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Using parasites to infer host population history: a new rationale for parasite conservation

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Abstract

Only one of the 5000 extant louse species (Phthiraptera) and no species of flea (Siphonaptera), parasitic helminth (Platyhelminthes), parasitic nematode (Nemata), mite, or tick (Acari) is listed as threatened by the IUCN, despite impassioned pleas for parasite conservation beginning more than a decade ago. Although they should be conserved for their own sake, past arguments, highlighting the intrinsic and utilitarian value of parasites, have not translated into increased attention by scientists or conservation managers, at least by the standard of listing for protection. Here, the use of estimated genealogies and population genetic patterns of parasites to illuminate their hosts' evolutionary and demographic history is advocated. Parasite DNA generally evolves more rapidly than their hosts', which renders it an underexploited resource for conservation biologists, particularly in cases where the hosts' genealogy or degree of population genetic structure is difficult to measure directly. Moreover, parasite gene flow may occur during host dispersal irrespective of host gene flow, revealing host movement through space and time. Parasite ecology and evolution may thus become another tool for the management of endangered vertebrate populations. This will result in the recognition of new host records, parasite species and cryptic lineages, which will help lift the veil of ignorance with respect to parasite biodiversity.

Parasites are the most diverse metazoan group on Earth. Despite the passing of more than a decade since the first articulation of impassioned pleas for parasite conservation (e.g. Windsor, 1990, 1995; Rósza, 1992; Holmes, 1993; Stork & Lyal, 1993; Durden & Keirans, 1996; Gompper & Williams, 1998; Koh *et al*., 2004), few are presently listed on the IUCN Red List of Threatened Species (IUCN, 2003). For example, only one of the 5000 species of louse (Insecta, Phthiraptera: Price, Hellenthal & Palma, 2003) is currently listed. While the listing of the pygmy hog sucking louse (Hematopinidae: *Haematopinus oliveri*) represents a victory for parasite conservation, no other lice have been given this designation, despite there being another 2323 potential host species (among the mammals and birds) listed (IUCN, 2003). Other parasites of vertebrates are similarly neglected, since no species of flea (Siphonaptera), parasitic helminth (Platyhelminthes), parasitic nematode (Nemata), mite, or tick (Acari) is listed despite the fact that 3524 vertebrate species are listed. Although Durden & Kerians (1996) identified 48 species of tick as candidates for endangered status, none are listed by the IUCN. Similarly, Perez & Palma (2001) suggested listing of the newly described host-specific louse *Felicola isidoroi* (Trichodectidae) from the Iberian lynx (*Lynx pardinus*), yet it presently remains unlisted. For some parasites, such as a potentially unique louse lineage (*Neotrichodectes minutus*) from the black-footed ferret (*Mustela nigripes*), or host-specific lice (*Colpocephalum californici*) of the California Condor (*Gymnogyps californianus*), it is too late, since parasites were intentionally killed during population management and captive breeding efforts (Gompper & Williams, 1998; Koh *et al*., 2004). These examples underscore the fact that formal protection of a host does not necessarily ensure protection of its parasites or other symbionts, which is not a novel observation. One study estimated that 200 'affiliate' species are now extinct due to their hosts' demise and that 6300 other affiliate species are co-endangered with their hosts (although most affiliates remain unlisted; Koh *et al*., 2004). Invertebrates may be particularly prone to extinction risk (Hadfield, 1993; Clark & May, 2002; Stein, Master & Morse, 2002), and since parasites are distributed in a negative binomial fashion among hosts (most hosts have few parasite individuals and few hosts have many parasite individuals: Crofton, 1971), they are particularly vulnerable to extinction when host populations are small or when natural dispersal is disrupted (*sensu* Templeton *et al*., 2001).

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Animal Conservation published no comparative or theoretical papers on invertebrate conservation in its first 5 years, during which it published 50 such studies on vertebrates (Reynolds *et al*., 2003). It seems that past arguments, highlighting the intrinsic and utilitarian value of invertebrate parasites, have not translated into increased attention by scientists or conservation managers, at least by the standards of publication or listing. This problem is not specific to the IUCN or *Animal Conservation*. It is the result of our general ignorance of invertebrate biology and diversity and we recognise that part of the problem is simply not knowing what to conserve. We urge funding agencies worldwide to increase the amount of monies available for cataloging biodiversity. Those who would argue for parasite conservation must address the fact that 'in order to care deeply about something important it is first necessary to know about it' (Wilson, 2000), yet we still know so little. Here, in this context, we propose a novel and pragmatic rationale for conserving parasites and pathogens, which may help to address all of these problems.

PARASITES AS INFERENTIAL TOOLS

Understanding the historical and contemporary relationships among fragmented vertebrate populations is important to conservation managers, for a variety of reasons (Avise, 1994, 1996; Templeton *et al*., 2001). Unfortunately, low genetic variability within and among populations of many vertebrate taxa obscures our ability to infer these historical genetic and contemporary demographic processes (e.g. cheetahs, Kieser, 1991; northern elephant seals, Hoelzel *et al*., 1993; killer whales, Hoelzel *et al*., 2002; Hainan Eld's deer, Pang *et al*., 2003). Population genetics of the parasites of these vertebrates may offer another avenue for illuminating their hosts' evolutionary history and current demographic processes, which buttresses arguments for conserving such host–parasite systems (if parasites contain more population genetic information than do their hosts).

Parasitologists have long used parasites to infer a host's evolutionary history (von Ihering, 1891, 1902; Fahrenholz, 1913; Eichler, 1942; Brooks, 1977, 1993; Brooks, Thorson & Mayes, 1981; Hoberg, 1997; Hugot, 1999, 2003). The key assumption is that parasites are transmitted vertically across generations and from parental to daughter lineages, in an ancestor-descendent fashion (Clay, 1949; Page, 2003). The root of this practice lies in the observation that morphological evolution within parasites proceeds more slowly than in their hosts (Klassen, 1992). Parasites may thus possess a 'conservative tendency that makes them useful as biological tags' (Ayala & Hutchings, 1974). Over the same time interval, while a pair of host sibling species may have undergone extensive morphological change since divergence from their common ancestor, the pair's parasites should have retained characters that are useful in the elucidation of their (and, by extension, the hosts') evolutionary history. The presence (or absence) on a host of a parasite taxon is therefore genealogical information in itself (Ronquist, 2003). For example, Gardner & Campbell (1992) used a phylogeny based on morphological characters of marsupial and monotreme cestodes (*Lintowia* spp.) to infer that this host–parasite system was in place before the break-up of Gondwanaland. The hosts' phylogeny was obscured by 'morphological divergence of marsupials in the Neotropical and Australian regions.' Thus, a monophyletic origin of the host lineages was recapitulated via phylogenetic data from their parasites, which were 'phylogenetic relicts' (*sensu* Brooks & Bandoni, 1988).

However, the advent of polymerase chain reaction (PCR), DNA sequencing and realistic phylogenetic and population genetic analytical tools (Avise, 1994; Templeton, 1998, 2004), has allowed evolutionary biologists to estimate genealogies and gene flow using organismal genes themselves. This has largely obviated the need for parasites in evolutionary inference. Here we argue, on other logical grounds, that this route of deduction still has conceptual merit and practical conservation application at the microevolutionary level, particularly in cases where the host's genealogy or population genetic structure is difficult to estimate directly.

There is growing evidence, across taxonomic boundaries, that the rate of molecular evolution is faster in parasite DNA relative to that within the homologous loci of their hosts (Hafner *et al*., 1994; Downton & Austin, 1995; Moran, van Dohlen & Baumann, 1995; Page *et al*., 1998; Clark *et al*., 2000; Funk *et al*., 2000; Paterson *et al*., 2000; cf. Ricklefs & Fallon, 2002). For example, Clayton & Johnson (2003) have shown that the rate of evolution in the mitochondrial DNA of avian lice is 10 times faster than that of their hosts. It is this property that has led several biologists to propose a new look at the use of parasites and other symbionts for inferring host evolutionary history (Funk *et al*., 2000; Page, 2003). Funk *et al*. (2000) noted that parasites' more rapid evolutionary rate, relative to that of their hosts, yields DNA sequence data that are 'comparatively informative sources of phylogenetic data.' Moreover, beyond consideration of mutation rates, the difference in generation time alone between most host–parasite pairs allows for the coalescent process to proceed much more rapidly in the latter, all else being equal (Rannala & Michalakis, 2003). Thus, not only can one expect more genetic variance to be present in the DNA or RNA of pathogens and parasites relative to their hosts, the analysis of how this variance is partitioned among host populations could reveal the hosts' evolutionary history before the host DNA has coalesced (Rannala & Michalakis, 2003). This is a powerful inferential tool indeed.

This logic was used to attack the difficult problem of characterising evolutionary relationships among human populations and historical human migration patterns. Genealogical relationships and gene flow patterns were inferred with success within and among populations of persistent human pathogens such as the ulcer-causing bacterium *Helicobacter pylori* (Ghose *et al*., 2002; Falush *et al*., 2003; Wirth *et al*., 2004) and urinary JC virus (Sugimoto *et al*., 1997). Moreover, comparisons between

H. pylori DNA sequences could 'distinguish between closely related human populations and are superior in this respect to classical human genetic markers' (Wirth *et al*., 2004).

INFERRING HOST GENEALOGY

Rannala & Michalakis (2003) provide a superb theoretical framework relating population genetic processes to co-phylogenetic patterns between hosts and parasites via coalescent theory, which is a useful context for the present discussion. Their analysis of host-tracking by parasites through time was split into three components (1) within-population, (2) between-population and (3) between-species.

Regardless of the level of analysis, inference of host genealogical history will be strongest when genetic data from vertically transmitted parasites or pathogens are used: 'The gene genealogy of a parasite with vertical transmission carries potential information about the genealogical relationships of infected hosts' (Rannala & Michalakis, 2003). However, some parasites and pathogens are transferred horizontally among host species and populations (host-switching). This may cloud the inference of host genealogy, causing problems analogous to those caused by horizontal transfer of genes (Page, 2003). These horizontally-transferred host–parasite pairs are useful in other contexts (Rannala & Michalakis, 2003; see below). Close attention should be paid to life-history differences among parasite lineages when they are used as evolutionary inferential tools.

In theory, N_e (effective population size) of hosts and parasites is extremely important in determining the level of population genetic structure in parasites (Nadler, 1995), and the degree of congruence between host and parasite lineages (Rannala & Michalakis, 2003). Moreover, the lineages of larger populations will arrive at reciprocal monophyly more slowly than smaller populations (Avise, 1994); lineage sorting may distort inferences of host history and result in host lineages coalescing before the parasite's, assuming equal generation times (Rannala & Michalakis, 2003).

Specifically, lineage sorting is a problem if 'surviving lineages in the parasite trace to phylogenetic splits either predating or postdating nodes in the host phylogeny' (Avise, 1994). Thus, as Rannala & Michalakis (2003) showed, lineage sorting can easily lead to incongruence between host and parasite trees within populations. Only if the parasite's N_e is 'very small' or if the hosts sampled 'are relatively distantly related, the parasite gene genealogy should provide a good estimate' of the ancestral infection graph (the actual history of parasite transmission, from host individual to individual, whether vertical or horizontal). Specifically, Rannala & Michalakis suggest that a parasite gene genealogy will reflect the history of transmission among hosts (and thus the host's history) if the number of generations (or parasite transfer events) between the hosts is > 10 times the parasite's N_e. Between populations, variance in migration rate, internal branch lengths within gene trees and N_e emerge as important determinants of whether host and parasite gene trees will accurately reflect population history. Simply stated, parasite species typified by relatively small population sizes and persistence on hosts separated by relatively longer periods of time, will yield more accurate information about host ecology or evolutionary history than the converse.

Given this and the important influences of life-history factors such as host range (Nadler *et al*., 1990), host sociality (Whiteman & Parker, 2004), parasite dispersal abilities (Johnson *et al*., 2002) and life cycle (Criscione & Blouin, 2004) on parasite population genetic structure, the examination of multiple parasite lineages within a particular host species may prove most useful, just as multiple loci should be used to increase the accuracy of phylogeny or population genetic structure estimates (Nadler, 1995; Johnson *et al*., 2002; Constantine, 2003; Criscione & Blouin, 2004). Hierarchical, comparative population genetic (Jarne & Theron, 2001) and phylogeographical approaches (Avise, 1996; Templeton, 1998, 2004) can then be used to infer both distant and recent population histories of multiple and phylogenetically independent parasite populations. Objectively differentiating among the various population genetic processes, such as range expansion, fragmentation, or isolation by distance is now at least technically feasible and hundreds of articles in the past few years alone have implemented statistical phylogeography, which is a testament to its broad appeal (Templeton, 2004). Such studies deepen our understanding of the vast interrelationships and interdependencies among taxonomically diverse lineages.

Focusing on permanent, directly transmitted parasites (those that generally depend on host–host contact for transfer, e.g. Phthiraptera) or pathogens that produce chronic and persistent infections (e.g. *Helicobacter*, helminths), may be one of the best strategies for implementing applied parasite population genetics. For example, host specificity of lice on birds and mammals is high, with each species occurring on an average of only two bird and 2.6 mammal species (Price *et al*., 2003). Lice are relatively easy to collect (Walther & Clayton, 1997; Clayton & Drown, 2001), and genotyping of large numbers of individuals is now routine (e.g. Johnson *et al*., 2002). Nadler *et al*. (1990), studying the lice of pocket gophers (*Thomomys bottae*), found that significant population genetic structure existed among louse populations and that this structure was broadly correlated with host gene flow. Barker *et al*. (1991*a*) and Barker, Close & Briscoe (1991*b*) also found significant structure among lice (*Heterodoxus octoseriatus*) from different colonies of their wallaby hosts and this was more broadly correlated with latitude, which in turn was correlated with the ranges of two different wallaby subspecies.

In our own preliminary work estimating the genealogical relationships among the nine extant island populations of the threatened Galapagos hawk (Aves, Falconiformes, *Buteo galapagoenesis*), its' ectoparasites (Insecta, Phthiraptera) have served as excellent markers of host population differentiation. Generally, we found much more population genetic structure in the parasite's

(Philopteridae, *Degeeriella regalis*) mtDNA (~1.5% maximum divergence within Galapagos) relative to that within the host's (~0.2% maximum divergence within Galapagos). Moreover, there was a greater degree of geographical partitioning of this variance among parasite populations than among their hosts (our unpublished data). This approach may be useful for inferring the population histories of other endemic Galapagos vertebrates, which, like other taxa inhabiting oceanic archipelagoes, are relatively genetically invariant (Tye *et al*., 2002).

INFERRING HOST POPULATION DYNAMICS

Population genetic studies of horizontally transmitted parasites and pathogens can provide information such as past host dispersal events that resulted in gene flow for the pathogen, but not the host (Criscione & Blouin, 2004; Whiteman *et al*., 2004). In other words, '[The gene genealogy] of a parasite with horizontal transmission carries potential epidemiological information about the patterns of parasite transmission among hosts' (Rannala & Michalakis, 2003). Tabor *et al*. (2001) advocated the use of this logic in a wildlife management context by suggesting using viral genetics as a means of inferring metapopulation dynamics of their lynx and mountain lion hosts. This, the authors argued, would help managers determine the location of natural corridors and areas where wild populations interact with domesticated animals. Host dispersal and demographic processes were illuminated via population genetics of lemming (*Dicrostonyx* spp.) cestodes (Wickström *et al*., 2003). The authors found evidence that population genetics of these parasites could serve 'as indicators of fine-scaled (temporal and geographical) events that are not (or not as clearly) apparent in the assessments of the biogeographical history of the hosts.' Similarly, dating the genealogical split between human head (*Pediculus humanus capitis*) and body lice (*P. h. corporis* or *humanus*) has given insight into when humans first started to wear clothing, since body lice require it for survival (Kittler, Kayser & Stoneking, 2003). Reed *et al*. (2004) have used parasite genealogies to infer that direct contact occurred between modern and archaic lineages of *Homo* (and corrected an error in Kittler *et al.*'s, 2003 study). From a wildlife perspective, Weckstein (2004) showed that louse lineages of sympatric, but unrelated, toucan hosts, were often each others closest relatives, indicating, perhaps, historic inter-specific host behavioural interactions (e.g. two species serially nesting in the same tree cavity hole) generated the observed patterns. At the population level, Whiteman *et al*. (2004) used a DNA barcoding approach in a simplified ecological setting to show that dispersal of lice from Galapagos doves (*Zenaida galapagoensis*) to Galapagos hawks (*Buteo galapagoensis*) occurred as a result of hawks feeding on doves.

Disease transmission within and among individuals *within* a population can reveal interactions among hosts. For example, population genetic data incriminated a physician who allegedly infected another person with an HIV-1 strain obtained from one of his patients (Metzker *et al*., 2002). A phylogenetic analysis revealed that the source of the strain could be identified, provided that the horizontal transmission event from source to recipient was recent enough for a paraphyletic relationship to remain between some of the source viral isolates and the recipient isolates (since the recipient often receives a genetic subset of the source's total number of genetic HIV isolates: Metzker *et al*., 2002). This logic could easily be applied to a conservation management context as well.

IMPACT ON PARASITE CONSERVATION

How, exactly, will this benefit parasite conservation? The careful genetic characterisation of parasite populations requires extensive sampling within and across host populations. Such basic distributional data of parasites themselves will begin to lift the veil of ignorance with respect to parasite biodiversity. Parasites comprise most of Earth's species (Windsor, 1998) and most of the species within the Insecta (Price, 1980), the most species-rich taxon on earth (Stork, 1988; Samways, 1994). Thus, examining fine-scale patterns of divergence among populations will help to unravel the processes responsible for the diversification of most of Earth's species. New host records will accumulate and new host-specific parasites will be discovered and named. The degree of fine-scale parasite population structure within hosts may be astoundingly high (e.g. Nadler *et al*., 1990; Johnson *et al*., 2002; McCoy *et al*., 2003); its description will invariably illuminate the presence of a multitude of cryptic evolutionary lineages within classically defined species of parasite or pathogen (e.g. Barker *et al*., 1991*a*, *b*; Hung *et al*., 1999; Jousson, Bartoli & Pawlowski, 2000; Perkins, 2000; Criscione & Blouin, 2004). The use of DNA barcoding approaches (Hebert *et al*., 2003*a*; Hebert, Ratnasingham & deWaard, 2003*b*) may further facilitate identification and classification of these lineages and provide insight into how parasites disperse between host individuals.

In view of the pragmatic value of parasites, managers of captive vertebrate populations may be encouraged to screen and genetically characterise the parasite populations of the vertebrates they manage, which will allow for a more informed discussion of host–parasite management options. If a population's parasites are eradicated before genetic characterisation can take place, a great deal of information, much of it of possible management value for the host, will be lost forever. Results from many studies generally support the argument that parasite population genetics can reveal host population biology (e.g. Mulvey *et al*., 1991; Blouin *et al*., 1995; Dybdahl & Lively, 1996; Demastes *et al*., 1998; McCoy *et al*., 2003; Wickström *et al*., 2003).

SPECIFIC RECOMMENDATIONS FOR MANAGERS

Do we suggest that managers indiscriminately sample parasites from small, threatened vertebrate populations? Obviously, this could result in the loss of a parasite population or species. Managers should consult with entomologists, microbiologists, parasitologists or other specialists before proceeding with large-scale sampling and genotyping. A large part of the problem of parasite conservation simply stems from not knowing what kind of diversity exists, given that parasites are the most diverse group on Earth. Thus, partnerships between managers/conservation biologists and parasite specialists will help to fill this gap in our knowledge while also alerting parasitologists to the presence of rare species.

When animals for captive rearing are first brought into captivity, or while being given wildlife health examinations, managers should not rush to control parasites. Instead we recommend they (in consultation with the appropriate specialists) make every reasonable effort to sample (e.g. through physical examinations by veterinarians, blood smears, faecal samples, pelage brushing, dust-ruffling of a limited number of hosts) parasites and then send such samples to experts for identification. Protocols for sampling parasites of mammals (Gardner, 1996), birds (see appendices in Clayton & Moore, 1997), amphibians, reptiles and fish (available online from the Ecosystem Monitoring and Assessment Network of Environment Canada: http://www.emanrese.ca/eman/ecotools/protocols) are all available. If unique parasites or other symbionts are found, they may be cultured in captivity on tissues of other host species (e.g. lice on feathers), or actually on other, more common host species ('purgatory hosts'), since many parasites are less host-specific in captivity. Although this may sound difficult to implement, researchers have developed this capability for some parasite taxa (e.g. lice: Clayton, Johnson & Al-Tamini, 2003). For smaller parasites (e.g. trypanosomes), cryopreservation of live samples is a viable option (Ndao *et al*., 2004). Such samples could be cultured and captive animals infected prior to release. Could wildlife biologists and veterinarians establish a parasite bank for endangered species? It has been done for parasites of human importance. The Malaria Parasite Bank of India, established in 1992, accumulates, identifies and cultures these parasites. Lice of the California condor now appear to be extinct (Koh *et al*., 2004); perhaps a culturing attempt may have saved them.

Will there be more parasites on the IUCN Red List of Threatened species a decade from now? Perhaps, if conservation managers begin to view applied parasite population genetics as another tool under the broader rubric of vertebrate conservation genetics. This could bring a revolution to the field of conservation biology because parasite conservation will become directly relevant to vertebrate conservation. To reiterate, however, we believe that parasites have intrinsic value and should be conserved for their own sake, not merely because they can be used as inferential tools. What we hoped to have accomplished is to illustrate what will be lost if vertebrate conservation biologists are not empowered to conserve parasites (Koh *et al*., 2004). It is through this new pragmatism, perhaps, that we may finally begin to live up to Wilson's (2000) lofty assertion that our conservation ethic is without taxonomic bias: 'The conservation biologist knows that each imperiled species is a masterpiece of evolution, potentially immortal except for rare chance or human choice, and its loss a disaster.' Lice and fleas, just like the lions and birds of paradise on which they live, are masterpieces of evolution, too. However, human taxonomic bias seems to fault even conservationists (Clark & May, 2002). Hopefully, the limelight will begin to shine on parasites and other symbionts, but it is up to us to make room for them on the stage.

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