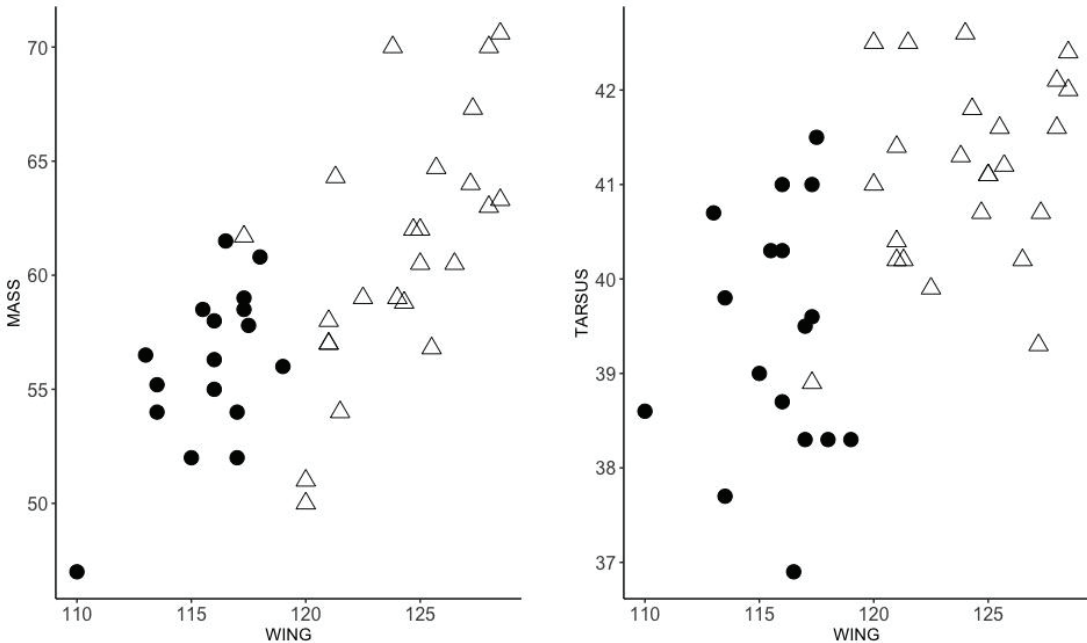


Figure 4. Plot of the measurements used as discriminant functions that best predict the sex of the Floreana mockingbird (*Mimus trifasciatus*). A) Wing length and mass and B) wing length and tarsus length measurements for male (open triangles) and females (closed circles) Floreana mockingbird. Wing length and tarsus length measures are given in mm, while mass is given in g.

an accurate classification. We concluded that male and female *M. trifasciatus* can be easily identified using simple morphological measurements. Our results confirm the assumption of Deem *et al.* (2011) that these monochromatic species present sexual dimorphism evident in morphological traits. Males were significantly larger than females in five of six morphological traits except beak width; similar features have been present in two other mockingbird species: *Mimus saturninus* and *Mimus*

triurus (Fuchs & Montalti 2016). However, while mass appeared to be the more dimorphic trait, when using the coefficient of sexual dimorphism mass differences were not enough to correctly assign sex using the DFA. Male and female *M. trifasciatus* can be distinguished with 98% accuracy using only wing length or a combination of wing length, mass, and tarsus length.

Despite the widespread use of DFA, some factors can affect its performance. One is the

Table 1. Morphological measurements of 41 Floreana mockingbirds (*Mimus trifasciatus*) sexed using molecular methods and percentage of dimorphism for each morphological trait. Unpaired *t*-test values (*t*), *df* and significant values are given for comparison of variables between sexes. NS = not significant. Length measurements are given in mm, while mass is given in g.

	Males			Females			Males vs Females			
	$\bar{x} \pm sd$	Range	n	$\bar{x} \pm sd$	Range	n	<i>t</i>	<i>p</i>	<i>df</i>	% Dimorphism
Mass	61.0 ± 5.5	50.0–70.6	24	56.0 ± 3.6	47.0–61.5	17	3.6	<0.001	38.9	8.2
Head-beak length	59.3 ± 1.2	56.2–61.0	24	57.9 ± 0.7	57.0–59.1	17	4.3	<0.001	37.0	2.1
Beak width	7.1 ± 0.5	6.4–8.1	24	6.9 ± 0.4	6.4–7.6	17	1.5	NS	38.1	2.7
Beak depth	6.6 ± 0.4	6.0–7.6	24	6.2 ± 0.3	5.7–7.0	17	3.9	<0.001	38.8	6.3
Tarsus length	41.1 ± 1.0	38.9–42.6	24	39.4 ± 1.3	36.9–41.5	17	4.6	<0.001	29.0	4.2
Wing length	124.1 ± 3.1	117.3–128.5	24	115.8 ± 2.2	110.0–119.0	17	9.9	<0.001	39.0	6.7

Table 2. Accuracy in assigning sex of Floreana mockingbirds (*Mimus trifasciatus*) using a discriminant function analysis, denoted by Wilks' Lambda (λ) using single variables and combined functions of the variables.

Variable	λ	Female (17)	Proportion correct		Proportion jack-knifing		
			Male (24)	Overall	Female	Male	Overall
Mass	0.78	0.58	0.83	0.71	0.59	0.75	0.68
Head-beak length	0.72	0.65	0.79	0.73	0.65	0.79	0.73
Beak width	0.95	0.41	0.83	0.66	0.41	0.83	0.66
Beak depth	0.74	0.82	0.75	0.78	0.82	0.75	0.78
Tarsus length	0.63	0.65	0.88	0.77	0.64	0.88	0.78
Wing length	0.31	1	0.96	0.98	1	0.95	0.98
Wing length + mass	0.28	1	0.96	0.98	1	0.96	0.98
Wing length + tarsus	0.29	1	0.96	0.98	1	0.96	0.98
Wing length + all beak	0.29	1	0.96	0.98	0.94	0.88	0.90
All variables	0.51	1	0.96	0.98	0.94	0.92	0.93

consistency of the observer when taking the morphometric measurements. Some studies of sexing by morphological traits have found that this parameter can create bias in the results (Henry *et al.* 2015). Here, our morphological measurements were taken by a single observer at both sites during the same period of time, thus reducing the likelihood of introducing bias to our analysis. Other disadvantages are that DFA can only be applied in populations with a small degree of geographic variation and only over a certain time frame due to the potential for temporal instability (Ruiz *et al.* 1998; Shealer & Cleary 2007). In the case of the *M. trifasciatus*, although there was a difference in morphology between populations (Reyes *unpubl. data*) most of the significant differences were related to the beak measurements, hence the usefulness of using wing length as a sexing method for both populations. However, our results may lose efficacy in the future if temporal variation in morphometric traits occurs, a factor that has been shown in other Galápagos passerines (Gibbs & Grant 1987).

We recommend the use of wing length as a simple trait because the accuracy of classification was over 90% and because this measurement has been widely used in other mockingbird species (Kinnaird & Grant 1982; Curry 1988; 1989; Curry & Grant 1989; Martínez-Gómez & Curry 1996; 1998; Fuchs & Montalti 2016). We note that tarsus has been used in other birds to classify sex but it was less effective in our study (Taylor & Jamieson 2007; Montalti *et al.* 2012). Additionally, we do not recommend the use of mass alone as a discriminant for sex because mass may vary daily and seasonally depending on, for example, reproduction status, resource availability, and time of day measurements are taken (Lehikoinen 1987). Nevertheless, the use of a single measurement has the added benefit of

minimising handling time which reduces stress, an important factor when handling endangered species (Dechaume-Moncharmont *et al.* 2011; Currylow *et al.* 2017). Moreover, the wing is an easy trait to measure because the landmarks are well-defined and because of its size in comparison with other morphological traits in passerine birds. Bigger traits are easy to measure regardless of the observer's experience reducing measurement errors (Yezerinac *et al.* 1992). One caveat is that our results can only be applied in non-moulting adults when using the wing only. Although not yet reported for *M. trifasciatus*, some passerine species show differentiation between the wing size of juvenile individuals, which have shorter and rounded wings when compared to adults (Norman 1997; Green *et al.* 2009).

The main goal of this study was to develop a tool that could be easily interpreted and applied by the personnel of the Galápagos National Park. This tool does not rely on equations of the discriminant functions but instead, a cut point on key measurements, easy to record and quick to apply on the field. Monitoring of *M. trifasciatus* and access to the study sites are restricted due to the conservation status of this species. Access to scientists only occurs for a limited number of days and a limited number of people. Meanwhile, parkrangers of the Galápagos National Park have free access to monitor the study sites and hence the need for an easy and quick tool for the monitoring of this endangered species.

ACKNOWLEDGEMENTS

We thank all the parkrangers from the Galápagos National Park for their valuable assistance in the field. Thanks to C. Burrige from the

Equine Parentage and Animal Genetic Services Centre, Massey University for the assistance in the molecular analysis. To D. Aguirre for statistics assistance and finally to L. Billows for proofreading the article and S. Giovanardi for the map artwork. This study is part of a wider research project that was financially supported by the Friends of Galápagos New Zealand (FOGNZ) to EMRR. This research was carried out under the permit 025-MAE-DPNG-IA, projects PC-63-18 and PC-27-19 of the Galápagos National Park and the Ecuadorian Ministry of Environment granted to LOC. Blood sample import permits to New Zealand were granted by the Ministry of Primary Industries under permit number CL8861 from MPI CUSMOD Release AF100101064522

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