

## **Missing the Forest for the Gene Trees: Conservation Genetics is More than the Identification of Distinct Population Segments**

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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 Pihans 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,<sup>1</sup> NICOLE K. BARKER,<sup>2</sup> and DANIEL J. MENNILL, *Department of Biological Sciences, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B3P4, Canada.* <sup>1</sup>Present address: *Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S5B6, Canada. E-mail: lauren.p.fitzsimmons@gmail.com.* <sup>2</sup>Present address: *Faculté de Foresterie, de Géographie et de Géomatique, Université Laval, 2405, rue de la Terrasse, Québec G1V 0A6.*

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**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

groups over many generations. In exhaustively detailing the pitfalls of using microsatellites with very little discussion of the strengths, however, Zink et al. (2010) appear to be making a strawman of our study. Barr et al. (2008) made no attempt to classify reciprocally monophyletic groups, as was the central focus of Zink et al. (2010). The stated purposes of both Zink et al. (2010) and Barr et al. (2008) centered around characterizing population structure in the Black-capped Vireo, whereas the scope of our study was rather more current: to ascertain the level of contemporary gene flow in the songbird given both extensive 20th-century habitat fragmentation and recent significant recovery of census populations. Zink et al. (2010:798) did note that “it is possible to detect fine-scale (i.e., recent) spatial structure with a set of highly polymorphic microsatellite loci,” a statement with which we certainly agree, given the aforementioned purpose of our study.

Zink et al. (2010) asserted that contemporary patterns of isolation and drift would be uniformly better detected by mtDNA because its smaller effective population size ( $N_e$ ) would impart a shorter coalescence time than that of microsatellites. This is not as clear to us. Though a smaller  $N_e$  would theoretically confer faster coalescence, the statistical power gained from using many microsatellite loci versus the inherently single-locus mtDNA likely outweighs the latter marker system’s presumed increased sensitivity to genetic drift and gene flow. This is especially important when studying processes that are necessarily very recent and, hence, would not be detected by an mtDNA tree, such as the effects of habitat fragmentation on gene flow. Furthermore, it is questionable whether the differences in coalescence time between mtDNA and microsatellites are as large as suggested by Zink et al. (2010). If, for example, the variance in reproductive success of males is much larger than that of females, the difference between the  $N_e$  of mtDNA and that of nuclear DNA (nDNA) will be small (Ballard and Whitlock 2004). Laporte and Charlesworth (2002) also illustrated that when males have higher variance in reproductive success and a lower dispersal rate than females, there is a sharp decrease in the difference in population structure expected between mtDNA and nDNA. Because males are more philopatric than females (Grzybowski 1995) and extrapair copulations are common in songbirds, this scenario is quite likely in Black-capped Vireos. There are, unfortunately, insufficient data to directly compare  $N_e$  between microsatellites and mtDNA here, but it would be surprising if the difference is as substantial as argued by Zink et al. (2010).

Zink et al. (2010) assessed how Barr et al. (2008) might be interpreted in the context of distinct population segments (DPSs). Barr et al. (2008) did not attempt to identify DPSs, and the criticisms of microsatellites and their use in this context should not be interpreted as damaging to our study. Barr et al. (2008) reported patterns of differentiation that suggest a recent decrease in gene flow between most of the major extant Black-capped Vireo aggregations in Texas and Oklahoma, as well as an inferred limitation on the Neotropical migrants’ dispersal capability. Solely regarding the mtDNA data in the absence of the microsatellite results might lead to the incorrect assumption that gene flow is currently high all across the Black-capped Vireo’s range, given that no geographically relevant patterns were detected (see Zink et al. 2010). On the basis of our microsatellite data, we could not agree more with the conservation recommendation by Zink et al. (2010:802) that future effort be made in connecting “high-value existing sites.”

Lost in the lengthy discussion of DPSs by Zink et al. (2010) are some potentially important inferences from the microsatellite data

for the conservation of the Black-capped Vireo. For instance, although their populations have dramatically recovered, the level of differentiation we detected suggests that individuals are not freely dispersing between major aggregations (Barr et al. 2008). We do not agree with Zink et al. (2010) that this structure can be explained solely by isolation-by-distance. In fact, we reported that “once distances separating pairs of populations exceed 100 km, there appears to be little evidence that genetic differentiation increases with greater distances” (Barr et al. 2008:3635). This pattern was the result of nearby aggregations having similar allele frequencies, a relationship that breaks down at >100 km, beyond which populations were all significantly differentiated with no evidence of isolation-by-distance (see Barr et al. 2008: figure 2). We suspect this to be a signal of localized dispersal, with genetic drift following founder events or otherwise small  $N_e$  obscuring isolation-by-distance at greater distances.

Zink et al. (2010) further criticized our conclusions that biologically significant differentiation exists on the basis of the low values of  $F_{ST}$  and “subjective” significance tests we reported. Although we appreciate the concerns about the potential problems associated with using  $P$  values as indicators of differentiation, the dismissal of small but significant levels of differentiation apparently results from a misunderstanding of the nature of estimating  $F_{ST}$  from highly polymorphic microsatellites. It is widely recognized that  $F_{ST}$  decreases with increasing marker polymorphism (Hedrick 1999, 2005; Jost 2008), and correcting for this problem remains a contested subject (see Whitlock 2011). At present, we can say that on the basis of Hedrick (2005) and Jost (2008), the  $F_{ST}$  values we reported probably underestimated the true differentiation, and that many pairs of Black-capped Vireo aggregations exhibited significant differences in allele frequencies. We also question the complete dismissal by Zink et al. (2010) of significant differentiation in allele frequencies at the level of  $\alpha < 0.001$  in a highly vagile passerine as having no biological relevance. Random sampling error is unlikely, and, as previously discussed, isolation-by-distance breaks down after a short distance. Ignoring these differences risks missing important processes that may be affecting Black-capped Vireo populations, such as habitat fragmentation and restricted dispersal over larger spatial scales.

In order to better explore the assertion that differentiation among sample locations separated by <600 km, as those studied in Barr et al. (2008), has no biological significance, we searched the literature for microsatellite-based studies of Neotropical migratory passerines for comparison. We identified eight relevant studies (Gibbs et al. 2000, Clegg et al. 2003, Veit et al. 2005, Ruegg et al. 2006, Davis et al. 2006, Lindsay et al. 2008, Winker et al. 2008, Bull et al. 2010), calculated the average  $F_{ST}$  between population pairs in these studies separated by  $\leq 600$  km, and compared these eight means to that of Black-capped Vireo population pairs using a one-sample  $t$ -test. The level of differentiation between major aggregations of other Neotropical migrants studied to date was significantly less than that of the Black-capped Vireo ( $t = -3.8$ ,  $df = 7$ ,  $P = 0.006$ ). Furthermore,  $F_{ST}$  between Black-capped Vireo aggregations was double, on average, that of other previously studied songbirds (data available on request). Considering these findings, we reject the opinion of Zink et al. (2010) that the level of differentiation we reported is biologically irrelevant.

In conclusion, Barr et al. (2008) analyzed patterns of allele frequencies over space and attempted to ascertain the underlying mechanisms driving or restricting gene flow on the basis of

geographic and ecological features—focal areas of study in conservation genetics. While noting that it was neither a goal nor a conclusion of our study, we applaud Zink et al. (2010) for applying mtDNA data to facilitate the management of the Black-capped Vireo by searching for potential DPSs—another, different focal point of conservation genetics. We are pleased that the mtDNA-based analyses, which illustrate little spatial structure over a deeper time-scale, are congruent with our suspicions that the restriction of gene flow between Black-capped Vireo aggregations is likely recent. It is our opinion that future investigators should continue recognizing the merits of both mtDNA and microsatellites and appropriately using both genetic markers in conservation genetics research.

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**Endangered species management and the role of conservation genetics: A response to Barr et al.**—Barr et al. (2011; hereafter BEA) are concerned that Zink et al. (2010; hereafter ZEA) did not appreciate the relevance of their (Barr et al. 2008) microsatellite results for conserving the Black-capped Vireo (*Vireo atricapilla*). Furthermore, they believe that a purpose of the ZEA paper was to discourage the use of microsatellites in phylogeography and conservation genetics. Both of these perceptions are correct.

The Black-capped Vireo has geographically restricted breeding and wintering ranges (Grzybowski 1995), and reconstruction of last glacial maximum and last interglacial distributions suggests that this has been true for at least the last glacial cycle (R. M. Zink unpubl. data; see also Vega Rivera et al. 2011). It would be unexpected to find major evolutionary subdivisions given the relatively small continental area occupied by the species. Nonetheless, we believe that the first step in assessing the genetic status of a species of conservation concern is to confirm whether it should be managed as one or more independently evolving entities. However, BEA state that answering this question “was neither a goal nor a conclusion of our study...” We fail to see how the