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Illuminating the Mysteries of the Smallest Birds: Hummingbird Population Health, Disease Ecology, and Genomics

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Keywords

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Abstract

Hummingbirds share biologically distinctive traits: sustained hovering flight, the smallest bird body size, and high metabolic rates fueled partially by nectar feeding that provides pollination to plant species. Being insectivorous and sometimes serving as prey to larger birds, they fulfill additional important ecological roles. Hummingbird species evolved and radiated into nearly every habitat in the Americas, with a core of species diversity in South America. Population declines of some of their species are increasing their risk of extinction. Threats to population health and genetic diversity are just beginning to be identified, including diseases and hazards caused by humans. We review the disciplines of population health, disease ecology, and genomics as they relate to hummingbirds. We appraise knowledge gaps, causes of morbidity and mortality including disease, and threats to population

viability. Finally, we highlight areas of research need and provide ideas for future studies aimed at facilitating hummingbird conservation.

1. INTRODUCTION

Members of the family Trochilidae, hummingbirds, share a fascinating and distinctive suite of traits, including exquisite iridescent feather colors, unique flight abilities, small body sizes, torpor, nectivory, insectivory, plant pollination, and a high number of species that radiated into nearly every habitat in the Americas. They capture the hearts and imaginations of nearly everyone who sees them zoom by; yet, they are understudied compared to other bird families. This is particularly true in the South American core of hummingbird species richness; thus, an important peril to species persistence and healthy populations includes the lack of scientific data on biology and threats.

With global environmental and climate changes accelerating, species in the Trochilidae family are among many birds in decline and at risk of extinction. The International Union for Conservation of Nature (IUCN) Red List (**https://www.iucnredlist.org/**) includes 191 hummingbird species as having decreasing population trends and 71 species with "unknown" trends as of July 2023. Two Caribbean hummingbird species are listed as Extinct, and other species likely were never identified to written science prior to their extinction. Despite the apparent ubiquity of several hummingbird species in suburban neighborhoods with feeders in North America, a range of hazards have caused many populations to decline. Lack of data for most species means their population viability status, and therefore their extinction risk, is unknown. Hummingbird conservation is hindered by knowledge gaps in population biology parameters such as vital rates, population sizes, genetics, normal versus stress physiology, disease prevalence, habitat requirements, and ecosystem status.

On an optimistic note, recent science and technological advancements in modeling, genomics, and pathogen detection are allowing discoveries and monitoring that are assisting bird conservation efforts. We review the disciplines of population health, disease ecology, and genomics as they relate to hummingbirds. We appraise gaps in knowledge, causes of morbidity and mortality including disease, and threats to population viability. Finally, we highlight areas of need and provide ideas for future research aimed at facilitating conservation.

1.1. Defining Terms

Because the terms population health, disease ecology, and genomics can be defined in various ways depending on use and context, we start with definitions as they are used here. We define a hummingbird population as a group of individuals of the same species that inhabit a common habitat. Migratory populations can have different subsets of individuals seasonally: wintering/nonbreeding populations, migratory populations that may travel through the same flyway in spring and late summer/autumn, populations that share breeding habitats during the spring and early summer, and post-breeding populations that include dispersing fledglings.

Hummingbird population health indicates that members of a population maintain biologic functions characteristic of the species and have fitness, survivorship, and recruitment of young adequate to maintain or increase the population size of the next generation. Prevalence of infectious and noninfectious diseases is included as a health factor, as is population genetic diversity, whereby, for example, inbreeding due to low population size and lack of mates can lead to poor population health. Healthy hummingbird populations are viable populations that contribute to the species' ability to adapt to its environment and evolve over time. Many factors influence population health,

including demographics and other life history traits, spatial and temporal distributions of members of its own species as well as other organisms that share its habitat, food and resource availability, and other environmental factors (1).

Disease ecology is a "subdiscipline of ecology concerned with how species interactions and abiotic components of the environment affect patterns and processes of disease" (2). Disease is "any deviation from or interruption of the normal structure or function of a part, organ, or system of the body as manifested by characteristic symptoms and signs" (3). The wildlife biology community increasingly recognizes that disease is an integral driver of population dynamics (4). The study of disease ecology in hummingbirds is challenging but vitally important for their conservation.

Genomics is one among a powerful set of tools that can be applied to evaluate hummingbird population health, disease ecology, and conservation biology (5). For the purposes of this article, we use the term genetics to describe the study of individual DNA sequences, including specific genes and their inheritance. Wildlife genetics historically has included tools such as microsatellites and mitochondrial DNA to estimate genetic variation within and gene flow across populations. We use the term genomics to encompass the study of an organism's DNA taken as a whole, including their genes and intervening DNA sequences. We also recognize the importance of other omics, including but not limited to epigenomics, transcriptomics, and proteomics, in the study of hummingbird population health and disease ecology. These are, respectively, studies of chemical instructions for genome function (6), RNA transcription and translation from DNA (7), and protein function and structure to understand an organism's biology (8).

1.2. Why Study Hummingbirds?

Hummingbirds have held fascination, wonder, and spiritual significance for people throughout known cultural histories of the Americas, including Aztec, Incan, and Mayan people (9). Their "jewel-like plumage" (10, p. 6), aerial flight acrobatics, and nectar-feeding flits among flowers are endearing to nearly everyone who interacts with them. Zooming among flowers; hovering to take a sip, but never too long; leaving a glint of bright iridescent color to the eye; then quickly flying in a beeline to the next floral stop: These wonders never cease to amaze, spike curiosity, and provide joy to those who feel honored to hold hummingbirds in hand, whether for wildlife rehabilitation (11) or during capture and release for research and population monitoring, as do the authors of this review.

Another reason for scientific focus on hummingbirds is their unique biology, ecological roles as pollinators, and unsurpassed metabolism, living the "sugar-charged lives of the world's smallest birds," as written by the authors of the Trochilidae species account in *Birds of the World* (12). With somewhere between 363 and 373 known living species, the hummingbird family is second only to the tyrant flycatchers (with at least 450) in species number (12; **https://www.iucnredlist.org**). They live in a wide range of habitats across the Americas, from deserts to rainforests, boreal forests, high mountains, islands, and urban landscapes. Most species inhabit the tropics of the Americas (12), whereas at the extremes of the continents are rufous hummingbird, *Selasphorus rufus* (13), breeding as far north as Alaska, and the green-backed firecrown (*Sephanoides sephaniodes*), breeding as far south as the Tierra del Fuego archipelago at the southern tip of South America (14) (**Figure 1**).

2. SPECIAL BIOLOGY, ECOLOGY, AND EVOLUTIONARY HISTORY OF HUMMINGBIRDS

Knowledge of hummingbird natural history, biology, ecology, and evolutionary history is vital to the study of population health, disease dynamics, and genomics. With their unique biology,

Figure 1

Approximate range of the family Trochilidae, the hummingbirds, throughout North, Central, and South America, during one or more of their breeding or summering, nonbreeding, and year-round ranges. Note that lack of data, and effects of climate change and human-caused alterations of habitats, may shift ranges from those shown. Information for this figure came from multiple sources including Reference 10, eBird (**https://www.ebird.org**), BirdLife International (**https://www.birdlife.org/**), and Birds of the World (**https://birdsoftheworld.org**). Figure adapted from images originally created in BioRender.com.

hummingbirds have extended into ecological niches and evolved into more than 360 species and filled a diverse array of habitats in the Americas (**Figure 2**). Hummingbirds obtain energy primarily from sugars in flower nectar, protein mainly from insects, and other nutrients supplied by both nectar and insects.

The smallest bird on earth, at 1.6–2.6 g (the weight of a US dime, or half the weight of a Cuban peso), is the bee hummingbird (*Mellisuga helenae*) (15), followed closely by other tiny hummingbirds, such as the vervain hummingbird (*Mellisuga minima*) (16). The largest species is the giant hummingbird (*Patagonia gigas*) of western South America; at ∼20 g, it weighs approximately 10 times more (17) and can look more like a swallow or swift than a hummingbird in flight (18). Their small body size of only a few grams houses a racing metabolic engine needing an estimated 4–10 calories per day (19). This would be the equivalent of 68,000–170,000 calories per day for a 68-kg person! To evade predators and travel quickly from flower to flower, hummingbirds fly fast and with unmatched avian agility. Their ability to fly in prolonged hovering (unlike any other vertebrate) (20) allows them to access energy nutrients via "dedicated nectivory" (21). Their wings can beat up to ∼3,000 times per minute (22), and their muscle is rich in ATP, powering flight of up to 30 km/h (20). Delivering the energy and nutrients to flight muscles is a cardiovascular and respiratory system with heart rates of up to ∼1,300 beats per minute (20). Specialized wing

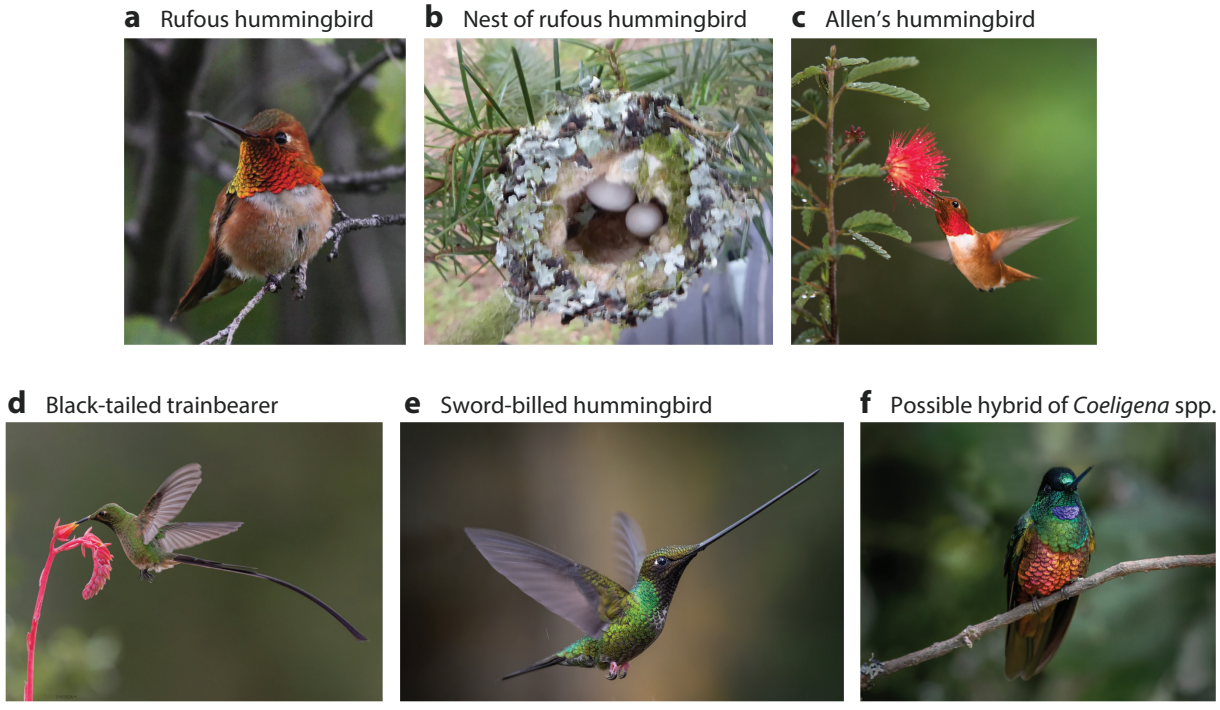

Figure 2

Hummingbirds. (*a*) Rufous hummingbird, British Columbia, Canada. (*b*) Nest of rufous hummingbird, British Columbia, Canada. (*c*) Allen's hummingbird, California. (*d*) Black-tailed trainbearer (*Lesbia victoriae*) lives in habitats of 2,500–3,800 m in Colombia, Ecuador, and Peru. (*e*) Sword-billed hummingbird (*Ensifera ensifera*) occurs in montane forest and edges in the Andes of South America. This species is characterized by its long bill and has coevolved with Andean plant species of *Passiflora* with long nectar tubes. (*f*) Possible *Coeligena* spp. This mysterious hummingbird was registered in Monserrate, a 3,000-m-high mountain and prominent tourist attraction in Bogota, Colombia. This individual might be a cross between the golden-bellied starfrontlet (*Coeligena bonapartei*) and the blue-throated starfrontlet (*Coeligena helianthea*); however, it disappeared before it could be studied. Photos courtesy of (*a*) Mark Habdas, (*b*) Stan Olson, (*c*) Scott Logan, and (*d–f*) Diego Emerson.

structures, including fused bones, allow the fast wing beats and hovering, upside-down, and backward flight.

Hummingbirds can visit thousands of flowers per day. These floral visitations provide pollination services necessary for plant reproduction. They eat hundreds of insects per day, providing another ecological service by dampening insect numbers, including mosquitoes and others that can harbor zoonotic pathogens. Their characteristic long bill houses a slender tongue unique to the animal kingdom (23), which can flick 15 times per second and corresponds evolutionarily to flowers sharing its shape (24). To discriminate a variety of flower colors and make optimal choices for nectar feeding, hummingbirds can see a wider spectrum of light than humans, including ultraviolet (25). With an enlarged hippocampal brain region, they have exceptional spatial and episodic memory, a type of memory for specific events and experiences (26).

Energy needs are the highest when hummingbirds are migrating, breeding, or molting feathers. Adaptations for fat deposition and metabolism allow survival during long migrations between summer and winter habitats. Ruby-throated hummingbirds migrate between eastern North America and Mexico, with some flying nonstop for days across the Gulf of Mexico (27). As the longest documented hummingbird migrant, the rufous hummingbird travels as much as

6,000 km each way on trips between the northern-most summering range in Alaska and their wintering range along the southwestern coast of Mexico (13). The migrating species build layers of fat on their chins and abdomens, often adding up to 44% of their body weight (28). Once the hummingbirds arrive at their destinations, fat, and even muscle, is depleted. Torpor, a slowing of metabolism and reduction in body temperature, enables hummingbirds to survive in colder climates or other adverse conditions by temporarily reducing metabolic needs (29). Well-meaning people sometimes "rescue" birds in torpor, believing they are sick or dying (30).

A nagging and unanswered scientific question is how contemporary hummingbirds arrived to the Americas, when their pre-Pleistocene fossil record comes only from Europe (31). Fossils of ancestral forms of hummingbird have been unearthed in Oligocene and Eocene epochs in the Old World, including Germany (*Eurotrochilus inexpectatus*) (32), France (31), and Poland (33). A general paucity of bird fossils, especially hummingbirds, is attributed to their small size and fragile hollow bones. This lack of fossils hinders the determination of evolutionary relationships of hummingbirds to other avian taxa; however, genomics is providing key insights.

Hummingbirds are grouped in the clade Strisores, along with swifts and nightjars, through evidence from the study of morphology (skull, humerus, and radial wing bone anatomy), phylogenetics, genomics, and paleontology (34). However, phylogenies remain contested in the literature. Phylogenetic studies suggest that hummingbirds diverged from their sister group, the swifts and treeswifts, ~42 million years ago in Eurasia and dispersed across Beringia and North America, reaching South America between ~40 and ~22 million years ago (35). Extant hummingbirds originated in South America (36) and radiated into nine main clades: topazes, hermits, mangoes, brilliants, coquettes, the single-species clade *Patagona* (the giant hummingbird), mountaingems, bees, and emeralds (37–39). Hummingbirds thrived in the lowlands of South America and expanded across diverse habitats across the Americas and the Caribbean. With the uplift of the Andes in South America during Pleistocene glaciations, topographic complexity and climatic stratification across elevation gradients played a key role in the diversification of hummingbirds and other vertebrates in the neotropics (40).

Most of the diversification of coquettes and brilliants, which together contain ~30% of hummingbird species, took place during the period of quickest Andean uplift (38). Similarly, several hermits, mangoes, bees, and emeralds originated from or arrived to the Andes when Andean uplift was underway (38). The topographic and ecological complexity of the Andes has enabled hummingbirds to pack and maximize geographic space through habitat partitioning and might explain the small geographic ranges that Andean hummingbirds occupy.

3. RESEARCH METHODS: SPECIAL TECHNIQUES FOR SPECIAL BIRDS

Researching hummingbirds requires special techniques, permitting, innovations, and attention to assure the welfare of the tiny bird in the hand (41–43) (**Figure 3**). Some of the methods are common to the study of other bird taxa, such as the use of stable isotopes to infer diet, origins, or migratory patterns (44) and the use of citizen science data (**https://ebird.org**) to identify hummingbird distribution, abundance, habitat use, migratory patterns, and temporal and spatial trends (e.g., 45). Breath, excreted urine and feces, and feathers provide minimally invasive sample sources for stable isotopes (46), pesticides (47), heavy metals (48), hormones (49), parasites (50), and genetics (51).

Bird banding, and its associated data and sample collection, has been an integral research tool for hummingbird disease ecology and genomics. In North America, banding data have allowed researchers to assess sex-specific disease prevalence (52), species hybridization rates (53), longevity, and migration ecology. Hummingbird leg banding (using a 1-mm-wide aluminum band

Figure 3

Hummingbird field work. (*top left to right*) Feeder net: Sides are released down via a line by a nearby biologist to enclose a hummingbird when it enters the feeder net; the biologist reaches in to gently capture the hummingbird in the net. A researcher examines the head and bill with a magnifying glass (female broad-tailed hummingbird, Wyoming). (*center left to right*) Wing length is measured and a federal ID band is placed (just above finger tips on leg); a band is shown in fingers. Hummingbirds are offered sugar water before release (most drink readily). A male broad-tailed hummingbird is ready for release back where it was netted. (*bottom left to right*) A magnifying loop, band placement pliers, and bands on sticks—band sizes shown are every 0.2 mm from 5.4 mm to 6.4 mm. A scale to weigh hummingbirds and a US nickel weighing 5.1 g, more than most North American hummingbirds. HummMobile, a mobile lab; two hummingbird field biologists inside HummMobile examine, band, and sample hummingbirds in Colorado. Two lower-left equipment photos courtesy of L.A. Tell; other photos courtesy of H.B. Ernest, Mark Gocke, and Jessica Grant.

stamped with a letter and five numbers) gained prominence for population research and monitoring starting in the 1960s. Between 1960 and 2022, data for 1,027,188 banded hummingbirds were reported to the US Geological Service Bird Banding Lab (BBL) (54; our analysis of the data). More than 96% of banding (>993,000) was focused on 7 species inhabiting North America, and of those, nearly half were ruby-throated hummingbird (*Archilochus colubris*, a common species of eastern North America); the other 6 species were those common in western North America [black-chinned (*Archilochus alexandri*), Anna's (*Calypte anna*), calliope (*Selasphorus calliope*), rufous, broad-tailed (*Selasphorus platycercus*), and broad-billed (*Cynanthus latirostris*) hummingbirds]. The BBL focuses mainly on birds in the United States and Canada, and the 7 commonly banded species represent only 2% of hummingbird species diversity, with the core of species diversity lying in tropical South America. However, these BBL data provide a foundation and model for species in Mexico and Central and South America. Individual researchers and monitoring networks, such as the Hummingbird Monitoring Network (**https://www.savehummingbirds.org**), collect more detailed data than required by BBL for specific research purposes, while also submitting data to

the BBL. BBL-permitted hummingbird banders share information and tips via an email listserv (as mentioned in 53). The Western Hummingbird Partnership (**https://westernhummingbird.org**) provides networking resources and education among North American federal agencies, nongovernmental organizations, individual researchers, and conservation advocates.

Use of telemetry for locating and tracking movements of hummingbirds has lagged its use in larger birds; however, miniaturization of radio transmitters is allowing expanded use with less impact on flight and behavior. Radio telemetry studies have allowed assessments of the effect of tropical deforestation on landscape movement patterns (55), patterns of habitat use (56), hummingbird interactions with nectar-producing plants (57), and migration ecology (58). Radio-frequency identification using passive integrated responders overcomes the weight, size, and durability limitations of radio transmitters and has proven useful to assess behavioral interactions at feeders (59) and phenology and migratory behavior (60). As technology and methods to attach telemetry improve, such as the development of a lightweight backpack harness (61), satellite and variations of GPS telemetry will soon provide even more valuable data.

4. POPULATION HEALTH AND DISEASE ECOLOGY

Hummingbird population health encompasses not only the prevalence or absence of infectious and noninfectious disease (62) but also population demographics (63); genetic and phylogenetic factors (35); and influences of its biotic and abiotic environment (57) on fitness, recruitment, and survivorship [for example, rufous hummingbird (64)]. The main proximate and ultimate causes of the declines of hummingbird populations are diverse and not well understood. Hazards to populations are mounting with climate change, wildfires, habitat degradation and loss, and exposure to toxins and non-native predators (including outdoor cats) and vary by species and ecosystem. Those physiologic stressors and exposures exacerbate disease risk, which can threaten population viability.

Detecting morbidity and mortality at population levels is a challenge in hummingbirds, which have solitary lifestyles; are small, aerial, and highly mobile; and require special expertise and permits to handle and tag with marking devices. Larger birds more readily can carry the weight of telemetry devices necessary to track them over extended time and space (e.g., raptors) and live in large groups (e.g., water fowl) and in habitats frequented by people, who more readily find and submit sick or dead birds (e.g., urban crows). In contrast, when these small, solitary birds get sick or die, their bodies are hidden in foliage and decompose quickly. Lacking adequate methods, the earliest disease studies were necessarily anecdotal and had insufficient sample sizes to conduct population-level epidemiology [a hummingbird disease and injury report in 1957, for example (65)]. Historically, reports in hummingbirds focused mainly on diseases that were documented previously in well-studied animals; showed externally obvious clinical signs; or were diagnosed via samples such as blood [hemoparasites in avian "malaria" (66)], feathers, and birds found dead or in captive settings.

Until recently, scant information was available to enable studies of population dynamics, epidemiology, pathogen exposure, and presence of disease in hummingbirds. Such data are necessary to document and develop strategies to mitigate the hazards that threaten hummingbird populations, including climate change, habitat loss, and urban dangers (window strikes, domestic cat predation). In addition to research studies focused on wildlife biology, surveys to monitor diseases of importance to humans or domestic animals sometimes opportunistically sample hummingbirds. Examples are dead bird monitoring for West Nile virus (67) and avian influenza virus (AIV); however, hummingbirds are not typically the main focus of those projects. They provide presence-only (not presence/absence) data and therefore are insufficient for hummingbird disease epidemiology.

For a review of history and summary of hummingbird disease discoveries, see Reference 62. Two groups of disease-causing pathogens infecting hummingbirds and featuring prominently in publications are *Avipoxvirus* and Haemosporidia ("malarial" blood parasites). These two groups of pathogens exemplify the importance of historical research foundations and contemporary disease studies, as well as applications of polymerase chain reaction (PCR)-based diagnostics and state-of-the-art research methods, in determining population impacts to hummingbirds. They also can serve as models for studies on other diseases in hummingbirds that may be important drivers in population declines.

4.1. *Avipoxvirus*

Among the earliest published observations of infection and disease in hummingbirds were of "foot pox" (68). Long before the cause of the disease was identified as members of the *Avipoxvirus* genus, diverse wild and domestic bird taxa were seen with "poxes" [sometimes referred to as "tumors" in older references (69)], wart-like growths on feather-free areas of skin, around eyes, on legs, and on tissues on and near the bill. The lesions were described as crusty nodules on feet, as well as bills and wings. Avian poxvirus (genus *Avipoxvirus*, family *Poxviridae*) infections are found in diverse bird species throughout the world and are primarily mosquito transmitted (70). Two basic presentations in birds other than hummingbirds are the wet form (diphtheritic) and the dry form (cutaneous) (70). *Avipoxvirus* was definitively diagnosed via electron microscopy and the characteristic histopathology findings in pox-like lesions in five Anna's hummingbirds (71). The wet form has not yet been documented in hummingbirds, possibly due to limited access to freshly dead wild hummingbirds for necropsy. Although publications, birders, and avian researchers historically referred to such lesions as pox, multiple etiologies may cause the disease presentation, and visual inspection or histopathology alone was found to underestimate avian pox prevalence (52).

Advancements in diagnostic techniques and epidemiology of *Avipoxvirus*-caused disease in hummingbirds include a minimally invasive method (52) using quantitative real-time PCR (72). This molecular approach for assessing the disease in live birds detected more pox virus infections in living birds than previous detection methods (52). Avian poxvirus was found to be one of the more common causes of disease in a retrospective study of hummingbirds presented for veterinary care to two California wildlife hospitals (73). Anna's hummingbird is one of the few species that is expanding its range (74); it will be interesting to see if avian pox (and its requisite insect vector) follows the species northward or affects additional hummingbird species.

4.2. Haemosporidians (Blood Parasites)

According to Moens et al. (75, p. 1235), "The explosive radiation of hummingbirds provides an ideal system to investigate the evolution of host specificity among avian Haemosporidia." Haemosporidian (blood) parasites belonging to the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* infect birds through insect vectors (76). They have long been known to cause severe disease in certain bird taxa and demographic groups, such as in raptor chicks (as reviewed in 77), and in some cases are an extinction threat in island birds (78).

All three genera infect hummingbirds in South America (79, 80). Only the genus *Haemoproteus* has been documented in hummingbirds of North America, including low-elevation hummingbirds sampled in California (81) and high-elevation broad-tailed hummingbirds sampled in the Rocky Mountains of Colorado and Wyoming (82). These North American species are migratory, spending winters in Mexico or Central America. The disease dynamics and pathogenesis of Haemosporidia in the Trochilidae are not well understood. With high metabolism and oxygen requirements (20), hummingbirds have greater red blood cell counts than other birds (83, 84); thus, one expectation is that carrying a parasite load in the blood would be detrimental. Research

in the Andes of Ecuador has shown that high prevalence is related to higher elevation (>2,145 m) and lower temperature (<16.5°C) and that parasite diversity is higher at mid-elevations (80). High prevalence and numerous parasite lineages suggested the presence of endemic infections in high-altitude Andean hummingbirds; however, an effect on health parameters has not been assessed. Anecdotally, in the authors' experience, North American hummingbirds we determined to have Haemosporidia in their blood typically had no signs of disease when examined in the field. Research providing keys to the identification (such as 85), improved pathogen diagnostic methods, insect vector ecology, and access to detailed hummingbird health data sets with which to conduct epidemiological analyses will provide insights into blood parasite disease mechanisms and causation.

4.3. Population Health and Disease Ecology in Mexico, Central America, and South America

Much of the research in disease and threats to hummingbirds has centered on the few species in the United States and Canada. Few studies have addressed hummingbird health in Mexico, Central America, and South America; therefore, there is insufficient information to determine the impact of pathogens and diseases on hummingbird populations. A study screening for AIV of 600 land birds in Peru included 75 hummingbirds across 3 species, of which 3 tested positive (86). The authors reported that this was the first broad-scale survey for AIV in South America and the first detection of AIV in hummingbirds. A few studies conducted in these regions identified a virus or bacterial infection in individual birds, as reviewed in Reference 62, such as a Venezuelan equine encephalitis virus (*Togaviridae*) in a long-tailed hermit (*Phaethornis superciliosus*) in Mexico in 1972 and a gram-negative bacteria (*Serratia marcescens*) in a swallow-tailed hummingbird (*Eupetomena macroura*) showing weakness, breast muscle atrophy, and an inability to swallow in Brazil. One-off pathogen identifications do not provide clues of disease ecology at population levels, although they can serve as early sentinels for a later epidemic. Ectoparasites such as lice and mites have been reported in hummingbirds throughout the Americas; however, their effect on hummingbird health and fitness is largely unknown. In an example where a model hummingbird species might inform South American research, a detailed study of *Proctophyllodes huitzilopochtlii* mites on 753 California hummingbirds found an abundance of mites on Anna's hummingbirds. A question remains as to whether a heavy burden of mites might have a flight fitness or mate attraction reproductive cost (50). Diptera fly larvae detected in hummingbird nests were studied in Brazil and Mexico. The larvae can reduce nestling development and survival depending on the fly species, infestation intensity, and nestling susceptibility (87, 88).

5. GENOMICS AND GENETICS: MOLECULAR APPROACHES TO HUMMINGBIRD POPULATION HEALTH AND DISEASE ECOLOGY

> 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.
>
> —DeCandia et al. (5, pp. 799–800)

Advances in genomics and conservation genetics are progressing hummingbird biology, disease ecology, and conservation applications (**Figure 4**). This is important because compared with stable populations, populations in decline have greater loss of genetic variation (genetic drift) and higher inbreeding rates. This can lead to loss of fitness, including lowered immunity against

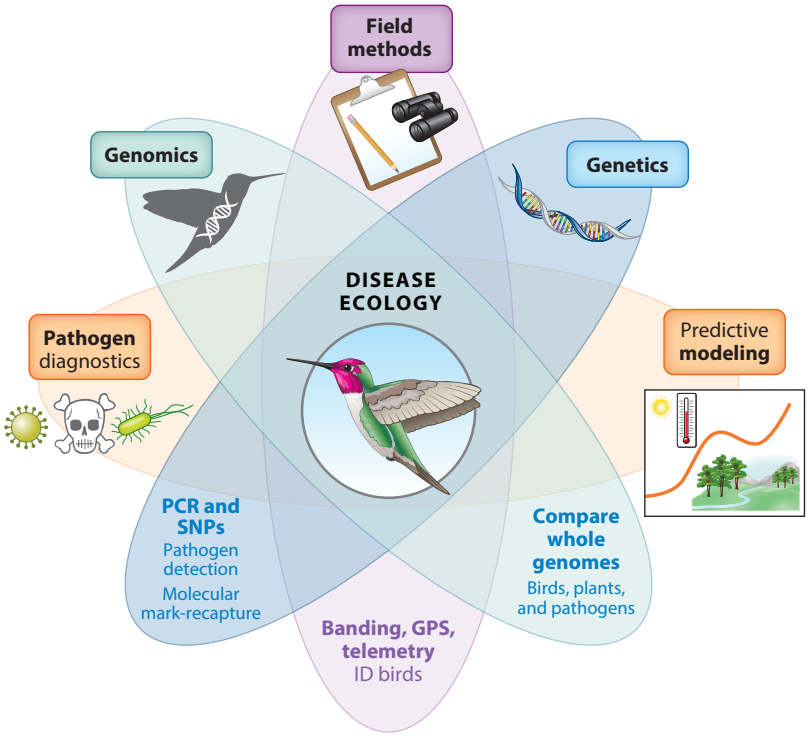

Figure 4

Some of the interdisciplinary sciences necessary to determine the status of population health of hummingbirds. Abbreviations: PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism. Figure adapted from images originally created in BioRender.com.

disease and degraded ability to adapt to changes in the environment. Genomics is a powerful tool, particularly when paired with machine learning and bioinformatics methods to handle large data sets and geographic information systems for spatial analyses. Diverse questions are now more tractable, ranging from individual bird identification to taxonomic phylogenetic relationships of hummingbirds and the pathogens infecting them.

5.1. A Brief History of Genetics and Genomics

The recognition that genetics plays a vital role in the conservation and population biology of organisms began to capture broader attention from researchers and conservation biologists in the 1960s and 1970s. Emerging then were ways to study genetic inheritance of traits involved in organism morphology and health (indirectly through allozymes). Published in 1966, heart allozyme data supported the phylogeny of hummingbirds and swifts together in Apodiformes (89). Another study tested whether hummingbird genera defined taxonomically by morphological characters were similarly categorized by allozyme data (90). The authors found "substantial" differences, eliciting new questions regarding how hummingbird species and other avian taxa are phylogenetically related. Allozymes require protein samples that prohibit sampling of a living hummingbird.

Methods including DNA–DNA hybridization led a revolution in phylogenetics, specifically in how bird orders and families were taxonomically categorized, including relationships among hummingbirds and swifts (37, 91). Mitochondrial DNA sequencing of genes such as *cytochrome*

b, *ND2*, and *ND5* led to new views of how phylogenies of hummingbirds in specific habitats are related, such as nine species in the genus *Metallura* inhabiting the Andes Mountains, some at the treeline and some at lower-elevation forests (92). Technology breakthroughs in PCR and use of oligonucleotides (short sections of DNA or RNA) ushered the "molecular revolution," described by Parker et al. (93) as (paraphrasing) "advancing fields across the biological sciences, from human medicine to diverse processes in the biology of natural populations." Earlier discoveries of restriction enzymes and recombinant DNA led to such work as the cloning and characterization of a gene important in hummingbirds' ability to reduce metabolic expenditure during torpor (94).

Studies in wildlife molecular population genetics expanded rapidly through the 1990s with the discovery and development of microsatellites (also called short tandem repeat DNAs and variable number tandem repeats) in concert with improved PCR methods. For the first time, noninvasive, minimally invasive, and small degraded samples could yield DNA data in wildlife. These tools "provided much more powerful means to describe genetic variation at nuclear loci, including the ability to detect past bottlenecks and estimate current effective population size with a single temporal sample" (95, p. 420). Microsatellite panels for hummingbirds developed in the 2000s included Jamaican streamertail hummingbirds (*Trochilus polytmus*) (96) and broad-tailed hummingbirds (97). This tool allowed detection of first-generation hybrids and backcrosses and the identification of contact zones between hummingbird species [for example, the endangered Chilean woodstar (*Eulidia yarrellii*) and a recently expanded Peruvian sheartail (*Thaumastura cora*) (98)]. The population structure of rufous hummingbirds was elucidated for their range in western Canada using microsatellites (99).

5.2. The Start of the Genomic Era

As powerful as microsatellites are for population studies, wildlife geneticists felt hindered by problems including the expense of developing marker panels, the species-specific nature of the genetic markers, and lack of reproducibility across labs. The genomic era of the twenty-first century brought many solutions and a rapid progression of genomic technology innovations, each often making the predecessor obsolete—which is a challenge in itself (lack of comparability with legacy data sets, keeping up with latest bioinformatic methods, the expense of upgrading sequencing equipment, for example). Comparative genomics for evolution, conservation genetics, taxonomy, disease ecology, and more across populations, related species, and widely divergent species was now not only possible but practical and available to broader communities of scientists globally. The first bird genome was sequenced in 2004 in jungle fowl, the ancestor to chicken (100). This first bird genome allowed aligning, comparing, and annotating of hummingbird sequences even before the first hummingbird genomes were sequenced.

The genomic era for hummingbird science began in earnest with the sequencing of an Anna's hummingbird genome (101). Subsequent research employed those sequences, and additional genome assemblies since published include, at the time of this writing, three more for Anna's hummingbird and those for three other hummingbird species [ruby-throated hummingbird, black-breasted hillstar (*Oreotrochilus melanogaster*), and long-tailed hermit (*P. superciliosus*) (2 assemblies)] (102). A study using low-coverage whole-genome sequencing examined gene flow and the role that evolution played in the expansion of Anna's hummingbird, one of the few species without populations in decline (74). This species has a well-defined ecological history of its expansion out from its breeding range in California north into Canada and east into New Mexico and Texas, USA. The researchers found that the species has high genetic diversity, low levels of population genetic structure, and little evidence of evolutionary selection during the species expansion.

The hummingbird and its genome are also of great interest to basic science and researchers outside of the wildlife realm due to its small genome size, unique sugar and fat metabolism,

heterothermy (torpor), plant–animal ecology, and utility for comparative biomedicine. Comparative studies of hummingbird genomes with other taxa, including hominid lineages, are providing insights into diverse aspects of biology across vertebrates that were unattainable prior to the genomic era, such as the physiology and mechanics of taste perception (103) and fat and sugar metabolism (104–106). Research that focused on comparative evolution of hummingbirds with other birds, reptiles, and mammals has discovered the loss of a gene that encodes for epithelial cilia important for clearing mucus (107) and elucidated the highly conserved and gene-rich microchromosomes, the "building blocks of chromosomes" (108).

Interest in the special traits of the hummingbird genome attracted the attention of the Earth Biogenome Project (109) (**https://www.earthbiogenome.org/**), G10K consortium's Vertebrate Genomes Project (**https://vertebrategenomesproject.org**), and the Telomere-to-Telomere Project (T2T). Because of its small genome size, and the ability to sample the same individual as the first hummingbird genome (101), T2T chose an Anna's hummingbird genome as their first organism to sequence and to optimize workflow and pipelines for subsequent organism genome assemblies (110).

Genomic science has opened opportunities to study pathogen epidemiology and to better understand disease ecology in hummingbirds. An example is the aforementioned *Avipoxvirus*, a serious health problem across domestic and wild birds, including Anna's hummingbird (71). Long-read technology facilitated whole-genome sequencing of avipoxviruses (111) directly from pox lesions without the need for extra steps to isolate the virus. This sampling and sequencing provides faster, more affordable pathogen surveillance, with poultry as the intended beneficiary; however, research in wild birds will benefit as well. The first genome of an avian blood parasite genome was published in 2016 (112). This was seminal: Human and other primate *Plasmodium* genomes had been available, but none up to that time were for bird blood parasites. The genome helped root and improve the phylogenetic tree of bird blood parasites, including those of hummingbirds. Genomic sequence capture methods were developed to detect low levels of parasitemia in birds, including hummingbirds in South America (113). This work will increase our understanding of low-prevalence endemic disease and its epidemiology.

5.3. Microbiome Studies and the Metagenome

The microbiome, that is, host-associated microbial communities that live on or within parts of the vertebrate body, is integral to vertebrate population health (114). Microbiome composition, stability, and community interactions influence and are influenced by host diet, physiology, fitness, and environment. Whereas the microbiome has been studied extensively in humans, model organisms, and food-producing animals, very little is documented for free-living birds, and particularly hummingbirds.

PCR and sequencing of a bacterial ribosomal RNA gene in Anna's hummingbird samples provided data to characterize bacterial components of the hummingbird microbiome and test differences across sample types and gastrointestinal regions (115). The researchers discovered differences in microbial composition in fecal versus intestinal samples and among demographic factors of hummingbird age and sex. Future studies will be important to characterize interactions and effects of the microbiome on hummingbird health, disease, and resilience to environmental hazards including climate change.

6. GLOBAL ISSUES AND THREATS TO HUMMINGBIRD POPULATIONS

Multiple lines of evidence support declines of 48% of bird species globally (116) and nearly 60% of North American birds (117). A 2022 assessment of all birds on the IUCN Red List

(**https://www.iucnredlist.org/**) shows that 1,481 bird species (13.5% of 10,994 recognized extant species) were listed as threatened with global extinction. Among hummingbirds, 60 species have IUCN high-risk categories of Near Threatened (22 species), Vulnerable (11 species), Endangered (17 species), Critically Endangered (9 species), and Data Deficient/Unknown (1 species). As one example of worsening conditions, the extinction risk for the sapphire-bellied hummingbird (*Chrysuronia lilliae*) increased recently from Endangered to Critically Endangered. At least 4 North American hummingbird species are reported as experiencing population declines. The discipline of conservation genetics and its quantitative metrics for genetic variation are gaining traction as a necessary data layer for assessing extinction risk. However, genetics is completely unused or not employed uniformly in published lists of threatened and endangered species, such as the IUCN Red List (118). If genetic diversity were used as a factor in the Red List, even more populations and species would be included.

6.1. Emerging Threats to Population Health

Causes of hummingbird declines are multifactorial and often additive in layering threat upon threat in the pattern of an extinction vortex (119). Loss of fitness and reduced recruitment in a population can be caused by any combination of factors that degrade or pollute habitat, food, air, and water or cause increased risk of disease or mortality. Depending on the habitat and hummingbird population, these include agriculture; climate change; logging and forestry practices; hunting, trapping, and illegal trade (120); building and window collisions; invasive non-native species, including cat predation; disease; and ultimately the small population risks of reduced ability to find mates and lower genetic diversity (116). Disease outbreaks and declines in genetic diversity increasingly result from these stressors and small population effects.

Climate change impacts some species differently than others. Anna's hummingbird is expanding its range into areas that were too cold previously, whereas hummingbirds inhabiting montane regions will be pushed higher in elevation into increasingly smaller habitats. Pathogen and vector (e.g., mosquitoes, midges) dynamics are shifting due to changes in temperature and humidity (121).

Impacts of intensive farming practices and rapid loss of wetland ecosystems are devitalizing their associated nectar-producing plants and insects that are critically important for the hummingbird species that depend on them (122). Adding to these issues is the near nonexistence of coordination between protected natural areas and habitats for neotropical migratory birds; for example, currently less than 6% of land important for migrating birds in the Andes of Columbia is included in a national land restoration plan (123).

6.2. South America and Mexico

In South America, agricultural expansion for crops and cattle pasture, pesticide use, and climate change are some of the major direct threats affecting the conservation of hummingbirds and other pollinators (124). Landscape heterogeneity has shown a positive effect on hummingbirds' taxonomic and functional diversity in montane forest in the Andes of Ecuador; however, a heterogeneity threshold likely exists beyond which there are negative effects on diversity (125). In contrast, declines in hummingbird diversity in montane forest in Costa Rica were related to increased deforestation and habitat fragmentation (126). Variation in functional diversity in response to human-modified landscapes seems to be more complex. In Ecuador, diversity increased with landscape heterogeneity but decreased with native vegetation cover, which suggests that maintaining significant coverage of native vegetation is key for hummingbird conservation across fragmented landscapes and to maintain high-quality pollination services (125).

Under different climate change scenarios, models predict effects on the altitudinal and latitudinal distribution of hummingbirds, contractions or expansions of current ranges, or extirpations of local populations (127). However, recent analysis of trends in rufous hummingbird adult survivorship does not indicate a climate factor affecting this vital rate, but information on effects of climate change on juvenile recruitment is not yet known (64). Mexican hummingbirds are likely to face shrinkages in suitable range size due to increases in average global temperature and decreases in annual precipitation (128). Specialists with narrow niche breadth and range sizes, which are less likely to colonize novel conditions in the future, are expected to be more affected by range shrinkage and have higher extinction risk. Long-term climate-resilient hotspots identified under climate change scenarios in western Mexico overlapped with unprotected forested areas located mostly in Oaxaca, Guerrero, and Chiapas; the hotspots contain 93.9% of hummingbird species richness and nearly 90% of endangered species. The protection of those regions is key for hummingbird conservation under climate change scenarios (128).

Whereas species in temperate regions are likely to persist by moving to higher latitudes, high-elevation tropical species, including hummingbirds, have responded to temperature increase by shifting their distribution upslope, increasing their vulnerability to climate change. Slope distribution shifts in montane species in response to temperature increase have resulted in shrinkages of the elevation gradient and the area inhabited by these species, declines in abundance, and local extirpation of mountaintop species (129). Research areas with ramifications for hummingbird population health and disease ecology include pesticide exposures, wildfire effects, and human provisioning of feeders.

6.3. Pesticides

Multiple studies have documented the negative effects of agriculture on nature's biodiversity and, specifically, as a main cause of bird declines in Europe and North America (122). Not all agriculture harms bird populations: Impacts vary across modes of agricultural activity (type of crop or livestock, level of mechanization and chemical treatment), ecosystem type (boreal versus temperate forests, for example), and regions [eastern versus prairies and Pacific Canada (122)]. Among the important impacts to pollinators including hummingbirds and bees is the application of pesticides used in agricultural and forestry practices (47). The introduction of organochlorine insecticides such as DDT (dichlorodiphenyltrichloroethane) began in the 1940s to reduce agricultural and malaria-carrying insects, which then initiated a cascade of harmful effects to birds and other life forms, as Rachel Carsen (130) documented in the 1962 book *Silent Spring*. Although DDT is banned in the United States, it remains in use in South America, and since DDT was developed, a burgeoning next generation of pesticides has further endangered birds (131) through effects on avian health and loss of invertebrates as a food source. Furthermore, the impact of plant protection products such as glyphosate and related chemicals can affect reproductive parts of flowers, which may affect nectar production and insect populations (132).

The introduction in the 1990s of systemic insecticides that infiltrate all parts of plants, including nectar and pollen, has changed the way in which wildlife and the types of wildlife that can be exposed to insecticides. Earlier iterations of insecticides could bioaccumulate in food webs, especially if the chemical was highly persistent and fat soluble. Generally, bioaccumulation occurred through a vertebrate food chain, and those compounds did not concentrate in plants because they were less hydrophilic. Neonicotinoid insecticides and related systemic insecticides now being introduced to the market are more hydrophilic but still retain some organophilic characteristics to allow them to persist in soil for two to three years. Following applications to crops such as berries, for example, neonicotinoid and butenolide insecticides persist in soils for years. They have been

detected in hummingbirds (cloacal fluid), bees, honey, bee and flower nectar, pollen, and water in natural areas sampled adjacent to sprayed fields and in these wildlife living several kilometers from agricultural sites, probably due to the widespread use of these systemic compounds in nursery products, pet medications, and forestry (47, 133, 134). In California, 44 of 64 hummingbirds tested positive for various pesticides using a newly developed feather rinsate method (135). The pesticides detected in Anna's hummingbird and black-chinned hummingbird were imidacloprid, acetamiprid, and thiacloprid insecticides from homogenized carcass samples (136) and carbaryl, malathion, dinotefuran, imidacloprid, thiamethoxam, and acetamiprid in feather samples.

Metabolic effects including lowered energy expenditure occur in ruby-throated hummingbirds exposed experimentally to the neonicotinoid imidacloprid (137). Lifestyle traits of hummingbirds make them particularly susceptible to harmful effects of pesticides through contact with thousands of flowers and insects per day; flight through airborne plumes; and, for most hummingbirds in the United States and Canada, migration and stopovers across vast landscapes (137). A data set and model (Pop-GUIDE) was developed to help users evaluate risks of pesticides on birds (132). The model used ruby-throated hummingbirds and imidacloprid as an illustration and predicted adverse reproduction effects when hummingbirds fed in or near agricultural fields. Only in 2018 did it come to light that hummingbirds were exposed to and accumulated pesticides in and on their body. Now we realize that hummingbirds are exposed chronically and episodically to various environmental contaminants throughout their life span.

6.4. Window Strike and Domestic Cat Mortality

Collisions with buildings, especially windows, kill an estimated 500,000 to 1 billion birds annually in the United States, and many of those deaths are hummingbirds (138, 139). Cat attacks on hummingbirds are a top-ranking cause of mortality, especially for immature hummingbirds during spring and summer months (139).

6.5. Wildfires

Continuing drought throughout large portions of the United States and Canada has resulted in greatly increased wildfires during the past several years. By July 2023, Canada had experienced the worst wildfire season on record, with more than 80,000 km^2 burned in the first half of 2023 alone (**https://www.ciffc.ca/**). Thick wildfire smoke covered much of the United States and Canada for weeks, with noted public health effects. Wildlife health consequences of more frequent and higher-intensity fires are just beginning to be studied. A multiyear study of habitats in Mexico uncovered impacts to hummingbird populations including changes in nectar resources, from wildfire, prescribed fires, and restoration practices (140, 141).

6.6. Hummingbird Feeders

Feeders can benefit bird populations by engaging the public in bird watching and conservation and by augmenting nectar during drought. However, improperly implemented feeders can concentrate bird densities and drive ecological change (142), as well as serve as fomites (surfaces) for spreading pathogens that cause diseases in birds [such as mycoplasma conjunctivitis in song birds (143)]. Concerns with sugar water feeders include whether (*a*) they concentrate hummingbirds such that they facilitate pathogen transmission (71), cause window-strikes (144), and attract stalking cats (30) and praying mantises (145); (*b*) they are important sources of microbial and fungal infections or other toxins [ethanol (146)]; (*c*) the sugar mixture does not adequately match nature's own nectar recipe; (*d*) they include a toxic red dye that is commonly used in popular commercial feeder

solutions; and (*e*) they change population dynamics, species distributions, or migration phenology of migrating birds (46). The answers to these questions still require research; however, for each of these concerns, the answer is "probably yes." Hummingbirds host different microbial communities than the flowers and the sugar water feeders they drink nectar from (147). This indicates that feeders expose hummingbirds to a different microflora than flower nectar, with unknown consequences to their health. Cultivated non-native flowers and feeders were associated with northward expansion of Anna's hummingbird geographic distribution in North America (148). Another concern about feeders is the ecological consequences of providing artificial food sources to birds that pollinate plants. A resourceful study employed stable isotopes measured from hummingbird breath to quantify hummingbird use of feeders versus native flowers (46).

Red dye is ubiquitous in hummingbird feeders of the United States and Canada, as can be seen throughout suburban neighborhoods. Although sugar water for hummingbird feeders can be made cheaply and easily at home, many consumers instead fill their feeders with various commercially available sugar waters, including those that contain red dye. Toxicity studies have not been published for hummingbirds, but research in mammals and humans provides cause for concern regarding health effects of red dye in feeders. Whereas hummingbirds' attraction to red flowers has been documented, the influence of red-dyed nectar in attracting birds to a feeder is unknown (149). Also not well understood are the potential toxicological effects of red dyes used to color commercial sugar waters on hummingbird health. In an examination of 15 commercially produced hummingbird nectars, 9 products were found to use Allura Red AC as a coloring agent (150).

7. CONCLUSIONS AND FUTURE DIRECTIONS

Pleistocene climate and land form changes facilitated the evolution of biological adaptations and a radiation of hummingbird species into novel ecological niches throughout the Americas (35). They have a combination of abilities unlike any other animal: prolonged hovering flight, small size, special bill and tongue morphology, unique fat and sugar metabolism, ability to access nectar in flowers, and torpor. They are cultural icons, including the Aztec hummingbird deity Huitzilopochtli (9), and the subjects of contemporary awe. These characteristics also attracted centuries of killing and trading of hummingbirds for feathers in hats and a contemporary illegal trade in their use for "love charms" (120). Hummingbird feeders are ubiquitous in the Americas, especially the United States and Canada, with the positive effects of engaging people with wildlife and ideas of conservation. However, negative consequences of feeders include window collisions, exposure to potential toxins and microbes, and ecological displacement of native nectar sources. Industrial-scale agriculture, conversion and development on natural lands, and climate change, among other threats, are taking a toll on hummingbird populations. The extinction vortex paradigm is in action, reducing population sizes, with disease and degraded genetic diversity as heightened results.

Requirements for future work, especially in South American species, range from obtaining data on demography, population sizes and trends, genetic diversity, and disease prevalence to determining species-specific ecological needs and threats. One specific need is a method to determine the age of individual hummingbirds. Age structure in populations is vital for determining demographic trends. Individual banded hummingbirds are known to live up to 10 years or more, although most probably do not survive their first year of life. Field-caught hummingbirds are categorized as hatch year or after hatch year (adults of any age) by their bill and plumage appearance. Age can be determined for the few hummingbirds that are recaptured after being banded during their first year. However, recaptures are the exception rather than the rule: Most banded hummingbirds

are not seen again. One method with promise could be use of DNA-based age markers, such as DNA methylation (151). Once a lab method of aging is developed, perhaps a portable technology could be developed for the field, or the lab method could be tested with bird morphology traits to develop and validate a field method.

There are opportunities to extend the techniques and science gained from better-studied model hummingbird systems, such as Anna's, rufous, and ruby-throated hummingbirds, to understudied populations in Mexico, Central America, and South America. Several areas where additional research would be beneficial include cost-effective tracking methods; field- and backpack-friendly DNA analyzers for whole-genome and pathogen detection that are low-cost, readily available, and do not require freezing or special handling; and bioinformatic methods to address large and complex data sets. Improvements in telemetry technology would advance research in multiyear and long-distance movements of hummingbirds. Innovations in sampling for pathogens, pesticides, and heavy metals and remote data transmission via cell towers and satellites have allowed for new insights into hummingbirds at local, national, and international levels.

A growing confluence of researchers, government, and nonprofit biologists, along with cadres of hummingbird banders and community-based networks, are pooling resources and efforts. These networks generate and analyze data and provide the public education and social media exposure needed to garner conservation support and actions from governments and society. Another reason for optimism are the advances in disease ecology and conservation genomics. Underrecognized before this century, these disciplines now have formal graduate programs, working groups in wildlife biology and veterinary professional societies, and dedicated labs in government agencies, such as those in the United States and Canada. Nongovernmental groups, such as the Hummingbird Monitoring Network and the Western Hummingbird Partnership, bring together people and resources to advocate for conservation action. Products from such effort include the published Restoration Guides for Maintaining and Improving Habitats for Hummingbirds (152). These innovations in science, technology, and community support give us a sense of optimism in the face of the many daunting issues facing hummingbird populations.

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