Endangered Species Management and the Role of Conservation Genetics: A Response to Barr et al.

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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

microsatellite data can be interpreted correctly without knowing for certain how many historical units might exist.

Because of the slower coalescence time of nuclear loci (including microsatellites), there could be significant structure recovered by analysis of mtDNA but "missed" by microsatellites (Palumbi et al. 2001). Thus, ZEA's survey of mtDNA variation tested whether recently isolated evolutionary divisions were overlooked in Barr et al.'s (2008) microsatellite study. ZEA's mtDNA survey found no significant evolutionary divisions in the Black-capped Vireo. ZEA (p. 802) concluded that

> population management can operate free of taxonomic and genetic restrictions, focusing instead on demography and population viability to maintain large populations of Black-capped Vireos in several locations (as a buffer against disease, fire, changing climate conditions, and other factors).

On the issue of determining the number of historically independent units, the evidence is strongly in favor of using mtDNA, owing to the lower effective population size (N_e) of the mitochondrial genome. BEA state that

> 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.

Perceived statistical power does not trump elementary coalescence theory. There is no question that theory shows that coalescence times are a function of N_e, and the N_e of mtDNA is one-fourth that of nuclear loci on average. Although BEA cite circumstances that could theoretically alter this relationship, their claim lacks empirical merit for birds. Zink and Barrowclough (2008) showed numerous cases of geographically structured (but "shallow") mtDNA gene trees without microsatellite support, simply as a function of the longer coalescence times of nuclear loci (and not male-biased gene flow). BEA do not cite a single example to the contrary. What this means is that many populations or groups of populations of conservation concern have been isolated for more than 1 N_e generations (actually, $1N_{ef}$, the effective size of the female population) but fewer than 4 N_e generations. That is, they are of very recent evolutionary origin.

We agree that confidence intervals on estimates of gene flow (and other parameters such as time since divergence) are enhanced by use of multiple loci. But because many intraspecific taxa are recently diverged, an mtDNA survey should be a "must" for studies of conservation genetics. Surveys of nuclear loci only (e.g., Barr et al. 2008) run the risk of missing significant evolutionary signals (e.g., Lee and Edwards 2008), and we urge editors to reject phylogeographic studies based solely on microsatellite data.

ZEA suggested a stringent criterion for recognizing evolutionary distinctiveness, namely reciprocal monophyly on an mtDNA gene tree. Part of the reason for this suggestion is practical—there are about twice as many intraspecific mtDNA clades as there are species of North American birds (Zink 2004). By contrast, Barr et al. (2008) noted that all 12 of their population samples were statistically differentiated (albeit not diagnostically so) using microsatellite allele frequencies. We suggest that this is understandable from the nature of microsatellite data. In most birds, mating is not random throughout the range because constraints on dispersal create isolation-by-distance (e.g., Pruett et al. 2008), even in species of limited distribution such as the Black-capped Vireo. Microsatellite studies with large numbers of loci and individuals and geographically spaced sampling nearly always return statistically significant F_{ST} values because of this bias toward dispersal being higher among adjacent populations, reduced over longer distances, and, hence, nonrandom mating at the geographic scale. We stress that this sort of mathematically deduced structure does not equate with evolutionarily significant divisions. Given limited resources, it seems preferable to first establish the major evolutionary limits within avian species to serve as a framework for conservation, and we are not alone in suggesting that mtDNA is a useful tool for this task (Lerner et al. 2009). In the Black-capped Vireo, there is one logical historical unit (the species), not 12.

Apart from establishing the number of historically independent units in a species, what additional information could be derived from either mtDNA or nuclear loci (or both) to inform conservation planning? We might wish to know whether there are restrictions to gene flow, especially among populations in fragmented habitats. For example, BEA state that one of their goals was "to ascertain the level of contemporary gene flow" in the Black-capped Vireo. Although it is preferable to use mark and recapture methods to get truly contemporary estimates of dispersal, BEA made many inferences about gene flow from the microsatellite data. However, the words "gene flow" do not appear in the Results section of Barr et al. (2008). The reason is that to estimate gene flow from microsatellite data one has to make a number of assumptions that likely are not met (Whitlock and McCauley 1999, Aspi et al. 2009).

The more powerful coalescence approaches to estimating gene flow rely on knowledge of phylogenetic relationships among sequences, information that is lacking for microsatellites (without making tenuous assumptions). From the mtDNA sequence data, the average number of Black-capped Vireos exchanged between sample sites exceeds 5 per generation (R. M. Zink unpubl. data)), sufficient to prevent divergence via genetic drift. We are currently sequencing nuclear loci to provide confidence intervals on gene flow.

Although they have no direct measure of gene flow from their microsatellite data, BEA state that "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…." BEA apparently think that the mtDNA and microsatellite data are at odds and that the microsatellite data demonstrate that gene flow is insufficient to prevent genetic drift. BEA plotted $F_{ST}/(1 - F_{ST})$ versus the log of geographic distance (Rousset 1997). They found that nearby localities were statistically similar in microsatellite allele frequencies, but that at greater distances F_{ST} was significant but did not appear to increase linearly with increasing geographic distances (very low F_{ST} values occurred at all distance categories). BEA interpret this as a restriction on gene flow among localities separated by a given distance. However, this inference lacks statistical validation. Despite having to pool individuals, assuming genealogical relatedness within sample sites, we constructed a similar plot (Fig. 1) with mtDNA data from ZEA (which included Mexican breeding localities), also finding very low F_{ST} values at all distance categories, and a tendency for larger values at larger geographic distances. The plot was similar to that for microsatellites. Given recent increases in population size (Barr et al. 2008), it might simply be too early in the process of reestablishing the range for a typical isolation-by-distance pattern to emerge. We are less concerned with the shape

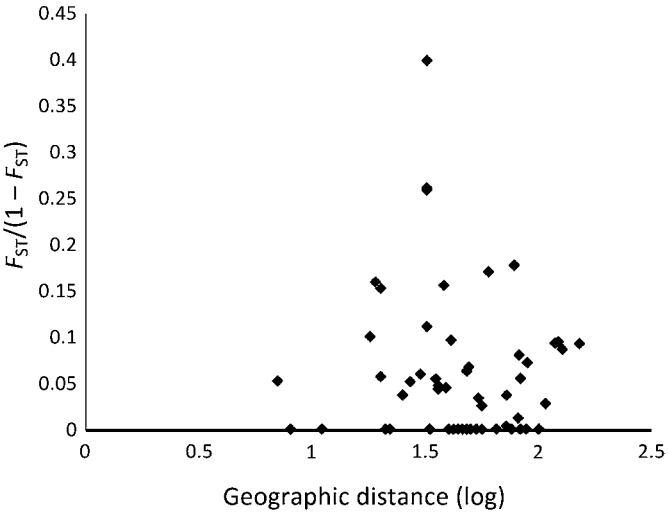

Fig. 1. Plot of genetic differentiation ($F_{ST}/(1 - F_{ST})$ versus log of geographic distance (scaled) for the mtDNA data of Zink et al. (2010). The plot shows low values of genetic differentiation at most geographic distances, and a general increase in genetic differentiation with increasing geographic distances.

of the plot than BEA are, given that geography explains very little of the genetic variance in the first place. Given that both analyses indicate some type of isolation-by-distance, it might be prudent to maintain viable populations within 100 km of each other.

BEA make a number of other comments in support of the use of microsatellites. For example, BEA state that

> 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.

Indeed, their confusion is understandable given that we said no such thing. But this brings up an important point from the literature, namely the misperception that mtDNA only recovers historical events and microsatellites recover information about current patterns of gene flow. We do believe that mtDNA finds historical divisions, those relevant for conservation, better than microsatellites. We are not sure what "contemporary" means in terms of N_e generations, the relevant time frame, but it is clear that microsatellites contain a mixture of current and historical information. Thus, this dichotomy is false.

BEA plead for equating statistical and biological significance. Between the point when two populations are first isolated and reciprocal monophyly is observed on mtDNA gene trees, significant geographic structuring can exist, as shown by significant geographic differences in haplotype or allele frequencies. However, where to impart biological significance on this continuous scale of genetic differentiation is unclear. BEA believe that because the alpha level associated with their F_{ST} value is <0.001, the observed structuring of 2.1% (or greater using methods that account for high allelic diversity) of the overall variation must have biological significance, whereas ZEA agreed with Björklund and Berget (2009) that one should not over-interpret such values. BEA compare their F_{ST} value to those from other hand-picked studies, finding theirs to be of greater magnitude and, therefore, biologically significant.

What, then, is meant by "biologically significant" in this case? Besides the level of differentiation, we consider it imperative to assess geographic pattern. The microsatellites do not indicate a coherent geographic pattern of restriction to gene flow (perhaps apart from the isolation of the Oklahoma population); rather, the pattern is idiosyncratic (see fig. 3 in ZEA). If there were a differentiable geographic pattern to the microsatellite data, we would consider it relevant, irrespective of the F_{ST} value. However, the current geographic structure lacks pattern and thus provides no management direction. The issue boils down to whether to consider the relationship of $F_{ST}/(1 - F_{ST})$ versus the log of geographic distance as biologically significant or an artifact of comparing relatively large numbers of loci, individuals, and geographic samples. Reviewing the microsatellite studies cited by BEA, we believe that the pattern they found is similar to patterns in species that are not fragmented or of any notable conservation concern and, therefore, that their plot yields no clear management implications, irrespective of the level of statistical significance. Our suggestion above to keep viable populations within 100 km of each other is precautionary, not a strong inference from either data set.

There is no question that phylogeography and conservation genetics would be drastically improved if we could sequence independent loci with the N_e of mtDNA and the mutation rate of microsatellites. Unfortunately, such loci do not exist. The question is how do we analyze nuclear loci, using allele-frequency based approaches such as microsatellites, or direct sequencing of nuclear loci, especially introns? We feel that the tradeoffs favor switching from allele-frequency approaches to sequencing studies (Zink 2010), especially given the potential to rapidly and quickly obtain new sequences. Thus, it is time to restrict the use of microsatellite data in studies of conservation genetics whenever evolutionary perspective is needed. The abandonment of allozymes in favor of mtDNA sequences was important and warranted because of the potential to use techniques of phylogenetics, and now coalescence, to derive inferences on population history. Analysis of gene sequences using coalescence analyses provides the most appropriate framework for both phylogeography and conservation genetics (Brito and Edwards 2009, Zink 2010).—Robert M. Zink (zinkx003@umn.edu),[1] Andrew W. Jones,[2] C. Craig Farquhar,[3] Michael C. Westberg,[1] and Jose I. Gonzalez Rojas.[4]

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