REVIEW ARTICLE

Testing local-scale panmixia provides insights into the cryptic ecology, evolution, and epidemiology of metazoan animal parasites

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SUMMARY

When every individual has an equal chance of mating with other individuals, the population is classified as panmictic. Amongst metazoan parasites of animals, local-scale panmixia can be disrupted due to not only non-random mating, but also non-random transmission among individual hosts of a single host population or non-random transmission among sympatric host species. Population genetics theory and analyses can be used to test the null hypothesis of panmixia and thus, allow one to draw inferences about parasite population dynamics that are difficult to observe directly. We provide an outline that addresses 3 tiered questions when testing parasite panmixia on local scales: is there greater than 1 parasite population/species, is there genetic subdivision amongst infrapopulations within a host population, and is there asexual reproduction or a non-random mating system? In this review, we highlight the evolutionary significance of non-panmixia on local scales and the genetic patterns that have been used to identify the different factors that may cause or explain deviations from panmixia on a local scale. We also discuss how tests of local-scale panmixia can provide a means to infer parasite population dynamics and epidemiology of medically relevant parasites.

Key words: cryptic species, mating systems, molecular epidemiology, transmission, population genetics.

INTRODUCTION

Testing the hypothesis of panmixia can provide valuable insight into the ecology and evolution of parasite populations. A panmictic population is one in which every individual has an equal chance of mating with another individual. Disruption of panmixia can happen at several scales, and ultimately leads to genetic structuring. For example, inbreeding (matings between relatives) increases homozygosity among individuals and isolation among geographical subpopulations will result in allele frequency divergence due to genetic drift. Because the pattern of genetic structuring depends on the ecological mechanism that generates the disruption, population genetics data can be used to elucidate these disruptive mechanisms and hence provide indirect inferences on population biology such as mating systems, dispersal patterns, or historical processes (e.g., population growth/decline). Consequently, population genetics data are of special relevance to parasitic organisms where the direct observation of population dynamics or even species identification is often precluded, and thus cryptic in nature, due to their small sizes, sites of infection on or in a host, life-cycle complexities, or limited intra- or interspecific morphological variability (Nadler, 1995; Criscione *et al*. 2005; de Meeûs *et al*. 2007*a*). Moreover, genetic structuring can affect the evolutionary potential of populations (e.g., inbreeding increases the efficiency of directional selection; Hedrick, 2005). Thus, population genetics data are needed to develop predictive hypotheses about parasite evolutionary dynamics (e.g., evolution of resistance to host immunity or anti-parasitic drugs). As in free-living organisms, non-panmixia in parasite populations may occur at the scale of individuals (non-random mating within populations) or among geographical locations (disruption of gene flow). What is unique and intriguing about the parasitic lifestyle is that panmixia can be further disrupted by 2 additional mechanisms that function within a localized geographical area: separation among sympatric host species and non-random transmission among individual hosts (i.e., infrapopulations; *cf*. Bush *et al*. 1997) of a single host species population.

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Here we provide a review of population genetic studies that address local-scale panmixia in metazoan parasites of animals. Certainly, many of the topics we cover are likely to be applicable to parasites of plants and protozoan parasites. The taxonomic restriction is largely a reflection of our laboratory's research interest rather than biological and is meant to provide a more concise review. It is clear that parasite life-history traits, host vagility, and/or historical processes (e.g., vicariance or colonization events) can disrupt panmixia over a geographical landscape (e.g., Criscione and Blouin 2004, 2007; Nieberding *et al.* 2006; Whiteman *et al.* 2007; Wielgoss *et al.* 2008; Štefka *et al.* 2009). However, it is probably less well-appreciated that factors other than inbreeding can lead to or explain non-panmictic patterns at local scales in parasites. By local scale we are referring to what one might consider to be a single host population or a small geographical area where a parasite (of a given life stage) infects sympatric host species. We highlight the evolutionary significance of non-panmixia on local scales and the genetic patterns that have been used to identify the different factors that may cause or explain deviations from panmixia on a local scale. Geographical patterns are only discussed if they help support the local-scale conclusions. We also discuss how tests of local-scale panmixia can provide a means to infer parasite population dynamics and epidemiology of medically relevant parasites.

At the heart of testing for deviations from panmixia is the principle of Hardy-Weinberg equilibrium (HWE). Deviations from panmixia will alter genotypic proportions expected under HWE. These deviations can be quantified with the fixation indices F_{IS} and F_{ST} that were designed by Wright (1969). For the sake of brevity, we refer readers to de Meeûs *et al.* (2007*a*) and Hedrick (2005) for in-depth explanations of these indices (see also definitions in Table 1). Briefly, these indices measure the proportional change in heterozygosity (expected under HWE) that is due to non-random union of gametes in a subpopulation (F_{IS}) or subdivision of a population into subpopulations (F_{ST}). F_{IS} can range from -1 to 1, where 0 indicates HWE within the subpopulation. Negative values (excess heterozygotes) can result from inbreeding avoidance, small population sizes, and asexual reproduction (de Meeûs *et al.* 2007*a*). Positive values (excess homozygotes) can be generated by matings between relatives (e.g., biparental inbreeding or inbreeding from self-mating in hermaphrodites) and when 2 genetically diverged populations (often unrecognized) are analysed as 1 (the Wahlund effect, Table 1). F_{ST} ranges from 0 to 1 where 0 indicates no genetic subdivision among subpopulations (e.g., equal allele frequencies among subpopulations) and increasing values indicate a trend toward fixation of different alleles in different subpopulations (see Meirmans and Hedrick (2011) for discussion on standardizing F_{ST}). F_{ST} is largely affected by genetic drift and gene flow and, in the case of metazoan parasites, can be influenced by non-random transmission among infrapopulations (Criscione *et al.* 2005). Because F_{IS} requires measures of observed and expected heterozygosity, our review is restricted to studies that use co-dominant, neutral genetic markers (e.g., allozymes, single copy-nuclear microsatellites).

We present the review in a hierarchical approach starting at the top (Fig. 1) because if one fails to recognize any upper level structure, then inference at the lower levels will be confounded. For example, one might incorrectly conclude that deviations from panmixia occurred due to non-random mating among individuals within subpopulations when in reality, deviations from HWE occurred because of unrecognized cryptic species in the sample (i.e., the Wahlund effect). Figure 1 outlines 3 tiered questions to ask at the local scale: is there greater than 1 parasite population/species, is there genetic subdivision among infrapopulations within a host population, and is $F_{IS} \neq 0$ (i.e., is there asexual reproduction or a non-random mating system) within the lowest level of subdivided units? Although we present our review as 3 discrete tiers and as discrete categories within tiers, we recognize that there is likely a continuum in patterns of genetic structuring once panmixia is disrupted. Thus, analyses of F_{IS} and F_{ST} among loci are often conducted concurrently. Moreover, structuring events at one level may not be mutually exclusive of dynamics at another level (e.g., inbred organisms also tend to show high genetic subdivision among subpopulations; Charlesworth, 2003).

IS THERE MORE THAN 1 POPULATION/SPECIES?

It is critical to identify the boundaries of a parasite population to correctly infer the ecological dynamics of parasite populations, to understand host-parasite interactions and co-evolution, and to identify mechanisms influencing the within population mating system. Moreover, failure to recognize the presence of distinct parasite species may lead one to draw incorrect conclusions on parasite epidemiology (including control strategies), pathogenicity, biodiversity, and systematics (see Nadler and Pérez-Ponce de León, 2011). We present 3 scenarios in which the local-scale structure would be influenced by the presence of more than 1 parasite population/species: (1) limited morphological variation precludes species or population recognition, (2) infection of sympatric host species results in divergence between host-affiliated parasite populations, and (3) recognized parasite species/populations become admixed.

Cryptic populations/species

It is increasingly recognized that the limited morphological characteristics of many parasitic taxa can

Table 1. Glossary of terms in review

Asexual reproduction	An individual produces new individuals that are genetically identical to the ancestor at all loci in the genome, except those sites that have had somatic mutations (de Meeûs *et al*. 2007*b*).
Clonal transmission	Refers to the manner (random or clumped dispersal) in which clonemates are transmitted from the asexual developmental stage to subsequent hosts for ensuing developmental stages.
Clonemate	Individuals that are the product of an asexual reproductive event of a progenitor individual and thus, are genetically identical. A clone refers to one unique multilocus genotype.
Clonemate-sampling	Incorrect estimates of F_{ST} among infrapopulations can be obtained if offspring of the adults that colonized the host are used to estimate allele frequencies. This is due to the fact that clonemates, which are genetically identical, could be sampled (i.e., pseudoreplication of individual adults). In the case of digeneans, incorrect estimates of the previous adult generation's mating system could also be inferred if F_{IS} is estimated without reducing the data set to one individual per unique clone (Prugnolle *et al*. 2005*a*).
F_{IS}	A measure of deviation from Hardy-Weinberg equilibrium within subpopulations (see text for a more formal definition).
F_{ST}	A measure of allele frequency differentiation among subpopulations (see text for a more formal definition).
Mating system	Refers to the manner (random or non-random) in which gametes are united to form a zygote. In this review, we focus on mating with respect to relatedness.
Mode of reproduction	Refers to whether offspring are products of asexual or sexual reproduction.
Null allele	In genotyping, this is an allele that fails to amplify. If an individual is a heterozygote with a null allele, that individual will appear as a homozygote for the allele that did amplify.
Sexual reproduction	For the purposes of this review, we define this as the union of gametes from one (self-mating) or two parents (outcross-mating) where gametes are products of recombination during meiosis.
Siblings	Individuals that share one or both parents and are the product of gamete union. Because gametes are products of recombination during meiosis, siblings are not genetically identical to one another or their parent(s).
Sib-sampling	Incorrect estimates of F_{ST} among infrapopulations can be obtained if offspring of the adults that colonized the host are used to estimate allele frequencies. This is due to the fact that sibling parasites, which share alleles, could be sampled (i.e., pseudoreplication of individual adults).
Sib-transmission	Refers to the manner (random or clumped dispersal) in which siblings are transmitted from their natal host to subsequent hosts for ensuing developmental stages.
The Wahlund effect	Refers to a reduction in the observed heterozygosity (relative to that expected under Hardy-Weinberg equilibrium) in a sample caused by subpopulation structure.

lead to an underestimation of the number of parasitic species (Pérez-Ponce de León and Nadler, 2010; Nadler and Pérez-Ponce de León, 2011; Poulin 2011). This underestimation is evident in studies that initially aimed to investigate the population genetics of a presumed single parasite species, but ended up discovering cryptic species (morphologically similar but genetically distinct). Below, we describe examples of such studies with emphasis on how deviations from panmixia revealed the cryptic species/populations.

Fixed allelic differences at allozyme loci led Renaud and Gabrion (1988) and Reversat *et al*. (1989) to conclude that there was more than 1 species in the taxon under investigation (cestode and digenean species, respectively). For example, at 2 loci with 2 alleles each, only homozygous individuals were observed among a sample of the presumed single cestode species *Bothrimonus nylandicus* (Renaud and Gabrion, 1988). These marine cestodes could be separated into 2 groups based on the homozygous genotypes (i.e., fixed alleles between species). Given the absence of heterozygotes in the 2 populations, they concluded that there was reproductive isolation between these morphologically indistinguishable groups (Renaud and Gabrion, 1988). A third locus with 2 alleles did show heterozygotes. Within the 2 designated groups, this locus was in HWE; however, a collective analysis of all samples showed significant reductions in heterozygotes (i.e., the Wahlund effect). Interestingly, both of these cryptic species can infect the same host species, but they show marked differences in their seasonal dynamics of infection. This latter result highlights how parasite ecology can easily be misinterpreted if one fails to recognize the presence of more than 1 species. Additional examples where fixed allelic differences have identified cryptic parasite populations are given in Nadler and Pérez-Ponce de León (2011).

Depending on the molecular markers and the time since divergence, fixed allelic differences, may not always be found. As a consequence, additional patterns have been used to identify cryptic populations. For instance, in an allozyme study on the marine digenean *Lecithochririum fusiforme*, F_{IS} values were highly variable across loci within infrapopulations and across infrapopulations for a given

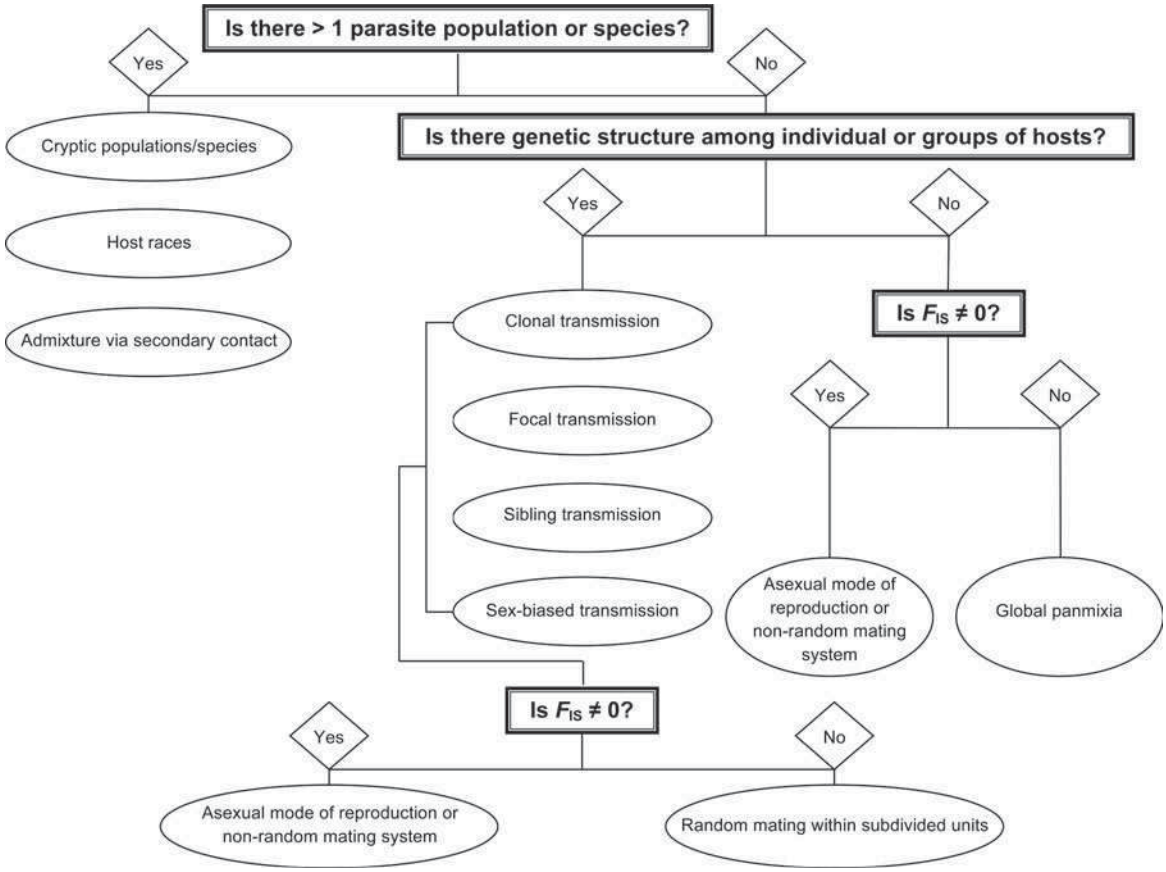

Fig. 1. Outline of the 3 tiered questions (in bordered boxes) to ask when testing for panmixia in local-scale analyses of parasite populations. Ovals below the 'Yes' answers designate the potential reasons behind the observed deviations from panmixia and are addressed in detail within each subheading of the review. F_{IS} quantifies the deviation from expected heterozygosity under Hardy-Weinberg equilibrium.

locus (Vilas *et al.* 2003). The authors postulated that the Wahlund effect might explain this variation as an inbred mating system was unlikely to produce such extensive variation in F_{IS} values among loci. This prediction was based on a qualitative correlation where allozyme loci with high average within-infrapopulation F_{IS} also had high F_{ST} among infrapopulations (see Criscione *et al.* 2011 for a discussion about what generates this pattern). Unfortunately, Vilas *et al.* (2003) could not test their hypothesis because the amount of tissue needed for allozyme typing precluded the ability to obtain multilocus genotypes (MLGs) for individual worms. Criscione *et al.* (2011) revisited the system with microsatellite loci and observed the same patterns among loci as Vilas *et al.* (2003). This time, the ability to obtain MLGs enabled the use of individual-based Bayesian and multivariate clustering methods to test for cryptic population structure (Fig. 2A). Indeed, most of the deviations from panmixia (e.g., variable average within infrapopulation F_{IS} and pairwise infrapopulation F_{ST}) were driven by the presence of 3 cryptic populations (possibly species) that had variable and intermingled distributions across 12 sampled infrapopulations. A different approach led Grillo *et al.* (2007) to conclude there was a cryptic species of the nematode *Teladorsagia circumcincta* in their samples. Nematodes were collected from 4 French goat farms. Among 3 farms there was no genetic differentiation ($F_{ST}=0$); however, the fourth farm was divergent from the other 3. A multivariate clustering method on the MLGs of individual worms demonstrated that this farm contained a mixture of mostly individuals of a cryptic species (as first suggested by sequence data in Leignel *et al.* 2002) and a few individuals of the 'standard' *T. circumcincta* species (Fig. 2B).

The examples above highlight how limited morphology can inhibit species discovery. Conversely, extensive intraspecific morphological variation or environmentally induced (e.g., host environment) phenotypic plasticity may lead to the over-designation of species (Perkins *et al.* 2011). Tests of panmixia among morphotypes can be used to determine whether a species status is warranted. For example, Grillo *et al.* (2008) found that 3 sympatric morphological variants in the nematode genus *Teladorsagia* (previously recognized as 3 species) did not show genetic differentiation among one another (Fig. 2C), thus indicating that the 3 morphotypes were of the same species.

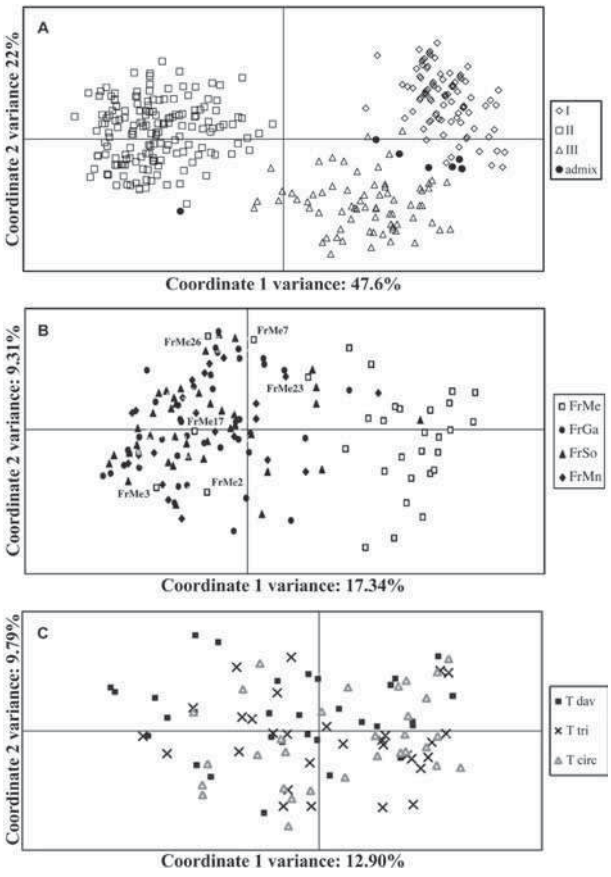

Fig. 2. Results from individual based Principle Coodinate Analyses (PCoA) used to test for possible underlying cryptic genetic strucuture in parasite populations. (A) PCoA revealed 3 clusters within a sample of trematodes (*Lecithochririum fusiforme*). Shapes correspond to the 3 cryptic populations identified by Bayesian cluster analyses. There is near complete agreement between the two analyses in the assignment of individuals to the 3 clusters. (B) Shapes correspond to the population of origin for *Teladorsagia circumcincta* collected from 4 French goat farms. Samples to the right of the figure separate out and represent the cryptic species. Notice that farm FrMe is largely composed of the cryptic species, but also has several worms that cluster with the 'standard' species. There was significant F_{ST} between all comparisons of farm FrMe to the other 3 farms, but there was no genetic differentiation among the other 3 farms. (C) Shapes represent the 3 morphotypes of *Teladorsagia circumcincta*. Notice there is no underlying pattern of structure between the 3 groups, indicating a single species. All 3 studies used nuclear microsatellite markers. Figures were reproduced with permission from Criscione *et al.* (2011); Grillo *et al.* (2007); and Grillo *et al.* (2008), respectively.

Host races

Many parasites can exploit more than 1 host species at a given developmental stage in their life cycle. When a panmictic parasite population becomes subjected to 2 or more sympatric host species, host-race associated subpopulations may evolve. Genetic subdivision among parasites in different host species could arise through extrinsic (e.g., ecological separation of life cycles) or intrinsic (e.g., host-induced selection against hybrid parasites) mechanisms (McCoy, 2003). Tests of parasite panmixia among multiple sympatric host species can help determine whether a presumed generalist parasite actually shows some degree of host affiliation.

In a series of publications centred on a seabird-tick system, McCoy and colleagues (McCoy *et al.* 2001, 2005; Kempf *et al.* 2009) determined the presence of morphologically indistinguishable, but genetically distinct tick subpopulations that were associated with different seabird host species in sympatry. Host-associated patterns were originally detected by comparing F_{ST} between ticks (*Ixodes uriae*) on sympatric host species with F_{ST} between allopatric populations of the same host species. They found that ticks infecting the same avian host species in allopatric habitats were more genetically similar to one another than ticks occupying different host species within the same habitat (McCoy *et al.* 2001). Subsequent studies demonstrated that this pattern of host race formation occurred multiple times and in different hemispheres, and is likely a recent phenomenon (McCoy *et al.* 2005; Kempf *et al.* 2009). Experimental host transplantations suggest these ticks have a quick local adaptive response, and thus may explain the recurrent host-race formation patterns (McCoy *et al.* 2002). Testing for deviations from panmixia has important epidemiological implications as these ticks are a vector for Lyme disease. Indeed, it was found that the different tick races had different infection intensities of bacteria of the *Borrelia burgdorferi s.l.* complex. After accounting for these differences among tick races, the overall estimated prevalence of the bacterial pathogen was higher than previously suspected (Gomez-Diaz *et al.* 2010). A similar rapid host-associated (deer and cattle) divergence pattern was detected for the cattle tick *Rhipicephalus microplus* (de Meeûs *et al.* 2010). In this study, F_{ST} based analyses coupled with genetic effective population size estimates and tests of population bottlenecks confirmed that the cattle tick was introduced to New Caledonia after the introduction of deer. Thus, the differentiation between ticks on cattle and deer had occurred in as few as 244 tick generations (de Meeûs *et al.* 2010).

Tests of parasite panmixia among host species also have an important epidemiological application for human parasites. For example, there is always concern that human parasites could be maintained in non-human hosts, such that these non-human hosts serve as a recurrent source (i.e., reservoir) of new parasite infections. Tests of genetic differentiation between human and non-human collected parasites can address whether non-human hosts are serving as reservoir hosts. In the Philippines, Rudge *et al.* (2008) carried out such tests with the blood fluke *Schistosoma japonicum* and found no evidence of

parasite genetic structure between humans and dogs. Thus, they concluded that future control programmes should account for transmission among dogs as well as humans. In contrast, similar studies in China revealed variable patterns in which some villages showed low but significant differentiation while others showed no differentiation among human and non-human hosts (rodents, dogs, cattle). Thus, in some villages rodents, dogs, and cattle may be reservoir hosts (Rudge *et al*. 2009). A related question of whether domestic (sheep) or sylvatic (macropods) intermediate hosts of *Echinococcus granulosus* harboured separate strains of the parasite was addressed by Lymbery *et al*. (1990). There was no genetic differentiation among parasites from intermediate hosts in sympatry.

Admixture via secondary contact

Above, we focused on addressing whether a presumed single parasite population was composed of cryptic species or host-associated populations. Here, we address studies that ask if there is potential secondary contact between 2 recognized parasite species/populations. Given there is secondary contact, an additional question of interest is whether this secondary contact leads to hybridization and possibly panmixia (i.e., homogenization) among the recognized parasite populations/species.

In Wielgoss *et al*. (2010), the potential for admixed parasite populations due to re-stocking of the eel host was tested. A previous analysis of geographical structure of the eel nematode *Anguillicola crassus* found significant genetic structure among 15 sampled locations in Europe (Wielgoss *et al*. 2008). This genetic divergence should therefore enable one to detect deviations from panmixia in a population where nematodes were introduced via eel re-stocking. Indeed, in a stream with natural eel recruitment, HWE was observed in the nematode population. In contrast, in a river with a history of re-stocking, there was significant positive F_{IS}, which could be driven by the admixture of genetically diverged nematode populations (i.e., the Wahlund effect). The hypothesis of admixture of nematode populations was supported via genetic assignment tests that provided evidence of first-generation migrant nematodes mixed among local population nematodes.

Relative to free-living organisms, hybridization studies among metazoan parasites have not received extensive attention (Detwiler and Criscione, 2010). The potential for gene introgression between the 2 populations/species is significant especially in relation to the potential for the introgression of novel host infectivity genes or genes that may play a role in drug-resistance evolution (Barton, 2001). We are aware of only 2 studies that used co-dominant markers to test for contemporary hybridization in sympatric populations. Sympatric populations of *Schistosoma mansoni* (blood fluke of humans) and *S. rodhaini* (blood fluke of rodents) were not panmictic, but rather showed high genetic divergence (Steinauer *et al*. 2008). Nonetheless, Bayesian clustering analyses with microsatellites confirmed contemporary hybridization where introgression appears asymmetric going from *S. rodhaini* to *S. mansoni*. The other example of contemporary hybridization is between human and pig-associated populations (which also show strong divergence) of roundworms (traditionally referred to as *Ascaris lumbricoides* and *A. suum*, respectively) (Criscione *et al*. 2007). Bayesian clustering methods revealed hybridization in sympatric populations within both Guatemala and China. These studies on *Ascaris* and *Schistosoma* highlight the epidemiological importance of panmixia tests in parasites with secondary contact. For instance, the results of both these studies indicate that although there is genetic divergence, there is still some contemporary interbreeding. Therefore, there is necessarily recent cross-transmission among host species.

IS THERE GENETIC STRUCTURE AMONGST INDIVIDUAL OR GROUPS OF HOSTS?

A common life-cycle feature among many metazoan parasites is that breeding adults are subdivided among infrapopulations (i.e., definitive hosts). The adults release offspring (eggs or larvae) from the definitive host into the external environment and, in general, do not multiply within or on their definitive host (Hudson *et al*. 2002). If transmission leads to a large mixing or dispersal of parasite offspring before infection of definitive hosts, then adult parasites will be randomly distributed among infrapopulations (i.e., F_{ST} among hosts will be 0). However, non-random transmission among individual or groups of hosts will cause a disruption of panmixia (F_{ST} among hosts significantly > 0) (Criscione and Blouin, 2005, 2006).

Testing for significant genetic structure among individual hosts is important for 2 reasons. First, the direct observation of parasite dispersal among individual hosts is nearly impossible. Tests for random transmission amongst hosts, however, provide an indirect means to infer the degree of mixing and the factors influencing among-host dispersal itself. Second, disruption of panmixia due to non-random transmission has evolutionary consequences for parasite populations. For example, under HWE the frequency of a rare homozygous genotype is q^2. However, Wright's (1969) models of population structure show that the overall frequency of a rare homozygous genotype approaches the higher frequency of q rather than q^2 when populations become subdivided. Indeed, this conclusion was supported by transmission simulations of Cornell *et al*. (2003) wherein parasite offspring from individual

infrapopulations were transmitted as a clumped unit (i.e., there was no mixing of offspring amongst infrapopulations) over multiple parasite generations. This increase in the frequency of the rare homozygous parasite genotype across the host population has critical implications for the evolution of drug-resistance (Cornell *et al*. 2003). In this section, we first discuss factors that may promote random transmission and then we examine 4 mechanisms that can generate non-panmictic dynamics amongst individual or groups of hosts of a single host population: clonal transmission, focal transmission, sibling transmission, and sex-biased transmission.

Random versus non-random transmission

How well parasite offspring are mixed during transmission will be influenced by such factors as host behaviour (e.g., territoriality), transmission environment (terrestrial *vs* aquatic), parasite life-cycle patterns, and the dispersal ability of free-living stages. In particular, complex life cycles, wherein the parasite transitions through several intermediate hosts before reaching sexual maturity in a definitive host, may increase the opportunity for mixing. There is likely to be a continuum among metazoan parasites along which various transmission processes lead to different levels of genetic differentiation among infrapopulations of a given host population. On one end of the continuum, Criscione and Blouin (2006) hypothesized that aquatic species with several intermediate hosts will have panmictic structure among infrapopulations (i.e., the aquatic mixing hypothesis). The logic being that the aquatic environment is conducive to parasite offspring dispersal whether as a free-living stage or during infection of an intermediate host. For instance, panmixia among hosts was observed in the freshwater digenean *Plagioporus shawi*, which cycles from an aquatic snail to an arthropod intermediate host to a fish definitive host (Criscione and Blouin, 2006). On the other end of the continuum, parasites that have direct life cycles and require physical contact between definitive hosts for transmission have limited opportunity for offspring mixing (Nadler, 1995). Indeed, significant genetic structure (F_{ST}=0·092) was observed among pocket gopher infrapopulations for the chewing louse (*Geomydoecus actuosi*), which can have multiple generations on a single host (Nadler *et al*. 1990).

It is important to note that to infer transmission among hosts one must sample the developmental stage that infects the host. Thus, to infer transmission among definitive hosts, adult parasites should be sampled. Unfortunately, in some parasites like human blood flukes (*Schistosoma* spp.), adult flukes cannot be sampled. As an alternative, fluke eggs have been used as the sampling unit. For example, Agola *et al*. (2009) detected significant F_{ST} for *S. mansoni* among 12 children in a localized area of Kenya. However, this structure could be due to the sampling of sibling parasites because parasite eggs were collected from individual hosts. As siblings share alleles, sib-sampling (Table 1) can distort the allele frequencies of the actual adult parasites that colonized the individual hosts (Criscione *et al*. 2005). Thus, one may falsely conclude there was non-random transmission among infrapopulations. For a thorough discussion on sib-sampling, we refer readers to Steinauer *et al*. (2010). One way to remove this effect is to do a hierarchical *F*-statistical analysis with infrapopulations nested in some higher level unit of substructure. Such an analysis still does not enable interpretation of F_{ST} among infrapopulations, but may allow for inference at higher levels of substructure.

Clonal transmission

Some metazoan parasites (digeneans and *Echinococcus* cestodes) have an obligate larval asexual and obligate adult sexual phase in their development. Thus, for these parasites, it is also of interest to discuss the mixing potential of identical clones (clonemates) from the asexual phase to the adult phase in definitive hosts. Clumped clonal transmission can have a profound effect on genetic structure among infrapopulations. The theoretical work of Prugnolle *et al*. (2005*a*, *b*) shows that as the variance in reproductive success among larval clones increases in an intermediate host, so does genetic differentiation (F_{ST}) among infrapopulations in subsequent intermediate or definitive hosts. The latter holds true if there is not complete mixing from the host with asexual parasite reproduction to subsequent hosts (i.e., $m_2 \neq 1$; see Prugnolle *et al*. 2005*a*). Moreover, Prugnolle *et al*. (2005*a*) stated that when using genetic differentiation among definitive hosts to infer offspring dispersal from definitive host to the host where there is asexual reproduction (i.e., m_1), multiple copies of a clone need to be reduced to a single representative within each definitive host. For methods to test whether repeated MLGs represent true clonemates, we refer readers to Gregorius (2005) and Arnaud-Haond *et al*. (2007).

Studies to date largely support the aquatic mixing hypothesis in that the variance in clonal reproductive success tends to be low in digeneans with full aquatic transmission or transmission involving open water systems (rivers, oceans) and bird definitive hosts. In part, this is due to the fact that few repeated copies of a clone have been found. Adult samples of *Plagioporus shawi* from salmon yielded 99% to 100% of unique clones among genotyped individuals (Criscione and Blouin, 2006). Thus, clonal reproductive success had no effect on among-host genetic differentiation. Among the 3 cryptic clusters identified with adult samples of *Lecithochirium fusiforme* from eels, the percentage of truly unique

clones among genotyped individuals was 97%, 89%, and 95% for Clusters I, II, and III, respectively (Criscione *et al*. 2011). Only in Cluster II was F_{ST} among infrapopulations significant due to the presence of repeated clones within hosts (Table 2). Other studies have been conducted at the second intermediate host level and reported values of 97% for *Diplostomum pseudospathaceum* in sticklebacks (Rauch *et al*. 2005), 98% in *Gymnophallus* sp. in cockles (Leung *et al*. 2009), and 97% and 98% for *Maritrema novaezealandensis* in crabs and amphipods, respectively (Keeney *et al*. 2007*a*, *b*). Keeney *et al*. (2007*a*) observed that crabs were occasionally infected by multiple copies of a clone, and this resulted in significant genetic differentiation among hosts (Table 2). However, they discussed that additional mixing to bird definitive hosts is likely to reduce the effect of clonal variance in reproductive success. For the above-mentioned aquatic trematodes (all of which require 3 or more hosts in their life cycle), when repeated clones are removed, genetic differentiation among hosts is no longer observed (Table 2). Thus, there is substantial mixing of parasite offspring prior to their asexual stage in the mollusk first intermediate host.

We are unaware of any examples where clonal transmission has been examined in fully terrestrial systems, but there are 3 examples from semi-terrestrial life cycles. Among rat hosts in marsh habitats, the percentage of unique clones was 80 to 85% in *Schistosoma mansoni* (Prugnolle *et al*. 2002, 2004*a*). The removal of repeated clone copies for both male and female worms reduced F_{ST} among hosts, but F_{ST} still remained significant (Table 2). In this 2-host life cycle, cercariae of *S. mansoni* are released from snails into shallow pools and directly penetrate the rat host. Therefore, a clumped transmission of clones may be likely if a rat remains temporarily stationary in a wet area. Furthermore, eggs of trematodes having terrestrial definitive hosts may often be deposited into a habitat unsuitable for transmission, thereby potentially causing greater reproductive skew among infrapopulations and low mixing potential of parasite offspring from rat to snail hosts. Similarly, clonal transmission was important for *Fasciola hepatica* (the percentage of unique clones was 0·577). F_{ST} amongst sheep infrapopulations on a farm was reduced after removing clones indicating clumped clonal transmission. However, F_{ST} remained significant after clone removal, thus indicating non-random transmission from definitive to snail hosts (Vilas *et al*. 2012). Like *F. hepatica, Fascioloides magna* also has a 2-host life cycle going from an aquatic snail to cercariae encysting on vegetation to a deer final host. Thus, it is likely that a deer host will ingest a clump of metacercariae consisting of many copies of the same clone. Indeed, Mulvey *et al*. (1991) found several deer that had more repeated MLGs than expected by chance. Amongst infrapopulations F_{ST} was not estimated, but simulations showed that F_{ST} amongst geographical hunt units was largely affected by the presence of repeated copies of clones. It would be interesting to test fully terrestrial parasites such as in *Echinococcus* or *Dicrocoelium dendriticum* to determine whether they too have clumped clonal transmission.

Focal transmission

One cause of non-random transmission is the presence of largely independent foci of infection among host groups in the host population. Such foci can lead to the partitioning of parasite genetic variation amongst infrapopulations. For example, if different groups of hosts had site fidelity to separate water sources and if parasite transmission occurred only in contact with water, then there is potential to have limited parasite gene flow amongst the different infection foci. Over several parasite generations, the infection foci would become genetically and demographically subdivided even in the presence of a panmictic host population.

Because parasite dispersal is almost impossible to observe directly, it is necessary to use population genetic methods to identify foci of transmission. Testing for foci of infection is of critical epidemiological importance especially with regards to transmission models that are used to evaluate control strategies. While classic models of transmission incorporate transmission heterogeneities such as different probabilities for individual host infection, they assume a single transmission unit (implicit in the measure of a single basic reproduction number, R_0) for a single human population. Stemming from these models is the 20/80 rule (20% host population is responsible for 80% transmission), which implies targeted treatment of the heavily infected can greatly reduce transmission (Woolhouse *et al*. 1997, 1998). Recent models indicate that if separate parasite populations exist in an interconnected network, then targeting high intensity infections may not improve effectiveness of control (Gurarie and Seto, 2009). Thus, it is important to test whether a single host population corresponds to a single parasite transmission unit.

Jones and Britten (2010) tested whether social colonies of the black-tailed prairie dog corresponded to focal points of transmission for the flea *Oropsylla hirsute*. No genetic structure was observed amongst colonies. The authors postulate that the use of multiple mammal species may contribute to dispersal. It is also important to note that in contrast to lice, fleas do not need to spend their entire life on a single host (compare to the louse genetic structure results of Nadler *et al*. 1990). Continuity in habitat may also limit focal transmission. For instance, amongst 5 locations of an 1800 km^2 region of Lake Victoria, cercarial samples of *S. mansoni* did not show

Table 2. Impact of clonal transmission on F_{ST} among digenean infrapopulations when copies of multilocus genotypes (MLGs) are included or reduced to one copy within a host

Species	F_{ST} with repeated copies of MLGs	F_{ST} with MLGs reduced to 1 copy per host	Habitat	Reference
Fasciola hepatica	0·224*	0·108*	Semi-terrestrial	Vilas *et al*. (2012)
Fascioloides magna[a]	0·011	0·006	Semi-terrestrial	Mulvey *et al*. (1991)
Lecithochirium fusiforme[b]			Aquatic	Criscione *et al*. (2011)
Cluster I	0·011	−0·003		
Cluster II	0·016*	0·004		
Cluster III	−0·011	−0·022		
Maritrema novaezealandensis	0·009*	0·004	Aquatic-seabird	Keeney *et al*. (2007*a*)
Plagioporus shawi[c]	N/A	0, 0, 0·002, 0·01	Aquatic	Criscione and Blouin (2006)
Schistosoma mansoni			Semi-terrestrial	Prugnolle *et al*. (2002)
Females	0·07*	0·045*		
Males	0·035*	0·024*		

[a] Analyses not based on actual data, but on re-sampling simulations. Also, analyses are for among deer hunt units and not among infrapopulations (Mulvey *et al*. 1991). Test of significance was not applicable in their simulations. Actual observed F_{ST} among hunt units was 0·016.
[b] Analyses conducted separately for the 3 cryptic clusters identified in the sample (Criscione *et al*. 2011).
[c] Among host structure was tested in 2 geographical populations and 2 time periods (4 population samples), hence four F_{ST} values are reported. Only one pair of flukes was identified as clonemates, so there was no variation in clonal reproductive success to impact among-infrapopulation genetic structure.
* Denotes significant genetic differentiation among infrapopulations.

any genetic structure (Steinauer *et al*. 2009). These results suggest that this continuous portion of the lake is a single source pool of infection. In contrast to the above studies, others have provided evidence for potential foci of infection. In a Brazilian village ($\sim 60\,km^2$) that lined a river drainage system, hierarchical *F*-statistical analyses revealed significant structure among hamlets (Thiele *et al*. 2008). On a similar scale, the tick *Ixodes uriae* had significant F_{ST} between topographical features on the breeding cliffs of the Black-legged kittiwake (McCoy *et al*. 2003).

The above studies test *a priori* delimited boundaries to determine whether they represent foci of infection within a host population. However, incorporation of molecular data into a landscape genetics framework can provide more detailed information on epidemiological correlates of the transmission process and can identify source pools of infection. The latter approach was used by Criscione *et al*. (2010) to examine the epidemiology of human roundworms (*A. lumbricoides*) in Jiri, Nepal. In this study, Bayesian clustering methods, which test for underlying structure based on HWE, were first used to determine whether there were non-panmictic dynamics at a very small scale ($14\,km^2$). There was strong support for local-scale genetic structuring. The results of the population clustering analyses were subsequently incorporated into multivariate regression methods to elucidate spatial, geographical, or epidemiological features associated with the partitioning of genetic variation in the sampled worms. These analyses revealed 3 key insights into *Ascaris* transmission in Jiri: there were separate foci of transmission at this local scale, households and nearby houses shared genetically related parasites, and people re-acquired their worms from the same source pool of infection over time. These results (along with those from Thiele *et al*. 2008) challenge the dogma that a single human community will correspond to a homogenous parasite population. Thus, in Jiri, multiple source pools of infection need to be considered when modelling parasite transmission, especially in relation to modelling drug treatment control strategies (Criscione *et al*. 2010).

Sibling transmission

We make a distinction between focal transmission where multiple parasite generations are cohesively cycled at a given source point of host infection to that of sibling transmission where parasite offspring are transmitted as a clump within a single life-cycle round. Focal transmission may certainly lead to sibling transmission. Nonetheless, we discuss sibling transmission as a separate mechanism for creating non-panmixia among infrapopulations as sibling transmission does not need to be tied to a specific spatial point of infection and its effects may be a transient phenomenon depending on local parasite population sizes and infection intensities. The significance of sibling transmission is that matings between relatives may increase if siblings are co-transmitted, thus leading to non-random mating dynamics within hosts (i.e., bi-parental inbreeding) (see *Mating systems*). Please note that our reference to

sibling transmission should not be confused with the issue of statistical artifacts generated by sib-sampling (as was discussed above, Table 1). Here we are concerned with studies that specifically test for related parasites within their samples.

An excellent example of sibling transmission is provided by Guzinski *et al.* (2009) where the within-host relatedness at each of 3 stages (larval, nymph, and adult) of the tick *Bothriocroton hydrosauri* was analysed. Female ticks deposit their egg clutch in a lizard refuge and the larvae remain clustered until a suitable lizard host is encountered. After a host is infected, engorged ticks detach over a number of days and possibly over multiple refuges. This process is repeated over subsequent developmental stages. Thus, the authors hypothesized that within-host relatedness of ticks should decrease over successive developmental stages. Indeed, this was the observed result, thereby illustrating how population genetics data can provide insight into the transmission process. The authors also indicate that sibling transmission can lead to inbreeding by showing that F_{IS} was significantly positive over all sampled adults; however, genetic structuring among infrapopulations will also contribute to this F_{IS} (i.e., the Wahlund effect). Thus, it would be more appropriate to partition the genetic structuring to estimate the average within-host F_{IS} and among-host F_{ST} to elucidate the effects of sibling transmission.

Sibling cohorts have also been implicated as causing significant F_{IS} within hosts in the ticks *Rhipicephalus microplus* and *Ixodes texanus* (Koffi *et al.* 2006; Chevillon *et al.* 2007; Dharmarajan *et al.* 2011). In these studies, no structure among infrapopulations was detected. However, Bayesian clustering (Chevillon *et al.* 2007) or parentage analyses (Dharmarajan *et al.* 2011) indicated that different sibling groups resulting from a strong variance in the reproductive success of females (i.e., a high variance in the number of sibs from a few clutches) might be present within the sample of ticks. Thus, to cause a positive F_{IS} within hosts, there must be admixed sibling groups on individual hosts where the mean sib-group size would have to be at least greater than 2 (the value assumed when modelling the genetics of ideal panmictic populations; e.g., Crow and Denniston, 1988). Dharmarajan *et al.* (2011) reported a mean sib-group size of 2 for all tested samples and stated that parentage analyses "regularly pooled individual ticks from different infrapopulations (<5% individuals within kin groups being from a single host)." These results indicate there was no admixture in infrapopulations, thus it remains unclear as to why there is positive within-host F_{IS} for *I. texanus*. Chevillon *et al.* (2007) did not give the among-host distribution of the potential sib-groups, but additional tests suggested that the positive F_{IS} was not due to non-random mating on hosts (see section on *Mating systems* below). They suggest that additional work is needed to verify the accuracy of Bayesian clustering methods to identify sibling groups.

Overall there has not been extensive work testing for sibling transmission in different environments or parasites with different life cycles. The largely panmictic dynamics detected in aquatic systems (see sections on *Clonal transmission* and *Mating systems*) indicate that co-transmission of parasite siblings is rare in aquatic systems. Similarly, in a semi-terrestrial system, Steinauer *et al.* (2009) showed that *S. mansoni* cercariae from co-infected snails were not more or less related than expected by the background levels of relatedness. It will be interesting to explore the potential for sibling transmission to definitive hosts in terrestrial flatworm systems such as tapeworms that release sibling offspring as clumps via intact gravid proglottids.

Sex-biased transmission

Amongst dioecious parasites, sex-biased transmission can lead to sex-specific genetic structure among individual hosts (Prugnolle *et al.* 2003). Variation in the dispersal potential of free-living stages, in host use, or immunological interaction with hosts may induce differences in the transmission patterns between male and female parasites (Prugnolle *et al.* 2003). Thus, tests of sex-specific genetic structure can help elucidate whether epidemiological studies need to consider males and females separately. Moreover, sex-biased dispersal may have evolutionary significance as a means of avoiding inbreeding, reducing local mate competition (e.g., the sex with higher mate competition disperses more) or reducing local resource competition (Prugnolle and de Meeûs, 2002). Also, in small populations, a difference in parental male and female allele frequencies increases offspring heterozygosity (and hence, a negative F_{IS} in a sample of offspring) (Balloux, 2004), which may have evolutionary and epidemiological significance in a host-parasite arms race (Prugnolle *et al.* 2003). For a review on methods to infer sex-biased dispersal see Prugnolle and de Meeûs (2002).

Prugnolle *et al.* (2002) tested for sex-biased dispersal with *S. mansoni* among rat infrapopulations and found that males were significantly more randomly distributed among definitive hosts than females. Building off of that study, Prugnolle *et al.* (2004*a*) found that female clones that were more heterozygous were more common than expected by chance. This in part explained why females were more structured than males as this correlation was not observed in males. Further, after only including single copies of clones in the dataset, there was still a sex difference in among-infrapopulation genetic structure (see Prugnolle *et al.* (2002) for discussion of possible mechanisms generating this pattern). Sex-biased dispersal with the males dispersing more has

also been observed in the tick *Ixodes ricinus* (de Meeûs *et al*. 2002; Kempf *et al*. 2010). Though this study was amongst geographical populations and not infrapopulations, we mention it as it is the only other known parasite example of sex-biased genetic structure. The authors suggest that maybe male ticks use more vagile hosts than female ticks. Other studies have tested for sex-biased genetic structure, but have not detected it or have not found overwhelming support (tick *R. microplus*, Koffi *et al*. 2006; tick *Dermacentor variabilis*, Dharmarajan *et al*. 2010; roundworm *A. lumbricoides*, Criscione, unpublished observations).

IS $F_{IS} \neq 0$?

At the lowest tier in Fig. 1, tests of panmixia are used to understand the mating interactions, or lack thereof, among individuals. Within-host estimates of F_{IS} provide an indirect means to infer the mode of reproduction (sexual *vs* asexual) and/or mating system (mechanism of gamete union) of the preceding adult generation (Table 1). Metazoan parasites display a wide diversity of reproductive modes including asexual or sexual, alternating sexual and asexual, and a mix of asexual and sexual (de Meeûs *et al*. 2007*b*). Further, there are monoecious (i.e., hermaphroditic) and dioecious species, even within a phylogenetically related group (e.g., Platyhelminthes). Thus, there are many opportunities for deviations from panmixia due to different mating systems or modes of reproduction among metazoan parasites. However, few parasite population genetics studies of metazoans have had the sole purpose of determining the mode of reproduction or mating system nor have many studies attempted to understand the underlying factors that influence the mode of reproduction or mating system in nature.

Mode of reproduction

Asexual reproduction will generate an excess of heterozygotes and thus drive F_{IS} to negative values (Prugnolle *et al*. 2005*a*, *b*; de Meeûs *et al*. 2006). Two theoretical models have been used to understand the effects of asexual reproduction on the population genetics of clonal diploids. In the first model, Balloux *et al*. (2003) considered monoecious organisms that, as adults, reproduce asexually with probability c and sexually with probability $1 - c$. Their model demonstrated that very high rates of asexual reproduction led to negative F_{IS}. Low levels of sexual reproduction caused F_{IS} to become zero; nevertheless, such low levels of sex still greatly increased the variance in F_{IS} among loci. Although not exactly the same, the life cycle of the nematode *Strongyloides ratti* approximates this model. Parthenogenetic females infect rat hosts and produce eggs that are introduced into the environment via the host's feces. These eggs may (1) develop into males and females that mate outside the host to produce infective larvae (heterogonic development), or (2) develop directly into infective female larvae (homogonic development). Thus, a certain proportion of adults will reproduce sexually and the others asexually as in the model of Balloux *et al*. (2003). In studies to understand dispersal of *S. ratti*, Fisher and Viney (1998) and Paterson *et al*. (2000) sampled parasite eggs from hosts and genotyped larvae at 2 allozyme loci. They found that the average within-host F_{IS} was negative, and that F_{ST} was significant among hosts (Paterson *et al*. 2000). However, this is a case where the effects of clonemate-sampling (analogous to sib-sampling discussed in *Random vs non-random transmission*, Table 1) should be considered. The negative F_{IS} in the larval sample is likely to be driven by the sampling of many clonal larvae originating from the parasitic parthenogenetic females. The sampling of many offspring that are clone-mates will result in infrapopulation allele frequency estimates that will be different from the true allele frequencies among the female worms that actually colonized the hosts. Thus, the estimated F_{ST} among infrapopulations is likely to be incorrect. In the UK, it was estimated that homogonic development predominates (Fisher and Viney, 1998, and references therein). It would be interesting to directly genotype the adult worms from rodents to determine whether the F_{IS} expectations for a highly clonal species (Balloux *et al*. 2003) can be observed in the UK relative to populations with greater heterogonic development. The Balloux *et al*. (2003) model may also be applicable in other species where adult parthenogenesis has been documented (e.g., in some digenean and cestode species; Whitfield and Evans, 1983).

In the second model, Prugnolle *et al*. (2005*a*, *b*) developed a theoretical model for parasitic organisms that undergo asexual reproduction in intermediate hosts, and obligate sexual reproduction in a definitive host. Digeneans and *Echinococcus* cestodes fit this model. Asexual reproduction within the intermediate host creates variance in the reproductive success of each unique genotype, which could ultimately cause deviations in HWE. As the variance in the number of copies produced by different clones increases, the within-host F_{IS} becomes increasingly negative and can reach a maximum of -1 if a single heterozygous clonal genotype colonizes the host (Fig. 3; Prugnolle *et al*. 2005*b*). Indeed, empirical data support this theoretical work in that when repeated copies of clones are removed F_{IS} values increase (e.g., Prugnolle *et al*. 2004*a*, 2005*b*; Criscione *et al*. 2011; Vilas *et al*. 2012). It is important to note that because multiple copies of a clone may be present, it is important to include only 1 copy of each clone (i.e., avoid clone-mate sampling) to correctly infer the mating system of the previous adult generation (Prugnolle *et al*. 2005*a*).

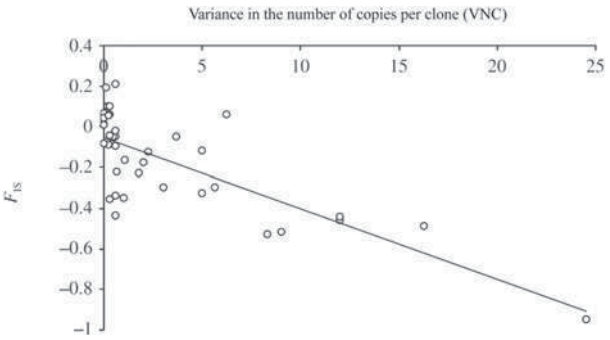

Fig. 3. Relationship between the variance in the number of copies per clone (VNC) observed in natural infrapopulations of *Schistosomes mansoni* and F_{IS}. Notice that as VNC increases F_{IS} becomes more negative. VNC was used as a proxy for the variance in reproductive success of clones. Infrapopulations where only 1 clone was present were omitted from this data set because it is not possible to compute VNC for such infrapopulations (see Prugnolle *et al*. 2005*b*). Reproduced with permission from Prugnolle *et al*. (2005*b*).

Mating systems

Given that there is sexual reproduction, the mating system refers to the set of circumstances under which pairs of gametes are united to form a zygote. We note that non-random mating may occur if there is a preference to mate with individuals of like phenotype (assortative mating) or unlike phenotype (disassortative mating), but only loci associated with those phenotypes would have genotypic proportions distorted from HWE expectations (de Meeûs *et al*. 2007*a*). Here, we are concerned with non-random mating with respect to relatedness. Inbreeding avoidance (mating with unrelated individuals) will result in excess heterozygotes ($-F_{IS}$). In contrast, inbreeding yields a positive F_{IS} value and is driven by matings between relatives (biparental inbreeding) and by self-mating in hermaphroditic species. Parasite inbreeding has significant effects on both parasite and host evolution. Parasite inbreeding increases homozygosity, which leads to more efficient directional selection (Hedrick, 2005). The latter is epidemiologically important as parasite inbreeding can radically increase the frequency of drug-resistant genotypes (e.g., Schwab *et al*. 2006). In addition, co-evolutionary models demonstrate that parasite inbreeding can impact the evolution of host mating systems (Agrawal and Lively, 2001).

Because self-mating is an extreme form of inbreeding, mating system studies have largely been focused on hermaphroditic species. Estimating the selfing rate and determining what factors influence the selfing rate are important goals in research on the evolution of mixed-mating systems in hermaphroditic plants and animals (Goodwillie *et al*. 2005; Jarne and Auld, 2006). Selfing and outcrossing rates can be estimated from F_{IS} with the equation $F_{IS} = S/2-S$ (where S is the selfing rate and $1-S$ is the outcrossing rate) if inbreeding equilibrium is assumed. Under this assumption, the selfing rate is constant and selfing is the only source of inbreeding, outcrossing occurs via random mating, and the effects of selection, genetic drift or gene flow have a negligible effect on genotype frequencies (Hedrick, 2005). One drawback to this method is that null alleles can artificially inflate F_{IS}. As another means to estimate selfing from a population sample, David *et al*. (2007) provide a method based on identity disequilibria (correlations of heterozygosity across pairs of loci), which is not affected by null alleles (see also Jarne and David, 2008).

Overall, most studies on hermaphroditic metazoan parasites have focused on aquatic life cycles, where either all hosts in the life cycle are aquatic, or the intermediate hosts are aquatic and the definitive hosts are birds feeding in aquatic habitats. For trematodes, studies have examined adult worms from fish definitive hosts, while the mating systems of species with bird definitive hosts have been inferred from larval stages that infect invertebrate intermediate hosts. HWE observed at either stage would indicate that random mating occurred among the adult trematodes in the previous generation. For example, Keeney *et al*. (2007*a*, *b*) found that the larval populations of the marine trematode *M. novaezealandensis* were in HWE in the snail first intermediate hosts and in the crab second intermediate hosts (birds are the final host). In a study on the freshwater trematode *Diplostomum pseudospathaceum* (life cycle: aquatic snail to fish to bird), several larval populations were panmictic (multilocus $F_{IS} = 0$), and the identity disequilibria method indicated that selfing rates were not significantly different from zero for all populations (Louhi *et al*. 2010). Thus, in both the marine and the freshwater species, random mating was occurring among the adult trematodes within the bird definitive hosts. Studies on trematodes with complete aquatic life cycles where adults were sampled have also observed random mating systems (e.g., Reversat *et al*. 1989; Vilas and Paniagua, 2004; Criscione and Blouin, 2006; Criscione *et al*. 2011). In the one semi-terrestrial digenean study (*F. hepatica*; Vilas *et al*. 2012) that we are aware of, average multilocus F_{IS} among sheep hosts was 0·16 and significantly different from zero.

Cestodes with aquatic life cycles also appear to have panmictic populations. Allozyme data indicated that cestodes from fish definitive hosts had panmictic populations with high outcrossing rates (Renaud and Gabrion, 1988; Šnábel *et al*. 1996). Microsatellite genotypes of *Ligula intestinalis* larvae from second intermediate fish hosts were used to test HWE, and S was estimated from F_{IS} in several populations (Štefka *et al*. 2009). Several populations had significantly positive F_{IS} with selfing rates from 0·19–0·4. However, using the identity disequilibria method

(David *et al*. 2007), none of the selfing rate estimates were significantly different from zero. This result suggested that unrecognized null alleles had artificially inflated F_{IS}. Thus, *L. intestinalis*, a cestode species with a mostly aquatic life cycle (bird definitive hosts), is another example of a largely outcrossing metazoan parasite from an aquatic habitat.

In contrast to the aquatic parasite species, high levels of inbreeding have been observed in the terrestrial cestodes of the genus *Echinococcus* (Lymbery *et al*. 1997; Knapp *et al*. 2008). For instance, high selfing rates were inferred from significantly positive F_{IS} values (close to 1) within populations of *E. granulosus* (Lymbery *et al*. 1997). In species of *Echinococcus*, inbreeding may result from self-insemination or by mating between identical clones, which is genetically equivalent to self-mating (Lymbery *et al*. 1997). Self-insemination could be promoted in low-density infections where the chance of encountering mates would be low, but may also occur when a membrane blocks the genital pore preventing outcrossing with other individuals (Smyth and McManus, 1989). Potential matings between clones is facilitated by the *Echinococcus* life cycle, which is similar to a digenean life cycle in that larval clones are asexually produced within the intermediate host. The difference, however, is that the clonal larvae do not disperse from this intermediate host. Thus, when a definitive host consumes the intermediate host, many individuals of the same genetic clone could be transmitted together. This dynamic increases the likelihood of matings between identical clones. Currently, there is only limited genetic evidence to suggest that clones of *E. multilocularis* are transmitted together when red foxes eat voles (Knapp *et al*. 2008). Stronger conclusions require multilocus genotypes to ensure proper identification of identical clones. Further, careful sampling will be required as worm burdens can be extremely variable and also generally high. Knapp *et al*. (2008) considered 10 000 or fewer worms to be a low-medium burden within a host, whereas greater than 10 000 parasites was considered high. With such high intensities of infection, large sample sizes are necessary to determine the frequency of repeated clonal genotypes. High levels of inbreeding ($F_{IS} = 0{\cdot}83$) were also detected in the gecko tapeworm *Oochoristica javaensis*, which has a 2-host, terrestrial life cycle going from arthropod to gecko (Detwiler and Criscione, 2011). However, *O. javaensis* does not have a clonal stage. Additional studies are underway to determine if the primary mating system alone can account for this high level of inbreeding or if non-random transmission among hosts also contributes to the high F_{IS} (Detwiler, *unpublished observations*).

What may promote outcrossing and random mating in hermaphroditic parasites? (1) Rauch *et al*. (2005) hypothesized that more complex life cycles (3-host life cycle) evolved to reduce the chances of matings between identical clones, which is equivalent to selfing, in the final host. Indeed their study showed that snail first intermediate hosts were primarily infected with a single genetic trematode clone, while fish second intermediate hosts had significantly higher clonal diversity with no or few repeated copies of clones within hosts. Thus, by the time that definitive hosts are infected, there will be few chances for identical clones to mate (see also section on *Clonal transmission*). (2) The frequency of contact with other individuals may also impact the selfing rate of hermaphrodites. Once within a host, site specificity and high-intensity infections may increase the likelihood of coming into contact with other individuals, and thus promote outcrossing (Šnábel *et al*. 1996). There is some evidence from natural populations which suggests that intensity of infection influences mating dynamics. Criscione and Blouin (2006) found that the number of single parasite infections corresponded to the selfing rate of the digenean *P. shawi* in naturally-infected fish hosts. Additional work is needed to determine how the intensity of infection directly relates to the primary rate of selfing in hermaphroditic parasites. (3) There may also be an interplay between the mating system within hosts and the transmission process itself including the habitat of transmission (e.g., aquatic *vs* terrestrial) and mode of transmission (e.g., free-living stages or the lack thereof). As noted above, most studies in aquatic systems have observed HWE within hosts, thus supporting the aquatic mixing hypothesis. This raises the interesting question of whether terrestrial systems promote sibling transmission, and thus biparental inbreeding. At this point, there are too few studies examining the mating systems of platyhelminth parasites to conclude that terrestrial life cycles promote more inbreeding than semi- or fully aquatic life cycles. In particular, the least is known about hermaphroditic parasites with terrestrial life cycles, although the few known examples suggest a trend towards high inbreeding (Lymbery *et al*. 1997; Detwiler and Criscione, 2011). (4) The life-history peculiarities of some parasite species also need to be considered when trying to elucidate what factors influence inbreeding. For instance, the digenean *Coitocaecum parvum* has a 3-host, aquatic life cycle (snail to amphipods to fish). In the amphipods, *C. parvum* has facultative maturation and sexual reproduction. However, worms remained encysted. Thus, even if more than 1 trematode infects an amphipod, individuals that precociously mature can only self-mate as they remain enclosed in the cyst. This precocious trematode has high levels of inbreeding with F_{IS} among 12 microsatellite loci ranging from 0·73 to 0·99 (Lagrue *et al*. 2009; we note the latter range excludes potentially duplicated loci in their data set; see Detwiler and Criscione,

2011). Even though *C. parvum* has a fully aquatic life cycle, its life-history strategy of early reproduction appears to play a larger role in determining its mating system than the environment of transmission itself.

The above studies estimated F_{IS} in the population sample, which leads to inference about the mating system of the previous adult generation. The mating system of the current generation can be elucidated by testing for pangamy, which is the random association of paired mates with respect to genetic relatedness (e.g., Prugnolle *et al*. 2004*b*; Chevillon *et al*. 2007). The null hypothesis of pangamy has been tested in dioecious parasites with terrestrial and semi-terrestrial cycles (e.g., ticks and schistosome trematodes). Chevillon *et al*. (2007) observed a positive within-host F_{IS} for the tick *R. microplus*. For these cattle ticks, it was predicted that members of the same sibling group might persist and mate together on a host individual, leading to the observed inbreeding on hosts. To test the hypothesis of non-random mating on hosts, they collected mating pairs of ticks from cattle and tested for a correlation between mating status and genetic relatedness. Their analysis revealed that tick pairs mated randomly with respect to relatedness. They postulated that the positive F_{IS} could be caused by the presence of admixed sibling groups due to a high variance in female reproductive success (see section on *Sibling transmission*). Amongst populations of another tick, *Ixodes ricinus*, variation in the mating system was observed (Kempf *et al*. 2009). Mating pairs from 2 populations were in pangamy, while non-random mate pairing with respect to relatedness occurred in 2 other populations. The authors hypothesized, but did not test, that the presence of cryptic host races might explain their results (i.e., mate pairings occurred between individuals of the same host race). If their hypothesis is true, then this reinforces our assertion (Fig. 1) that cryptic, upper levels of structure (e.g., presence of different species) need to be addressed prior to making inferences at lower scales (e.g., mating systems within a species). In contrast to the ticks, negative F_{IS} was found within rats for *S. mansoni* (Prugnolle *et al*. 2002). This study found that the negative F_{IS} was likely caused by sex-biased dispersal, which provides a potential means to limit inbreeding. Thus, it was hypothesized that the predominant mating strategy would be pangamy because there would be no need to evolve mechanisms to prevent inbreeding (e.g., avoidance of kin). Prugnolle *et al*. (2004*b*) directly tested for pangamy in a natural population of *S. mansoni* by comparing the observed relatedness between individual male-female pairs to the relatedness between all possible male-female pairs within a host. The prediction of pangamy was supported as no association was detected from naturally infected hosts.

CONCLUDING REMARKS

Our review is not meant to be exhaustive, but rather aims to highlight some major causes or explanations for why one might observe deviations from panmixia in a local-scale study of metazoan parasites. There may be other factors that affect parasite transmission and thus drive genetic differentiation among infrapopulations that we have not covered in depth such as ecological or immunological differences between host sexes or host ages (e.g., Caillaud *et al*. 2006; Dharmarajan *et al*. 2010). We also did not cover technical issues such as null alleles that can cause aberrant *F*-statistics, in particular the inference of inbreeding from positive F_{IS}. Likewise, other non-Mendelian factors such as duplicated loci and sex-chromosome linked loci can lead to the calculation of artifactual *F*-statistics and thus, erroneous population inferences (Detwiler and Criscione, 2011). We refer readers to de Meeûs *et al*. (2004) for methods to detect null alleles, but one quick check is to explore the variance in F_{IS} values among loci. An inbred mating system is unlikely to generate extensive variation, thus a high variance in F_{IS} among loci provides a clue that disruptions (technical or biological) of panmixia other than the mating system are at play. Also, our focus has been on the use of F_{IS} and F_{ST} to test for deviations from panmixia. However, linkage disequilibrium (non-random association of alleles among loci) patterns can also be informative. For example, admixed populations not only cause a deficit of heterozygotes (the Wahlund effect) at individual loci, but also increase linkage disequilibrium among loci. Indeed, when ignoring the 3 cryptic populations of *L. fusiforme* (Criscione *et al*. 2011), all possible pairwise loci combinations showed significant genotypic disequilibrium. Once separated into each of 3 clusters, only Cluster II showed significant overall genotypic disequilibrium. But, genotypic disequilibrium in Cluster II was actually caused by the presence of repeated copies of clones (see de Meeûs *et al*. 2006), and equilibrium was observed after reducing the data set to a single copy per clone.

We began this review by highlighting that population genetics data can be used to elucidate the ecological mechanisms that caused disruptions in panmixia. We do caution that different ecological mechanisms may lead to similar patterns of genetic structure. Indeed, our review is intended to provide a rough guideline to deal, in part, with this latter issue. Because direct observation of many parasite population processes such as transmission among hosts and direct mating interactions are often not possible, population genetics approaches may be the only viable option to elucidate certain parasite population dynamics. In some cases, genetic data can be used to make direct inference on parasite biology. For example, parent-offspring genotype data

can be used to directly estimate the mating system of a hermaphroditic parasite. In other cases such as the use of F_{ST} to elucidate transmission amongst hosts, indirect inference may only be possible. Such indirect methods are not without their caveats. For instance, significant F_{ST} amongst infrapopulations indicates allele frequency differences, and thus provides an assessment of some form of non-random recruitment. However, equating F_{ST} to some actual level of dispersal (i.e., the effective number of migrants per generation, $N_e m$) may be impractical because the transmission process itself may violate one or more assumptions (e.g., equilibrium between genetic drift and migration) in population genetics models (see Whitlock and McCauley, 1999). Another important aspect to mention is that population genetics methods are largely allele/genotype frequency based methods. Thus, appropriate sample sizes are needed to estimate F_{IS} and F_{ST}. It is difficult to provide a standardized sampling protocol because life-history variation in parasites is vast. Also, sampling will depend on the question. As a start, our own personal experience leads us to suggest the following to assess whether there is among-infrapopulation genetic structure. If mean intensities are low ($\sim < 10$), we suggest genotyping all individuals from about 20 to 30 infected hosts. If intensities are high, we would suggest genotyping 20–30 parasites per host for 10 or more hosts. It would be useful for future work to simulate transmission and mating dynamics under various parasite life histories (e.g., Prugnolle *et al*. 2005*a*, *b*) to not only assess appropriate sampling regimes, but also to better assess the impacts of sib-sampling on estimated *F*-statistics and population genetic parameter expectations under focal or sibling transmission.

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