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Conservation genetics of bees: advances in the application of molecular tools to guide bee pollinator conservation

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Bees (Hymenoptera: Apoidea) are a monophyletic group of insects comprising an estimated 20,000 species distributed in a wide range of latitudes, including all terrestrial ecoregions except for Antarctica. One of the special characteristics of bees is that they are insects that exclusively use pollen and nectar to feed their brood-except for a handful of necrophagous stingless bee species (Roubik 1982). As a result of their dependence on floral resources, bees are the most important pollinators for the majority of flowering plant species (>80%, Ollerton et al. 2011). The close interactions between bees and plants have shaped the deep evolutionary histories of bee families (Cardinal and Danforth 2013) and the demographic histories of bee species across natural, agricultural and urban landscapes (Dellicour et al. 2016; López-Uribe et al. 2016). In addition to managing bees for crop pollination, some bee species, including honey bees and stingless bees, have been used for centuries by humans for honey production (Crane 2013). An increasing number of bee species (e.g., *Bombus terrestris, Megachile rotundata, Osmia cornifrons*) are now being commercially produced for greenhouse and outdoor crop pollination in response to increases in the intensification of agricultural systems and related reductions in wild pollinator habitat (Aizen et al. 2009).

As a result of habitat loss and a number of additional potential causes, populations of both managed and unmanaged bees have been reported to be declining during at least the last decade in different parts of the world (Biesmeijer et al. 2006; vanEngelsdorp and Meixner 2010; Cameron et al. 2011; Bartomeus et al. 2013). Because of their important role as key pollinators, the possibility of bee decline has raised concern about the potentially disastrous impacts of their loss on ecosystem functioning, which could affect multiple trophic levels and threaten food security for humans (Allen-Wardell et al. 1998; Potts et al. 2016). Thus, the decline of bee populations is of great conservation concern, and efforts from researchers, educators and policymakers are focusing on developing effective strategies to preserve and restore bee populations across habitats types (Brown and Paxton 2009; Dicks et al. 2013). However, the development of effective conservation strategies requires an understanding of key evolutionary and ecological processes, at both historic and contemporary time scales, such as the drivers of speciation, the generation and conservation of genetic variation and the factors influencing demography, colonization, migration and foraging (Frankham et al. 2004; Epps and Keyghobadi 2015).

Molecular tools can provide information relevant for a number of research themes underlying bee conservation, ranging from phylogeography and genetic variation across landscapes, to paternity assignment for colony-level foraging analyses (reviewed in Woodard et al. 2015). For example, phylogeographic studies are necessary to better understand bee diversity and to identify groups of conservation concern. These studies may be particularly relevant for the conservation of bee groups that have experienced high diversification rates, but exhibit little morphological differentiation or substantial convergent morphology (e.g. sweat bees or bumblebees, respectively). Determining

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how genetic variability is distributed across a species' geographic range is critical to identify units for conservation efforts (Duennes et al. 2012; López-Uribe et al. 2014; Dellicour et al. 2015).

Another important research theme in conservation genetics is understanding how landscape composition and topographic complexity drive population structure. Bees are flying insects and can often forage long distances, but it is unclear which habitat features influence their patterns of dispersal. Population genetic studies can shed light on potential drivers of dispersal from inferred genetic structure (Jha 2015). At contemporary time scales, reduced genetic diversity in a population can increase the risk of inbreeding (Zayed and Packer 2005), and inbreeding depression can result in fitness declines, such as slower growth rates and lower reproductive rates (Whitehorn et al. 2009). In bees and other haplodiploid insects with single locus complementary sex determination, the risk of inbreeding depression associated with the loss of genetic diversity is higher than in diploid organisms because sterile diploid males are produced if the sex determination locus is homozygous. Therefore, estimating levels of male diploidy can reveal genetic degradation at the population level and could assist in the identification of populations of conservation concern (Zayed and Packer 2005).

The study of relatedness within nesting aggregations and across landscapes is another important theme in conservation genetics. Specifically, examining bee relatedness across spatial scales can indicate the most important factors influencing dispersal and nest fidelity (López-Uribe et al. 2015), as well as the potential drivers of social behavior (Soro et al. 2010) and colony-level foraging patterns (Jha and Kremen 2013). Lastly, the availability of next generation sequencing (NGS) tools now allows for the development of research programs that identify adaptive variation in bee populations and the potential role of symbionts (e.g., microbes) in adaptive processes. Across these many research themes, conservation genetics information could be used to enhance breeding programs for managed bees, identify areas of great conservation value and improve habitat restoration programs for threatened species.

In order to update researchers on these themes, in August of 2015 we organized a symposium on the "Conservation Genetics of Bee Pollinators" at the Ecological Society of America Meeting in Baltimore, MD (USA). The outcomes of this symposium exceeded our expectations in synergy and breadth and provided the inspiration for this Special Issue of *Conservation Genetics*. Here, we present studies that use both traditional and novel genetic approaches to advance our understanding of (1) *phylogeography*: how geography and climate have shaped the evolutionary history of wild bee pollinators, (2) *population structure, inbreeding and foraging*: how the distribution

of genetic diversity, ploidy and relatedness of individuals changes across landscapes, and (3) *genetic consequences of bee management*: how commercial bee management can influence patterns of genetic diversity and structure of key crop pollinators. These studies provide essential data for evidence-based recommendations regarding bee conservation and management strategies. Though this Special Issue is diverse and broad, it also highlights the critical need for additional studies on the genetic diversity of bees, the genetic structure of populations across space and the meaning of genetic diversity and structure for population demography and adaptation.

Phylogeography

Phylogeographic studies investigate spatial patterns of genetic diversity across large geographic scales to understand the role of geographic distance, topographic complexity and climate instability, among other potential factors, in mediating population connectivity. Koch et al. (2017) use a comparative approach to investigate levels of connectivity among four sympatric bumble bee (*Bombus*) species in the Pacific Northwest of the United States. They identify areas within the Pacific region that harbor isolated populations of the four bumble bee species and point out differences in ecological niches that may drive isolation by distance (IBD) patterns across sympatric species. Using coalescent approaches and Bayesian clustering methods, Cěrná et al. (2017) reconstruct the historical demography of two closely related species of Palearctic *Anthophora* bees. Their results show signatures of reduction in effective population size that coincide with increasing temperatures after glaciations, a result expected for cold-adapted species. Frantine-Silva et al. (2017) also provide insight on likely climate drivers of phylogeographic structure. Specifically, they analyze variability of mitochondrial genes in the neotropical orchid bee *Euglossa iopoecila* and find greater genetic diversity in central regions of the Atlantic forest (Brazil), where there was likely greater past climate stability, than in the southern regions, which exhibit signatures of recent population expansion. These results are consistent with the historical biogeography of other forest specialists in this ecoregion (Carnaval et al. 2009). In addition to exploring environmental drivers of phylogeographic structure using mitochondrial (COI) microsatellites, Duennes et al. (2017) add a morphometric analysis to their study of the Mesoamerican bumblebee *B. ephippiatus*. They find evidence of multiple evolutionarily significant lineages that are maintained by different geographic and environmental factors across the distribution of the species. Last, McKendrick et al. (2017) also investigate whether microsatellite nuclear markers support three cryptic species within the

European bumblebee *B. lucorum;* indeed, they detect three discrete groups that correspond to three mtDNA lineages previously identified.

Population structure, inbreeding and foraging

Understanding how anthropogenic changes shift the distribution and levels of genetic diversity in bee populations may elucidate important demographic mechanisms associated with possible population decline. Lecocq et al. (2017) investigate IBD patterns of multiple social and solitary European bee species in a comparative individual-based versus population-based differentiation framework. Their results show that individual based approaches have the potential for greater detection power of IBD patterns and thus suggest a reassessment of the exclusive use of population-based analyses when investigating signatures of dispersal. Schenau and Jha (2017) examine genetic structure and diploidy in males of *B. vosnesenskii* across mainland and islands, including landscapes that exhibit a gradient of habitat quality (availability of flowers and nesting sites). Their findings indicate high levels of male diploidy, especially in the mainland and in areas with limited nesting habitat. Soro et al. (2017) also focus on male bees and examine patterns of genetic diversity and genetic structure in the males of two orchid bee species (*E. dilemma* and *E. viridissima*) across different levels of human disturbance and between mainland and islands. They find significantly reduced genetic diversity on islands, but no relationship between habitat loss and genetic diversity, though they do document low levels of both population structure and male diploidy. In another study of euglossine male bees, Suni (2017) likewise finds weak differentiation for *E. imperialis* across fragmented landscapes in Costa Rica, despite their dependence on forested habitat for nesting. Similarly, Landaverde-Gonzalez et al. (2017) revealed very little genetic structure and no signatures of recent bottlenecks for the stingless bee *Partamona bilineata* in Guatemala. These three tropical bee studies and others (e.g., Jaffé et al. 2016) suggest that some species can be quite resilient to habitat degradation despite the high rates of fragmentation these regions are experiencing. At a finer spatial scale, one study in the Special Issue examines the relatedness and colony-identity among individuals across different floral resource landscapes. Pope and Jha (2017) develop a modeling approach to evaluate the efficacy of different sampling approaches to build molecular-based foraging clouds for social bees. They provide a quantitative comparison of the multiple sampling methods currently used by most scientists to map colony-level foraging and then make recommendations for future mapping of spatial foraging analyses.

Genetic consequences of bee management

The process of animal domestication is associated with dramatic changes in the phenotype and genotype of domesticated species. While humans have bred some bee species for honey production and crop pollination, these are not considered domesticated species because they do not significantly differ from their wild ancestors. Understanding how bee management has changed the frequency and distribution of neutral and adaptive genetic diversity in bees is critical for the conservation of important genetic variability that can be used for breeding programs in these species. Using microsatellite data, López-Uribe et al. (2017) compare feral and managed honey bee (*Apis mellifera*) genetic diversity and find that it positively correlates with immune response in feral, but not managed colonies. This study highlights the importance of wild populations as sources of genetic variability for breeding programs aimed at supporting healthier managed species. In another comparative study, Suni et al. (2017) investigate the population structure of *B. impatiens* in natural and agricultural areas of the Northeastern region of the United States, where managed bumblebee colonies are used for cranberry pollination. Based on Bayesian clustering analyses, this study shows that individuals from wild and commercial colonies are genetically distinct and suggest that managed colonies may not be successfully establishing in the areas where they are introduced. In one of the first molecular studies of the solitary managed alfalfa leafcutting bee *M. rotundata*, Strange et al. (2017) use microsatellite-based population structure analyses to reveal that it exhibits low population structure in the introduced range, likely due to frequent movement by humans. Further, the authors found no signatures of a founder event after its introduction to North America, suggesting that genetic diversity is being maintained by breeding facilities in Canada before shipping to the southern US. While a number of bee species are bred for management, so far only the honey bee *A. mellifera*, has been subjected to breeding programs to select for specific traits (e.g., efficiency in pollination, resistance to pathogens). However, establishing breeding programs may be necessary for other managed bees in the near future. In a quantitative meta-analysis, Koffler et al. (2017) gathered published heritability estimates for ants, bees and wasps and found that they are influenced by trait type, with morphological traits exhibiting the highest heritability estimates, and defense and metabolism-related traits showing the lowest estimates of heritability. This study suggests that artificial selection for some traits may be feasible within pollinator breeding programs, even though further research is necessary. Among other important traits that may be considered for optimal commercial bee management programs is their association with microorganisms, an interaction that can

often prove beneficial for bee health (reviewed in Engel et al. 2016). In this Special Issue, Graystock et al. (2017) examine the microbiota of two non-corbiculate bee species (genera *Ceratina* and *Megalopta*) and their relationship to pollen microbes. They report that, unlike *A. mellifera*, both species have similar microbiota that match their pollen provisions and only share one phylotype across all three bee species. They discuss their results in the context of the importance of considering healthy microbiomes for pollinator restoration projects.

Closing remarks

Even though we have made considerable progress in understanding the genetic factors that influence bee decline, the development of cost-effective high throughput sequencing technologies has opened new possibilities for the application of genomic approaches to conservation genetic questions in model and non-model bee species (Lozier 2014; Harpur et al. 2015). In the final piece of our Special Issue, Lozier and Zayed (2016) review this topic and discuss a conceptual framework for how different genomic tools can be applied to a wide range of important questions for bee conservation (Fig. 1, Lozier and Zayed 2016). Incorporating these new methods will greatly advance our knowledge and their application to enhancing conservation and restoration of wild and managed bee populations.

In summary, the field of bee conservation genetics has grown tremendously in the past decade since the first review of the field (Zayed 2009) and now spans an increasingly diverse set of research topics. This Special Issue highlights a number of key findings within these research themes. First, it reveals the importance of climate and geography in driving bee phylogeography. In particular, it highlights the role of climatic factors in current patterns of bee population structure. These results call attention to the potential impact of climate change on the stability of bee populations and species distribution. Conservation genetic studies that combine molecular data with climate niche models will continue to provide insights into predictions of population persistence and structure. A second important contribution of the studies in this special issue includes that tropical bee species are more resilient to anthropogenic change than traditionally expected. More case studies with a broader phylogenetic sampling are necessary to establish if this is a general pattern for all tropical bees, or if this finding is lineage specific and constrained to corbiculate bees of the wet forested Neotropics. Indeed, bees from temperate areas such as bumble bees seem to be much more dependent on habitat quality for enhanced nesting density, reduced inbreeding and lineage survival (Carvell et al. 2017). Last, a number of studies illustrate the promising

role of genetics/genomics as a correlate for key traits useful for enhancing wild and commercial bee health. These studies highlight the need of conserve genetic diversity in wild bee populations and understanding its structure and function to design successful breeding programs.

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