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Conservation Genetics and Genomics of Amphibians and Reptiles

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Abstract

Amphibians and reptiles as a group are often secretive, reach their greatest diversity often in remote tropical regions, and contain some of the most endangered groups of organisms on Earth. Particularly in the past decade, genetics and genomics have been instrumental in the conservation biology of these cryptic vertebrates, enabling work ranging from the identification of populations subject to trade and exploitation, to the identification of cryptic lineages harboring critical genetic variation, to the analysis of genes controlling key life history traits. In this review, we highlight some of the most important ways that genetic analyses have brought new insights to the conservation of amphibians and reptiles. Although genomics has only recently emerged as part of this conservation tool kit, several large-scale data sources, including full genomes, expressed sequence tags, and transcriptomes, are providing new opportunities to identify key genes, quantify landscape effects, and manage captive breeding stocks of at-risk species.

INTRODUCTION

Amphibians and nonavian reptiles comprise two of the largest, least well known, and most threatened groups of vertebrates on earth. As of this writing, 6,409/7,304 (92%) of the currently recognized species of amphibians and 4,204/9,909 (42%) species of nonavian reptiles have been evaluated for their conservation status by the International Union for Conservation of Nature (IUCN) (1). The results are not encouraging (**Figure 1**). For amphibians, 38% of the evaluated species fall into the threatened categories (extinct, extinct in the wild, critically endangered, endangered, vulnerable, or near threatened), 36% are of least concern, and the remaining 26% are data deficient; for nonavian reptiles, the numbers are 28%, 54%, and 18%, respectively. Most species, including the hyperdiverse tropical taxa, are poorly studied ecologically, and their nocturnal habits and cryptic behavior continue to hamper field studies that support conservation actions (**Figure 1**, data-deficient taxa). In addition, amphibians and reptiles present an amazing array of life history strategies and physiological conditions that affect their

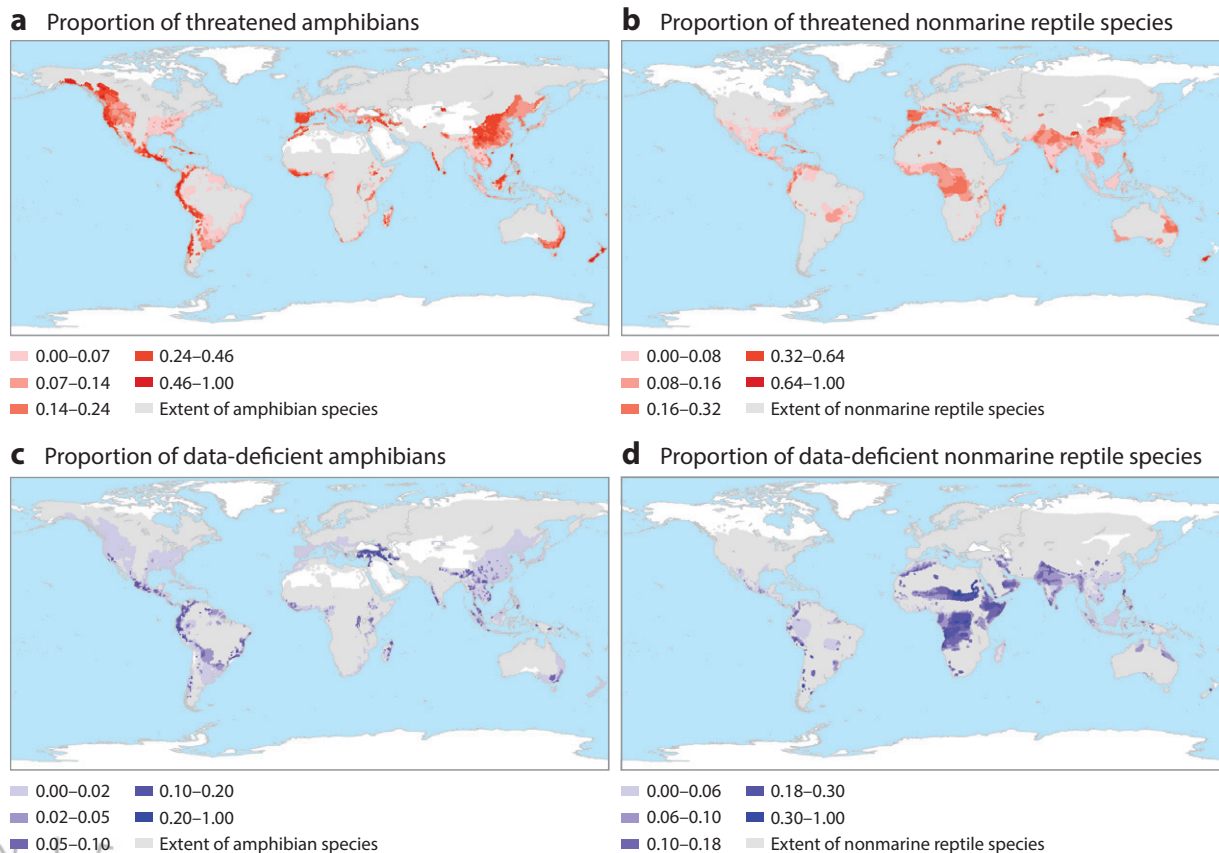


Figure 1

Globally threatened and data-deficient reptiles and amphibians per equal-area hexagonal cell ($\sim 4,500 \text{ km}^2$), expressed as a proportion of all species present (not including data-deficient species): (a) threatened and near-threatened amphibians and (b) threatened and near-threatened nonmarine (terrestrial and freshwater) reptiles, each expressed as a proportion of all species in the data set; (c) data-deficient amphibians; and (d) data-deficient nonmarine (terrestrial and freshwater) reptiles. All species distributions and threat assessments derived from the International Union for Conservation of Nature (1).

evolution, ecology, and conservation. Amphibians present diverse life history strategies and physiological adaptations, including biphasic life histories, direct development, and extensive parental care (2, 3). Most amphibians possess permeable skin and are susceptible to toxicological challenges, novel pathogens, and devastating disease outbreaks. Many reptiles are characterized by narrow thermal tolerances and temperature-dependent sex determination (4), raising questions about the effects of current and future climate change on population stability. Both amphibians and reptiles are generally low-vagility, sedentary ectotherms whose population biology may (but need not) be adversely affected by habitat fragmentation and anthropogenic alterations.

In this review, we summarize the ways that genetic data have contributed to our understanding of major threats and their solutions for the conservation of amphibians and reptiles (hereafter we use the term reptile to refer to nonavian reptiles, fully realizing that birds are well-nested phylogenetically within nonavian reptiles). Although they encompass the full sweep of ecological and evolutionary adaptations found across virtually all ectothermic vertebrates, reptiles and amphibians also share enough features that treating them in a single review makes sense.

We divide our review into three major sections that focus on the use of genetics to identify species and lineages of concern, set conservation prioritizations, and inform management actions. Throughout, we strive to succinctly summarize the most important recent literature and to provide a forward-looking view of the opportunities that larger-scale genomic analyses may offer to the conservation and management communities (Figure 2; Supplemental Figure 1, follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>). Our very strong view is that the emerging field of conservation genomics is especially well-suited to amphibians and reptiles, given the difficulty of making direct observations of most species.

USING GENETICS TO IDENTIFY SPECIES AND LINEAGES OF CONCERN

A truism of conservation biology is that you cannot protect what you do not recognize, and genetics has been used to identify populations, species, and clades of conservation concern at a wide variety of scales and levels. Genetic tools have been particularly effective in quantifying effective population size (N_e) in difficult-to-study species for which direct estimates of the number of breeding individuals are rare. Genetic methods are frequently the only way to estimate effective population sizes, as well as historical patterns of population expansion or decline (5–7). Somewhat more broadly, conservation planning at the regional and landscape levels requires information on past and current connectivity between adjacent populations. By combining genetic analyses that quantify migrant exchange among populations with spatial geography, several studies have successfully inferred those parts of the landscape that are most used by reptiles and amphibians, sometimes with surprisingly counterintuitive results (8–10). Finally, genetic analysis can help conservationists understand the history and distinctiveness of population segments within a declining species. For threatened vertebrates, including reptiles and amphibians, distinct population segments (DPSs) are eligible for protection under the US Endangered Species Act, and their determination necessarily relies on genetics. We outline common tests for delineating evolutionarily significant units (ESUs), which are frequently used for setting conservation priorities; present examples of where they have been critical for the conservation of reptile and amphibian species (11–13); and briefly highlight opportunities for improving estimates with genomic approaches.

Sequence accumulation through time in nucleotide and EST databases

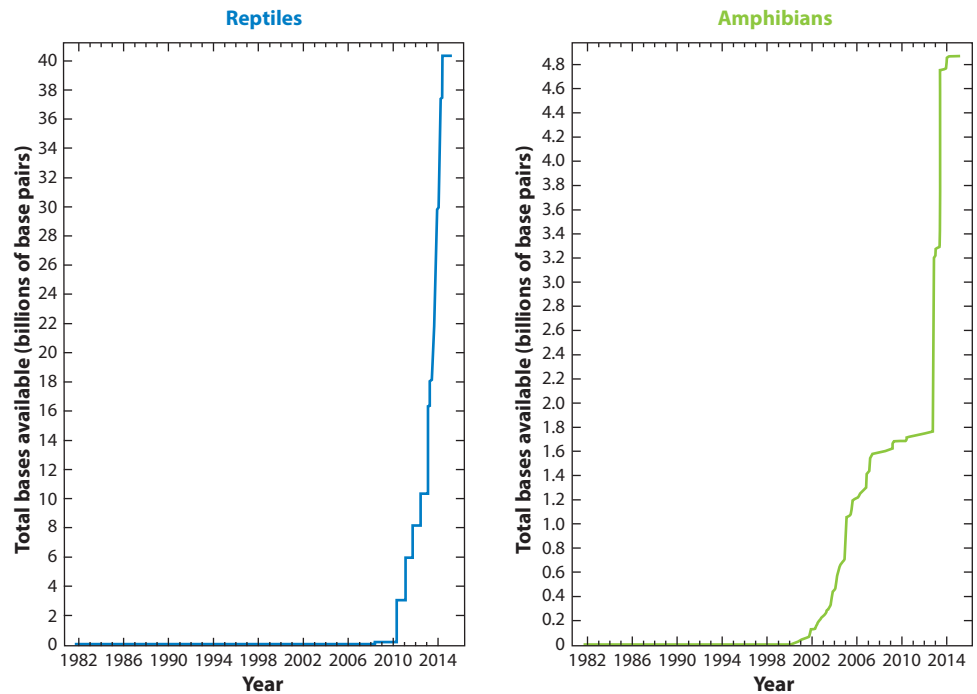


Figure 2

Cumulative base pairs available for reptiles and amphibians in the National Center for Biotechnology Information's (NCBI's) nucleotide and EST databases since the launch of GenBank in 1982. Note that the scales of the two graphs are different, with reptiles representing nearly 10 times as many base pairs as amphibians to date. Sequences from the nucleotide and EST databases were fetched using NCBI's E-Utilities and binned by month according to release date. The source code for data aggregation and figure generation can be found at <https://github.com/atcg/reptileAmphibianSeqGrowth>.

Population Estimation

Effective population size (N_e) of a population is a critical piece of information for its management (14, 15). Because reptiles and amphibians are often difficult to count in the wild, direct estimates of the number of breeding individuals are rare. Genetic methods offer a valuable alternative that allows researchers not only to estimate the effective breeding size of a population but also to infer historical patterns of population expansion or decline (6, 16, 17).

Populations that are small and isolated are negatively affected by inbreeding and genetic drift, and one central goal of conservation management is ensuring that populations remain large and maintain essential genetic diversity. A prevailing pattern in recent genetic studies has been that N_e as determined by genetic analysis is often surprisingly small. This was the case in a 1999 study of long-toed salamanders (*Ambystoma macrodactylum*) that found all six of the investigated populations to have an N_e under 100 (18). Similarly, Phillippsen et al. (19) inferred N_e of less than 50 individuals for all of the 64 investigated populations of *Rana pretiosa*, *Rana cascadae*, and *Rana luteiventris*. The same study, however, did find evidence that the N_e of some populations of *Rana* (*Lithobates*) *pipiens* ranged into the thousands.

Several studies have characterized the historical fluctuations of reptile and amphibian populations using genetic data, which can be useful for conservation efforts even when the inferences

made refer to a time period thousands of years ago. For instance, Myers et al. (20) analyzed mitochondrial DNA (mtDNA) from the northern desert nightsnake (*Hypsiglena chlorophaea*) and found that population sizes remained stable during the Pleistocene despite extensive glacial activity, suggesting that these snakes may be well adapted to a changing climate. Conversely, Guiher & Burbrink (21) found evidence that several populations of the venomous cottonmouth and copperhead (genus *Agkistrodon*) experienced rapid population growth following the last glacial maximum. For a conservation issue as complex as global climate change, inference of past performance in times of extreme climate change provides an important tool for assessing future risks. Unfortunately, many of these past studies have relied on one or a few genes, and most have exclusively used mtDNA. The use of genomic-scale data sets will greatly improve the precision of such estimates by averaging over many gene histories. Perhaps most importantly, genomic and transcriptomic approaches (see below) offer the opportunity to identify and protect the genetic polymorphisms that will enable adaptation in the face of rapid climate change.

Landscape Use and Landscape Genetics

Conservation plans for species are being implemented on regional and landscape levels with increasing frequency, and connectivity between adjacent populations is a critical contributor to the persistence of many threatened species (22). By combining genetic analyses that inform us about migrant exchange among populations with spatial geographic analyses to identify important landscape characteristics, researchers can infer which parts of the landscape are most used by reptiles and amphibians (10, 23). This knowledge is essential when designating conservation regions and corridors for threatened species.

A common theme in landscape genetic studies is determining whether effective distances as determined by the presence of suitable habitat between populations better predict genetic distances than do straight-line distances. In one such study, researchers found that genetic diversity in timber rattlesnakes (*Crotalus horridus*) was not predicted well by distance alone but was positively correlated with the amount of basking habitat available across the landscape (24).

Not only can spatial features of the landscape be compared against Euclidean distances between populations, but several landscape features can also be evaluated against one another to assess their relative importance in determining dispersal. For instance, Wang et al. (8) used least-cost path analysis to discover that chaparral is more conducive to California tiger salamander (*Ambystoma californiense*) movement than are grasslands, a surprising result given that these animals are typically associated with grassland habitat. In another amphibian study, Spear et al. (25) analyzed microsatellite variation in the closely related blotched tiger salamander (*Ambystoma tigrinum melanostictum*, sometimes classified as *A. mavortium melanostictum*) and found that some landscape variables (such as rivers) were associated with increased gene flow, whereas others (for example, elevation) were associated with reduced gene flow.

Genetically informed migration rate estimates can also be important for conservation planning, as they can tell us a great deal about the metapopulation dynamics of a region. High migration rates often indicate that local extinctions of populations are regularly rescued via natural recolonization, whereas low migration rates suggest that localized extinction events may be more permanent. With this in mind, Howes et al. (26) combined landscape and statistical genetic analyses to discover populations of Blanding's turtle [*Emys (Emydoidea) blandingii*] and black ratsnake (*Pantheropsis obsoletus*) at the edges of their respective ranges that acted as sources of genetic variation for the surrounding areas. Similarly, Manier & Arnold (27) and McCartney-Melstad et al. (28) found evidence of asymmetrical gene flow in garter snakes (*Thamnophis elegans* and *Thamnophis sirtalis* from western North America) and yellow anacondas (*Eunectes notaeus*

from Argentina), respectively. In these latter two cases, asymmetric gene flow from putative source to sink populations indicated the importance of maintaining natural metapopulation dynamics in the face of regular extinction-recolonization events. These studies emphasize the importance of source-sink dynamics for management, because sources of genetic diversity are likely to be more important for the preservation of species than are migratory sinks that have a greater chance of experiencing temporary localized extinction.

Many landscape genetic studies test for the impacts on dispersal of natural landscape features, such as topography. The impacts of these landscape features on genetic structure are often easier to assess than explicitly anthropogenic factors because natural features have usually been present for many generations, leading to landscape-level patterns that can often be identified even with modest amounts of population genetic data. For example, Funk et al. (9) found a high degree of interbreeding between Columbia spotted frog (*R. luteiventris*) populations in low-elevation ponds but low amounts of interbreeding between low- and high-elevation ponds. This suggests that elevation limits dispersal in this species and that populations along elevational gradients are important conservation targets. Similarly, Savage et al. (29) found that dispersal between ponds was extremely limited for southern long-toed salamanders (*Ambystoma macrodactylum sigillatum*), likely as a result of the topographically complex habitat of the Sierra Nevada region where they were studied.

Although the contributions of anthropogenic factors to spatial genetic patterns are often difficult to assess because they have affected gene flow for relatively few generations, researchers have begun to explicitly test for impacts of current and forecasted human factors on connectivity between populations of reptiles and amphibians. In one such example, Tolley et al. (30) combined landscape genetic analysis with future climate models to predict the impacts of impending climate change on three species of lizards in the Cape Floristic Region of South Africa. They found that future climate change scenarios would likely lead to reduced gene flow in both dwarf chameleons (*Bradypodion* spp.) and Burchell's sand lizard (*Pedioplanis burchelli*) but that there would likely be no such effect on the southern rock agama (*Agama atra*).

Management actions for reptile and amphibian species are spatially explicit decisions, so spatially explicit characterizations of genetic diversity are inherently important. As we begin to better understand the impacts of different landscape variables across many different reptile and amphibian species, we can more efficiently prioritize conservation lands to take into account connectivity among populations. Particularly as very large data sets become more accessible, landscape genomics offers tremendous potential to allow managers to better determine how to preserve critical dispersal corridors, even for cryptic, difficult-to-study amphibians and reptiles. The value of genomic data is particularly strong in situations where very low levels of differentiation resulting from recent anthropogenic land use changes are beginning to be expressed genetically (31).

Defining the Units of Conservation

Because conservation at the species level may not adequately protect intraspecific diversity, conservation priorities below the level of species are often a prerequisite for both conservation and recovery. For threatened species, the critical concern is most often the preservation of intraspecific genetic diversity, given its importance for current and future adaptive evolutionary potential. Unfortunately, there are no universally established and accepted methods for identifying these units, a situation that has often hampered effective management actions. Many definitions have been suggested to aid in delineating intraspecific units for conservation, primarily focusing on management units (MUs) and ESUs, but the implementation of these concepts is uneven across

studies and taxonomic groups. For threatened vertebrates, including reptiles and amphibians, DPSs are eligible for federal protection under the US Endangered Species Act, and the determination of what is or is not a DPS is often made on the basis of genetic patterns. However, there are also agency-specific criteria that must be met before a genetic unit can be formally considered a DPS and become a candidate for listing.

ESUs have become the prominent guiding concept in defining these units for the conservation community more generally, although in the almost 20 years since the concept's inception, an exact definition has yet to be agreed upon. The evolution of the ESU concept has been reviewed elsewhere (32, 33). The definition most frequently used for conservation is based upon Moritz's (34) criteria of protecting historically isolated units as identified by having significant divergence at nuclear loci and reciprocal monophyly of mtDNA (33, 34). For many study systems, this is the simplest definition to implement (33). For example, analysis of mtDNA of the Australian agamid lizard *Diporiphora* (previously *Amphibolurus*) *nobbi* divided the species into two ESUs (35). Because one of these units is found solely in small habitat remnants in an agricultural zone, the species as a whole has the potential to lose a significant amount of biodiversity (35).

MUs are also frequently used to designate units of conservation. Less stringently differentiated than ESUs, MUs are generally defined as demographically independent populations, as evidenced by divergence in allele frequencies, but which lack the evolutionary depth and longevity of ESUs (32, 34, 36). Two recent analyses of Blanding's turtle, *E. blandingii*, and the timber rattlesnake, *C. horridus*, found that although they do not meet the strict criteria for ESUs, populations east and west of the Appalachian Mountains were genetically differentiated and had sufficiently low gene flow between east and west populations to qualify as MUs (11, 24). Given that both species are in decline across parts of their respective ranges, the recognition of these MUs is a key element in regional management.

ESUs and MUs have traditionally been based on neutral (or at least perceived neutral) genetic variation and fail to recognize potential adaptive variation (32, 33, 37, 38). Thus, reciprocal monophyly in mtDNA may not be present in cases of recent evolutionary divergence where genetic differentiation may be coupled to ecologically important morphological or physiological divergence. A case in point is the IUCN critically endangered Panamanian golden frog (actually a toad, family Bufonidae) *Atelopus zeteki*, in which proposed ESUs are not reciprocally monophyletic but exhibit significant genetic and morphological differentiation (37). Perhaps the most important potential pitfall of a strict adherence to the ESU/MU criteria is that key life history traits are also often overlooked. Particularly for amphibians with diverse sets of life history attributes, this oversight is often unacceptable. In the Oklahoma salamander, *Eurycea tynerensis*, strict application of genetic criteria would ignore such life history traits (39).

The governmental agency overseeing the protection of endangered species often sets broad guidelines for determining conservation units, such as DPSs under the US Endangered Species Act. These units are typically loosely based on ESUs and MUs but may be broadened to include ecologically and distributionally relevant information not captured purely by population genetic differentiation. For example, a population can be defined as a DPS if it is geographically discrete from other populations, biologically or ecologically significant, and threatened or endangered (40). The first step in effective management is to identify such units, assess their conservation status, and bring that information to managers for consideration. An analysis of the Cascades frog, *R. cascadae*, identified two clearly delineated DPSs based upon genetic differentiation of mtDNA and nuclear DNA (nDNA). A third population exhibited deep divergence in mtDNA but not in nDNA, suggesting the possibility of gene flow in recent evolutionary history. However, the authors suggested that because this third population is currently physically isolated and inhabits a unique habitat, it should also be considered as a separate DPS (41).

One of the relatively few species for which the DPS designation has been used in a regulatory framework is the federally and state-endangered California tiger salamander, *A. californiense*. Based on range-wide sampling of 696 individuals, Shaffer et al. (42) demonstrated reciprocal mtDNA monophyly of three geographically isolated sets of populations, which was then used by the US Fish and Wildlife Service to identify three DPSs (Sonoma, Santa Barbara, and Central). One of the key features of such an approach is that individual DPSs can be managed independently—in the case of *A. californiense*, the Sonoma and Santa Barbara DPSs each have restricted ranges and multiple threats and are currently classified as endangered, whereas the Central DPS, with a more extensive range, is listed as threatened. Such flexibility can accommodate variable, appropriate actions with species and can be an important, genetically enabled tool for resource managers.

Virtually all of these studies have relied on a small handful of presumably neutral markers, and genomic approaches should provide much greater resolving power in the near future. As recently emphasized by Funk and coauthors (43), genomic data offer the opportunity to delimit MUs and conservation units with neutral and outlier loci that are subject to strong natural selection. Although this approach has not yet been applied to any amphibians or reptiles for conservation purposes, the potential for managing for both past history and adaptive variation should be a key element of future genetic management practice.

USING GENETICS TO SET CONSERVATION PRIORITIES

Genetic analyses, in combination with field-based demographic studies, have been crucial in developing conservation priorities across a wide range of amphibian and reptilian taxa. The ways in which genetic data have affected conservation and management priorities, and those for which genomic data may add additional insights, range from shallow-time population genetic to deep-time phylogenetic prioritization. Hybridization, genes that matter with respect to natural selection and future adaptation, and environmental and genetic gradients all present the opportunity to prioritize populations and landscapes with genetic and genomic data. For example, given that hybrids are often differentially valued compared with nonhybrid members of a threatened clade (e.g., under the US Endangered Species Act), studies of hybridization dynamics have helped define the boundaries of endangered taxa, resolved the extent and spread of anthropogenic and natural hybrid zones, and played a central role in setting conservation priorities for several well-studied amphibian and reptile study systems. This work, in turn, has provided resource managers and conservationists with the data that they require to set policy on the role of hybridization (44–46).

In general, understanding the interplay of genetic, behavioral, and morphological variation over ecological scales is essential to understanding how species survive across heterogeneous landscapes. Such studies have been undertaken in amphibians and reptiles with traditional ecological and quantitative genetics, transcriptomic analyses that explicitly examine functional genomic regions that are critical for local adaptation, and analyses of environmental gradients in generators of adaptive variation in nature (47–55). We are particularly excited about the role of transcriptomics in conservation prioritization, given the accessibility of quantitative RNA analyses in nonmodel amphibian and reptile systems. Finally, genetics and genomics continue to be the key drivers in developing regional and global phylogenies that allow the conservation community to set phylogenetic priorities for amphibians and reptiles based on patterns of phylogenetic relatedness and evolutionary uniqueness. Although formal adoption of phylogenetic prioritization by any conservation agency has not, to our knowledge, taken place, it clearly offers a way of triaging species conservation in the near future (56, 57).

Hybridization

One of the key areas where genetics has unambiguously aided conservation efforts has been in the clarification of hybridization between endangered and nonendangered amphibians and reptiles. In many countries, including the United States, either hybrids are not protected by law or protection is ambiguous, and genetics has been used across a wide range of taxa to clarify the extent of hybridization in nature. In one of the most striking cases to date, the endangered Florida bog frog, *Rana (Lithobates) okaloosae*, with a total known distribution of less than 20 km², was found to consist of 5–10% hybrids with the widespread bronze frog *Rana clamitans* based on nine microsatellite loci analyzed for 350 frogs (58). Given the limited number of available markers, it was unclear from this analysis whether all hybrids were F1 or whether more extensive hybridization may have occurred, but the possibility may exist for the entire nominal species to be subsumed into a hybrid swarm.

Among salamanders, hybridization has been identified as a high conservation priority in two sets of taxa in the tiger salamander (*Ambystoma tigrinum*) complex. Natural hybrids were inferred based on a combination of mitochondrial and microsatellite data between endangered *Ambystoma tigrinum stebbinsi* and the widespread barred tiger salamander *Ambystoma tigrinum mavortium* (45), raising a conservation concern for the narrowly distributed *A. t. stebbinsi* in southern Arizona. In a series of papers, our laboratory has demonstrated that nonnative *A. t. mavortium* introduced into central California in the 1950s led to the formation of a hybrid swarm in approximately 25% of the range of the federally and state-protected California tiger salamander (*A. californiense*); another 25% has been invaded by a smaller set (3/68 marker loci) of super-invasive genes that are spreading more rapidly (46, 59, 60). At both the phenotypic and genetic levels, these hybrids generally have higher fitness than pure native salamanders, raising serious conservation concerns for the endangered *A. californiense* (61–64).

Among reptiles, recent work has demonstrated hybridization between rare endemics and more common congeners across every major clade examined, sometimes at surprisingly high levels. Using a panel of 10 microsatellite loci, Rodriguez et al. (65) found that nearly 40% of crocodiles from the Yucatan peninsula (Mexico) were hybrids between endangered *Crocodylus acutus* and *Crocodylus moreletii*. An analysis of a sea snake species assemblage in northwestern Australia revealed that up to 95% of the critically endangered *Aipysurus fuscus* were hybrids with the more widespread *Aipysurus laevis* and that only a single reef still supports pure *A. fuscus* (66). Much more rarely, hybrids were identified between endangered spiny-tailed iguanas (*Ctenosaura bakeri*) and a widespread congener (*Ctenosaura similis*) on the island of Utila (Honduras), suggesting that hybridization may not pose an immediate threat to the single island–endemic *C. bakeri*, as initially hypothesized (67). Finally, turtles appear to be particularly sensitive to threats from hybridization, and both human-mediated and natural hybrids between endangered and widespread species have been uncovered in many clades and geographic regions (68, 69). Given the rate and extent of the trade of turtles at a global level, and their status as the most endangered major clade of vertebrates on earth, hybridization is a threat to the genetic integrity of many species that must be considered in management and conservation.

Ecological Genetics and Ecological Gradients

Biotic and abiotic ecological factors often act as selective agents on phenotypes, resulting in population genetic differentiation (70). This differentiation in turn generates phenotypic diversity and perhaps new species (50). From a conservation perspective, such diversity may well be the key to current and future ecological success, and its maintenance is a critical factor in setting conservation priorities to avoid demographic collapse and extinction.

Diversification in heterogeneous habitats is likely to occur if differential natural selection is strong enough to overcome the homogenizing effects of gene flow (71). Adaptive variation resulting from differential selective regimes has been observed in Caribbean lizards (49, 72), a tropical African lizard (*Trachylepis affinis*) (51), Panamanian golden frogs (37), and dendrobatid frogs (73), among other taxa. This relationship between environmental heterogeneity and genetic and phenotypic diversity indicates the action of strong natural selection leading to local adaptation that may be important in the speciation process (70). As such, these areas with heterogeneous habitats capture ecological and evolutionary processes that are fundamental in maintaining and generating diversity (74).

Niche conservatism is the degree to which species retain their ancestral ecological characteristics (75, 76), and many amphibians and reptiles are conservative ecologically over long periods of evolutionary time. As a consequence, purely ecological factors may act as barriers to dispersal and gene flow (77), leading to regions with high species richness and many localized endemic taxa. For example, North American plethodontid salamanders (*Desmognathus* and *Plethodon*) inhabiting similar climatic conditions in the Appalachian Highlands are unable to tolerate intervening lowland environments, leading to geographic isolation and frequent allopatric speciation among populations that are incapable of traversing subtle ecological gradients (76). Although this pattern of genetic differentiation can occur in any taxon, it appears to be particularly prevalent in terrestrial salamanders, perhaps because of their specialized ecological needs and extremely limited dispersal capabilities (78).

Of course, not all species are evolutionarily conservative with respect to ecological adaptation, and some amphibians and reptiles respond to environmental heterogeneity with local adaptation and genetic change. Environmental gradients are often viewed as one important driver of intraspecific adaptive variation that may allow species to persist through changing environments and be a source of variation for ecological speciation and intraspecific adaptive variation (51, 79). In combination with environmental data layers from geographic information systems, genetic analyses of population structure and gene flow have provided insights into the role of ecological factors in promoting divergent selection and speciation in amphibians (77), phenotypic divergence in *Anolis* lizards (80), and convergent morphological evolution consistent with ecological speciation in three lizard species in the extreme White Sands desert of the southwestern United States (81). Morphologically similar but genetically distinct amphibian lineages sampled across climatic gradients have also shown that divergent selection maintains distinct lineage distributions in the salamander *Aneides flavipunctatus/niger* in western North America (82). A key insight from these studies is that preserving environmental gradients may be the key to maintaining species during climate change, both historically and in the future (83), and may be of potential importance for conservation management.

Genes that Matter and the Promise of Transcriptomics

A central biological focus of our time is to determine how and to what extent the nucleotides that make up an organism's genome are responsible for the different physical characteristics of that individual. A related key area of modern organismal biology is a mechanistic understanding of the ways that natural selection interacts with standing genetic variation producing local adaptation (84). The combination of these two questions is important in a management context because an organism's ability to persist in an environment is constrained by its physiological limits as determined (at least partially) by its genetic makeup (85). As such, detection of the genetic signatures of local adaptation can be a key factor in making decisions about translocations and forward-looking reserve allocations, particularly in a changing world.

Shaffer et al.



Previously unavailable for most nonmodel organisms, modern sequencing methods now allow for unprecedented investigations into the sequence and architecture of a species' genome. Although few reptile and amphibian genomes are currently available, transcriptome sequencing is now an affordable approach to evaluate genetic variation in functional regions of the genome for virtually any species. Although still in its infancy, transcriptomics clearly has the potential to shape future research in reptile and amphibian conservation genomics.

Two general approaches exist for finding important genes for species management: candidate gene approaches and analysis of genome or transcriptome data to quantify patterns of differential expression between different environments and/or outliers in rates of gene evolution. Candidate gene approaches investigate one or a few genes that have been implicated in another species (often a model organism) as being important to some biologically important attribute of interest. A candidate gene framework has been used to study several reptile and amphibian systems. In one such experiment, the melanocortin-1 receptor (*MC1R*) gene was found to be an important factor in adaptive coloration differences among populations of the lesser earless lizard (*Holbrookia maculate*) and the little striped whiptail (*Aspidoscelis inornata*), a pattern previously found in mammals and birds (86). Understanding the underlying variation in a population's *MC1R* gene could therefore be important in assessing the feasibility of translocation of a population to an environment with differently colored substrates.

The timing of metamorphosis may be an important factor determining how the native California tiger salamander (*A. californiense*) will fare in the face of increasing hybridization with the invasive barred tiger salamander (*A. t. mavortium*) (87). To investigate the genetic control of metamorphic timing in ambystomatid salamanders, Voss et al. (88) tested whether two thyroid hormone receptor genes (which were previously found to mediate development from larvae to adult in the species) could be responsible for controlling the timing of metamorphosis in crosses between the Mexican axolotl (*Ambystoma mexicanum*) and eastern tiger salamander (*A. tigrinum*). In this case, Voss et al. found a significant relationship between a thyroid-hormone receptor alpha genotype and metamorphic timing. Follow-up work (89, 90) used genomic scans to explore the quantitative relationship between QTL associated with the timing of metamorphosis and the threshold at which a salamander metamorphoses or breeds as a paedomorphic (larval) adult. This information can now be used to assay wild California tiger salamander populations to determine if animals harbor nonnative genotypes at thyroid-hormone receptor loci, allowing managers to make better-informed decisions about which populations are the biggest threat to spreading more nonnative alleles throughout the landscape. Another candidate gene study investigated thyroid hormone receptor beta and found that the gene was significantly associated with developmental timing in the moor frog, *Rana arvalis* (91). Again, this work has considerable potential for landscape management decisions, particularly in the face of climate change and shortened periods for larval development.

Genomic- and transcriptomic-scale analyses focusing on outliers in evolutionary rates and differing gene expression profiles between groups are another class of functional gene studies that can be important for determining genes and genomic regions that might be most important for population persistence. One early study evaluated 392 amplified fragment length polymorphisms (AFLPs) in the common frog (*Rana temporaria*) along an elevational gradient to help understand adaptation to higher elevations. The authors identified several AFLPs that were significantly associated with elevation (55). As physical and genetic maps become more widely available, these early studies can be used to identify genetic regions and allelic variants that may be critical for populations to persist if they are unable to move upward in elevation under a scenario of global warming.

Because many reptile and amphibian species do not yet have sequenced genomes, transcriptome sequencing can be an important means of generating first-pass genomic-scale data for

these species. Additionally, such data can be used to generate genomic capture probes for sequencing exons and surrounding introns (92). Several reptile and amphibian transcriptomes have been sequenced (93–95), but as of yet most of the work in RNA sequencing of reptiles and amphibians has necessarily been descriptive, and its use for discovering genes that matter in natural populations and applying them to conservation work is a developing field.

Transcriptome-based studies are, however, increasingly contributing to our knowledge of reptiles and amphibians at the fundamental molecular level. For instance, transcriptome data can be useful in searching for the presence or absence of certain functional genes in groups of organisms, as was the case in a study by Bar-Yaacov et al. (94) that found that *COX8*, a gene involved in cellular respiration, was likely missing from iguanid lizards. Transcriptomics has also been extremely valuable in characterizing the genes that respond to infection by *Batrachochytrium dendrobatidis* (*Bd*) in ranid frogs and the generality of this response across different species (48). And as an early example of using comparative transcriptomics to find genes that may be important for local adaptation to extreme environments, Yang et al. (96) found a list of 14 genes that may have been involved with adaptation of the Plateau brown frog (*Rana kukunoris*) to high elevation. As more functional gene studies are conducted in reptile and amphibian systems, we will continue to understand the molecular factors that are most important for populations to adapt to a rapidly changing world. Assuming that this field continues to grow (and we see no reason why it should not), the knowledge gained from these studies will form a key component of forward-looking conservation planning in the decades to come.

Phylogenetic Prioritization

With the advent of inexpensive genetic sequencing and increased computational power, well-supported multilocus phylogenetic trees are becoming increasingly common for many groups of vertebrates, including amphibians and reptiles. Phylogenetic trees can play two separate but related roles in conservation. The first is the identification of lineages (or failure to do so; see References 97–100) that are prone to extinction, and the second is the consideration of phylogenetic diversity (PD), a measure of evolutionary distinctiveness of species useful in prioritizing conservation needs independently of taxonomy. PD is based on the amount of branch length subtending a species or node of a tree. In essence, a species subtended by a long branch has no living close relatives and thus represents a pool of genetic information that differs substantially from other extant species. Following from this, the conservation of PD is often considered to be a proxy for conserving unmeasured but existing functional diversity, or for evolutionary potential (101). The concepts and methods of measuring PD have been discussed at length elsewhere (102); here we review the use of PD in empirical studies of reptiles and amphibians.

Consideration of reptile and amphibian PD began, unsurprisingly, with the tuatara of New Zealand. Using allozymes, Daugherty et al. (97) generated a phylogeny of tuatara populations and found greater among-lineage diversity than had been suspected previously, leading them to recognize both *Sphenodon punctatus* and the most divergent island population, *Sphenodon guntheri* [although a recent study by Hay and colleagues (99) has recommended returning to the single-species view of tuatara]. Although their paper was published before the first formal treatment of PD by Vane-Wright et al. (103), Daugherty et al. (97) made clear reference to the distinctiveness and subsequent conservation value of *Sphenodon* among squamates and the consequences of splitting the species on squamate PD as a whole. Given its status as the only living member of the order Rhynchocephalia and its sister-group status to all other squamates, tuatara is a poster child for the importance of PD in conservation prioritization.

Incorporation of evolutionary history and distinctiveness of individual lineages within ecological communities or bioregions may better maintain functional diversity than considerations



relying on species richness or rarity (102). Many biologists consider PD (or taxonomic distinctiveness as a proxy for PD) a necessary addition to conservation prioritization methods that consider rarity alone, arguing that PD accounts for the loss of unique and distinct lineages and the potential maintenance of unique ecological niches, whereas rarity and species loss may disproportionately preserve more speciose but less ecologically divergent clades (101). For example, Crawford and colleagues (104) found that 41% of species were extirpated by the fungal disease chytridiomycosis in a Panamanian amphibian community, but only 33% of the PD was lost. They also examined phylogenetic patterns in species loss but found that extirpated species were randomly distributed in the community phylogeny. This is in contrast to the results of Smith and colleagues (105), who examined chytridiomycosis-affected amphibian communities in Central and South America and found that local endemics were disproportionately extirpated such that remnant amphibian faunas were homogenized, exhibiting a substantial loss of both taxonomic and ecological diversity; it would be of considerable interest to repeat the work of Smith et al. (105) using a strict phylogenetic analysis to examine PD loss in this system. In a similar vein, Corey & Waite (106) conducted a global analysis of amphibians and found that enigmatically declining and critically endangered status were clustered within hyloid frogs; although they did not specifically test for PD, they analyzed three available phylogenetic topologies for amphibians and found in all cases that shared evolutionary history was a key explanation of intrinsic vulnerability. In one of the most complete analyses yet conducted, Isaac and colleagues (57) used a combination of amphibian species' threat status and their evolutionary distinctiveness to generate phylogenetically informed conservation priorities from a tree including 5,713 species; the end result was a list of "evolutionarily distinct and globally endangered" (EDGE) species. Finally, in a meta-analysis of the IUCN Red List conservation status of the world's reptiles (*Sphenodon* excluded), Böhm and colleagues (107) found that threat was not evenly distributed across reptile families but instead was concentrated in the turtle families Geoemydidae and Chelidae; the true crocodiles (Crocodylidae); and the lizard families Pygopodidae, Xantusiidae, and Iguanidae. Once again, repeating this analysis using PD would provide additional insights into the distribution of branch length loss in reptiles. It is also worth noting that IUCN Red List status may not adequately capture functional and phylogenetic diversity, a limitation that may be addressed with EDGE prioritization (108).

At the level of policy, conservation of PD is absolutely reliant on accurate taxonomy: Without accurate identification of species within lineages, unnamed cryptic diversity is at significant risk of being lost before it is even recognized and legally protected. Engstrom and colleagues (98) constructed a mitochondrial DNA tree of the southeast Asian softshell turtle genus *Chitra*, a widespread lineage that was often considered to be monotypic. They identified three deeply divergent, reciprocally monophyletic lineages, at least one of which was critically endangered. Another southeast Asian turtle species, *Heosemys spinosa*, which is widely collected for the pet trade and was already considered threatened, was thoroughly reviewed by Spinks and colleagues (100). Using one mitochondrial and eleven nuclear loci, Spinks et al. identified two distinct lineages within *H. spinosa*, emphasizing the need for conservation actions for each.

Although theoretical arguments for its utility in conservation exist, it remains unclear if PD will emerge as a useful measure for the conservation of reptiles and amphibians in practice. As it stands, the use of PD is contingent on conservationists valuing the preservation of evolutionary history as a key element of prioritization. The links between PD and ecological processes, evolutionary potential, and ecosystem services are poorly understood and certainly deserve additional attention. In one study that explicitly looked at functional diversity loss in amphibian communities impacted by logging in West Africa, Ernst and colleagues (109) found a significant loss of functional diversity with no significant loss of PD. Clearly, if PD is to become an integral component of conservation prioritization, reptile and amphibian communities must be examined



individually with an eye toward greater understanding of the relationships between ecological, functional, and phylogenetic diversity. Of course, it goes without saying that estimates of phylogenetic trees and branch lengths will only gain in accuracy and resolution as phylogenomic data are brought increasingly to bear in reptile and amphibian phylogenetics.

USING GENETICS TO INFORM MANAGEMENT ACTIONS

One of the most direct roles of genetics in informing amphibian and reptile conservation actions involves identifying and managing the human activities that affect and endanger wild populations. Of the many topics that fall into this broad area of research, we have identified four that were particularly relevant: the trade of threatened species, captive breeding and disease, ecotoxicology and genotoxicology, and the design of natural reserves and parks. The trade of reptiles and amphibians for food and the global pet industry is a major component of species decline, and genetics has helped identify the sources of traded species, the reduction in genetic diversity associated with declining population numbers, and the patterns of movements and reintroductions that have occurred for several key species (110–112). When *ex situ* captive breeding is necessary, as has been the case for several of the world's most threatened amphibians and reptiles, genetics has contributed to successfully preventing inbreeding and outbreeding depression, creating breeding programs that mimic natural mating systems, and managing levels of genetic variation to maximize the probability of successful reintroductions into the wild (113–118).

Although wildlife disease certainly stands as an independent area of research, we consider it with breeding because the release and spread of diseased animals is a persistent management problem that genetics has played a crucial role in managing. Genetic analyses of the immune systems of infected and healthy host populations, and of diseases themselves, show some promise in helping avoid future wholesale extinctions owing to novel emerging pathogens and the diseases that they cause. Unlike most other wildlife diseases, which affect individual species or populations, both fungal and viral infections have led to the wholesale collapse of entire amphibian faunas, and genetic analyses may help the conservation community as they plan for the repatriation of disease-resistant strains back into nature (47, 48, 118, 119).

Ecotoxicological pollutants have potentially devastating effects on amphibian and (perhaps to a lesser extent) reptile populations at local and global scales. In addition to these direct effects, amphibians and reptiles are often physically contaminated by heavy metals, pesticides, and fertilizers, which can damage DNA, leading to conservation concerns ranging from developmental defects to increased disease susceptibility (120–123). The field of genotoxicology is still in its earliest stages with many species of reptiles and amphibians, but it is an area that will certainly benefit from genomic perspectives.

Finally, we briefly discuss the composite role of many of the aforementioned sections in the design of natural reserves for the protection of amphibians and reptiles. Current practices center on critical habitat for endangered species, with little consideration of the efficacy of those reserves in the face of climate change; disease spread; and the interplay between natural selection, gene flow, and genetic drift. Genetics can and should play a critical role in the identification and configuration of reserves, and we end our review with our collective views on reserve design.

Trade

The exploitation of reptiles and amphibians for consumption, medicinal use, and the pet trade has been identified as a major component of some species' declines (124). The impact of trade can be examined from a genetic perspective by (a) identifying the sources of traded species, (b) assessing

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the loss of genetic diversity associated with declining population numbers, and (c) identifying the movements and reintroduction of captive animals into extant populations or the establishment of new populations. As both transcriptomic and whole-genome data become increasingly available, these will add tremendously to all three of these research agendas.

Identifying the geographic origins of traded species may help managers identify at-risk populations and trade routes. Using genetic markers that are variable between populations, forensic methods can identify source regions and authenticate taxonomic judgments by comparing the genetic markers of confiscated animals to a database of known haplotypes (125). For example, Welton et al. (112) used population genetic networks, phylogenetic inference, and DNA barcoding methods to accurately identify the source island of illegally traded *Varanus* monitor lizards in the Philippines when compared with a molecular data set of samples from known localities. The authors showed that animals were often from a different origin population than that reported by the trader (112). Similarly, the population of origin was determined via haplotype network analysis for captive and wild-caught European pond turtles, *Emys orbicularis* (110). Results indicate that both captive and wild animals represent widespread translocations owing to the pet trade and to incidental catch and release by tourists (110).

Loss of genetic diversity can occur as a consequence of harvesting and collection for trade and lead to a reduction in N_e (126). This reduced population size and associated loss of genetic diversity is frequently detected as a population bottleneck signature (127, 128). A signal of this human-induced bottleneck was identified in the olive Ridley turtle, *Lepidochelys olivacea*, following intense harvesting of these marine turtles at nesting sites between 1960 and the late 1980s (approximately 1.5 turtle generations) (129). Interestingly, although a population bottleneck was detected across all nesting sites, there was no demonstrable loss of heterozygosity (129). Monitoring loss of diversity owing to overharvesting is also an important conservation goal. However, Peery et al. (128) caution that because sample sizes are often quite low for species of conservation concern, standard tests to identify bottlenecks (e.g., the M -ratio test) often do not detect bottlenecks, even in populations where they are known to have occurred (128). Future studies should strive to expand sample sizes if possible to more effectively track emerging population trends.

Finally, the genetic signature of introductions may be important when common, widespread taxa become invasive in nonnative introduced landscapes. In California, the source of nonnative barred tiger salamanders [*A. (tigrinum) mavortium*] was confirmed from multiple sources across the Great Plains of the central United States (130). Direct verbal communication with individuals who moved tiger salamanders from Texas and New Mexico to the Salinas Valley in central California partially confirmed this inference, but detailed mtDNA analyses of all nonnative populations established in California, Nevada, and Oregon, and a wide range of potential source populations from across much of North America, further demonstrated that several different introduction sites had contributed to the introduction history of these salamanders. Given their devastating impact, both via hybridization with native endangered California tiger salamanders and as much more effective apex predators in their breeding pond habitats, understanding the source of introductions is an important step in their possible control (46, 62, 131). Similarly, in both Italian wall lizards [*Podarcis siculus* (132)] and brown anole lizards [*Anolis sagrei* (133)], mtDNA sequence data have suggested that invasive populations in nonnative habitats may harbor more genetic variation than any population from original source areas. Because these lizards are collected for the pet trade, in which color and morphological variation are often highly prized, animals from diverse source areas have been introduced to the same sites, leading to increased fitness associated with genetically variable, admixed lizards.

Both for historical demography (including bottleneck and population expansion) and for admixture analyses of invasive species, genomic data offer much greater precision in the inferences



that can be drawn. The tradeoff between number of individuals and number of markers sampled is well known, and even with single individuals, historical demography can be estimated accurately with genomic data (134). Similarly, as the number of loci increases, key genes in admixed populations of invasive species of reptiles and amphibians can be identified and targeted for functional analyses and potential eradication (59, 60).

Captive Breeding and Disease

Since its first introduction in the 1970s, captive breeding has become an important tool for zoos and conservationists in pursuit of species recovery initiatives (115, 135). Among the many methods and goals implemented in successful captive breeding programs, the maintenance of genetic diversity has been of primary importance. Assessing the genetic variation of founder populations in captive breeding programs can prevent inbreeding and outbreeding depression, ensuring that offspring suffer from neither (113, 117). In addition, analyzing the genetic makeup of captive-bred offspring can improve our understanding of mating systems, which in turn can help predict genetic diversity patterns (136). For example, genetic analysis of Galapagos tortoises (*Chelonoidis nigra* species complex) and tuatara (*S. punctatus*) revealed low genetic diversity associated with mating dominance of a few males and females (136, 137). These insights should encourage genetic intervention of a captive breeding group, with the goal of managing matings to avoid the further loss of genetic variation. Additionally, genetic sampling efforts of wild animals could assist managers in identifying the most important locations and populations for introduction of captive stock (115, 118), ensuring the maintenance of genetic lineages, particularly in isolated or locally adapted populations.

Emerging infectious diseases, such as chytridiomycosis in amphibians, inclusion body disease in snakes, and upper respiratory tract disease in tortoises, are inevitable threats to the conservation of amphibians and reptiles (138–141). An important step in controlling and limiting disease outbreaks is maintaining sufficient genetic variation, both in the immune system and more generally, so that populations can successfully overcome these disease challenges (47, 118). Higher genetic diversity is correlated with a greater capacity to recover from a diverse array of pathogens, whereas low genetic diversity is associated with susceptibility (47, 118). Thus, in the case that an emerging infectious disease is endangering the survival of a species, such as the Aruba Island rattlesnake (*Crotalus durissus unicolor*) (142), genome analyses, including expression studies of specific organs or the immune system, can help identify how the immune system is being impaired or the immune response function necessary to suppress the disease (48).

One of the most insidious emerging diseases to affect amphibians at a global level has been the global spread of the chytrid fungus *Bd* and its associated disease, chytridiomycosis. Although considerable progress has been made in our understanding of the spread and pathology of this fungus, implementable approaches to reduce or eliminate its devastating effects have remained elusive. A recent genetic meta-analysis of disease-impacted populations identified genes that help with resistance to the emerging pathogen, including the examination of the genetic response to infection (48). Decreased expression of immunogenetic markers during the onset of *Bd* infection was linked to frog mortality (48). Such data can and should be useful to conservationists and land managers in their efforts to translocate disease-resistant individuals to pathogen-vulnerable populations, given the prediction that resistance genes would be under very strong selection and would likely spread rapidly (48, 119).

Recent analyses of transcriptome data from two anuran species, one tropical and one temperate, demonstrated that transcripts of both the innate and acquired immune system were expressed in multiple tissues and were highly expressed, suggesting that such targeted analyses of



immune system transcripts should help identify individuals and populations that have been exposed to, and survived, *Bd* and other diseases (143). Such information may be critical for identifying high-priority populations for conservation and as sources for reintroductions. However, a recent transcriptomic analysis of the highly *Bd*-susceptible toad *A. zeteki* comparing previously exposed and naïve individuals indicated that immunosuppression by *Bd* appears to be occurring, leading the authors to suggest that ineffective immune pathway activation and timing, rather than a lack of immune response, may be the cause of *Bd*'s effectiveness; if true, it remains unclear what the most effective conservation strategies may be in battling this devastating fungal pathogen (144).

Ecotoxicology and Genotoxicology

As the environment is continuously loaded with exogenous chemical substances released by anthropogenic activities, these chemicals often affect directly or indirectly the gene pool of natural populations, including those of reptiles and amphibians. It is well known that chemical agents may alter the genetic variability of an exposed population by mutagenesis in somatic and germ cells, demographic declines, alteration in migration rates, population bottlenecks, or other selective agents (145, 146).

There are few ecotoxicological studies on the effects of contaminants on amphibian and reptile populations (122, 123, 147), and analyses of how these contaminants may directly alter their DNA are sparse compared with other taxa. The most common methods for genotoxicity testing on amphibians to detect DNA damage are chromosome aberration assays, micronucleus assays, sister chromatid exchange assays, and so-called comet assays (single cell gel electrophoresis) (120). More recent approaches, including chromatin immunoprecipitation, have provided important insights into epigenetic alterations in male crested newt (*Triturus cristatus*) spermatogenesis owing to environmental exposures (148); additional research on other taxa would be a welcome addition to our understanding of toxicological exposure in amphibians. Comet assays, which visualize breaks in denatured DNA, have also been used to analyze genotoxicological effects in reptiles and amphibians to a limited extent. For example, Poletta et al. (121) used comet and micronucleus assays to examine the genotoxicity of a widely applied herbicide on the broad-snouted caiman (*Caiman latirostris*). Similarly, Yin and colleagues (149) observed significant, concentration-dependent DNA damage in erythrocytes of Chinese toad tadpoles (*Bufo gargarizans*) exposed to sublethal concentrations of four different herbicides via comet assay. Several other recent studies have used these assays to establish baseline data and examine genotoxicological effects on an increasingly wide range of taxa, ranging from tropical anurans and lizards to amphisbaenians and marine turtles (150–153). Given the conservation status of many amphibian and reptile species, it is crucial to build a larger knowledge base on this subject to better understand if and how ecotoxic agents are affecting populations. Genomic scans, including the identification of candidate genes responding to the novel selection pressure of ecotoxicological contaminants, are a promising area of future research to identify candidate populations for reintroductions and assisted migrations.

Reserve Design

Finally, we turn our attention to one of the largest-scale applications of genetic data to amphibian and reptile conservation—the effective design of ecological reserves. We take it as a guiding principle that understanding the ecological and evolutionary processes that maintain and generate biodiversity is essential for designing effective natural reserves (74). Reserve design for amphibians and reptiles should consider natural selection, genetic drift, and gene flow in combination with disease mitigation and basic natural history.

To capture evolutionary processes in reserve design, PD (101), ESUs (34), and environmental gradients (83) have all been proposed as critical tools in the identification of the most important landscapes for protection. PD may fail to capture intraspecific adaptive variation, especially with respect to recent events of local adaptation and isolation (154); instead, intraspecific (rather than interspecific) variation associated with habitat heterogeneity may help to capture recent evolutionary processes that lead to the origin of new species. ESUs may capture this intraspecific variation, at least at recent evolutionary timescales (155). This has been demonstrated in three species of endemic Florida lizards (the mole skink *Plestiodon egregious*, the scrub lizard *Sceloporus woodi*, and the sand skink *Neoseps reynoldsi*), in which high levels of genetic divergence occur between regions, suggesting that conservation of genetic diversity requires the protection of several isolated populations (156). On the Cape Verde Islands, a recent study demonstrated that areas currently targeted for preservation, which are based upon species diversity and human recreational use, fail to effectively protect ESUs of some species in the herpetofaunal community on several of the islands, while sufficiently protecting them on others (157).

To maximize the efficacy of reserves that focus on ESUs, these reserves should ideally also protect adaptive variation, which is exceptionally important to maintain in the face of differential selection in changing environments (37). The Panamanian golden frog, a critically endangered species, exhibited concordant variation in genetic and phenotypic traits, which corresponds with environmental differences in natural populations (37); combining such analyses with transcriptomic scans across populations in the same species (e.g., 143, 144) should become the next logical step in employing genomic-level analyses to quantify the efficacy of reserves at conserving historical population divergence and the actions of natural selection at the genic level.

FUTURE DIRECTIONS

If the past was conservation genetics, the future is clearly conservation genomics. At this point, three traditional limitations to the application of genomic approaches to ecological, evolutionary, and conservation-relevant research for nonmodel systems are all being resolved. The first, and by far the most important, is the availability of a broad set of reference genomes that span the tree of life. Bold new initiatives, among them the Genome 10K Project, that seek to enable the sequencing of 10,000 vertebrate genomes (158) are rapidly closing the genome gap. Reptiles have enjoyed far greater success than amphibians in terms of reasonably high-quality genome assemblies, and as of this writing, four turtles (159, 160), four crocodylians (161, 162), four squamates (163–166), and one amphibian (167) have been released and are available to the community. The second is the analysis pipeline, particularly for low-coverage genomic data. Although still a challenge, new software (168, 169) is making it possible to apply very low-coverage data ($1\times$ or possibly lower) to important landscape and conservation genomics research. And finally, cost is coming down—our lab is currently sequencing 270 desert tortoises (~ 3 -Gb genome) at $1\times$ coverage for under \$300 each. Amphibian genome sizes (particularly salamanders) are often in the tens of gigabases, and their assembly remains a challenge, but long-read technologies should ultimately resolve this issue. And as these data come in, they can and will be applied to help us learn about barriers to movement, mating patterns, assisted migration, and a host of other critical conservation concerns, enabling stronger science and better management of endangered amphibians and reptiles globally.

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