

## INVITED REVIEW

# Molecular ecology of parasites: elucidating ecological and microevolutionary processes

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## Abstract

We review studies that have used molecular markers to address ecological and microevolutionary processes in parasites. Our goal is to highlight areas of research that may be of particular interest in relation to the parasitic lifestyle, and to draw attention to areas that require additional study. Topics include species identification, phylogeography, host specificity and speciation, population genetic structure, modes of reproduction and transmission patterns, and searching for loci under selection.

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## Introduction

Parasitism is one of the most common lifestyles among eukaryotes (Poulin & Morand 2004). Yet, molecular ecology studies on parasites have lagged far behind those on free-living organisms. Molecular markers offer great tools for studying processes such as transmission, the evolution of host specificity and patterns of speciation. Molecular ecology studies also have very practical applications such as in studying the evolution and control of drug resistance. Another reason to study parasites is that they display a huge diversity of life cycles and lifestyles (modes of reproduction, dispersal abilities, effective sizes, and so on). These provide great opportunity for comparative studies to test parasite-specific questions or questions about evolution in general. Nevertheless, parasitologists have been slow to adopt the methods of molecular ecology, and for some reason, the field of parasitology has yet to attract many evolutionary biologists.

In this review we highlight studies that used molecular markers to address questions about parasite ecology or evolution. We do not provide an exhaustive review, but instead use selected examples to illustrate areas of research that may be of particular interest in relation to the parasitic lifestyle and highlight areas that require additional study. To facilitate discussion, we limit our review to organisms typically covered in parasitology texts (protozoan and

metazoan parasites of animals) (Poulin 1998; Bush *et al.* 2001). Phylogenetic studies on recognized species or studies on cospeciation between parasites and hosts certainly provide insight into parasite evolution; however, phylogenetics is beyond the scope of this review.

## Species identification: the fine line between population genetics and phylogenetics

Delimiting species of parasites is often difficult owing to their limited morphological characters. Additionally, the primary samples may consist of unidentifiable egg or larval stages from a particular host. Thus, genetic identification of species is by far the most utilized application of molecular techniques in parasitology (McManus & Bowles 1996). Here we briefly draw attention to three key uses of molecular markers in species identification.

First, the identification of known species from morphologically indistinguishable life stages (e.g. eggs, larvae) is important for disease transmission studies and clinical diagnostics. Faecal samples can be screened for eggs to assess the parasite diversity in the host (e.g. Schnieder *et al.* 1999; Wimmer *et al.* 2004). Such screening may be a necessity in hosts where invasive sampling of adult parasites is not an option (e.g. humans, endangered species). Correct identifications are critical for designing control programs or applying drug treatments. The study by Singh *et al.* (2004) illustrates the importance of correct identifications. Malarial infections in humans from Malaysian Borneo were previously identified via microscopy as *Plasmodium malariae*, which is

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**Table 1** Glossary of parasite terms

**Abundance:** the number of individuals of a particular parasite in/on an individual host regardless of whether or not the host is infected.

**Component population:** all individuals of a parasite species in a specified life history phase (e.g. mature adults) at a particular place and time.

**Definitive host:** the host in or on which a parasite sexually reproduces.

**Infrapopulation:** all individuals of a parasite species in or on an individual host at a particular time.

**Intensity:** the number of individuals of a particular parasite in/on a single infected host.

**Intermediate host:** the host in or on which a parasite undergoes some developmental change, but does not reach sexual maturity.

**Macroparasite:** multicellular parasites such as nematodes, platyhelminthes, acanthocephalons, pentastomes, and arthropods.

**Prevalence:** is the number of hosts infected with one or more individuals of a parasite species divided by the number of hosts examined for that parasite species.

**Paratenic host:** host in which development does not occur, but which may serve to bridge an ecological gap in a parasite's life cycle.

**Reservoir host:** host in which a parasite can survive and reproduce, but is not considered the normal host.

morphologically very similar to *Plasmodium knowlesi* (a parasite of macaque monkeys). Small subunit rDNA sequences obtained from infected patients, however, were identical to *P. knowlesi*. This identification now enables research to focus on potential transmission dynamics between humans and macaque monkeys (Singh *et al.* 2004).

A second use of species identification is to elucidate parasite life cycles (e.g. Cribb *et al.* 1998; Jousson *et al.* 1999; Bartoli *et al.* 2000). For example, which species serve as the *intermediate hosts* (see Table 1 for glossary) for larval stages of a parasite? Traditional methods for working out the life cycle, such as experimental infections, may be hampered if potential hosts cannot survive in the laboratory. PCR detection techniques (e.g. use of species-specific primers) or sequencing provide a rapid means to screen potential hosts as illustrated by the study of Jousson *et al.* (1999). Adult specimens for 16 known species of trematodes and unidentifiable larval specimens were collected from fish *definitive hosts* and mollusc intermediate hosts, respectively. Sequences of the internal transcribed spacer rDNA matched many of the larval specimens to their corresponding adult form, thus, identifying the natural intermediate hosts.

A related use of molecular markers is the search for cryptic species (morphologically similar, but genetically distinct) (e.g. Hung *et al.* 1999; Jousson *et al.* 2000; Leignel *et al.* 2002). Here the goal is not to use markers to distinguish among known species, but to prospect for new species

(Blouin 2002). The finding of cryptic parasite species has become very common as more phylogeographical and genetic structure studies are carried out on parasites (Anderson *et al.* 1998). For instance, in studies on trematodes in salmon (Criscione & Blouin 2004) and on avian malaria parasites (Bensch *et al.* 2004), the observation of complete disequilibrium between nuclear and mitochondrial markers proved that cryptic species can co-occur in the same individual hosts.

DNA-based identification and discovery of parasite species also has implications for our understanding of global biodiversity (Brooks & Hoberg 2000; Poulin & Morand 2004). The limited morphological characters of many parasitic helminths and protozoans have probably resulted in a gross underestimation of the true number of species in biodiversity surveys (e.g. Bensch *et al.* 2004; Westenberger *et al.* 2004). For instance, mtDNA analyses suggest that the number of species of avian malaria approaches 10 000 rather than the 175 that were previously identified via morphology (Bensch *et al.* 2004).

An emerging area using molecular methods for species identification is molecular epidemiology and epizootiology (the study of disease transmission in humans and wildlife). For example, one can ask if a species of human parasite infects a reservoir host (host in which a parasite can survive and mature, but is not considered the normal host; Bush *et al.* 2001). *Reservoir hosts* are significant because they may maintain parasites in the absence of the primary host. Anderson (2001) concluded that cross-infections were rare between *Ascaris* nematodes of humans and pigs in sympatric areas. Thus, control programs for human *Ascaris* need not concentrate on infection control in pigs. Other molecular epidemiology applications include detecting mixed species/strain infections or subpatent infections, surveying wildlife for human parasites, and estimating *prevalence*, (percent of hosts infected) (e.g. Perkins *et al.* 1998; Dubey *et al.* 2004; Njiru *et al.* 2004).

## Phylogeography

Reconstructing the historical biogeography of populations (e.g. vicariance events) and identifying major genetic subdivisions within species are major objectives of phylogeographical analyses (Avice 2000). Furthermore, comparative phylogeography can identify historically and evolutionary independent geographical regions (Bermingham & Moritz 1998). There have been relatively few traditional phylogeographical studies on parasites (Table 2). Given that parasites are closely tied to their hosts, it might be expected that parasites and their hosts would share similar phylogeographical patterns. Indeed, congruence between host and parasite phylogeography has been found, although it is not always perfect (Table 2). The degree of congruence will depend on which host in a parasite's life cycle is

**Table 2** Summary of phylogeographical studies of nonhuman parasites (see text for human parasite examples)

| Parasite species                                                           | Host species                                   | Reference                       | General conclusions and phylogeographical breaks                                                                                                                                                                                        |
|----------------------------------------------------------------------------|------------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Gyrodactylus salaris</i> (monogene)                                     | <i>Thymallus thymallus</i> (European grayling) | Meinila <i>et al.</i> (2004)    | The distribution of mtDNA haplotypes was consistent with postglacial recolonization patterns of the host.                                                                                                                               |
| <i>Heligmosomoides polygyrus</i> (nematode)                                | <i>Apodemus sylvaticus</i> (field mouse)       | Nieberding <i>et al.</i> (2004) | Congruence with major host breaks (western Europe, Italian, and Sicilian), but parasite also had further breaks within some regions. Host and parasite recolonized northwestern Europe from Iberian refuge.                             |
| <i>Leptorhynchoides</i> spp. three cryptic species found (acanthocephalan) | <i>Lepomis</i> spp. (sunfish)                  | Steinauer (2004)                | Significant phylogenetic associations with previously reported breaks (east–west of the Apalachicola River and Gulf–Atlantic drainages) for several taxa and the parasite's hosts (see Avise 2000). Data suggest allopatric speciation. |
| <i>Paranoplocephala arctica</i> (cestode)                                  | <i>Dicrostonyx</i> spp. (collared lemmings)    | Wickstrom <i>et al.</i> (2003)  | Nearctic and Palaearctic clades, which correspond with major division of the hosts, but parasite lacks complete congruence with host relationships.                                                                                     |
| <i>Perkinsus marinus</i> (protozoan)                                       | <i>Crassostrea virginica</i> (American oyster) | Reece <i>et al.</i> (2001)      | Atlantic–Gulf Coast break; a pattern also seen in its host (see Avise 2000).                                                                                                                                                            |
| <i>Plasmodium azurophilum</i> (malaria)                                    | <i>Anolis</i> spp. (lizards)                   | Perkins (2001)                  | Patterns not consistent with lizard hosts. Vector mediated dispersal may contribute to discordance.                                                                                                                                     |

compared and on the nature of the transmission dynamics between the host and parasite (Nieberding *et al.* 2004). For instance, malaria parasites of Caribbean lizards showed complex patterns of genetic fragmentation that were inconsistent with those of their lizard hosts (Perkins 2001). Population extinctions and dispersal by the vector host may contribute to this discordance (Perkins 2001).

Phylogenetic studies that test for cospeciation between parasites and hosts have expanded our understanding of parasite evolution (Page 2003). Likewise, much can be gained by testing whether a single species of parasite and its host have similar phylogeographical structure. As an example, Nieberding *et al.* (2004) illustrates how cophylogeographical patterns between a parasite and its host can be used to calibrate a molecular clock for parasites. Additional comparative phylogeographical studies between hosts and parasites are needed to help illuminate the microevolutionary processes that result in interesting macroevolutionary patterns such as cospeciation.

Phylogeographical data can also be used to understand the history of colonization by an exotic parasite. These data could indicate potential source populations or the timing of invasion, or could be used to test whether multiple introductions have occurred. Knowing the origin of the parasite and levels of genetic diversity in its native range may be useful for implementing control measures or for evaluating the potential for drug resistance in introduced locations. For example, molecular data suggest that the human malarial parasite, *Plasmodium falciparum*, has a most recent common ancestor that dates back c. 100 000 years, and then underwent a recent population expansion in Africa followed by separate colonization events into South America and

Asia. (Hartl *et al.* 2002; Mu *et al.* 2002; Joy *et al.* 2003). There appear to have been multiple independent introductions into South America (Anderson *et al.* 2000; Joy *et al.* 2003). The human filarial nematode *Onchocerca volvulus* also recently colonized South America. Molecular data suggest that New World populations originated from an African savannah strain during the slave trade (Zimmerman *et al.* 1994). Likewise, mtDNA data support a recent introduction of the trematode *Schistosoma mansoni* into South America from Africa (Despres *et al.* 1993).

### Host-specificity and speciation

Parasites are interesting systems in which to study patterns and mechanisms of speciation. Adaptation to different tissues or body locations in a single host species could select for reproductive isolating mechanisms between groups of parasites. Such may be the case for two species of *Plasmodium* that infect lizards (Perkins 2001). One infects red blood cells while its sister species infects white blood cells in the same hosts (Perkins 2001). Adaptation to different sympatric hosts could also select for intrinsic barriers to reproduction. Alternately, nonoverlapping ecologies between sympatric host species (distinct transmission cycles) could result in simple physical separation between groups of parasites that ultimately leads to speciation (McCoy 2003). We are not aware of any molecular studies that explicitly test mechanisms of speciation in parasites. However, Trouve *et al.* (1996, 1998) observed potential assortative mating and hybrid breakdown as a result of crossing *Echinostoma caproni* collected from Egypt and Madagascar. Mechanisms of reproductive isolation that

result from host specificity can potentially be tested in the laboratory by running the life cycle of a parasite through different intermediate or definitive host species for several generations. As far as elucidating patterns of speciation, some genetic studies have found parasite populations that are subdivided between host species in sympatry. Examples include ticks on communally nesting seabirds (McCoy *et al.* 2001) and lice on doves (Johnson *et al.* 2002). However, these studies only show that host-race formation in sympatry is a potential diversifying force in parasites. More work is needed to determine the importance of extrinsic vs. intrinsic barriers, and to rule out other explanations such as geographical speciation followed by secondary contact of host species.

### Population genetic structure

The ability of parasites to adapt to local environments or hosts, to evolve drug resistance, or to speciate will be affected by gene flow among populations and genetic drift within. Thus, having estimates of migration rates and effective population sizes ( $N_e$ ), and understanding what factors control those parameters in parasites, are key for understanding microevolutionary processes in these taxa.

#### Gene flow

Host vagility should be a major determinant of parasite gene flow because many parasites lack free-living stages (e.g. malaria) or have low dispersal capability in their free-living stages (Price 1980; Nadler 1995). For example, three freshwater trematode species that cycle exclusively in aquatic hosts are much more subdivided than another trematode species from the same locations but whose life cycle includes highly mobile terrestrial hosts (Criscione & Blouin 2004). Two other studies that compared the genetic structures of parasites having hosts with different dispersal capabilities also supported host movement as a key determinant of parasite gene flow (Blouin *et al.* 1995; McCoy *et al.* 2003). A corollary hypothesis is that gene flow in a parasite with a complex life cycle will be controlled by the most mobile host (Jarne & Theron 2001; Prugnolle *et al.* 2005b). Other possible determinants of gene flow such as the dispersal ability of free-living larval stages or the degree of host specificity (e.g. many reservoir hosts may increase dispersal chances; Nadler 1995) have not been tested.

Much can be learned from comparing genetic structure in parasites and in their hosts. For example, host-parasite systems can be used to study the interaction between gene flow and the ability of natural selection to promote local adaptation. Theoretical models show that co-evolution between host and parasite is affected by the relative rates of gene flow in the parasite and in the host (Lively 1999; Gandon 2002). These models predict that local adaptation

by parasites to their host is facilitated by higher parasite migration (because parasite migration can import novel alleles that may counteract a host's evolutionary response to the parasite). Support for this prediction was found in two snail-trematode systems in which there was greater parasite gene flow and locally adapted parasites (Dybdahl & Lively 1996; Davies *et al.* 1999). Studying movement in parasites and hosts is also important for understanding the evolution of drug resistance. For example, the widespread occurrence of drug-resistant *Plasmodium falciparum* was shown to result from the recent spread of a few selected alleles, rather than from the independent evolution of new resistance alleles in multiple locations (Nair *et al.* 2003; Roper *et al.* 2003). Finally, genetic structure in parasites could potentially be used to inform us about genetic structure in their hosts. Parasites have been used as biological tags for stock identification of migratory marine organisms (reviewed by MacKenzie 2002). Here the presence or absence of parasite species is used to identify the geographical origin of hosts that carry those parasites. If a parasite is more finely subdivided than its host, then one could potentially use the genotypes of a single species of parasite to assign hosts to their population of origin with higher probabilities than by using the host's own genotypes. Whether a parasite is more or less subdivided than its dispersing host should depend on factors such as the *intensity* and prevalence of infection in migrating hosts, and on the effective sizes of host and parasite populations.

#### Genetic diversity within populations and effective population size

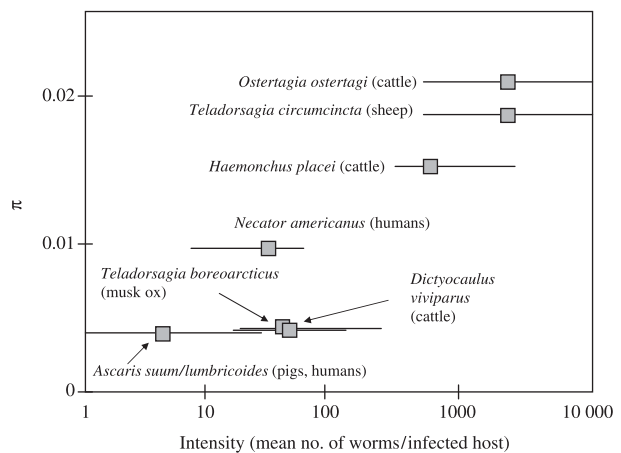
Price (1980) predicted that parasite populations would be largely homozygous and have low genetic diversity. His prediction stems from the idea that hosts are ephemeral habitats and represent patchy environments for the parasite. Therefore, parasite populations should be subject to large population fluctuations and chance colonization events that promote inbreeding and fractionated gene pools. However, most animal *macroparasites* (helminths and arthropods) have levels of allozyme and mtDNA diversity that are similar to or higher than what has been reported in free-living animals (Blouin *et al.* 1992; Bush *et al.* 2001; Criscione & Blouin 2004). Why then do we see deviations from Price's predictions?

Price's perception may have stemmed from the idea that all parasites of a given species within or on an individual host (i.e. an *intrapopulation*; Bush *et al.* 1997) undergo genetic drift like a traditional deme. Many of Price's examples were phytophagous insects that can have many recurrent generations on a single host plant. In contrast, most animal macroparasites release offspring into the external environment. Offspring are mixed and then recruited back into new definitive hosts. So the question of whether the

component population (all the parasites of a given species in an entire host population; Bush *et al.* 1997) or the infrapopulation is best considered the relevant unit of evolution has been raised repeatedly (Lydeard *et al.* 1989; Nadler 1995; Sire *et al.* 2001). In reality there is probably a continuum. If offspring are well mixed, then the transmission process only separates adult breeders into infrapopulations each generation but does not result in recurrent generations within individual infrapopulations. On the other end of the continuum, if offspring reinfect their natal host (e.g. lice, pinworms) or if offspring are transmitted as a clump from host to host over several generations, then the component population behaves more like a traditional subdivided population with infrapopulations as demes. Such species would be more likely to fit Price's predictions. For instance, lice on pocket gophers recruit back to their natal host, and infrapopulations of these lice have low heterozygosity and are highly subdivided (Nadler *et al.* 1990).

$N_e$  has a large influence on overall levels of genetic diversity in populations, and on the fate of alleles under selection. Therefore,  $N_e$  affects local adaptive potential, including the ability of a parasite population to evolve drug resistance. Yet  $N_e$  is an almost completely ignored parameter in parasitology, and we know almost nothing about what factors control the  $N_e$  of parasite populations. Prugnolle *et al.* (2005a) modelled how several features of the complex life cycle of trematodes affect the distribution of genetic variation within and among infrapopulations. In particular, they showed how an increase in selfing rate and in the variance in the reproductive success between clones reduces  $N_e$  of the component population, while clumped transmission of offspring from host to host increases  $N_e$  (by creating nontransient population subdivision). Criscione & Blouin (2005) present a conceptual model for making demographic estimates of  $N_e$  in parasites. This model highlights several factors such as aggregated distributions, crowding effects, and host immunity that can influence the  $N_e$  of parasite component populations via effects on sex ratios and variation in reproductive success. An interesting result is that several features of parasite life cycles probably act in concert to reduce  $N_e$  below that expected in a single free-living population of equivalent census size. Conversely, dormant stages (e.g. long-lived nematode eggs) or *paratenic* hosts (hosts in which a parasite does not develop, but can be maintained for long periods until reaching a definitive host) may buffer the effects of genetic drift (Nadler 1995; Nunney 2002).

As a starting null hypothesis, one might expect a simple relationship between  $N_e$  and the census number of adult parasites in definitive hosts. A crude estimate of the standing number of breeders at any one time would be the number of hosts times the mean *abundance* (number of parasites per host examined; Bush *et al.* 1997). Interestingly, mtDNA diversity in outcrossing nematode species



**Fig. 1** Average population mtDNA diversity ( $\pi$ ; average number of substitutions per site) vs. mean infection intensities for several species of nematodes. Host species are in parentheses. Horizontal bars give the range of typical intensities for that species. mtDNA diversities based on whole-molecule RFLP or on *Cox1* sequences are reported as given. mtDNA diversities based on *Nad4* gene sequence were adjusted down by 20% to account for the higher substitution rate of *Nad4* (Blouin 2002). RFLP: *Ascaris suum/lumbricoides*; *Cox1*: *Dictyocaulus viviparus*, *Necator americanus*; *Nad4*: *Haemonchus placei*; *Ostertagia ostertagi*; *Teladorsagia boreoarcticus*; *Teladorsagia circumcincta*.

that have direct life cycles appears to be a simple function of mean intensity, which should be correlated with the number of parasites in the component population (Fig. 1). However, many parasites have much more complex life cycles. Thus, more comparative studies among species or populations would be useful (e.g. Blouin *et al.* 1999). For example, comparisons of short-term genetic estimates of  $N_e$  among parasite populations that differ in key traits (e.g. levels of aggregation or abundance) would help identify the ecological determinants of  $N_e$ . Unfortunately, we are not aware of any short-term  $N_e$  estimates for parasites that utilize techniques such as the temporal method (Schwartz *et al.* 1998). There are a handful of long-term estimates based on DNA diversity (Blouin *et al.* 1992; see Hartl *et al.* 2002 for malaria references). However, these are crude estimates of long-term species-wide or metapopulation-wide  $N_e$ , and require an estimate of the mutation rate. Such long-term estimates can be interesting, but they provide little insight into current ecological determinants of  $N_e$ .

The effects of mating systems on within-population genetic diversity (reviewed by Charlesworth 2003) have also received little attention in parasites. Among plants and snails, outcrossing species tend to have higher levels of diversity than selfing species (Jarne 1995; Charlesworth 2003). Nematode parasites appear to have a similar pattern, but this conclusion is based on only a handful of studies (Blouin 1998). More comparative studies are needed to generalize these conclusions among parasites. It is worth

noting that almost any variation in reproductive mode (e.g. asexual, monoecious, haplodiploidy, dioecy; Bush *et al.* 2001) can be found among animal parasites. Therefore, numerous opportunities exist to test how life history variation influences genetic diversity.

### Modes of reproduction, mating systems, and transmission patterns

The interplay between transmission dynamics, mode of reproduction (sexual vs asexual), and mating system (selfing, biparental inbreeding or outcrossing) in parasites is important for several reasons (see also Prugnolle *et al.* 2005a). First, transmission and mating patterns influence inbreeding rates (see Christen *et al.* 2002; Christen & Milinski 2003; Prugnolle *et al.* 2004a for how inbreeding may affect parasite infection success). For example, clumped recruitment of siblings into a definitive host ('sib-transmission') can increase the chance of biparental inbreeding (Anderson *et al.* 1995; Nadler 1995). This effect will be exacerbated if the effective number of breeders per infrapopulation is small (Anderson *et al.* 1995). Populations of hermaphroditic parasites that have low mean intensities may have a higher rate of selfing than populations with high intensities. Likewise, transmission that leads to clones in the same definitive host can increase the effective selfing rate (e.g. as in *Plasmodium falciparum*, Anderson *et al.* 2000; or in trematodes where asexual multiplication in the snail leads to the synchronous release of many genetically identical cercariae that could infect the same definitive host). Indeed, it has been hypothesized that trematodes keep second intermediate hosts in their life cycle, rather than going directly from snail to definitive host, as an inbreeding avoidance mechanism (Rauch *et al.* 2005). Here the second intermediate host 'collects' different cercarial genotypes over time, and these different genotypes then mate when a definitive host ingests the second intermediate host (Rauch *et al.* 2005). Second, mode of transmission and mating system also control the opportunity for kin selection. Recent models predict that relatedness among parasites within a host is a key determinant of optimal strategies of parasite growth, manipulation of host behaviour, or virulence (Frank 1996; Brown 1999; Parker *et al.* 2003). These predictions remain untested.

#### Inferring modes of reproduction and mating systems

Molecular markers can be very useful when the mode of reproduction is still in doubt. For example, Viney *et al.* (1993) and Viney (1994) demonstrated where sexual reproduction occurs in the life cycle of the nematode *Strongyloides ratti*, and showed that the parthenogenetic phase was mitotic rather than meiotic. Populations of protozoan parasites are frequently classified as being clonal (limited genetic exchange that is not sufficient to erode linkage

disequilibrium), panmictic (frequent sexual recombination), or epidemic (basic panmictic structure, but some genotypes expand clonally due to favourable conditions) (see MacLeod *et al.* 2001). Populations of the same protozoan species often show different degrees of clonality (reviewed by Tibayrenc & Ayala 2002). For example, populations of African trypanosomes in wildlife and livestock are mostly epidemic in genetic structure. However, clonal structure has arisen several times in human hosts (MacLeod *et al.* 2001). Similarly, *Toxoplasma gondii* has a mix of clonal and sexual propagation that may be associated with domestic and wild environments (Ajzenberg *et al.* 2004).

There have been relatively few studies that used molecular markers to infer mating patterns. Mixed mating systems with high rates of outcrossing were demonstrated using progeny-array analysis from experimental infections in a trematode, *Echinostoma caproni* (Trouve *et al.* 1999) and from *in vitro* matings in a cestode, *Schistocephalus solidus* (Luscher & Milinski 2003). Deviations from Hardy-Weinberg equilibrium have been used to estimate outcrossing rates in natural populations of flatworms. The trematode *Lecithochirium rufoviride* and cestode *Proteocephalus exiguus* had inferred outcrossing rates of 70% and 84%, respectively (Snabel *et al.* 1996; Vilas & Paniagua 2004), whereas the cestode *Echinococcus granulosus* had a very low inferred rate of outcrossing (1.1%) (Lymbery *et al.* 1997).

Of course multiple factors can cause deviations from Hardy-Weinberg or linkage equilibrium within parasite infrapopulations, so in many cases inferring the mating system from just Hardy-Weinberg considerations can be problematic. Large and variable (often negative) deviations from Hardy-Weinberg equilibrium may appear if clones transmit together and are not identified as such (Prugnolle *et al.* 2005a). Hosts that sample from spatially or temporally separated pools of infective larvae could show a Wahlund effect in their infrapopulations (Vilas *et al.* 2003). Clumped transmission of sibs can produce variable, and often negative values of  $F_{IS}$  if samples consist of a few sibships (Balloux 2004). Clumped transmission of sibs can also produce positive  $F_{IS}$  owing to inbreeding in subsequent generations if offspring continue to transmit together.

#### Inferring the ecology of transmission

Data on the nonrandom distribution of genotypes among infrapopulations or social host groups can be very useful for understanding the ecology of transmission from host to host.

For example, humans in Guatemala were infected with ascarid nematodes having identical mtDNA haplotypes more often than expected by chance alone (Anderson *et al.* 1995). Thus, transmission may be clustered within human households, rather than occurring randomly throughout each village. The distribution of clones within and among hosts can be informative about transmission in some parasites

(e.g. trematodes). For example, Theron *et al.* (2004) studied the transmission of the trematode *Schistosoma mansoni* between its snail intermediate host and rat definitive host in Guadeloupe. Individual snails carried an average of 1.1 unique genotypes, while individual rats carried an average of 34. Thus, rats are infected from about 30 snails, rather than in one or a few infection events. Similarly, only a few clones of the trematode *Diplostomum pseudospathaceum* are normally found in each snail, while a fish second intermediate host can harbour dozens of unique genotypes (Rauch *et al.* 2005).

To date most studies have relied on  $F_{ST}$  (or related analogs) to describe patterns of genetic differentiation among hosts (Nadler *et al.* 1990, 1995; Anderson *et al.* 1995; Fisher & Viney 1998; Davies *et al.* 1999; Paterson *et al.* 2000; Brouwer *et al.* 2001; Hawdon *et al.* 2001; Sire *et al.* 2001; Curtis *et al.* 2002; Prugnolle *et al.* 2002; Vilas *et al.* 2003; Braisher *et al.* 2004; Theron *et al.* 2004). The usual explanation for a significant  $F_{ST}$  is genetic drift, but for many parasite species infrapopulations may not behave as traditional demes. Instead, variation in allele frequencies among infrapopulations informs us more about recruitment patterns and aspects of the mating system (Sire *et al.* 2001; Prugnolle *et al.* 2005a).

There are, however, several methodological concerns when interpreting estimates of  $F_{ST}$  among infrapopulations. First, small sample sizes of parasites per host can lead to imprecise or biased estimates of  $F_{ST}$  (Waples 1998). Unfortunately, small sample sizes may be unavoidable because some parasites naturally have low mean intensities or highly aggregated distributions (i.e. few hosts are heavily infected, but most hosts have few parasites; Shaw *et al.* 1998). In the latter case, researchers could sample only from large infrapopulations (e.g. Vilas *et al.* 2003), but then sampling from a nonrandom sample of hosts may be an issue. Second, many studies rely on samples of offspring (e.g. excreted eggs) because collecting adult parasites from each infrapopulation is impractical (e.g. Fisher & Viney 1998; Patterson *et al.* 2000; Brouwer *et al.* 2001; Curtis *et al.* 2002). The problem here is that one usually does not know the effective number of breeders that contributed those offspring. Sampling a large number of sibs from a limited number of breeders can inflate the apparent  $F_{ST}$  among infrapopulations because of chance allele frequency differences among families (Allendorf & Phelps 1981). One way around this problem that has never been applied to parasites is to partition offspring into sibships using marker data, and to then replace sibships with reconstructed parental genotypes for the purposes of estimating allele frequencies (Blouin 2003 and references therein). Note also that this approach could be used to estimate the number of breeding adults infecting a host when direct counts are impossible (e.g. how many adult schistosomes occur in an infected human?). Such data can be essential for parameter

estimation in epidemiological studies and for estimating the effective size of parasite populations (Criscione & Blouin 2005).

Even if we ignore the above methodological concerns, interpreting the causes of among-infrapopulation  $F_{ST}$  in the absence of other information remains problematic. If parasite isolation among geographical locations or host social groups is not accounted for,  $F_{ST}$  among infrapopulations will be inflated (e.g. Nadler *et al.* 1995; Curtis *et al.* 2002). Multiple copies of the same clone in infrapopulations can also inflate the among-infrapopulation  $F_{ST}$  (Theron *et al.* 2004; Prugnolle *et al.* 2005a). Assuming one has adjusted for clones and simple geographical isolation, a nonrandom distribution of parasite genotypes among infrapopulations could still be caused by several processes such as sib-transmission or even host factors that select against certain parasite genotypes (Anderson *et al.* 1995). Presumably it is processes such as these that one is interested in studying, but simply demonstrating significant  $F_{ST}$  among infrapopulations does not distinguish among them.

Much more could be gained by using polymorphic markers and modern analytical techniques such as methods for pedigree reconstruction (Blouin 2003; Jones & Ardren 2003) and methods for a posteriori assignment of individuals into natural groupings (Pritchard *et al.* 2000; Dawson & Belkhir 2001). Pedigree methods can directly assess the degree to which sibs are transmitted together and will be essential for testing hypotheses about kin selection in parasites. One could test whether deviations from Hardy–Weinberg equilibrium within infrapopulations result from the presence of related individuals, of inbred but unrelated individuals, or of groups of unrelated individuals from distinct gene pools (Ritland 1996; Amos *et al.* 2001; Castric *et al.* 2002). As some studies have shown that parasites are capable of mate choice (Trouve *et al.* 1999; Luscher & Milinski 2003), it will also be interesting to test if sib-transmission results in the evolution of inbreeding avoidance behaviours (Prugnolle *et al.* 2004b).

### Searching for loci under selection

Many methods are available for identifying the signature of selection in genomes (see Conway & Polley 2002 and Anderson 2004 for recent reviews). These methods can provide important contributions in two areas of parasitology. One is the identification of potential drug resistance genes (Anderson 2004). For example, intense selection on a resistance gene in *Plasmodium falciparum* was inferred from patterns of linkage disequilibrium and heterozygosity around the target locus (Wootton *et al.* 2002; Nair *et al.* 2003). A second application is the identification of genes involved in host–parasite interactions. For instance, several loci encoding antigens in *P. falciparum* have been found to be under balancing selection, thus, indicating potential

interactions with host immunity (Conway & Polley 2002). Searching for genes involved in host–parasite interactions is an exciting and growing field that should benefit greatly from the availability of entire sequenced genomes of several hosts and parasites (e.g. El-Sayed *et al.* 2004; Ghedin *et al.* 2004).

### Concluding remarks

The disciplines of molecular ecology and parasitology have much to offer each other. Molecular methods can elucidate ecological and microevolutionary processes in parasites, and parasites are unique study organisms for evolutionary ecologists. For example, the interactions between hosts and parasites raise interesting questions about the evolution of parasitic lifestyles and host defenses. The fitness effects of parasites on hosts are of obvious concern to medical science and commercial industries (e.g. aquaculture, livestock). Parasites are also extremely diverse (Poulin & Morand 2004). By diversity, we include not only the myriad of taxa that have independently evolved a parasitic lifestyle, but also the diversity in life cycles, modes of reproduction, host species, and ecosystems utilized by parasites. For example, hermaphroditic, parthenogenetic, and asexual modes of reproduction are frequent and have evolved independently among several parasite taxa. This diversity should captivate biologists because parasites present numerous opportunities for the study of ecological and evolutionary theory. Nevertheless, there has been relatively little work on parasites using the methods of molecular ecology. Indeed, even traditional phylogeography studies have been done on only a handful of parasite species. Parasite molecular ecology is still in its infancy, but it promises to be a rewarding field for those who embrace it.

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