Further Analysis Supports the Conclusion that the Songs of Screaming Pihas are Individually Distinctive and Bear a Lek Signature

Authors: Fitzsimmons, Lauren P., Barker, Nicole K., and Mennill, Daniel J.

Source: The Auk, 128(4) : 790-792

Published By: American Ornithological Society

URL: https://doi.org/10.1525/auk.2011.128.4.790

Sommer 2007:965). (2) The authors appear to have used the discriminant function to classify the same songs that they used to compute the function; this kind of circular analysis vastly increases the likelihood that the function will seem to correctly classify the songs, and is simply bad practice (Tabachnick and Fidell 2007). (3) The function "assigned 76.4% of songs to the correct lek, well above the 25% level of correct assignment expected by chance"; statistical significance for the function is implied, yet no such test was done, and it seems incorrect to simply assert a 25% chance level of assignment when, to complicate matters, 50% of the songs come from one lek. Preferably, one determines *a priori* the chance classification probability for each category and then determines how close the classification comes to those probabilities (Tabachnick and Fidell 2007).

The third problem is in interpretation. I provide one example: "Our finding that Screaming Pihas sing individually distinctive songs adds to growing evidence that there may be a learned component to song in some suboscines" (Fitzsimmons et al. 2008:913). This statement is in the final sentence of the paper, the place where an author wants to leave the reader with a lasting impression about the significance of a study, yet the statement is nonsensical and, even worse, misleading, because songs in a wide range of species (most likely all species) are individually distinctive whether the songs are learned or not. In nonlearning flycatchers (*Empidonax* spp.), for example, songs are individually distinctive, perhaps best documented by the two papers the authors cite about the Alder Flycatcher (*E. alnorum*; Lovell and Lein 2004a, b); the birds even use the variation to discriminate among individuals. Even if individually distinctive songs had been demonstrated for the pihas, such a finding would have no bearing on whether the songs were learned or not.

Given the paper's problems in sampling, analysis, and interpretation, Fitzsimmons et al. (2008) cannot reach any valid conclusions about whether songs are individually distinctive. Nor do they present valid evidence of songs differing from lek to lek. Nor are the findings relevant to the question of vocal learning in suboscines.

When papers like this appear in print, authors rightly share blame with others who facilitate the publication process, including reviewers and editors. How this extended responsibility can fail is illustrated not only by the initial publication of Fitzsimmons et al. (2008) but also by the reluctance of those involved in the review process to share my desire that a severe, but fair, review be published. This cavalier attitude toward the design of research and the collection and analysis of numbers is unacceptable, because such permissiveness undermines the very science we claim to be doing. The present case is not unique, and such flawed papers can do considerable damage if they go unchallenged. If the research model is emulated by others and if the conclusions and logic are accepted as reported, progress in understanding birds is confused and stymied (for additional discussion, see Byers and Kroodsma 2009). We deserve better from each other, and we should hold each other to a higher standard.

Acknowledgments.—I thank K. Yasukawa and editors S. Sealy and M. Murphy for their advice on the content of this letter, and two reviewers whose advice I sought but who chose to remain anonymous.—Donald Kroodsma, *36 Kettle Pond Road, Amherst, Massachusetts 01002, USA. E-mail: dekroodsma@yahoo.com*

Literature Cited

Byers, B. E., and D. E. Kroodsma. 2009. Female mate choice and songbird song repertoires. Animal Behaviour 77:13–22.

Fitzsimmons, L. P., N. K. Barker, and D. J. Mennill. 2008. Individual variation and lek-based vocal distinctiveness in songs of the Screaming Piha (*Lipaugus vociferans*), a suboscine songbird. Auk 125:908–914.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Kroodsma, D. E. 2005. The Singing Life of Birds: The Art and Science of Listening to Birdsong. Houghton Mifflin, Boston.

Kroodsma, D. E. 2009. Birdsong by the Seasons: A Year of Listening to Birds. Houghton Mifflin Harcourt, Boston.

Lovell, S. F., and M. R. Lein. 2004a. Neighbor-stranger discrimination by song in a suboscine bird, the Alder Flycatcher, *Empidonax alnorum*. Behavioral Ecology 15:799–804.

Lovell, S. F., and M. R. Lein. 2004b. Song variation in a population of Alder Flycatchers. Journal of Field Ornithology 75:146–151.

McGregor, P. K., C. K. Catchpole, T. Dabelsteen, J. B. Falls, L. Fusani, H. C. Gerhardt, F. Gilbert, A. G. Horn, G. M. Klump, D. E. Kroodsma, and others. 1992. Design of playback experiments: The Thornbridge Hall NATO ARW Consensus. Pages 1–9 *in* Playback and Studies of Animal Communication (P. K. McGregor, Ed.). Plenum Press, New York.

Mundry, R., and C. Sommer. 2007. Discriminant function analysis with nonindependent data: Consequences and an alternative. Animal Behaviour 74:965–976.

Saranathan, V., D. Hamilton, G. V. N. Powell, D. E. Kroodsma, and R. O. Prum. 2007. Genetic evidence supports song learning in the Three-wattled Bellbird *Procnias tricarunculata* (Cotingidae). Molecular Ecology 16:3689–3702.

Tabachnick, B. G., and L. S. Fidell. 2007. Using Multivariate Statistics, 5th ed. Allyn & Bacon, Boston.

Received 2 July 2009, accepted 30 July 2010.

The Auk 128(4):790–792, 2011

Further analysis supports the conclusion that the songs of Screaming Pihas are individually distinctive and bear a lek signature.—The conclusion of our field study (Fitzsimmons et al. 2008) was that three complementary methods of analysis demonstrated significant differences in song features between individual male Screaming Pihas (*Lipaugus vociferans*) and, to a lesser degree, distinctiveness based on the lek at which they were recorded. Kroodsma (2011) presents many criticisms of our paper, related to our sampling approach, our acoustic measurements, our analytical approach, and our interpretation. Here, we address these criticisms and provide additional data and analyses in support of our conclusions. We argue that, despite some deficiencies, our investigation provides an interesting contribution to the literature on suboscine songbirds.

Sampling approach.—Kroodsma's (2011) first main criticism is related to our sampling approach. He points out that each male's songs were recorded during only one recording session, and he suggests that our observed differences are due to differences between recording sessions rather than differences between individuals. As

is the case for many field studies, particularly in remote locations, our sampling approach reflected a tradeoff between the amount of time available to sample each individual and the amount of time to sample different individuals; we chose to maximize the number of individuals recorded within our time-limited field expedition. We agree with Kroodsma (2011) that this sampling approach is not ideal, but we do not agree that this approach invalidates the conclusions of our paper, for at least three reasons.

First, we evaluated whether Screaming Piha song structure changes between recording sessions and found no evidence to support this idea. We recorded three male Screaming Pihas singing on the same song perches on different days and used spectrogram cross-correlation to compare songs across recording sessions. For these three males, cross-correlation scores tended to be higher between songs recorded from the same individuals on different days (mean ± SE = 0.58 ± 0.07) than between songs recorded from different individuals (0.53 ± 0.01). We removed these data from our paper because of a reviewer's concern over the small sample size, which is too small for statistical analysis. Nevertheless, these data are consistent with the idea that Screaming Piha song structure is less variable between recording sessions than between individuals. Further research, preferably with a large population of banded individuals recorded during multiple recording sessions, would provide a clearer answer to the question of whether an individual Screaming Piha's songs change over time.

Second, although we agree that acoustic recordings can vary with factors such as a bird's motivation or recording conditions, we have no reason to believe that the songs of Screaming Pihas are affected by systematic variation among our recording sessions. We minimized potential variation between recording sessions by collecting recordings at the same time of year in the same context: males performing spontaneous songs from their positions on leks. There is no suggestion that Screaming Piha song structure changes over the course of the day, either in our field experience or in the literature on this species (e.g., Snow 1982, Nemeth 2004, Snow 2004). Kroodsma (2011) cites two examples of diel variation in the fine structure of bird song from his popular book (Kroodsma 2009), but we are not aware of any study showing diel variation in structure within a song type in a suboscine bird. By contrast, studies of suboscine flycatchers have revealed remarkably little variation over time (e.g., Lovell and Lein 2004, Lein 2008). It is generally thought that suboscine songbirds have remarkably consistent songs (e.g., Seddon and Tobias 2007), and Screaming Pihas appear to fit this pattern.

Third, Kroodsma's suggestion that all of the measured variation can be reduced to variation among recording sessions is too strong a statement. We agree that there is potential for variation to arise from differences between individuals and differences between recording sessions, and it is possible that the differences we described were influenced by both sources of variation. Very few studies have addressed this question by systematically quantifying variation in songs between recording sessions of an individual (Ellis 2008). One recent study demonstrated that variation within a recording session is less than the variation between recording sessions, but that the variation between recording sessions of the same individual is still significantly less than the variation between individuals (Wilson and Mennill 2010). The same study used playback to evaluate whether birds respond to these sources of variation (between-recording-session vs. between-individual variation); birds did not respond to between-recording-session variation but responded to between-individual variation (Wilson and Mennill 2010). These results provide direct evidence that birds categorize together songs recorded from the same individuals across different recording sessions. Although the study species was an oscine songbird, the Black-capped Chickadee (*Poecile atricapillus*), the results demonstrate that salient inter-individual variation in song can be quantified from a single recording session (Wilson and Mennill 2010).

Our original investigation was motivated by an intriguing study of another cotinga, the Three-wattled Bellbird (*Procnias tricarunculata*), by Kroodsma and his colleagues (Saranathan et al. 2007). We were unable to follow the protocol used in that study because the authors did not describe their acoustic sampling approach in detail in the paper presenting their results (Saranthan et al. 2007). Nevertheless, we think that our approach was appropriate for sampling the vocalizations of 26 individuals from four leks, and we maintain that our data provide evidence of individual-level variation in Screaming Pihas. We echo Kroodsma's (2011) suggestion that future research on this topic should attempt to quantify variation across recording sessions as rigorously as possible, and we recommend that future studies sample individuals across multiple recording sessions as an improvement on the design of our study.

Bioacoustic measurements.—Kroodsma (2011) questions the validity of the methods we used to measure sound spectrograms of our field recordings. Modern bioacoustic software facilitates precise and fine-scale measurements of field recordings that may not be obvious by visual inspection of sound spectrograms. Automated parameter measurements, such as those used in our study, provide objective, repeatable, empirical measurements of recordings. As we stated in our original paper (Fitzsimmons et al. 2008), all sounds were normalized to the same amplitude prior to measurement, and time measurements were collected in relation to a standard threshold. Although our sound recordings include reverberation—as one should expect for field recordings of the loud songs of rainforest birds (Nemeth et al. 2006)—our standardized, automated measurements permit careful quantification of subtle acoustic differences without reliance on subjective assessment.

Analytical approach.—Kroodsma identifies pseudoreplication in our canonical discriminant analysis between leks involving 10 songs from each of 26 males at 4 leks. To address this constructive point of criticism, we reran our analysis using a resampling approach. We conducted canonical discriminant analysis on one randomly selected song from each individual (n = 26 songs from 26 individuals from 4 leks) and repeated this procedure 100 times. This analysis revealed the same pattern we reported in Fitzsimmons et al. (2008); discriminant analysis assigned songs to the correct lek 91.7 ± 0.6% of the time (range: 73–100%). Variables with strong loading on the first and second canonical axes for this lek-level analysis were length of the *pee* syllable, bandwidth of both frequency-modulated portions of the *haw* syllable, and frequency of maximum amplitude (FMA) at the end of the *haw* syllable, as well as FMA of the end of the last introductory syllable (as in the analysis in our original paper). Therefore, an improved approach that avoids pseudoreplication yields the same pattern as our original paper.

As Kroodsma (2011) points out, there are alternatives to the simplistic null model we used in our original paper (i.e., 25% level of correct lek assignment expected by chance). Given that the four

leks we studied differed in size, the chance of any given song being classified to the correct lek is influenced by the size of the lek where it was recorded. A more conservative null model for this analysis is to use a 34% level of correct assignment expected by chance (the weighted mean of the probability of each individual song being classified to the correct lek). Comparing our discriminant analysis to this new value using prior probabilities of group membership, we find the same pattern reported in our original paper; 91.7% is significantly higher than the 34% level of correct assignment expected by chance (Binomial test: $P < 0.001$).

Implications.—Kroodsma's final main criticism concerns our interpretation, drawing attention to the last sentence of our paper. Here Kroodsma has distorted our conclusions by presenting a single sentence out of context. Contrary to the implication of his critique, this sentence was not intended as a major conclusion of our paper. Rather, it was included in the final paragraph to connect our results to recent studies that suggest the occurrence of vocal learning in suboscine songbirds and to encourage further research on this topic. In our original paper, we made the same important point that Kroodsma (2011) has articulated in his critique: "Individual differences in song features cannot necessarily be interpreted as evidence of vocal learning" (Fitzsimmons et al. 2008:912). In our final paragraph, we further explained that

> although recent evidence is compelling, raising young birds in a laboratory environment in isolation of tutors, following the classic protocol of Kroodsma, is an important area for further evaluation of whether songs are learned or innate in cotingas. Future studies should record individuals over multiple years and at different geographic sites and conduct playback experiments to determine whether Screaming Pihas discriminate between individuals using vocal cues…. Much more research is needed on this suborder before we can begin to fully understand the evolution and origins of vocal learning. (Fitzsimmons et al. 2008:913)

In summary, the conclusions of our original paper stand up to scrutiny and further analyses: Screaming Piha songs are individually distinctive and, to a lesser degree, they bear a lek signature. We thank Don Kroodsma for bringing his concerns to our attention and for continuing to scrutinize research in the field of bird song. There are many challenges inherent in field studies, and we and other researchers must continue to be mindful of the potential pitfalls; advanced planning, pilot studies, thorough sampling, and a rigorous but respectful peer-review process will help to improve the quality of ornithological research.

Acknowledgments.—We thank R. Lein, an anonymous reviewer, and editors S. Sealy and M. Murphy for comments that improved this letter.—Lauren P. Fitzsimmons,[1] Nicole K. Barker,[2] and Daniel J. Mennill, *Department of Biological Sciences, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B3P4, Canada.* [1]*Present address: Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S5B6, Canada. E-mail: lauren.p.fitzsimmons@gmail.com.* [2]*Present address: Faculté de Foresterie, de Géographie et de Géomatique, Université Laval, 2405, rue de la Terrasse, Québec G1V 0A6.*

Literature Cited

Ellis, J. M. S. 2008. Decay of apparent individual distinctiveness in the begging calls of adult female White-throated Magpie-jays. Condor 110:648–657.

Fitzsimmons, L. P., N. K. Barker, and D. J. Mennill. 2008. Individual variation and lek-based vocal distinctiveness in songs of the Screaming Piha (*Lipaugus vociferans*), a suboscine songbird. Auk 125:908–914.

Kroodsma, D. E. 2009. Birdsong by the Seasons: A Year of Listening to Birds. Houghton Mifflin Harcourt, Boston.

Kroodsma, D. E. 2011. Neither individually distinctive songs nor "lek signatures" are demonstrated in suboscine Screaming Pihas. Auk 128:X–X.

Lein, M. R. 2008. Song variation in Buff-breasted Flycatchers (*Empidonax fulvifrons*). Wilson Journal of Ornithology 120:256–267.

Lovell, S. F., and M. R. Lein. 2004. Song variation in a population of Alder Flycatchers. Journal of Field Ornithology 75:146–151.

Nemeth, E. 2004. Measuring the sound pressure level of the song of the Screaming Piha *Lipaugus vociferans*: One of the loudest birds in the world? Bioacoustics 14:225–228.

Nemeth, E., T. Dabelsteen, S. B. Pedersen, and H. Winkler. 2006. Rainforests as concert halls for birds: Are reverberations improving sound transmission of long song elements? Journal of the Acoustical Society of America 119:620–62.

Saranathan, V., D. Hamilton, G. V. N. Powell, D. E. Kroodsma, and R. O. Prum. 2007. Genetic evidence supports song learning in the Three-wattled Bellbird *Procnias tricarunculata* (Cotingidae). Molecular Ecology 16**:** 3689–3702.

Seddon, N., and J. A. Tobias. 2007. Song divergence at the edge of Amazonia: An empirical test of the peripatric speciation model. Biological Journal of the Linnean Society 90:173–188.

Snow, D. W. 1982. The Cotingas. British Museum (Natural History) and Oxford University Press, London.

Snow, D. W. 2004. Family Cotingidae (cotingas). Pages 32–109 *in* Handbook of the Birds of the World, vol. 9: Cotingas to Pipits and Wagtails (J. del Hoyo, A. Elliott, and D. A. Christie, Eds.). Lynx Edicions, Barcelona, Spain.

Wilson, D. R., and D. J. Mennill. 2010. Black-capped Chickadees, *Poecile atricapillus*, can use individually distinctive songs to discriminate among conspecifics. Animal Behaviour 79:1267–1275.

Received 6 January 2011, accepted 25 June 2011.

The Auk 128(4):792–794, 2011

Missing the forest for the gene trees: Conservation genetics is more than the identification of distinct population segments.—Zink et al. (2010) reinterpreted Barr et al. (2008) with the apparent agenda of espousing the use of mitochondrial DNA (mtDNA) over microsatellites in conservation genetics. In doing so, Zink et al. (2010) poorly represented both Barr et al. (2008) and the general value of microsatellites in population genetics research. We are compelled to correct some of the misconceptions that may have been created by Zink et al. (2010), and to underscore the value of microsatellite data in the conservation of the endangered Black-capped Vireo (*Vireo atricapilla*).

We agree with Zink et al. (2010) that mtDNA can be an excellent marker choice for characterizing phylogenetic structure, especially that resulting from disruption of gene flow between