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PERSPECTIVE

The conservation utility of mitochondrial genetic diversity in macrogenetic research

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Introduction

As soon as molecular genetic data began to accumulate, population geneticists started to address questions about the nature of genetic variation across species (Soulé 1976). Early approaches to multi-species population genetics relied on harvesting population genetic information from the literature and merging it with other data to address multi-species scale questions (Soulé 1976; Loveless and Hamrick 1984; Nevo et al. 1984; Frankham 1996). The recent accumulation of open molecular genetic data in repositories such as GenBank and DRYAD has vastly increased the power, scope, and types of multi-species population genetic questions we can ask because raw data can be used for new purposes. Perhaps unsurprisingly then, we have seen an increased interest in this area of research (Miraldo et al. 2016; Lawrence and Fraser 2020; Manel et al. 2020; Millette et al. 2020; Schmidt et al. 2020a; Theodoridis et al. 2020). This new work has led to a coalescence of ideas around the emerging subdiscipline of *macrogenetics* (Blanchet et al. 2017). *Macrogenetics* has come to encompass population genetic research that repurposes genetic data, whether collected from the literature or harvested raw data, to address questions about the ecological and evolutionary causes and consequences of genetic variation across multiple species.

Having recently identified macrogenetic processes as a subject matter worth dedicated study, we are only beginning to identify the phenomena that fall under its purview. A recent focus has been the mapping of broad-scale patterns of genetic diversity and the exploration of its relationships with environments and species richness (Miraldo et al. 2016; Manel et al. 2020; Theodoridis et al. 2020). This line of inquiry is exciting with important implications for our understanding of biodiversity and its conservation. Miraldo et al. (2016) were the first to explore global patterns of genetic diversity by harvesting georeferenced publicly available mitochondrial DNA (mtDNA) sequences for mammals and amphibians. They detected a latitudinal gradient in mtDNA diversity in mammals and amphibians that mirrored species richness patterns. Manel et al. (2020) and Theodoridis et al. (2020) used similar methodological approaches focusing on fish and mammals respectively, also finding that latitudinal gradients in mtDNA diversity reflect species richness patterns.

Each of these papers highlights the need for the multi-layered conservation of biodiversity at the genetic and species levels and recognize that describing broad-scale patterns in genetic diversity will be necessary for this. Each paper also notes that our understanding of the processes underlying biogeographic scale genetic diversity patterns would be greatly enhanced by incorporating analyses of nuclear genetic markers. This is easier said than done. Raw nuclear genetic data suitable for estimating genome-wide genetic diversity is not programatically accessible in centralized data repositories; however, mtDNA is—hence the early emphasis on mtDNA diversity patterns. Our goal is to delve further into the caveats associated with the use of mtDNA markers for macrogenetics studies as noted by the authors of Miraldo et al. (2016), Manel et al. (2020), and Theodoridis et al. (2020). We expand on the potential drawbacks of mtDNA sequence data for macrogenetic studies and its interpretation for conservation decision-making within that context. The evolution of mitochondrial genomes across species is notably "capricious" (Galtier et al. 2009a). This makes linking mtDNA diversity patterns to population-level processes (Zink and Barrowclough 2008; Edwards and Bensch 2009; Bohonak and Vandergast 2011) and thus the conservation utility of mtDNA diversity gradients, fraught. We first describe the disconnect between mtDNA variation

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and adaptive potential—the quantity of interest for conservation. We then discuss the mismatch between patterns and population-level processes due to idiosyncrasies in mtDNA evolution. We conclude with potential future directions for the continued study of mtDNA patterns in macrogenetics.

Measuring genetic diversity that is relevant for conservation

The target when conserving genetic diversity is "genetic material of actual or potential value" (CBD and UNEP 2010). Genetic material of potential value refers to the genetic variation underlying a population's capacity to adapt—that is, the additive genetic variance in fitness in that population (Fisher 1930). Quantifying additive genetic variation requires the direct measurement of fitness across a large number of relatives for multiple generations, which is difficult for wild animal populations. Theory predicts that neutral estimates of genome-wide diversity should be proportional to additive genetic variation (Falconer and Mackay 1996) and empirical evidence suggests there is indeed a weak positive correlation (Mittell et al. 2015). It is thus notable that the first macrogenetic analysis of neutral nuclear genetic diversity found that it was negatively correlated with species richness (Schmidt et al. 2020b). This contrasts with the consistent positive correlation between mtDNA diversity and species richness. If patterns of nuclear DNA diversity—which is positively correlated with adaptive potential—trend opposite those of mtDNA, multi-species gradients in mtDNA diversity are not capturing genetic diversity of conservation value in a straightforward way. This is not to say that mtDNA does not have conservation value for some species-specific applications. For example, it can be useful for revealing glacial refugia or identifying cryptic lineages, and high levels of mtDNA diversity are rarely found in highly inbred populations.

mtDNA diversity patterns

Typical approaches for identifying mtDNA diversity patterns divide the globe into grid cells, then summarize diversity within cells by calculating the average nucleotide diversity for each species and finally averaging nucleotide diversity across species (Miraldo et al. 2016; Manel et al. 2020; Theodoridis et al. 2020). However, this diversity metric is hard to interpret because mtDNA mutation rates are highly variable across taxa (Nabholz et al. 2008b; Allio et al. 2017). For example, in mammals mitochondrial mutation rates can vary 100-fold across species (Nabholz et al. 2008b). Furthermore, not all grid cells contain the same species. Multi-species cell-wise averages thus seem likely to strongly depend on what species are in the cell, making comparisons of diversity across cells difficult to interpret. We suspect averages of mtDNA diversity taken across species likely obscure intraspecific spatial variation. We note that this is not a criticism of mtDNA per se, as the biological meaning of multi-species averages of nuclear genetic diversity would also be unclear. Mutation rate variation can be accounted for by treating species as a random effect in multilevel models (as in Millette et al. 2020; Schmidt et al. 2020a). We are uncertain of the extent to which multi-species averages can precisely capture patterns of mtDNA diversity.

Inferring processes

There has been considerable debate surrounding the use of mtDNA as a sole marker for inferring population and species-level pattern and process in other areas (Ballard and Whitlock 2004; Rubinoff and Holland 2005; Zink and Barrowclough 2008; Edwards and Bensch 2009; Bohonak and Vandergast 2011). There is now a general consensus among advocates and detractors of the various uses of mtDNA that it is most useful for inferring patterns (e.g., phylogenies), but alone it is often not sufficient for inferring processes shaping population history (Zink and Barrowclough 2008; Edwards and Bensch 2009). This is because the bulk of evidence suggests mtDNA diversity is not systematically or strongly related to ecology, demography, or genome-wide diversity (Bazin et al. 2006; Nabholz et al. 2008b; Galtier et al. 2009b; James and Eyre-Walker 2020). In practice maintaining a disconnect between pattern and process when interpreting our analyses is difficult because we are inherently interested in process (Edwards and Bensch 2009).

Identifying the common causes of biodiversity at species and genetic levels would considerably advance our basic evolutionary knowledge in addition to laying important groundwork for the joint conservation of species and genetic diversity. To varying extents, Miraldo et al., Manel et al., and Theodoridis et al. each interpret mtDNA diversity patterns in terms of processes related to ecology and demography. As noted above, the link between pattern and process in these cases is tenuous. The authors interpret their mtDNA diversity gradients in terms of established hypotheses for the origins of the species richness gradient. Hypotheses with mechanisms that might produce genetic diversity gradients positively correlated with species richness include evolutionary speed, climatic stability, and energy availability. Evolutionary speed hypotheses suggest that higher temperatures in the tropics cause higher metabolic rates and shorter generation times, leading to increased mutation rates and faster rates of population divergence and speciation. The climate stability hypothesis posits that environmental

instability causes recurring bottlenecks that limit both species and genetic diversity. Energy availability hypotheses suggest that high energy regions support larger populations and communities with high genetic diversity and species richness due to greater chances of population persistence. Reviews of these hypotheses can be found in Currie 1991; Mittelbach et al. 2007; Pontarp et al. 2019. These hypotheses hinge on ecological and demographic processes.

With respect to the evolutionary speed hypothesis, mutation rates in mtDNA are not strongly correlated with nuclear mutation rates—indeed, there is some evidence that mtDNA nucleotide diversity measured at silent sites (approximately neutral) is correlated with nuclear diversity only after applying corrections for differences in mutation rate (Allio et al. 2017). Furthermore, the relationship between metabolic rate and mtDNA mutation rates are complex and appear to not be consistent across taxa (Lanfear et al. 2007; Galtier et al. 2009a). One idea is that mutagenesis is driven by the increased production of reactive oxygen species in the mitochondria when metabolic rates are high, but oxidative damage is likely not the primary contributor to high mtDNA mutation rates (DeBalsi et al. 2017). Regardless, reactive oxygen species produced in the mitochondria during cellular respiration do not cause oxidative damage to nuclear DNA (Hoffmann et al. 2004). Thus it is unclear whether higher mtDNA or genome-wide diversity towards the equator is the expected pattern under the evolutionary speed hypothesis. Climate stability and energy availability hypotheses depend on environmental limits on population size. Yet, the relationship between mtDNA diversity and population size, or ecological and life history correlates of population size, is unclear and perhaps too weak to be useful (Bazin et al. 2006; Nabholz et al. 2008b; James and Eyre-Walker 2020). Given the peculiarities of mtDNA evolution and its likely non-neutral status it is not certain whether a general positive relationship with population size is the null expectation. Even so, relationships between mtDNA diversity at silent sites and commonly used proxies of population size do not vary in consistently expected directions (James and Eyre-Walker 2020). This lack of consistent relationship between mtDNA diversity and demography makes it ill-suited for testing general relationships between conservation relevant genetic diversity, environments, and species richness.

To illustrate this issue, we can take the well-founded prediction that human activity and urbanization should reduce genome-wide diversity by decreasing population sizes due to habitat fragmentation (Johnson and Munshi-South 2017). Synthetic analyses of genome-wide diversity of mammals through space and time consistently agree with this prediction (DiBattista 2008; Li et al. 2016; Leigh et al. 2019; Schmidt et al. 2020a). However, this relationship appears not to hold in general for mammalian mtDNA diversity (Miraldo et al. 2016; Millette et al. 2020; Theodoridis et al. 2020). Using mtDNA in this instance seems to miss declines in nuclear genetic diversity relevant for conservation.

Moving forward

The wealth of raw genetic data now available is exciting because of the new opportunities for exploring previously hidden levels of biodiversity it brings, and its value as a conservation tool. But the use of mtDNA as a metric for conservation-related decisions should be done with care. We note that Miraldo et al., Manel et al., and Theodoridis et al. do not make explicit conservation recommendations based on their findings, but the potential use of global maps of mtDNA diversity for the preservation of biodiversity is clear. For example, protected areas are a critical conservation tool, and the integration of genetic diversity patterns into protected area designation and management is needed for the maintenance of genetic diversity. Discussions about just how genetic diversity patterns could be integrated into international biodiversity conventions are underway (Hoban et al. 2020). Given our current understanding of the conservation utility of macrogenetic patterns of mtDNA diversity, decisions whether to integrate them into policy should be made carefully, with explicit presentations of the shortcomings of the marker. After discussing the caveats and nuanced interpretations of mtDNA gradients, we feel the case for its use should be strongly argued, not taken for granted. Indeed, the general targeting of regional conservation actions based on global patterns of interspecific mtDNA variation could inadvertently capture regions of low adaptive capacity, contradicting our conservation goals (Schmidt et al. 2020b). Thus, mtDNA diversity gradients should not be used uncritically to provide general conservation guidance, nor to test general links between conservation relevant genetic diversity and ecological or environmental processes. mtDNA variation is an important element of genetic heritage, but its variation will primarily be related to cellular respiration and does not reflect genome-wide diversity well.

Beyond concerns about its relevance for conservation, we reiterate that macrogenetic patterns in mtDNA are not uninteresting and provide an opportunity to test other hypotheses. The longevity hypothesis, for example, posits that selection in long-lived species acts to lower mtDNA mutation rates and reduce oxidative damage to the mitochondrial genome which may contribute to ageing (Nabholz et al. 2008a). The clear spatial relationships between body size and environmental temperature (Bergmann's Rule), and life history correlations between body size and longevity (Stearns 1992) suggest a possible mechanism capable of producing the consistently identified broad-scale gradients in mtDNA diversity that positively correlate with species richness. It

would also be worthwhile to more directly test purported links between temperature, metabolic rate, and mitochondrial mutation rate. For instance, whereas metabolic rates in ectotherms increase with environmental temperature, this relationship is more complicated for endotherms. If a causal connection between temperature, metabolism, mutation rate, and mtDNA diversity exists, we would expect it to be more apparent in ectothermic species. Importantly, both of these ideas require focusing on those species which have enough data for intraspecific tests to identify and compare patterns.

mtDNA can clearly inform conservation decision-making in some species-specific contexts. It is however, unclear that multi-species macrogenetic patterns of mtDNA variation are useful conservation tools. We echo the calls of Miraldo et al., Manel et al., and Theodoridis et al. to continue exploring these patterns with multiple marker types. In the meantime, we call for a very careful presentation of just what mtDNA data can tell us about the type of genetic biodiversity we want to conserve.

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Compliance with ethical standards

Competing interests The authors declare no competing interests.

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