Toward an integrative molecular approach to wildlife disease

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Abstract: Pathogens pose serious threats to human health, agricultural investment, and biodiversity conservation through the emergence of zoonoses, spillover to domestic livestock, and epizootic outbreaks. As such, wildlife managers are often tasked with mitigating the negative effects of disease. Yet, parasites form a major component of biodiversity that often persist. This is due to logistical challenges of implementing management strategies and to insufficient understanding of host–parasite dynamics. We advocate for an inclusive understanding of molecular diversity in driving parasite infection and variable host disease states in wildlife systems. More specifically, we examine the roles of genetic, epigenetic, and commensal microbial variation in disease pathogenesis. These include mechanisms underlying parasite virulence and host resistance and tolerance, and the development, regulation, and parasite subversion of immune pathways, among other processes. Case studies of devil facial tumor disease in Tasmanian devils (Sarcophilus harrisii) and chytridiomycosis in globally distributed amphibians exemplify the broad range of questions that can be addressed by examining different facets of molecular diversity. For particularly complex systems, integrative molecular analyses present a promising frontier that can provide critical insights necessary to elucidate disease dynamics operating across scales. These insights enable more accurate risk assessment, reconstruction of transmission pathways, discernment of optimal intervention strategies, and development of more effective and ecologically sound treatments that minimize damage to the host population and environment. Such measures are crucial when mitigating threats posed by wildlife disease to humans, domestic animals, and species of conservation concern.

Keywords: epigenetics, genetics, microbiome, transcriptomics, wildlife disease management, zoonoses

Hacia una Estrategia Molecular Integrada para las Enfermedades de Fauna Silvestre

Resumen: Los patógenos presentan amenazas serias para la salud humana, la inversión agrícola, y la conservación de la biodiversidad debido al surgimiento de zoonosis, el paso de estos hacia el ganado doméstico, y los brotes epizootícos. Por esto, los manejo de fauna comúnmente tienen que mitigar los efectos negativos de las enfermedades. A pesar de esto, los parásitos forman un componente importante de la biodiversidad que generalmente persiste. Esto se debe a los obstáculos logísticos de la implementación de estrategias de manejo y al poco entendimiento de las dinámicas bospeadero - parasito. Abogamos por un entendimiento incluyente de la diversidad molecular en la causa de las infecciones parasitarias y los estados variables de los hospederos en sistemas de fauna y examinamos papeles de la variación microbiana genética, epigenética y comensal en la patogénesis de las enfermedades. Estos papeles incluyen mecanismos subyacentes de la virulencia parasitaria y la resistencia y tolerancia del hospedero, así como el desarrollo, regulación y subversión parasitaria de las vías inmunes, entre otros procesos. Estudios de caso de la enfermedad del tumor facial de los demonios de Tasmania (Sarcophilus harrisii) y la quitridiomycosis en anfibios con distribución mundial ejemplifican la amplia gama de preguntas que pueden abordarse examinando las diferentes facetas de la diversidad molecular. Para sistemas particularmente complejos, los análisis moleculares integrados presentan una frontera prometedora que puede proporcionar conocimiento crítico necesario para elucidar las dinámicas de las enfermedades que operan a lo largo de las escalas. Este conocimiento permite la evaluación más precisa del riesgo, la reconstrucción de las vías de transmisión, el discernimiento de las estrategias óptimas de intervención, y el
Introduction

Integrative molecular approaches have yielded novel insights into human disease at a variety of scales. Independent and concurrent analysis of genetic, epigenetic, and commensal microbial diversity has elucidated proximate and ultimate drivers of disease state and transformed understanding of pathogenesis, transmission, and treatment (Pacis et al. 2014; Hand 2016). Similar analyses have only recently appeared within the context of wildlife disease and conservation science. We argue that adoption of diverse molecular methods in wildlife disease will provide more thorough appreciation of mechanisms that underlie host disease state and ultimately translate into more effective population monitoring, management, and conservation.

Wildlife disease can pose significant threats to human health, agricultural investment, and biodiversity conservation through the emergence of zoonoses, spillover to domestic livestock, and epizootic outbreaks (Daszak et al. 2000). Initially, theoretical models suggested that disease-mediated declines would halt transmission before host extinction could occur (Anderson & May 1979). Although this is true for many pathogens with density-dependent transmission, these well-defined thresholds are often nonexistent for host–parasite systems with more nuanced transmission structures (Getz & Pickering 1983). Complications include free-living parasite stages, endangered hosts sharing parasites with abundant reservoir populations, and systems with frequency-dependent transmission (Lafferty & Gerber 2002; Lafferty et al. 2015). Consequently, managers are tasked with mitigating wildlife disease in systems of varying complexity. Yet, elimination of harmful parasites remains elusive due to logistical challenges of reaching adequate proportions of wildlife, limited monetary and veterinary resources, and insufficient understanding of the numerous drivers underlying host disease state.

Environmental, immunological, behavioral, demographic, physiological, and molecular mechanisms contribute to the expression of host disease phenotype (Fig. 1). Although many of these factors have been reviewed elsewhere, comprehensive understanding of molecular diversity in the context of wildlife disease is lacking. In many studies, molecular diversity is considered synonymous with genetic diversity, but this fails to consider 2 additional components critical to proper immune functioning in hosts: epigenetic gene regulation and commensal microbial communities.

We reviewed recent advances in understanding the molecular underpinnings of wildlife disease and examined how molecular methods can inform management of disease dynamics operating across scales. We considered numerous examples to highlight questions that can be addressed with each molecular data type (i.e., parasite genetics, host genetics, epigenetic gene regulation, and commensal microbial variation). Two pathogens serve as our primary examples: devil facial tumor disease (DFTD) in Tasmanian devils (Sarcophilus harrisii) and chytridiomycosis in globally distributed amphibians (Table 1). These diseases lie at the frontier of applying an integrative molecular approach to wildlife disease, and demonstrate the numerous insights that can be gleaned from adopting these methods.

Genetic Analyses of Wildlife Disease

Two central concepts have emerged in the history of genetic analyses and disease: parasite virulence and host
resistance and tolerance are genetically based traits, and hosts and parasites are locked in a coevolutionary arms race, constantly evolving to obtain a mutational advantage over one another. Although this race is often asymmetrical due to the shorter generation time of pathogens, variation is the key driver of evolutionary change for both players. Thus, the application of molecular genetic techniques to the study of wildlife disease has typically focused on characterizing genetic diversity in parasites and their hosts and leveraging that information to discern selection on parasite transmission and host immunity. Technology has evolved from microsatellite markers through gene sequences to genomic data sets, but the core questions remain. It is the ability to address these questions that is considerably enhanced by large-scale data sets and the increasing array of programs with which to analyze them.

Parasite Genetics

The study of parasite genetics has moved from broad-brush pathogen identification (McManus & Bowles 1996) to more nuanced elucidation of complex transmission dynamics (Webster et al. 2016). This is particularly important when managing endangered populations threatened by generalist parasites. Under these scenarios, multihost transmission can be the difference between population persistence and local extinction. For example, disease remains a primary threat to endangered Ethiopian wolves (*Canis simensis*) (Randall et al. 2004; Gordon et al. 2015).

Though their population is too small to sustain pathogens such as canine distemper virus and rabies, the large population of dogs nearby is consistently identified as the source of disease outbreaks through viral sequencing. This renders both wolves and dogs critical targets for management.

Studies of parasite genetics can also be used to identify the subtle mechanisms underlying infection and virulence or damage to the host through parasite interactions (Schmid-Hempel 2011). These mechanisms can arise through mutation or horizontal gene transfer and facilitate processes such as toxin production, host cell invasion, and immune evasion that enable parasites to infect, propagate, transmit, and in some cases switch hosts (Hacker & Kaper 2000; Geoghegan et al. 2016). Avian influenza, for example, undergoes frequent mutation to sequentially infect the host respiratory tract and avoid sites of infection that limit transmission (Reperant et al. 2012). For *Mycoplasma gallisepticum*, the bacterial pathogen that causes conjunctivitis in House Finches (*Haemorhous mexicanus*), quantitative genetic tools paired with disease phenotype data suggest rapid evolution of increased transmission potential and virulence upon emergence in a new host population (Hawley et al. 2013).

By elucidating the specific molecular mechanisms underlying infection and virulence, one can pursue refined diagnostic tools, vaccination strategies, and therapeutic interventions (Johnston et al. 1999). Genomic analyses comparing pathogenic *Batrachochytrium* spp.,
the causative agent of chytridiomycosis, with free-living relatives, for example, reveal evolutionary adaptations for pathogenicity and infection strategies employed by different species (Farrer et al. 2017). This represents a promising direction in chytridiomycosis research because parasite variability has been implicated in driving disease outcomes in hosts. Similar analyses of *Plasmodium* spp. suggest mechanisms of immune evasion in mosquito vectors (Molina-Cruz et al. 2015) and have identified loci associated with antimalarial drug resistance under positive selection (Shen et al. 2017). Genetic tools have also located hotspots of polygenomic malaria infection, where hosts harbor multiple parasite strains simultaneously (Rice et al. 2016). Considered together, this information enables better monitoring where malaria transmission and polygenomic infection rates are high and suggests possible molecular mechanisms for disease control.

To pursue these research and management goals, genetic analyses have been conducted on a wide range of parasite taxa (Supporting Information). Going forward, it is critical to focus on additional pathogens in multihost systems occurring in geographically diverse populations, particularly where spillover is likely. Further, it is important to perform comparative analyses in the contexts of host phenotype, genotype, and environment (Lazzaro & Little 2009).

### Host Genetics

Studies of host genetics tend to focus on resistance, tolerance, and diversity. Resistance can be defined as the ability to eliminate parasites from the host, whereas tolerance imposes limits on the parasite’s negative effects (Best et al. 2008, 2009; Råberg et al. 2009). Host fitness is intimately coupled with these traits and forms a significant component of their underlying mechanistic and molecular processes. In both cases, the prevailing paradigm is based on the assumption that genetic diversity at immune loci buffers hosts from disease risk. As such, demography resulting in genomic diversity loss (e.g., inbreeding) is often associated with increased likelihood of disease-mediated population decline (Spielman et al. 2004).

Early researchers examining host genetics in wildlife disease adopted a broad definition of genetic diversity. Employing neutral microsatellite markers, they calculated summary statistics to determine whether host genetics correlated with disease state. Although many reported significant relationships (Coltman et al. 1999), others failed to identify strong associations between neutral diversity and disease (Schwensow et al. 2007). This led many to question the efficacy of using neutral markers as a proxy for genome-wide variation (Váli et al. 2008) and ultimately shifted the field toward a more targeted immunogenetic approach.

In vertebrate systems, the major histocompatibility complex (MHC) dominated the literature. Critical to adaptive immunity, this hypervariable gene family encodes for MHC molecules that bind to antigens and display them on cell surfaces to initiate an immune response. This renders MHC variation a convenient target for candidate gene approaches. For example, analyses of
Tasmanian devils threatened by DFTD suggest that low MHC diversity in hosts may provide means of immune evasion for this transmissible cancer (Siddle et al. 2007). Similar analyses in amphibians show associations between MHC diversity and survival in frogs experimentally infected with the pathogenic fungus \textit{Batrachochytrium dendrobatidis} (Bd) and host species known to be Bd resistant (Fu & Waldman 2017). The MHC-based explorations of host disease state consider only one aspect of immunity, however. Many other genes (e.g., Toll-like receptors) also aid host defense (Acevedo-Whitehouse & Cunningham 2006). Further, specific genes often associate with disease state more strongly than summary statistics of genetic variation (Bateson et al. 2016); thus, treating MHC diversity as a proxy for larger scale immunogenetic variation may eventually prove too reductionist.

Genomic methods are increasingly used by conservation scientists to pursue more comprehensive analyses. Since publication of the first human genome, technological advances have rendered whole genome sequencing accessible for wildlife studies. Even when whole genomes are unavailable, restriction enzyme-based methods rapidly and affordably generate genome-wide data (Davey & Blaxter 2010). For example, genomic analyses confirmed low levels of standing variation in Tasmanian devils, identified geographic structuring of host populations, enabled reconstruction of DFTD emergence and transmission pathways, and supported allograft transmission of this contagious cancer (Murchison et al. 2012; Grueber et al. 2015; Morris et al. 2015; Hendricks et al. 2017). Although in accordance with earlier MHC-based studies, genomic analyses enabled deeper understanding of the roles host and cancer genomics play in DFTD pathogenesis. They additionally positioned genetic diversity maintenance as a top conservation priority for wild and captive devil management.

We hope that more systems will benefit from similarly comprehensive analyses. Although the trend toward genome-level data comes with inherent difficulties, it provides critical insight into complex disease dynamics. By integrating the study of parasite and host genomics, wildlife managers can leverage better understanding of parasite virulence, transmission, and host disease state toward more effective prevention and management.

**Host Gene Regulation in Immunity**

For the immune system to properly function, genes must produce their corresponding antigen binding, recognition, and signaling proteins. Gene regulatory variation therefore arises as another contributor to host disease state. If an underlying genotype for host immunity is transcriptionally silenced, infection becomes likely. For example, suppression of inflammatory response genes in bats may allow them to asymptotically harbor viruses that are highly damaging to nonvolant mammals (Brook & Dobson 2015; Banerjee et al. 2017).

Epigenetic mechanisms (e.g., DNA methylation, histone modification, etc.) can alter the expression of immune genes in response to environmental stimuli, thereby inducing or preventing an immune response (Morandini et al. 2016). Thus, effective immunity requires action from both genetic and epigenetic dimensions of the host genome: the genetic capacity to recognize and respond to a diverse array of parasites, the concerted expression of those genes, and the ability to overcome immune escape efforts.

The functional link between epigenetics and immunity is a burgeoning field. Although many studies focus on tumor growth, autoimmune disorders, and developmental changes, increasing attention is being paid to epigenetic mechanisms operating in infectious disease (Pacis et al. 2014; Zhao et al. 2015). From the perspective of resistant hosts, fine-tuned regulation of immune genes and tissues targeted by pathogens may enable early detection and eradication of infecting parasites, as seen in some frogs experimentally infected with Bd (Ellison et al. 2014). Where tolerance is preferred, suppression of these genes and their resultant processes (e.g., strong inflammatory responses) may ultimately lessen morbidity, as seen in bats infected with viruses (Banerjee et al. 2017).

Yet, it often appears that epigenetic alterations worsen disease outcomes for hosts. In accordance with the co-evolutionary arms race, many parasites manipulate host epigenomes to facilitate immune evasion and within-host proliferation (Paschos & Allday 2010). For example, \textit{Leishmania} protozoans are hypothesized to alter DNA methylation patterns in macrophages to downregulate host defenses and promote parasite survival (McMaster et al. 2016). Similarly, infection with influenza viruses and coronaviruses may induce histone modifications that alter immune signaling in favor of infecting parasites (Schäfer & Baric 2017). Such phenomena remain active areas of research, as scientists seek to uncover the diverse mechanisms used by parasites to circumvent host immunity (Supporting Information).

Within wildlife systems, the field of ecological epigenetics has focused primarily on changes induced by behavior, diet, and environmental conditions. Only a few researchers have linked these changes to immune processes (Isaksson 2015), and fewer still have examined differences in gene regulation between host populations. In 1 example, Ellison et al. (2014) compared transcriptome data from 4 sympatric frog species with different Bd susceptibility. When exposed to a pathogenic strain of Bd, resistant species exhibited downregulation of skin inflammatory pathways and upregulation of genes pertaining to skin-barrier integrity and cell-mediated immune responses. These patterns suggests that
resistant hosts maintain skin and immune functioning during infection. This contrasts susceptible species, which often succumb to immunosuppression and disease-mediated disruption of the skin barrier. Together, these results reveal important processes involved in chytridiomycosis morbidity and mortality across species.

Perhaps the strongest example of an integrative genomic, transcriptomic, and epigenetic approach to understanding disease pathogenesis occurs at the intersection of cancer and infectious disease with DFTD. Although genomic analyses revealed insights about host diversity and disease transmission, they have not elucidated specific mechanisms underlying emergence and immune evasion of this transmissible cancer. To address these questions, Murchison et al. (2010) considered functional variation alongside traditional genetic markers. Host and parasite genetics confirmed that tumors were genetically distinct from hosts and clonal in origin. Analyses of devil and tumor transcriptomes suggest that DFTD first arose in a mutated Schwann cell. Even though these cells function in local immunity, the Schwann cell origin of DFTD does not sufficiently explain the capacity of tumor cells to evade the immune system. More likely mechanisms appeared in analysis of expression profiles, which revealed upregulation in molecules associated with immune evasion and downregulation in molecules associated with tumor suppression. Considered alongside MHC-based and genomic analyses (Grueber et al. 2015; Morris et al. 2015), it seems that low immunogenetic diversity in hosts decreases the immune system’s probability of detecting tumors that fail to display MHC class I molecules on their cell surfaces (Siddle et al. 2007, 2013). This failure appears to result from epigenetic changes that induce downregulation of antigen-processing genes in cancerous cells, which then allows tumor cells to slip under the immune system’s radar. Critically, this may be reversible through epigenetic or immunological manipulation, which suggests a promising new direction for treatment (Siddle et al. 2013).

In the case of DFTD, chytridiomycosis, and other wildlife diseases, analyses of gene regulation provide a powerful tool for identifying mechanisms underlying pathogenesis and pursuing novel targets for treatment (Kungulovski & Jeltsch 2016). Yet, questions remain about the factors that operate alongside hosts and parasites to induce these changes. Recent evidence suggests that epigenetic mechanisms interact with commensal microbial communities inhabiting hosts, thus participating in regulatory cross-talk between the immune system, epigenome, and microbiome (Levy et al. 2015; Celluzzi & Masotti 2016). The importance of this cross-talk has yet to be elucidated, but mounting evidence suggests that a more complex understanding of immune regulation is needed.

Commensal Microbes and Dysbiosis

Studies of humans and model systems increasingly extol the importance of microbiomes in immunity. Commensal microbes have been implicated in developing the host immune system, shielding hosts from infection by competing with invaders, regulating immune responses through altered gene expression and immune signaling, and aiding resolution of responses once parasites are cleared (Supporting Information). Extreme examples are the fungal microbiomes of many plants that have self-organized into symbiotic immune systems that possess all the classic features of vertebrate immune systems (e.g., self- and nonself recognition and short- and long-term memory) (Berendsen et al. 2012).

Unlike other aspects of host biology, microbial communities are both stable and flexible, rendering them particularly useful to immunity. Core microbiota, or resident commensals that consistently colonize certain body sites, are largely determined by host genetics (Goodrich et al. 2014). Healthy community composition is often taxon specific and putatively results from long-term coevolution (Colston & Jackson 2016). Transient and temporary resident microbes primarily derive from environmental and behavioral sources (Candela et al. 2012). These can rapidly change to reflect different external conditions or parasitic infections and may even function in larger scale adaptation of hosts (Shapira 2016).

In the absence of infection, commensal microbial communities aid homeostasis and are critical to host defense (Honda & Litman 2016). Yet, microbes do not present an impenetrable front. Termed dysbiosis, disruption of healthy microbial communities is often associated with autoimmunity and disease pathogenesis (Petersen & Round 2014). As a result, microbial analyses of human diseases have skyrocketed in the last decade as researchers seek to elucidate mechanisms underlying pathology and design novel treatments (Knight 2015).

The need for studies examining the role of microbial communities in wildlife disease is increasingly recognized (Redford et al. 2012). Many primarily seek to characterize microbiome structure in healthy individuals. For example, Cheng et al. (2015) sequenced microbial communities from wild and captive Tasmanian devils and noted distinct differences between them. This establishes a baseline for comparison with communities disrupted by captivity and DFTD. In other systems, such as chytridiomycosis, research has moved away from descriptive studies toward manipulative experiments seeking novel treatment options. Many amphibian species naturally harbor skin bacteria known to secrete metabolites harmful to Bd. Commensal microbial communities have therefore been associated with differing disease outcomes in host populations (Jani et al. 2017). In an effort to commandeer this phenomenon for disease management, treatment of Rana muscosa with Bd-resistant bacteria drastically
By focusing on community structure and function, adoption of a multiomic approach may mitigate this difficulty through design of more effective taxon-specific probiotic therapies (Rebollar et al. 2016). In the case of probiotic-resistant species, microbial bioaugmentation may not be universally applicable as frog species harbor unique communities that differ in response to manipulation (Kueneman et al. 2014; Küng et al. 2014). In the case of probiotic-resistant species, community composition may act as a sentinel for estimating susceptibility across populations (Becker et al. 2015). 

**Applying an Integrative Molecular Approach to Conservation**

When examined in isolation, genetic, epigenetic, and microbial variation can provide key information about wildlife disease ecology that informs conservation. Although implementing these methods in wildlife comes with the inherent challenges of limited sampling opportunity, lack of reference genomes for nonmodel organisms, and high start-up costs of learning new laboratory and analytical techniques, it remains a worthwhile and versatile endeavor. Depending on the resources available, ability to collect samples, complexity of the host-parasite system, and questions most relevant to effective conservation, managers can pick and choose the methods that best address their needs (Fig. 2). Further, decreased mortality in experimental trials (Harris et al. 2009). Similar results were reported for experimental manipulations of *Anaxyrus boreas* microbial communities, where the loss of Bd-inhibitory bacteria increased infection rates and the application of resistant bacteria increased survival (Kueneman et al. 2016). However, probiotic bioaugmentation may not be universally applicable as frog species harbor unique communities that differ in response to manipulation (Kueneman et al. 2014; Küng et al. 2014). In the case of probiotic-resistant species, community composition may act as a sentinel for estimating susceptibility across populations (Becker et al. 2015).

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**Figure 2.** Steps in the application of molecular methods to wildlife disease. In step 1, the question of interest must be identified. In step 2, the molecular method selected needs to address the chosen question. In step 3, samples need to fit the selected method and financial and logistical constraints of sampling the host–parasite system. In step 4, the molecular dataset must be appropriate for addressing the question with the collected samples. For complex systems characterized by multiple questions, relevant methods are combined to adopt an integrative molecular approach. See Supporting Information for case studies in which molecular techniques informed management of wildlife disease.

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- **Step 1: Identify Question**
  - What parasites infect hosts?
  - How many strains are there?
  - Where did emergence occur?
  - What are common transmission pathways?
  - Which hosts are reservoirs?
  - Are specific parasite genes associated with virulence?
  - Do hosts possess high levels of immunogenetic variation?
  - Are there specific host genes associated with morbidity, mortality, or treatment efficacy?
  - Is disease driving local adaptation or demographic changes in hosts?
  - Are gene regulatory changes associated with disease state?
  - If so, what genetic mechanisms drive morbidity, mortality, or treatment efficacy?
  - Are these mechanisms reversible through epigenetic intervention?
  - What microbial communities typically inhabit healthy hosts?
  - Is microbial dysbiosis associated with disease state?
  - Do certain bacterial strains often co-occur with infection?
  - Can probiotic bioaugmentation improve disease outcomes?

- **Step 2: Choose Method**
  - **Parasite Genetics**
    - Microparasites (isolated from swabs, cultures, blood, saliva, feces, infected tissues)
    - Macroparasites (directly removed from hosts or collected from host excrement)
  - **Host Genetics**
    - Noninvasive samples (e.g., scat, hair, feathers, saliva) can be used with methods that accept low input DNA (such as microsatellite genotyping)
    - Invasive samples (e.g., tissue, blood, bone) produce high-quality DNA suitable for most analyses
  - **Host Epigenetics**
    - Invasive samples (e.g., tissue, blood, bone) produce high-quality DNA suitable for most analyses
    - For epigenetic / transcriptomic analyses, site-specific tissue (e.g., skin for skin disease) is often required, though blood can serve as an adequate proxy
  - **Host Microbiome**
    - Scat or anal swabs (gut)
    - Saliva or cheek swabs (oral)
    - Skin punches or swabs (skin)
    - Additional site-specific swabs or tissues, depending on question and focal host-parasite system

- **Step 3: Collect Samples**
  - **Samples**
    - Microsatellites (low input DNA)
    - Targeted gene sequencing
    - Whole or partial genome sequencing
    - Targeted epigenetic sequencing
    - Reduced representation bisulfite sequencing
    - Whole methylome sequencing
    - Gene expression profiling
    - Whole transcriptome sequencing
  - **16S ribosomal RNA (rRNA) sequencing**
    - Metagenomic sequencing

- **Step 4: Generate Data**
  - **Samples**
    - Microsatellites (low input DNA)
    - Targeted gene sequencing (e.g., MHC or TLR genes)
    - Restriction-enzyme based sequencing
    - Whole genome sequencing
    - Microsatellites (low input DNA)
    - Whole transcriptome sequencing
  - **Data**
    - Whole or partial genome sequencing
    - Targeted epigenetic sequencing
    - Reduced representation bisulfite sequencing
    - Whole methylome sequencing
    - Gene expression profiling
    - Whole transcriptome sequencing

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Conservation Biology
Volume 32, No. 4, 2018
sequencing costs and increasingly user-friendly analytical pipelines have rendered these methods accessible to studies of nonmodel organisms.

When multiple questions arise and resources allow, applying an integrative molecular approach represents an exciting new frontier in wildlife disease ecology. Concurrent examination of interconnected factors within molecular diversity can elucidate patterns and processes relevant to complex wildlife disease systems. As evidenced in case studies of DFTD and chytridiomycosis, analysis of genetic, gene regulatory, and microbial variation provides important insight into pathology, transmission dynamics, and novel avenues of treatment. For DFTD, genetic and transcriptomic analyses were needed to distinguish host from parasite, confirm allograft transmission and Schwann cell origin of the disease, and discover means of immune evasion through suppression of immune signaling amid minimal immunogenetic diversity in hosts. These insights have since informed management strategies, including ongoing treatment development and management of captive insurance populations (Pye et al. 2016). In the case of chytridiomycosis, genetic and transcriptomic analyses have furthered understanding of immune processes involved in species-specific resistance to fungal infection, and microbial analyses have provided promising new treatment options through bioaugmentation with naturally Bd-resistant microbes (Bletz et al. 2013; Woodhams et al. 2016).

Overall, application of molecular methods will enable more effective monitoring and management of at-risk populations. Depending on questions and data types examined (Fig. 2), understanding of wildlife disease prevalence, pathology, and persistence can be improved. Parasite genetics can refine diagnostic techniques, reconstruct transmission pathways, and identify genes underlying virulence. Similar analyses in hosts can characterize host population structure, patterns of immunogenetic diversity, and specific genes associated with resistance and tolerance strategies. Further examination of gene regulatory diversity can identify specific mechanisms underlying successful parasite invasion and host morbidity that may serve as targets for epigenetic intervention. Finally, commensal microbial analyses can elucidate patterns of dysbiosis associated with host disease state and suggest probiotic bioaugmentation as a novel treatment strategy. Especially when considered alongside other drivers of disease state (Fig. 1), molecular analyses present innumerable opportunities to advance management of wildlife disease and mitigate threats posed to human and domestic animal health, agricultural systems, and wildlife populations. From examination of the human literature, it is evident that the technology for molecular analyses exists. The urgency of many conservation problems suggests that it is time these methods are applied to studies of wildlife disease.

**Acknowledgments**

This material is based on work supported by the National Science Foundation Graduate Research Fellowship under grant DGE1656466. We thank E. S. Almberg and D. R. Stahler for many insightful discussions.

**Supporting Information**

Tables containing additional citations serving as examples of factors affecting host susceptibility (Appendix S1), genetic analyses of parasites (Appendix S2), epigenetic, microbial, and integrative molecular analyses in human and model systems (Appendix S3), and molecular analyses informing wildlife conservation (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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