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# Visually sexing Loggerhead Shrike (Lanius ludovicianus) using plumage coloration and pattern

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**TITLE:**

# Visually Sexing Loggerhead Shrike (*Lanius ludovicianus*) Using Plumage Coloration and Pattern

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**KEYWORDS:**

Laniidae, sexing, plumage, primary feather, loggerhead shrike, rachis, primary coverts

**SHORT ABSTRACT:**

We present a protocol to characterize the sex of loggerhead shrike visually based on the coloration and pattern of the sixth primary wing feather.

**LONG ABSTRACT:**

The loggerhead shrike is a small sexually monomorphic passerine bird using grassland habitats across North America. Based on Breeding Bird Survey data, the species has undergone a drastic decline since the mid-1960s. The cause of decline is unknown, and research is actively underway to address this knowledge gap. These efforts are hindered by an inability to sex the species in hand, which to date was only possible using molecular markers. Here, we present a protocol to sex loggerhead shrikes by visually analyzing the coloration and pattern in the sixth primary feather. The application of the method will facilitate our ability to identify threats on a finer scale than has been possible to date and to address various ecological and evolutionary hypotheses. The methodology is simple and results reliable–we encourage including this method for research of both in situ and ex situ populations.

**INTRODUCTION:**

The loggerhead shrike (*Lanius ludovicianus*) is a North American passerine with a broad geographical range encompassing most of North America and a variety of habitats that can generally be described as grassland[1]. It is one of only two species of shrikes (Order Passeriformes) that occur in North America. Shrikes are best known for the unique raptor-like bill, which allows them to take vertebrate prey, and their unique behavior of impaling food items on thorns or other sharp objects. The loggerhead shrike is the only species of 'true shrike' (Family Laniidae) endemic to the continent. Shrikes breeding above 40°N are generally obligate migrants[1–3], with wintering grounds almost entirely encompassed within that of non-migratory conspecifics[1,4].

North American Breeding Bird Survey data[5] for loggerhead shrike indicate a significant (3.18% $yr^{-1}$) range-wide population decline. The loggerhead shrike is one of 24 "Common Birds in Steep Decline"–i.e., those that have lost more than 50% of their populations over the past 40 years, but which lack other elevated vulnerability factors that would warrant higher "Watch List" status[6]. Habitat loss due to succession and human development likely contributed to the initial declines[4,7], but continued population declines are outpacing habitat loss in the breeding season, suggesting other limiting factors, in particular in areas where the species is an obligate migrant[4,8]. Results of a population viability analysis conducted for the critically endangered population of loggerhead shrike in Ontario suggests that over-wintering success of birds in their first year of life is a driver of the population trends[9,10]. Results further indicate that the conservation breeding effort, which is augmenting the wild population, has kept the species from extirpation in this area[9,10].

Understanding sex differences is an important component of both ecological and evolutionary hypotheses. The plumage of loggerhead shrikes (*Lanius ludovicianus)* is sexually monochromatic and therefore individuals cannot reliably be sexed in the hand. However, based on a method applicable to the northern shrike (*Lanius excubitor*)[11,12], it has been shown to be possible to sex at least some populations of adult loggerhead shrikes using the coloration pattern in the sixth primary wing feather[13]. We have revised this methodology[13] to include consideration of a second variable, specifically the extent of pigmentation in the rachis of the sixth primary, which allows reliable identification of sex in the majority of individuals in eastern populations, and tested its application (previously only applied to adult birds) to fledged young of the year. The method requires no specialized equipment or costly lab assays, and no measurements are required that would be subject to observer bias. Based on our results, the method is easily learned and, once mastered, highly accurate. We present detailed instructions on how to sex shrike in the hand using our method and discuss the wider implications of including sex assessment in future research and conservation efforts for this unique and enigmatic species of conservation concern.

**PROTOCOL:**

The research protocol presented herein complies with African Lion Safari's Animal Care Committee guidelines.

**1. Sexing Loggerhead Shrikes by Color and Patern of the Sixth Primary Wing Feather**

NOTE: Shrikes can be sexed in hand based on the coloration and pattern in the sixth primary wing feather (P6). In brief, the technique requires the observer to visually extrapoloate a line along the lower edge of the primary wing coverts, and then to assess how far the brown extends in the rachis (shaft) through the white portion of the feather visible below the primary coverts.

1.1. Hold the bird firmly in the banders grip and carefully extend one wing to allow the sixth primary wing feather to be viewed (**Figure 1**). Do not over-extend as this can cause harm to the bird's musculature.

1.2. Locate the P6 feather: Loggerhead Shrikes have 10 primary feathers, with the last ($10^{th}$) being the most distal (outermost) feather and reduced in size compartive to the other 9 primary feathers. Unlike the secondary feathers, and with the exception of the reduced $10^{th}$ primary feather, all primaries have a degree of white coloration. It is often easier to count backwards from the $10^{th}$ primary than to locate the first primary and count forward to locate the P6.

1.3. Assess the brown in the rachis (hereafter shaft): Does it extend at least half way through the white, and touch, or nearly touch the distal end (furthest from the body and point of emergence) of the primary covert feathers as they lay naturally over the primary feathers? If "yes", the bird is female (**Figure 2**). If "no", then the bird is male (**Figure 2**).

1.4. The pattern and extent of coloration in the primary feathers varies among individuals. If unsure of whether the brown in the shaft extends at least half way through the white patch to the distal tips of the primary coverts, or if the brown is somewhat indistinct, use a secondary technique. Specifically, assess the symmetry of the brown to white transition on each side of the shaft (**Figure 2**). If the brown at the line where the coloration changes to white meets at the same spot on either side of the shaft, the bird is male. If there the line meets asymetrically at the shaft, creating a step or notch, the pattern indicates that the bird is a female (**Figure 2**).

1.5. If there is still uncertainty as to the individual's sex, examine the angle of the transition between the brown and the white coloration. If the brown has a steep angle where it meets the white, forming an upside down "V", the pattern indicates that the bird is a female (**Figure 2**). If the pattern is more that of an "M", it indicates a male bird (**Figure 2**).

**REPRESENTATIVE RESULTS:**

Male and female plumage is, overall, monomorphic in the loggerhead shrike. However, it has been established that sex can be discerned based on the pattern of coloration in the $6^{th}$ primary in the population that occurs on mainland California[13] and the northern shrike[12]. We tested Sustaita et al.'s (2014) protocol[13] to determine if it was applicable to northeastern populations of loggerhead shrike and to younger age cohorts. We developed a modified version of the protocol, with the goal of developing an accurate and easy to use method suitable for use in the field. We tested our modified protocol by developing a Citizen Scientist survey project. The survey was sent to African Lion Safari's (ALS) e-newsletter database, which consists of subscribers from Canada, the United States, and around the world. In addition, we also posted the survey on ALS's Facebook page, which reached 5,604 people. In total, 399 people reviewed the survey and 120 participated. Participants were asked to read a brief instruction on how to sex using feather pattern (similar to that presented here) and then to sex a series of 26 shrikes (n = 13 female and n = 13 male) based on a photograph showing the pattern in the $6^{th}$ primary. Photographs were presented in the same order for each viewer, with photos of males versus females randomly ordered. The gender of the shrikes had previously been confirmed using genetic sexing methods[14]. All photographs were of 2017 fledged Hatch Year birds that produced at a conservation breeding facility in Ontario, Canada, and originating from the same founder lines, representing only one subspecies and one population in eastern Canada. Only photos from birds in which the flight feathers were fully emerged were included in the study. All photos were taken

opportunistically as birds were being handled for routine care and management (e.g. for vaccination). Photos were selected based on their image quality – i.e. resolution, focus and if the presentation of the 6th primary was unobstructed – rather than on how well they represented the ideal for each sex.

Respondents were asked to rate their prior knowledge of birds and whether they felt the scoring became easier as they reviewed photos. Only 4% of respondents rated themselves as experts regarding birds. The remaining respondents were fairly evenly split, rating themselves as either having no experience (43%) or as an amateur (46%).

Our Citizen Scientists averaged a 77% (range = 70% to 85%) correct assessment for females, and 77% for males (range = 67% to 86%) (**Table 1**). Scoring was consistent among photos suggesting that the patterns are likely fairly consistent within each sex. Seventy one percent of our volunteers responded that they felt scoring became easier as they went along. However, the average number of correctly scored photos was nearly identical for the first ten (78% scored correctly) versus second ten (77% were scored correctly) photos reviewed, suggesting that scoring itself did not become easier or more accurate with practice, but that the participants were more at ease with the methodology.

We tested the hypothesis that accuracy increases with training and experience. Ten individuals were trained in person on our method, rather than having them read instructions. Each individual was shown 5 pictures of shrikes, which were not included on the survey. The sexing method was reiterated verbally by the trainer for each photograph. The trainees were then asked to conduct the same assessment as our Citizen Scientists. All individuals trained one-one-one assessed 12 out of 13 photos of females and 11 of the 13 photos of males correctly. One trainee incorrectly scored a female bird's wing as being that of a male. Two trained individuals incorrectly identified 2 photos of a male wing as being female. Our results suggest that one-on-one verbal training, which in our case was accomplished using photographs, was highly effective and increased accuracy of designations compared to written instructions. Training could also be conducted in the field with a bird in hand. Regardless of the method, we recommend one-on-one individual training whenever possible over use of written instructions prior to data collection.

Our results indicate that with a modest amount of practice, that does not have to rely upon having birds in hand, sexing loggerhead shrike using the coloration and pattern of the 6th primary provides a highly accurate method by which the sex of shrike can be discerned. However, future research is required to determine if this methodology works universally among loggerhead shrike populations and in other subspecies. We also suggest that future research assess the degree of difference in the P6 pattern and coloration between left and right wings, and in subsequent and repeated molts. We would also recommend that future research assess the repeatability of the method by the same observer. Regardless, given the results of our own and previous research, it would appear that this technique has the potential for broad-scale utility within the species.

**FIGURE AND TABLE LEGENDS:**

**Figure 1. Wing of loggerhead shrike extended in preparation of assessing the 6th primary feather.**

**Figure 2. Example of sixth primary feather coloration and pattern in female (A and B) versus male (C and D) loggerhead shrike.** The dark pigmentation in the rachis is touching, or nearly touching the distal tip of the primary coverts, the brown feather vane coloration is asymmetrical on either side of the shaft where it transitions to white, and there is a steep "V" angle at transition in females. The dark pigmentation in the rachis is no more than half way to the distal tips of the primary coverts and the brown in the feather vane on either side of the shaft at the transition to white is symmetrical and forms a shallow "M" angle at the transition point in males. A solid black line has been superimposed on the pictures to demonstrate the distal tip of the primaries. In photos B and C, a blue line has been superimposed on the photos to demonstrate the "V" versus "M" angles. The fourth primary (P4) and sixth primary (P6) are both labelled to indicate the order in which primary feathers are numbered.

**Table 1. Ratio of correct responses by Citizen Scientists reviewing photographs of female (n = 13) and male (n = 13) loggerhead shrike wings to determine sex based on the color and pattern in the 6th primary feather.**

**DISCUSSION:**

Herein, we describe a simple and efficient method whereby loggerhead shrike can be sexed based only on visual cues and provide an assessment of the method's accuracy. Our simple method is easily and quickly undertaken, with results indicating a high accuracy rate that increases with a small amount of training. Our results support those of previous work[13] that indicated the method originally developed for use in northern shrike[12] had utility for sexing adult loggerhead shrikes in mainland California. We have extended this research to demonstrate that the technique also works elsewhere in the species' range, in a different subspecies[15], and for young of the year with fully emerged flight feathers. We have simplified the approach[13] to focus only on the pattern of a primary wing feather, as our goal was to determine if the method could be used as a user-friendly and easily learned technique to sex shrike in hand in the field, which has previously only been possible for a short period of time during the breeding season[11].

The ability to determine sex in loggerhead shrike will facilitate examination of a greatly expanded set of ecological and evolutionary hypotheses for the species. Sex-biased differences in demographic and life-history traits can impact the effectiveness of conservation actions, and data on these biases is needed to adapt management programs to best meet the species' needs. Research on other avian species indicates that demographic and life history traits, including molt[16,17], social structure and reproduction[18], dispersal and gene flow[19,20], mortality[21–23], migration[24] and carry-over effects[25], body condition[26], habitat choice and use[27–30], stress-induced pathways[31,32], parisitization[33,34], and response to various environmental stressors[35] can vary based on sex. Sex-biases in populations can have important implications for population demography, breeding, and even social biology[36]. Work on the population of loggerhead shrike in Midewin National Tallgrass Prairie in northern Illinois suggests that male-biased mortality may be negatively impacting the population's trend[37]. With the advances in the use of exogenous

markers, such as stable isotopes and nuclear genetic microsatellites[38], migrant shrikes can be discerned from residents. The ability to easily sex shrike as part of similar research will facilitate research into the cause of sex biased mortality and other demographic factors that could be driving the population trends in this species.

Conservation breeding has proven a critical management tool for the population of loggerhead shrikes in Ontario[9,10]. The augmentation of the wild population using young of the year bred in human care has kept this population from extirpation, essentially "buying time" for research to better understand the cause of decline. To date, more than 1000 young birds have been released into the wild in Ontario since the program was initiated in the year 2000, with young migrating and returning to breed in subsequent years[39,40]. Each year, a few individuals are held back to ensure future breeding can occur. Ideally, the ratio of males to females would be equal. A rapid and inexpensive method would facilitate this aspect of the management of the ex situ population. With more than 100 young birds released in most years[41], the cost of DNA sexing is cost prohibitive. As a result, it is not possible to determine if return rates for each sex are proportional to that released, or if sex-biased mortality is occurring. Again, an inexpensive and easily implemented methodology by which young of the year can be sexed would assist in management and conservation of the species in Ontario and help to broaden the scope of possible research to include, for example, sexual size dimorphism and nestling survival seasonal brood sex ratios, etc.

While molecular sexing methods are available that are a widely applicable for use with birds[14,42], and have proven effective with loggerhead shrikes[38,42], they require a tissue sample from which to extract DNA, specialized equipment, and expertise and are not as cost effective as our method. Other sex-determination methods using external characteristics such as vent sexing are less effective for loggerhead shrike, which only exhibit a cloacal protuberance for short periods of time (Chabot, unpublished data). Morphometrics suitable for sexing the closely related northern shrike (*L. excubitor*)[12] have not been as effective in loggerhead shrikes[43–45]. Our accurate and easily implemented method of sex determination has broad utility, not only for informing comparative demographic and life history studies, but also for research and conservation activities that require sex-specific data.

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**DISCLOSURES:**

The authors have nothing to disclose.

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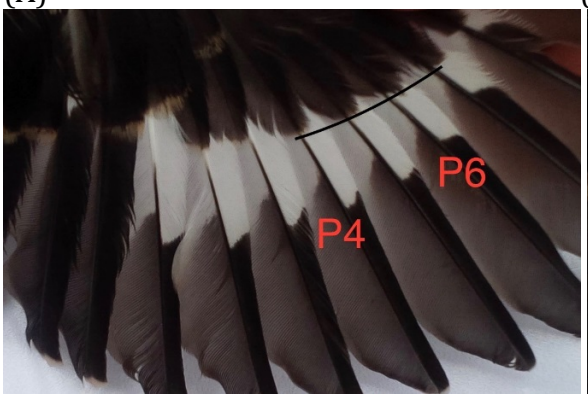
Figure

Click here to access/download;Figure;JOVE CHABOT et al June 24 2019 Figure 1.pdf

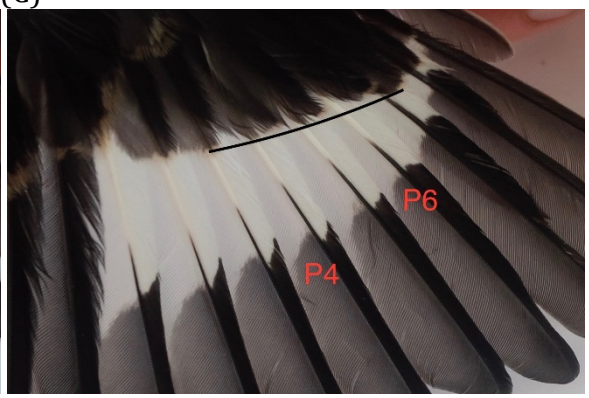
Figure

Click here to access/download;Figure;JOVE CHABOT et al June 24 2019 Figure 2 (sexes together).pdf

(A)




(C)

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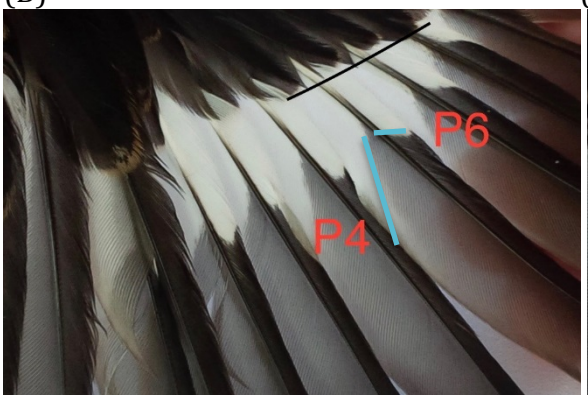


(D)

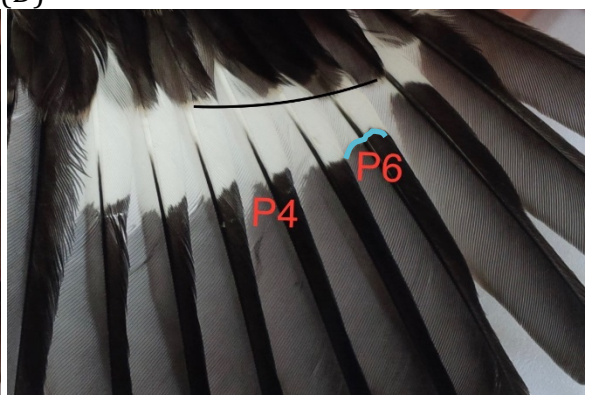

Table 1



| Ratio of correct resp | Females | Males |
|---|---|---|
| 60% to 69% | 0 | 1 |
| 70% to 79% | 7 | 7 |
| 80% to 89% | 6 | 5 |
| 90% to 100% | 0 | 0 |
| Total pictures | 13 | 13 |

Table of Materials

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Title of Article: Assessing Age, Sex and Molt in Logger head Sharks

Author(s): Amy Chabot , Gareth Morgan

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**CORRESPONDING AUTHOR**

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| Date: | Jan 21. 2019 |

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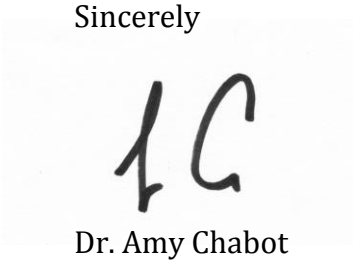
13 August 2019

Dear Dr. Steindel,

Please find attached a summary of the revisions requested to our submission JoVE59713R1 "Visually sexing loggerhead shrike (Lanius ludovicianus) using plumage coloration and pattern". Each of the editorial and peer review comments have been addressed as noted.

Sincerely



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**Editorial comments:**
General:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. **We have proofread the final revised clean copy.**
2. Please ensure that the manuscript is formatted according to JoVE guidelines–letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps. **We have checked formatting.**
3. Please revise lines 39-50 to avoid textual overlap with previously published work. **We have revised the text.**
4. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s). Please number references by their appearance in the manuscript. **References have been revised.**

Protocol:
1. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. **We have included a statement to this effect.**
2. For each protocol step, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps. **We have reviewed the protocol steps and feel they provide the reader with the ability to answer "how do I do this".**

Results/Figures:
1. While you do discuss efficacy of this protocol, it would be best to have a visual representation of this, either another figure or a table. This would be put in the results section of the video. **We have added a table summarizing results.**

References:
1. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. **We have revised the references as needed.**
2. Please do not abbreviate journal titles. **We have revised the references as needed.**

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol. **We have reviewed the TofM. Our method requires no materials other than a bird in hand.**

**Reviewers' comments:**
**Reviewer #1:**
Manuscript Summary:
The authors describe a technique of sexing Loggerhead Shrikes.

Major Concerns:
None

Minor Concerns:
None

**Reviewer #2:**

Manuscript Summary:

This is a well-written paper that provides and interesting approach for visual sexing of loggerhead shrikes. Overall the protocol appears easy to use, and small changes to the Figures would greatly facilitate the reader's understanding of this approach. The ability to accurately sex individuals with visual characteristics would greatly facilitate research with this declining species, however it is unlikely that the results can be generalized to other species.

Major Concerns:

L116-118 - Were observers shown the photos in the same order? If so, the reporting of sexing getting easier with practice is confounded with the presentation order. Statements around this approach getting easier with practice are not founded. Can you show how the accuracy rate changes with the number of photos observed? **We have reviewed results and assessed if accuracy in the first ten versus second ten photos and added text summarizing these findings. Note, we did also assess this by sex and for varying number (e.g. first 5, next 5) and results were the same. Thus, we opted for a simple first versus second ten.**

L118 - How many were juveniles vs adults? Among juveniles, were young in the nest also considered or only those that fledged? **We have revised text to clarify that all individuals were fledged, hatch year birds with fully emerged feathers.**

L126-128 - Were there particular photos that were consistently mis-identified across observers? If so, this might suggest some shrikes have characteristics that do not fit well within the criteria described. **We have reviewed the scoring for each photo and added text to our results to address this comment.**

L135-137 - could a training dataset of photos also help practice before the approach is used in the field? If so, recommendations on the number of photos to achieve higher accuracy? **We do make a clear statement that a training dataset of photos helps. We did not test how many photos would be the best and do not feel we can make a recommendation to this effect.**

Figures 2 and 3

-These figures should be combined into a single figure and the panels should be clearly marked as female or male and which criteria is being used - a grid approach could be helpful here.

-The blue line in these figures draws attention away from the shaft; a legend indicating what the different lines represent would be helpful.

-It's also unclear why P4 is indicated on the Figures.

**We have revised the two figures into one panel as suggested and added text to our figure caption, rather than including a legend, which we felt had the same effect, to describe the purpose of the lines and why we labeled P4. We did not include a grid as our intent is for the method to be "field friendly" and do-able with a bird in hand, in which case a grid will not be applicable.**

Minor Concerns:
L83 - indicate that P10 is the outermost feather. **We have revised the text.**

L172-174 - Be more specific here about the implications. **We feel our discussion already includes a range of implications and uses and are unsure how to be more specific.**

L192 - "REF" - missing reference? **This has been fixed.**

**Reviewer #3:**

Manuscript Summary:

The authors used citizen helps to sex diagnostics in a fascinating birds species, loggerhead shrike.

Major Concerns:

I have any. OK, maybe I'm so big fan of shrikes, and I like nearly everything dedicated to that bird species, but seriously it is very important and useful job.

Minor Concerns:

In page 9 - I suggest to say, that even small differences in morphology - like described in the paper - may have strong consequences for foraging and even survival; it was tested with other shrike species: Hromada, M., Kuczynski, L., Kristin, A., & Tryjanowski, P. (2003). Animals of different phenotype differentially utilise dietary niche-the case of the Great Grey Shrike Lanius excubitor. Ornis Fennica, 80(2), 71-78.

You can also discus repeatability - if you check it between observers - I guess between binary values can be even higher that in morphological traits - see also shrikes example: Kuczynski, L., Tryjanowski, P., Antczak, M., Skoracki, M., & Hromada, M. (2002). Repeatability of measurements and shrinkage after skinning: the case of the Great Grey Shrike Lanius excubitor. Bonner Zoologische Beiträge, 51(2/3), 127-130.

Page 11- Panov, E. N., 2011. The true shrikes (Laniidae) of the world: Ecology, behavior and evolution. Pensoft Publishers. Moscow. - suggest differences in diagnostic traits (as you describe in secondaries) between populations.

**While the reviewers additional references were of interest and their insights excellent, we were not able to access either journal article via Queen's University library services or on-line. Panov (2011) was available for purchase, but at a high cost ($250 US) and with a shipping time of one or more months. As such, we did not include it among our references. As such, we did not feel we could comment upon the impact of a morphological difference such as our results indicate exists between sexes or among populations. We did not assess repeatability, and thus cannot comment on it. But, as noted, it is a binary trait and we assume it would be fairly high. We did add additional text recommending future research to expand upon the work, including repeatability.**

**Reviewer #4:**

Manuscript Summary:

This manuscript describes a technique for determining the sex of Loggerhead Shrikes in-hand, in the field. The technique is an adaptation of previously-described approaches based on the coloration of the 6th primary flight feather, involving the extent of pigmentation within the feather rachis, the symmetry of the coloration of the barbs on either side of the rachis, and the angle formed by the pigmentation where the dark and light colors meet. The technique is simple to apply by amateurs and experts alike, and has proven to be very effective for discriminating between genetically-verified male and female shrikes. The ease of implementation and accuracy of the method present a vast improvement over previous methods, and makes a strong contribution to ornithological field methods.

Thank you for the opportunity to review for JoVE, I very much enjoyed reading the manuscript. I hope that my 2-cents below are of any use.

Major Concerns:

The manuscript is succinct, clear, and very well-written. In addition to the methods, the authors did very well to provide ample background, relevance, and short- and long-term implications of their protocol. My only (not-so-)major concerns were:

1) In regards to how the 22 shrikes were chosen for testing (e.g., lines 117-118); were these selected at random, or haphazardly, from a larger pool of shrikes, or do they represent the 'best-case' scenarios of what male and female shrikes (should) look like? In lines 65-66 it was stated that the protocol was tested on adults and YOY; how were these age groups represented in the test sample? Were all the shrikes from the same geographic locality? The measured success of the protocol - as well as extrapolation of the technique - sort of depends on this. **We have revised text to clarify the sample group chosen for testing.**

2) The survey certainly seems innocuous enough, but the authors might consider looking into (if they have not already) whether or not IRB approval is required to report on the results. The authors might also consider including the actual survey used as an appendix/ supplemental material. **As we obtained photos only while handling birds for routine procedures such as vaccination, rather than specifically for the study, we do not need IRB approval.**

3) The authors might consider the caveat (e.g., in lines 209-212) that this technique may or may not work as well on other populations/ subspecies of shrikes unless/until proven so. For mainland California populations, Sustaita et al.'s (2014) version of the technique worked better in conjunction with a few additional quantitative measurements. **We have added text to clarify that our results are specific to our population and suggested future research should extend the work to other populations. We chose not to note the addition of other quantitative measures as our intent was to determine if this simply, somewhat quantitative but visual methodology was sufficient to sex shrike, and how accurate it was.**

Minor Concerns:

Line 35-36: "to compensate for the lack of raptorial talons" has been perpetuated in the literature as the sole driving force for impaling, but may or may not be entirely accurate - consider citing Yosef & Pinshow's (2005) 'multi-functional' perspective of impaling. **We have removed reference to the reason for impaling, and chose not to expand fully on the range of purposes of this trait.**
Line 36: My understanding is that being in the family Laniidae is what makes them 'true shrikes' (as opposed to bushshrikes, helmetshrikes, etc.), and I am not aware of any subfamily Laniinae; perhaps this is very old (or very new?) taxonomy. **We have removed reference to subfamily.**
Line 51: not sure 'Population Viability Analysis' needs initial caps, since it's spelled out. **We have removed capitalization.**
Lines 61-62: Consider citing Brady et al. 2009 (for NOSH) here as well - they deserve the credit for discovering the p6 gem! **We have ensured Brady et al. are cited adequately throughout.**
Line 77: in regards to "up"; consider more descriptive terms (if even in parentheses); something to the effect of 'proximally' and/or 'toward the wrist' **We have revised text to be more clear.**
Line 88: in regards to 'distal tips'; the average reader probably does not understand distal/proximal, so consider adding something to the effect of 'i.e., toward the trailing edges of...' in parentheses. **We have revised text for clarification.**
Line 108: Consider citing Brady et al. 2009 as well **We have done so.**
Line 111: Consider 'to be undertaken' OR 'to undertake' **The grammar has been corrected.**
Line 208: Should be 2009, rather than '1999' **The citation has been corrected.**
Line 241: Should be 2009, rather than '1999' **The citation has been corrected.**
Line 272: Should be Carzzaolo, rather than 'Caaarolo' **The citation has been corrected.**