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Population genomics of helminth parasites

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Abstract

Next generation sequencing technologies have facilitated a shift from a few targeted loci in population genetic studies to whole genome approaches. Here, we review the types of questions and inferences regarding the population biology and evolution of parasitic helminths being addressed within the field of population genomics. Topics include parabiome, hybridization, population structure, loci under selection and linkage mapping. We highlight various advances, and note the current trends in the field, particularly a focus on human-related parasites despite the inherent biodiversity of helminth species. We conclude by advocating for a broader application of population genomics to reflect the taxonomic and life history breadth displayed by helminth parasites. As such, our basic knowledge about helminth population biology and evolution would be enhanced while the diversity of helminths in itself would facilitate population genomic comparative studies to address broader ecological and evolutionary concepts.

Introduction

Over the past four decades, molecular population genetics has proven to be a fruitful field in addressing fundamental questions regarding the population biology of helminth parasites. Although its implementation by parasitologists initially lagged behind those in other fields (Criscione, 2016), molecular population genetic methodologies have since been used to help elucidate the ecology, epidemiology/epizoology and evolution for a number of parasitic helminths (Nadler, 1995; Criscione *et al.*, 2005; de Meeûs *et al.*, 2007; Cole & Viney, 2018). Moreover, the field has been integral in overcoming inherent challenges (e.g. laboratory maintenance of complex life cycles, need to dissect hosts, etc.) by allowing indirect inferences on population biology (de Meeûs *et al.*, 2007). By sequencing one or a few targeted loci (e.g. a region of the mitochondria or ribosomal DNA) or genotyping a panel of five to 20 codominant genetic markers (e.g. allozymes or microsatellites), parasitologists could begin to elucidate previously unknown or inaccessible helminth population dynamics. For example, molecular markers have proven essential in parasite studies on cryptic species (Nadler & de Leon, 2011), hybridization (Detwiler & Criscione, 2010), modes of reproduction (Tibayrenc & Ayala, 2013), mating systems (Detwiler *et al.*, 2017), contemporary or historical effective population sizes (N_e) (Archie & Ezenwa, 2011; Strobel *et al.*, 2019), phylogeography (Nieberding *et al.*, 2006), local scale transmission (Criscione *et al.*, 2010) and anthelmintic drug resistance (Gilleard & Beech, 2007).

Molecular population genetics has helped advance our knowledge about parasites; nonetheless, this 'first generation' of parasite population genetics often required large time investments to collect data and/or monetary constraints that largely restricted studies to the use of one or a few targeted loci. Many molecular ecological questions can be addressed with a few genetic markers, but inferences in some areas remain limited or intractable. For instance, drug resistance studies may have missed novel loci due to a focus on candidate gene approaches (Gilleard & Beech, 2007; Doyle & Cotton, 2019), historical demographic estimates may have been less accurate (Putman & Carbone, 2014), evolutionary history conclusions based on a single marker could reflect the history of the locus rather than the species (Casillas & Barbadilla, 2017), or mito-nuclear discordance patterns might be inconclusive in disentangling incomplete lineage sorting from hybridization (Joly *et al.*, 2009; Perea *et al.*, 2016).

In 2005, the first next generation sequencing (NGS) technologies were developed (Margulies *et al.*, 2005; Pareek *et al.*, 2011). Numerous genetic markers across the genome, for example, thousands of single nucleotide polymorphisms (SNPs), could now be obtained in a short time frame and at a reduced cost per locus (Leshchiner *et al.*, 2012). Thus, emerged the field of population genomics, which as stated by Charlesworth (2010) 'is a new term for a field of study that is as old as the field of genetics itself, assuming that it means the study of the amount and causes of genome-wide variability in natural populations.' While the primary benefits of population genomics may not necessarily be the generation of novel questions (Charlesworth, 2010), Luikart *et al.* (2018) discussed how population genomics has led to 'conceptually novel approaches to address questions intractable by traditional genetic methods by using high-density genome-wide markers (e.g. DNA, RNA, epigenetic marks),' especially

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questions centred on adaptive evolution. Thus, genomic approaches have increased the accessibility of the population genetics field to further our understanding of parasite ecology and evolution.

The advantages in population genomics data lie in two non-mutually exclusive areas. First, NGS enables researchers to assemble the genomes of target organisms with no existing or limited genomic resources (Luikart *et al.*, 2018). In turn, an assembly, especially a highly contiguous assembly, allows annotation to characterize functional and structural variants in the genome (Thomma *et al.*, 2016). Second, the availability of additional genetic markers improves the ability to accurately predict population genetic parameters (e.g. demography), provides a backdrop to identify loci under selection (i.e. outlier-based tests) and allows tracking genetic changes along genomic regions (i.e. inferences are along haplotypes vs. individual SNPs) (Allendorf *et al.*, 2010; Luikart *et al.*, 2018). For example, reductions in nucleotide diversity along chromosomal segments help identify strong selective sweeps (Stephan, 2019; Garud *et al.*, 2021); the frequency and length of stretches of homozygosity (runs of homozygosity (ROH)) estimate inbreeding and demographic history more accurately (Ceballos *et al.*, 2018); and the length of haplotypes with derived alleles (extended haplotype homozygosity (EHH)) have greater ability to detect soft selective sweeps (Garud *et al.*, 2021). Such data enable greater access to complex evolutionary questions related to the genetic architecture of adaptive traits or differentiation, loci affecting phenotypic variation or fitness, hybridization and adaptive introgression and associations of landscape/environmental features to genomic variation (Charlesworth, 2010; Luikart *et al.*, 2018; Rochus *et al.*, 2018; Orteu & Jiggins, 2020). Taken together, the rapidly expanding field of population genomics presents new opportunities to elucidate parasite population biology by giving a more complete picture of the genome.

Here, we review population genomics studies on parasitic helminths of animals. Although interesting, between-species comparative genomics (e.g. synteny comparisons) is beyond the scope of our review (see Coghlan *et al.*, 2019). We also do not review various statistical methodologies for population genomics analyses (we refer readers to reviews by Hahn, 2018; Luikart *et al.*, 2018; Bourgeois & Warren, 2021). Rather, a primary goal is to highlight how current studies are applying NGS and population genomics to study helminth population biology and microevolution. We cover many classical topics in molecular ecology outlined in earlier reviews (Nadler, 1995; Criscione *et al.*, 2005; Detwiler & Criscione, 2010; Gorton *et al.*, 2012) including species identification, phylogeography, demography, hybridization and loci under selection. As an additional goal, we discuss these topics with renewed interest in the expanded inferences that can be drawn from population genomics data. We also cover the fields of parabiome research and linkage mapping given that these areas often have interrelated objectives and/or methods with population genomics. Finally, we provide quantitative summaries of the taxa studied in the recent literature to not only emphasize the current application of population genomics, but to also identify where we believe major strides can be made using population genomics data to address the breadth of parasitic helminth life cycles, life histories and ecological and evolutionary diversity.

Parabiome

Helminth species do not exist in isolation within a host, rather, a host is a microcosm of an ecosystem for which there may be a community of microbes (bacteria and viruses), protozoans, or helminths concurrently infecting a host (Pedersen & Fenton, 2007; Graham, 2008). It is recognized that the makeup of a microbial community can impact both the host and the 'ecosystem' which the host represents (see Ha *et al.*, 2014 for a review). Likewise, infection by multiple helminths and/or protozoan parasites is a major concern in livestock and wildlife given that co-infections have the potential to exacerbate morbidity or mortality of the host (Graham, 2008; Ezenwa & Jolles, 2011; Griffiths *et al.*, 2011; Gorsich *et al.*, 2014; Lee *et al.*, 2014; Ezenwa, 2016). Thus, knowing the helminth and protozoan parasite community composition in a host (what we describe as the parabiome) is critical given the potential for the community composition to influence the host immune response, other organisms residing in the host (e.g. dysbiosis), as well as the variable pathogenicity of parasites (Supali *et al.*, 2010). Moreover, characterization of a parabiome is useful in understanding broader community ecology questions concerning species interactions, diversity and abundance (see Poulin, 2001, 2014; Ezenwa & Jolles, 2011; Titcomb *et al.*, 2019). We note that the term parabiome represents a subset (i.e. helminths and protozoans of a host) of the term holobiont, which is defined as a metazoan organism with all associated microorganisms living on or in it (Hodžić *et al.*, 2023).

With regard to helminths, parabiome work has largely centred on nematodes (also known as the nemabiome; Avramenko *et al.*, 2015). Prior to the use of molecular markers, morphological analysis of third larval stage (L3) larvae was used for livestock nematode identification, which was largely limited to the genus level (Roeber *et al.*, 2012; Roeber & Kahn, 2014; Avramenko *et al.*, 2015). Taking advantage of the ability of NGS to generate large amounts of sequence data, Avramenko *et al.* (2015) conducted a proof-of-concept study to determine if deep amplicon sequencing, that is, metabarcoding, of nematode *ITS-2* (internal transcribed spacer unit 2 of the rDNA complex) could be used to identify L3 larvae to species as well as ascertain species composition in a host. Using eight laboratory-reared species of nematodes, they found that *ITS-2* reliably differentiated species. However, when pooling L3 larvae of different species, sequencing produced species-specific amplification biases. Applying a correction factor to account for the biased amplification, their method was able to detect species at low frequencies within samples and access species proportions. In addition, their method identified species that were difficult to identify morphologically. For example, the sequence data revealed L3 *Haemonchus contortus*, whereas morphological identifications only reported *Haemonchus placei*.

Subsequently, several studies have used the nemabiome approach for various metabarcoding applications (table 1). A primary focus is survey work of domestic farm animals to determine species composition and identify pathogenic nematodes. For example, Avramenko *et al.* (2017) found lower nematode species diversity in Canadian cattle herds, which were dominated by *Ostertagia ostertagi* and *Cooperia oncophora*, compared to herds in the central/south-eastern United States and São Paulo, Brazil, where *Cooperia punctata* and *H. placei* were more common. In addition, the proportion of *Cooperia* spp. increased and *O. ostertagi* decreased following macrocyclic lactone treatment in Canadian herds. Unexpectedly, *C. punctata*, a highly pathogenic nematode thought to be better adapted to warmer climates, was found in Ontario (central Canada). In a subsequent study across western Canada, De Seram *et al.* (2022) also found a high proportion of *C. punctata* in Manitoba cattle herds and hypothesized a range expansion due to a combination of animal movement,

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Table 1. Parabiome.

Host species	Study objective	Brief summary
Cattle [1]	Ground truthing of *ITS-2* metabarcoding for identification of livestock nematodes	Species identification with *ITS-2* metabarcode data was congruent with morphological-based identification. Method able to detect species in low abundance and tease apart species that are morphologically indistinguishable
Cattle [2]	Nemabiome characterization in herds from Canada, United States and São Paulo, Brazil. Impact of anthelmintic treatment on community composition in cattle	Higher nematode species diversity in herds from central/south-eastern United States compared to Canada. The proportion of *Ostertagia ostertagia* decreased and *Cooperia* spp. increased after macrocyclic lactone treatment
Bison [3]	Nemabiome characterization in Canadian commercial (plains bison) and conservation herds (plains bison and wood bison)	Commercial bison communities had higher nematode diversity compared to cattle at similar latitudes with *Haemonchus placei*, not typically found so far north, also being more common. Conservation plains bison had a high proportion of *Trichostrongylus axei* whereas wood bison had a high proportion of *Orloffia bisonis*, a parasite thought to have coevolved with bison
Cattle [4]	Nemabiome characterization of herds in western Canada	*Ostertagia ostertagi* and *Cooperia oncophora* are common in Alberta and Saskatchewan herds. However, the predominant species in Manitoba herds was *Cooperia punctata*, which is typically not associated with cold climates
Cattle [5]	Compares nemabiomes of faecal pats on pastures before and after winter	*Cooperia oncophora* and *O. ostertagi* were common and *Nematodirus helvetinaus* and *T. axei* were rare both before and after winter. *Haemonchus placei* was rare before and not detected after winter. Overwintering leads to significant pasture contamination for grazing cattle in the spring/summer
Sheep [6]	Validation of method and assessment of the nemabiome in sheep	Using archived samples, the nemabiome approach provided more detailed community composition data compared to a previous species-specific polymerase chain reaction approach
Roe deer [7]	Validation and characterization of the nemabiome in roe deer	Using samples collected from two isolated roe deer populations, identified a higher proportion of cervid-specific species compared to generalist nematode species
Sheep and roe deer [8]	Comparison of nemabiomes and nematode exchange between domestic sheep and wild roe deer	The most prominent nematode in sheep and roe deer was *Haemonchus contortus*. There was no evidence of host associated genetic structure based on follow-up mtDNA sequence data. *Ostertagia leptospicularis*, a nematode of wild cervids, was at low frequency in roe deer. Authors suggest roe deer parasites likely displaced by decades of sheep farming
Horses [9]	Validation of method and assessment of nemabiomes in horses	Identified 33 different species and confirmed repeatability across complex infections greater than ten species
Large mammal herbivores [10]	Tested the role of host traits and phylogenetic relatedness in structuring the nemabiome of 17 sympatric species of large mammalian herbivores in central Kenya	Key associations to nematode community composition included host identity, host phylogeny and host gut structure. Host body size, social-group size, or feeding height did not correlate with nematode composition
Primates [11]	Survey of the parabiome (helminths, fungi and protozoan) across 11 non-human primates' species using faecal samples and 18s rDNA marker	Parasites classified to family level. Metabarcoding identified at least one parasite family not previously reported from each host species. Parabiome similarity was higher for more closely related primate species and those from the same continent

[1] Avramenko *et al.* (2015); [2] Avramenko *et al.* (2017); [3] Avramenko *et al.* (2018); [4] De Seram *et al.* (2022); [5] Wang *et al.* (2020); [6] Redman *et al.* (2019); [7] Beaumelle *et al.* (2021); [8] Beaumelle *et al.* (2022); [9] Poissant *et al.* (2021); [10] Titcomb *et al.* (2022); [11] Gogarten *et al.* (2020).

changes in climate and anthelmintic treatment. In a survey of sheep and neighbouring roe deer populations, Beaumelle *et al.* (2022) examined if livestock farming was modifying the roe deer parasite community composition. They found higher infection intensities and prevalence of generalist nematode species (e.g. *H. contortus*) compared to wild-deer specialist species (e.g. *Ostertagia leptospicularis*) in roe deer. Their findings were in contrast to a previous roe deer nemabiome study wherein nematodes commonly associated with cervids were mostly found in two isolated roe deer populations (Beaumelle *et al.*, 2021). Beaumelle *et al.* (2022) hypothesized that the nematode community of roe deer near sheep farms had been displaced by generalist livestock parasites after several decades of sheep farming. Additional parabiome approaches have been extended to horses (Poissant *et al.*, 2021), and have included applications such as comparison of community composition of faecal pats on pastures before and after winter (Wang *et al.*, 2020) and comparison among cattle, commercial bison and wild bison populations (Avramenko *et al.*, 2018) (table 1).

Other parabiome studies have focused on foundational parasite biodiversity and community ecology questions. For example, Gogarten *et al.* (2020) used a 18s rDNA region to provide family-level identification of helminth, fungi and protozoan parasites across 11 non-human primate species. They found previously unreported families from some primates and that closely related primate species had greater parabiome similarity. Titcomb *et al.*

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(2022) tested for associations between the nemabiome and host traits or phylogenetic relatedness across 17 species of sympatric mammalian herbivores in Kenya as well as assessed parasite-sharing networks among hosts. Key findings included 53% of the nemabiome dissimilarity among faecal samples explained by host species, significant congruence between host and parasite phylogenies, and that host gut morphology predicted nematode community composition. The parasite-sharing analyses indicated that most nematode species were host specific, but a few did have broad host ranges suggesting potential for exchange between wildlife and livestock. Additionally, they suggested that central host species (i.e. hosts that shared parasite species with many other hosts) could be targeted for management strategies where deworming these hosts might limit the spread of parasites to threatened wildlife (Titcomb *et al.*, 2022).

A primary utility of a parabiome approach is in estimating parasite richness, especially regarding the detection of low frequency and morphologically indistinguishable species. However, both Avramenko *et al.* (2015) and Titcomb *et al.* (2022) have noted important caveats about quantifying parasite abundance. In particular, cell number, rDNA copy number, variation in primer binding efficiency across species and amplification efficiency of sequence variants are factors that can skew true abundance. Controlled experiments can be used to correct for some of these latter factors (see Avramenko *et al.*, 2015), but many natural systems may have undescribed species or species not yet represented with sequence data (e.g. Titcomb *et al.*, 2022). Also, as source material are parasite offspring from faecal samples, abundance inferences need to be restricted to what will be seeding the next generation of infections. For example, the presence of a highly fecund species may lead to a higher proportion in faecal samples relative to the adult population in the host itself. Lastly, the need to collect L3 larvae is also a logistical issue if rapid species detection is critical (e.g. agricultural producers). Using eggs, Francis & Šlapeta (2022) recently developed a quick and simple nemabiome protocol. Such a protocol may facilitate the parabiome approach to a wider range of host–parasite systems.

Hybridization

Hybridization between genetically diverged groups of populations can lead to the formation of stable hybrid zones, reinforcement, speciation, or homogenization of the groups (Runemark *et al.*, 2019; Moran *et al.*, 2021). Moreover, hybridization is a potential source of genetic variation via introgression into the diverged genetic backgrounds of the interbreeding groups (Barton, 2001). For parasites in particular, introgression events could influence parasite pathogenicity, virulence, fitness, drug resistance and host specificity (Ziętara & Lumme, 2002; Detwiler & Criscione, 2010; King *et al.*, 2015). For example, hybrid offspring may be able to colonize novel hosts or be able to infect both host species for which the parental parasite lines were host specific (Henrich *et al.*, 2013). As such, detection of helminth hybrids is critical from both an evolutionary viewpoint and an epidemiological perspective.

The use of molecular markers to study parasite hybridization dates to the late 1970s (e.g. Bullini *et al.*, 1978; Vrijenhoek, 1978). Many pre-NGS population genetic studies inferred hybridization via nuclear-mitochondrial discordance (primarily comparing a rDNA region such as *ITS-1* to a mtDNA region such as cytochrome c oxidase subunit 1). The caveat with these data is that one cannot tease apart incomplete lineage sorting, historical introgression, or contemporary hybridization (Detwiler & Criscione, 2010). Also, only a couple of studies have inferred natural contemporary helminth hybridization via a small panel of microsatellite loci (Criscione *et al.*, 2007; Steinauer *et al.*, 2008). Population genomics data and analyses greatly improve inferences of hybridization by enabling detection of cryptic species/species complexes, timing of hybridization events, as well as introgressed genomic regions that are likely to confer a selective advantage in their new 'species background' (Payseur & Rieseberg, 2016; Moran *et al.*, 2021). Moreover, there are genomic methods that enable teasing apart lineage sorting from historical introgression (Bourgeois & Warren, 2021).

The use of population genomics to study helminth hybridization has largely been conducted among schistosome species. Indeed, interest in schistosome hybridization dates to the 1950s (reviewed in Southgate *et al.*, 1998; Morgan *et al.*, 2003; Detwiler & Criscione, 2010; Steinauer *et al.*, 2010; Leger & Webster, 2017). Hybridization between *Schistosoma haematobium* and *Schistosoma bovis* has received the most recent attention. Oey *et al.* (2019) compared a *de novo* genome assembly of *S. bovis* to the assembly of an Egyptian isolate of a *S. haematobium*. The genomes were highly similar with 97% sequence identity. However, there were also a few distinct genome regions with >99% sequence identity. Oey *et al.* (2019) concluded that the Egyptian isolate of *S. haematobium* most likely contained *S. bovis* DNA via hybridization.

Subsequently, Platt *et al.* (2019) compared the *S. bovis* genome to multiple *S. haematobium* samples from Niger and Zanzibar. Analyses assessing ancestry proportions did not reveal any admixture in the Zanzibar samples, but 0.1 to 2.7% of *S. bovis* and *Schistosoma curassoni* (the latter two are closely related) ancestry was detected in several Nigerien samples. Using lengths of introgressed haplotype blocks, admixture was estimated to occur between 108 and 612 years ago. There was no evidence of filial 1 (F1) or early generation hybrids. Moreover, these admixed regions in the Nigerien *S. haematobium* samples showed fixed or nearly fixed *S. bovis* alleles. Analyses of allelic differentiation and EHH provided strong evidence of selection in these regions. In particular, one region on chromosome 4, annotated to contain an invadolysin gene, showed a large reduction of nucleotide diversity indicating a selective sweep. Interestingly, this region is also one of the introgressed regions identified in the Egyptian isolate of *S. haematobium* by Oey *et al.* (2019). Overall, Platt *et al.* (2019) provided evidence of ancient, regional hybridization along with selection for an introgressed invadolysin gene, which they speculated may be involved in tissue penetration or immune evasion of the mammalian host. Additional sampling by Rey *et al.* (2021a) supports an ancient introgression where the same *S. bovis* invadolysin-containing tract was found in isolates of *S. haematobium* from Egypt, Corsica and Mali; Madagascar samples did not show evidence of introgression. Interestingly, the Corsican sample had 77% *S. haematobium* and 23% *S. bovis* genomic content (Kincaid-Smith *et al.*, 2021), which is an admixture pattern consistent with more recent hybridization such as a mating between a F1 hybrid and *S. haematobium* parent (but see Rey *et al.*, 2021a for other explanations).

The above work on *S. curassoni*/*S. bovis* x *S. haematobium* hybrids currently suggests ancient introgression, but it is important to note that contemporary hybridization can easily go undetected (especially if hybrids are selected against) by sparse sampling typical of genomic studies (e.g. one to ten samples per country or broad geographical region). Targeted local scale

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sampling in areas of documented sympatry of potentially hybridizing species may yield new insights. Indeed, based on prior survey and nuclear-mtDNA discordance data (Webster *et al.,* 2013), Berger *et al.* (2022) used genomic data to test if there was contemporary hybridization between *S. bovis* and *S. curassoni* from naturally infected cattle in Northern Senegal. Using miracidia and adult parasites from four hosts, they identified bidirectional hybridization with admixture results consistent with F1 hybrids and backcrosses (between F1 hybrids and *S. curassoni*). Ongoing hybridization was inferred in this system as hybrid-identified samples came from multiple hosts and stages and showed no evidence of relatedness.

In additional studies, RADSeq data suggested another possible contemporary hybridization between *S. haematobium* and *Schistosoma guineensis* in samples from Cameroon (Landeryou *et al.*, 2022). Recent genome assemblies of other *S. haematobium* isolates from Africa have shown evidence of introgressed regions matching other species in the *S. haematobium*-group (Stroehlein *et al.,* 2022). As noted by Stroehlein *et al.* (2022), the *S. haematobium*-group may be a complex genetic landscape resulting from a history of genomic admixture. If such reticulate evolution characterizes this group, the concept of a 'pure' *S. haematobium* isolate becomes obscure.

Population genomics of hybridization has received little attention in other helminths with just a few studies on human-associated and pig-associated roundworms (*Ascaris*) and monogeneans in the genus *Gyrodactylus*. For the former, species status and the interbreeding capabilities of ascarid worms infecting humans (*Ascaris lumbricoides*) and pigs (*Ascaris suum*) has been the subject of debate for some time (Peng & Criscione, 2012). It is clear that in sympatry human-roundworm and pig-roundworm samples show genetic differentiation (reviewed in Peng & Criscione, 2012; Wang, 2021). Nonetheless, it has also been shown that sympatric samples from both hosts can be genetically more similar than they are to their host-associated counterparts from a distant location (Criscione *et al.*, 2007). Based on results from pre-NGS population genetic studies, Peng & Criscione (2012) proposed the hypothesis that geographical isolation along with multiple host colonization (i.e. host jump followed by differentiation) events may characterize the evolutionary history of human and pig *Ascaris*.

Zhou *et al.* (2020a, b) conducted population genomics analyses of sympatric *Ascaris* samples from China based on autosomal genome-wide SNPs and mito-genomes, respectively. Zhou *et al.* (2020a) found clear genetic differentiation between host-associated samples, but a small sample size (six nematodes per host species) may have precluded detection of hybrids. Moreover, while the mito-genome study of Zhou *et al.* (2020b) included pre-identified hybrid samples (based on prior microsatellite and *ITS* genotypes), Zhou *et al.* (2020a) only included pre-identified non-hybrid samples (see their methods). Unfortunately, mtDNA alone is not sufficient to address hybridization. As demonstrated by Easton *et al.* (2020), the inclusion of both mtDNA and nuclear DNA provides additional insight into possible hybridization between human and pig *Ascaris*. They sampled 68 roundworms from human hosts in Kenyan villages, where pig husbandry is rare. In a comprehensive analysis of existing mtDNA data from around the world, there was no consistent pattern of mtDNA clade with host or geographical association (e.g. their Kenyan samples from human roundworms had haplotypes falling in the clade predominantly comprising pig-sampled roundworms). Moreover, the genomic analysis of autosomal SNPs among the Kenyan samples in comparison to an *A. suum* reference genome showed a mosaic of *A. suum*-like or *A. lumbricoides*-like inheritance patterns. Overall, these patterns are consistent with an interbred *Ascaris* species complex and are consistent with a history of multiple host colonization events. Nevertheless, to test the latter hypothesis, global sampling along with concurrent sympatric sampling remains necessary.

Population genomics has also provided an interesting perspective on the mode of reproduction and hybridization in monogeneans of the genus *Gyrodactylus*. Gyrodactylids have a unique 'Russian nesting doll' mode of reproduction wherein they give birth to a fully developed offspring that already contains a developing embryo. First-born are produced asexually, second-born through parthenogenesis and possible sexual reproduction or parthenogenesis thereafter (Cable & Harris, 2002; Bakke *et al.*, 2007). As such, there is the potential for the propagation of clonal lines. In the context of asexual reproduction, 'hybridization' occurs between two diverged clonal lines as observed in some protozoan parasites (Detwiler & Criscione, 2010). The extent to which *Gyrodactylus* spp. have clonal lines that persist and hybridize in nature had limited support from a study on *Gyrodactylus salaris* showing fixed heterozygosity (hypothesized to have resulted from a hybridization event) at a single nuclear marker (Kuusela *et al.,* 2008). Two recent population genomics studies on *Gyrodactylus* spp. provide evidence of hybridization between diverged lineages (Konczal *et al.*, 2020, 2021).

Konczal *et al.* (2021) sampled 30 *Gyrodactylus turnbulli* across multiple rivers from Trinidad and Tobago. Relative to the samples from Tobago, the samples from Trinidad ($N = 14$) had low levels of genome-wide heterozygosity and were more similar to one another and a reference genome generated from parasites of commercial guppies. In contrast, only one sample from Tobago had comparable low heterozygosity with most samples ($N = 13$) from Tobago having much higher heterozygosity. Consistent with hybridization of diverged lineages, the highly heterozygous Tobagonian samples consisted of two divergent haplotypes: one similar to the low heterozygosity Tobagonian sample; and the other similar to the reference assembly. There were also two additional samples from Tobago that had mosaic patterns of heterozygous and homozygous blocks (of each 'parental' haplotype), indicating recombination after the initial hybridization event.

In Konczal *et al.* (2020), a reference genome of *Gyrodactylus bullatarudis* originating from Tobago was compared to 10 samples from Trinidad. Inference of hybridization was inferred based on bi-modal distribution of divergence across the genome from the Trinidad samples compared to the reference. In Konczal *et al.* (2020, 2021), the authors invoke greater hybrid fitness to the 'hybrid' genotypes in their sampling. We advise caution in these adaptive inferences for the following reasons. First, genetic drift alone could explain the greater frequency of hybrid individuals. A single hybrid individual could colonize a new location (i.e. a founder event) where clonal reproduction subsequently maintains highly heterozygous genotypes. Indeed, Konczal *et al.* (2021) discuss how the short generation time of *G. turnbulli* likely leads to mostly first and second births, both of which produce clonal offspring. Second, in Konczal *et al.* (2020), the *G. bullatarudis* reference genome could in itself be the 'hybrid' genome rather than all of the samples from Trinidad.

Population structure across multiple scales

A central aim in population genetics is to determine the factors that shape the amount and distribution of genetic variation within

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and among units at different scales (e.g. individuals, subpopulations and vicariant geological features). As such, topics may range from how hermaphroditic mating systems increase individual homozygosity to estimating current rates of gene flow among subpopulations to determining if past geological/environmental events altered population growth or influenced the presence of lineages across a landscape (i.e. what shaped the phylogeography of a species; Avise, 2004). As sexually mature adults of many helminth parasites are further subdivided among individual hosts or host species, parasitologists have also utilized population genetics to elucidate transmission foci or the presence of host races, respectively (Criscione *et al.*, 2005; Huyse *et al.*, 2005; Gorton *et al.*, 2012). More recently, population genetics has been applied to monitor the effectiveness of helminth control programmes via changes in genetic diversity (N_e) (Criscione, 2013; see review on schistosomes in Rey *et al.*, 2021b). The nature of population genetic questions has largely remained the same among population genomic studies (table 2). Nevertheless, the larger amounts of data afforded by NGS along with various genomic-based analyses enable finer resolution of parameter estimates such as relatedness as well as more accurate historical inference (e.g. Wang *et al.*, 2016; Terhorst *et al.*, 2017).

Various local scale applications of helminth population genomics have included studying the potential for transmission foci, the potential impacts of chemotherapeutic control measures and assessment of reservoir hosts (table 2). An example addressing transmission dynamics is provided by Shortt *et al.* (2021) where miracidia of *Schistosoma japonicum* were collected from 12 villages (maximum distance ~25 km) in Sichuan, China, in 2007, 2008, 2010 and 2016. Using model-based and non-model-based clustering analyses, individuals from the same village largely belonged to the same cluster, regardless of the time-period sampled. Also, the proportion of rare alleles shared among villages declined as distance increased. These patterns, which indicate that transmission is primarily restricted to within villages, held in a reduced data set correcting for the possibility that miracidia from a host may be siblings (see Steinauer *et al.*, 2013). Indeed, several instances of full or half-sibling parasites were found among miracidia from the same host and a few cases between hosts in the same village. The latter finding is suggestive of clonemate adults (resulting from the asexual stage in snails) in different hosts and thus, denotes the same source of infection for these hosts. Sibling miracidia were also found in pre-praziquantel and post-praziquantel treatment of the same host, indicating that individuals likely retained infections of adult flukes. Collectively, the results of Shortt *et al.* (2021) parallel findings from a microsatellite-based landscape genetics study on the roundworm *A. lumbricoides* (Criscione *et al.*, 2010) in that there are local parasite transmission foci and that these foci are stable over time and after drug treatment.

In contrast to the focal transmission of *S. japonicum*, *S. mansoni* in shoreline and island villages of Lake Victoria (~100 km apart) show markedly less population structure (Berger *et al.*, 2021; Vianney *et al.*, 2022). Although, a nearby inland district (~40 km from the shoreline villages) was genetically distinct. At the individual host level, Berger *et al.* (2021) did not find evidence of highly related *S. mansoni* miracidia from individuals in shoreline villages of Lake Victoria. Vianney *et al.* (2022), however, did find some related *S. mansoni* miracidia from island villages of Lake Victoria using the relatedness measure of Shortt *et al.* (2021). The relatedness measure of Shortt *et al.* (2021) is a within-study relative measure as the proportion of shared alleles cut-off to determine relatedness differs in Vianney *et al.* (2022); thus, a direct comparison between the *S. japonicum* and *S. mansoni* studies is not possible. Nonetheless, a face value comparison suggests that human hosts of *S. mansoni* around Lake Victoria harbour more breeding adults compared to human hosts of *S. japonicum* in the villages of Sichuan.

Berger *et al.* (2021) and Vianney *et al.* (2022) also examined whether praziquantel treatment impacted the local population structure of *S. mansoni*. Berger *et al.* (2021) found no difference in nucleotide diversity and no evidence of genetic differentiation between one round of pre-praziquantel and post-praziquantel treatment samples of *S. mansoni* from shoreline villages of Lake Victoria. Moreover, despite nine rounds of mass drug administration in these villages, N_e has remained stable and large (~33,000) in recent history. In mild contrast, *S. mansoni* from the island villages of Lake Victoria did show a slight reduction in nucleotide diversity, 0.00325 to 0.0032, in one round of pre-treatment vs. post-treatment, respectively (Vianney *et al.*, 2022). In addition, island villages with drug treatment four times per year had slightly lower nucleotide diversity compared to villages treated one time per year; N_e was estimated to be 100,000 (Vianney *et al.*, 2022). Collectively, mass drug administration with praziquantel is having a mild impact, at best, on reducing the N_es of *S. mansoni* around Lake Victoria, supporting conclusions from pre-NGS population genetic studies (reviewed in Rey *et al.*, 2021b).

The use of reservoir hosts by human parasites is an important epidemiological consideration as reservoirs could maintain a local parasite population despite the reduction in human infections. Indeed, extensive eradication efforts since 1986 have drastically reduced Guinea worm, *Dracunculus medinensis*, infections in humans in Chad, but there has been a concurrent rise in reports of dog infections (Durrant *et al.*, 2020). Both mitochondrial and nuclear genome data show that human and dog samples of Guinea worms are part of a single population. These authors acknowledge that their historical (>2000 thousand years ago) N_e estimate of ~31,000 in Chad may not capture a population bottleneck in the past few decades. Nonetheless, the high level of nucleotide diversity (0.0252) still indicated a currently large N_e despite the near absence of human infections in the past two decades. Hence, these authors advise vigilance by surveying both dogs and humans as control programmes continue.

On broader geographical scales, topics of assessing ecotypes and historical migration/colonization patterns have been addressed in helminth population genomic studies. For example, the genomics data of Choi *et al.* (2016) showed genetic distinction between the savanna and forest ecotypes (possibly associated with blackfly host specificity) of the filarial nematode *Onchocerca volvulus* in West Africa. Several helminths show a history of dispersal that reflect human movement (table 2). Crellen *et al.* (2016) found that *S. mansoni* has an East African origin and diverged from *Schistosoma rodhaini* approximately 107.5–147.6 thousand years ago, which is in line with the time frame for the origins of human fishing in this region. Additionally, the data indicated that *S. mansoni* spread to the Americas between the 16th and 19th centuries, which coincides with the transatlantic slave trade. Additional sampling by Platt *et al.* (2022) also found an East African origin for *S. mansoni* with an expansion, and reduction in genetic diversity, into West Africa and then to the Americas. Similar levels of nucleotide diversity in West Africa and Brazil may suggest repeated colonization events that occurred during the transatlantic slave trade. Population genomic evidence

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Table 2. Population structure.

Parasite species	Analysis methods	Brief summary
Baylisascaris schroederi [1]	Coalescent historical-N_e. Population structure assessed with model and non-model clustering, and phylogenetic methods	240 samples from captive pandas (Sichuan subspecies) and 26 samples from wild pandas (Qinling subspecies). Historical changes in parasite N_e lagged behind changes in host N_e, which was always much lower than parasite N_e. Host N_e decline, followed by parasite N_e decline, coincided with the last two Pleistocene glacial periods. Population structure analyses showed that parasites from wild and captive panda populations are distinct with no evidence of admixture ancestry
Dracunculus medinensis [2]	Coalescent historical-N_e and population separation history. Population structure assessed with model and non-model clustering, and phylogenetic methods	Population structure analyses showed samples separated by geography rather than host species. Coalescent model testing favoured an early split (20,000 years ago around last glacial maximum when Africa was arid) between West African samples, which had the lowest genetic diversity, and East African and Chad samples (the latter split around 4000 years ago). This model also favoured a single Chad population, that is, no distinction between human and dog samples. This works indicates that dogs act as a reservoir
Haemonchus contortus [3]	Coalescent historical-N_e. Population structure assessed with model and non-model clustering, and phylogenetic methods	Analyses of global samples showed high genetic diversity and geographical structuring of subtropical African isolates, Atlantic isolates, and Mediterranean and Oceania isolates. Current N_e ranges from 0.6 to 1.05 million. Simulations indicated 3 migration events: (1) non-African isolates are a subset of African diversity consistent with migration out of Africa; (2) connectivity between West Africa and American isolates support parasite spread during trans-Atlantic slave trade movement; and (3) intermixing of samples and admixture patterns of Australian, South-African and Mediterranean isolates suggest connectivity resulting from British colonization of Australia
H. contortus [4]	Population structure assessed with non-model clustering	Confirms findings from Sallé *et al.* (2019). Additional samples from Pakistan fall out between South African and Indonesian samples; United States and United Kingdom samples found among global samples likely due to modern human movement
Onchocerca volvulus [5]	Population structure assessed with model clustering, absolute differentiation (F_{ST}) and phylogenetic methods	Clear population structuring among Ugandan, West African and Ecuadorian isolates. Elevated Tajima's D in Ecuador consistent with founder effect. Additional differentiation between savanna and forest samples in West Africa is consistent with two-strain hypothesis. Evidence of unidirectional gene flow from savanna to forest based on admixture ancestry
O. volvulus [6]	Population structure assessed with pairwise comparison of F_{ST} and non-model clustering methods	Used Pool-seq data to characterize genetic diversity within and between 592 adult female worms from Cameroon and Ghana that were naive to/sparingly treated with ivermectin (NTL), had good response to ivermectin (GR), and sub-optimal response (SOR) to ivermectin. Population structure analyses found that there was significant structuring between the two countries. Unexpectedly, the NTL were more genetically similar to the SOR worms than the GR worms, despite being from geographically distinct areas within each country. GR worms in both countries have greater differentiation to SOR and NTL worms, which is consistent with transmission restriction of GR worms
Schistosoma japonicum [7]	Population structure assessed with non-model clustering and phylogenetic methods	Presented methods for using whole genome amplification and double digest restriction site associated DNA sequencing for single nucleotide polymorphism (SNP) discovery. SNP data from eight miracidia showed samples clustered by village and host individual within village
S. japonicum [8]	Population structure assessed with model and non-model clustering and phylogenetic methods. Kinship/relatedness analysis	Using methods of Shortt *et al.* (2017), SNP genotyped 200 miracidia across 12 villages in Sichuan, China, and four time points from 2007 to 2016. Developed a relative relatedness measure based on the proportion of shared alleles. Allele sharing decreased with geographical distance. Samples clustered by village, not time, indicating stable transmission foci of genetic clusters even after repeated drug treatments. Miracidia within hosts tended to have higher relatedness than between hosts within a village
S. japonicum [9]	Population structure assessed with non-model clustering and phylogenetic methods. Kinship/relatedness analysis	Presented methods for using whole genome amplification and short-read next generation sequencing data to identify SNPs. From Sichuan, China, genotyped 16 miracidia from ten people of one village and six miracidia from one person in another village. Samples clustered by village. Using relatedness measure of Shortt *et al.* (2021), allele sharing tended to show higher relatedness for miracidia from same hosts

(*Continued*)

Table 2. (*Continued.*)

Parasite species	Analysis methods	Brief summary
Schistosoma mansoni [10]	Coalescent historical-N_e. Population structure assessed with model and non-model clustering. Kinship/relatedness analysis. Assessed genetic diversity and differentiation in relation to drug treatment	SNPs genotyped across 198 miracidia from three schools (31 children) in Mayuge district along the shoreline of Lake Victoria, Uganda and one school (three children) in inland Tororo district. The three Mayuge schools are largely panmictic and slightly differentiated (F_{ST} ~0.023) to inland district. No evidence of sibling miracidia in the overall sample. Negative Tajima's D in all school samples indicative of population expansion. N_e estimates about 26,000 and 33,000 in inland and Lake Victoria schools, respectively. Nucleotide diversity is similar across all schools even though inland schools only had one round of treatment vs. nine rounds in Lake Victoria schools. A single round of treatment in Lake Victoria samples did not really affect differentiation or nucleotide diversity. Overall, drug treatment did not appear to impact population structure
S. mansoni [11]	Population structure assessed with non-model clustering. Kinship/relatedness analysis. Assessed genetic diversity and differentiation in relation to drug treatment	SNPs genotyped across 174 miracidia from eight villages (four with intensive drug treatment of 4 × /year and four with standard 1 × /year) on islands in Lake Victoria (Mukono district). These island samples clustered more closely with the Lake Victoria shoreline vs. the inland samples used in Berger *et al.* (2021). Differentiation was low between island villages (maximum F_{ST} = 0.0067), but villages on same island tended to be less differentiated. Relatedness, as estimated in Shortt *et al.* (2021), tended to be higher for miracidia from the same host as compared to between hosts. Island samples had more genetic diversity and higher N_e estimate (~100,000) compared to samples in Berger *et al.* (2021). After a single round of drug treatment, post-treatment samples had slightly lower nucleotide diversity. Villages with intensive treatment also had lower nucleotide diversity compared to villages with standard treatment, suggesting a small effect of praziquantel treatment
S. mansoni [12]	Coalescent historical-N_e and population separation history. Population structure assessed with non-model clustering, and phylogenetic methods	Using *S. rodhaini* as an outgroup, analyses indicated that *S. mansoni* emerged 126,500 years ago and in East Africa. Authors suggest the adoption of fishing by humans coincided with the emergence of *S. mansoni*. Decline in N_e between 20,000 and 90,000 years ago followed by expansion across African continent. Split between Guadeloupe and West African samples dates 1117–1742 AD, consistent with the time of transatlantic slave trades
S. mansoni [13]	Population structure assessed with model and non-model clustering, admixture tests and phylogenetic methods	High genetic diversity in East Africa samples not due to hybridization with *S. rodhaini* has no signature of admixture. Analyses largely delineated East African, West African and American samples. East African samples had higher genetic diversity and lower linkage disequilibrium than West African and Brazilian samples; the latter two had similar genetic diversity and linkage disequilibrium. Nuclear genomic data do not support a bottleneck during colonization of Brazil. Data not conclusive if single or multiple colonization events into Americas. Brazilian source of *S. mansoni* inferred to be from a region between Benin and Angola
Trichuris trichiura [14]	Coalescent historical-N_e. Population structure assessed with model and non-model clustering, admixture tests and phylogenetic methods	17 samples of *T. trichiura* were collected from ancient latrines in Denmark and the Netherlands, seven modern samples came from captive non-human primates at zoos in Spain and Denmark and 37 modern samples came from humans in Uganda, Cameroon, Tanzania, Denmark, Holland, Lithuania, Spain, Honduras and Guangdong, China. Non-model clustering with all data showed three main clusters: samples from China; samples from Honduras; and the remaining samples. Demographic analyses were consistent with an African origin and subsequent movement out of Africa in association with human migrations. An isolation-by-distance pattern was identified with the ancient samples. Using a subset (N = 31) of high-quality samples in model clustering showed the following clusters: two baboon samples with two ancient samples; Honduras samples; China samples; and a cluster of Ugandan samples with mixed ancestry of the China cluster and baboon-ancient DNA cluster
Wuchereria bancrofti [15]	Coalescent historical-N_e. Kinship/relatedness analysis	Mosquitos fed on three infected people from Papua New Guinea and 13 third larval stage (L3) larvae from these mosquitos used for genomics. Inferred full and half-sibling relationships within individual hosts. This result is reflective of the fact that microfilaria could be siblings of the mating adults within the human host. Mitochondrial DNA supports a shared mother. Changes in N_e showed population growth then a decline starting about 1500 years ago. Authors discuss how some of the historical changes in N_e may correspond to changes in mosquito populations

Wuchereria bancrofti [16]	Coalescent historical-N_e, Population structure assessed with model and non-model clustering and phylogenetic methods. Migration model testing using allele frequency spectrum	Analyses delineate samples from Haiti, Mali, Kenya and Papua New Guinea according to their sampling location. Model testing favoured strict isolation among populations since divergence. Divergence among all samples was estimated to be 37,000 to 57,000 years ago. The split of 400–500 years ago between Haiti and Mali is consistent with the period of transatlantic slave trade

[1] Han *et al.* (2022); [2] Durrant *et al.* (2020); [3] Sallé *et al.* (2019); [4] Doyle *et al.* (2020); [5] Choi *et al.* (2016); [6] Doyle *et al.* (2017); [7] Shortt *et al.* (2017); [8] Shortt *et al.* (2021); [9] Nikolakis *et al.* (2021); [10] Berger *et al.* (2021); [11] Vianney *et al.* (2022); [12] Crellen *et al.* (2016); [13] Platt *et al.* (2022); [14] Doyle *et al.* (2022a); [15] Small *et al.* (2016); [16] Small *et al.* (2019).

of helminths transported to the Americas via the slave trade also exists for *H. contortus* (Sallé *et al.*, 2019), *O. volvulus* (Choi *et al.*, 2016) and *Wuchereria bancrofti* (Small *et al.*, 2019). Global sampling of *H. contortus* also revealed reduced genetic diversity among isolates outside of Africa, an additional link reflecting British colonization of Australia and some phylogenetic clustering that likely reflects mixed-origin sheep breeding (Sallé *et al.*, 2019). In a non-human example where the parasite parallels host history, Han *et al.* (2022) found that changes in the N_e of the roundworm *Baylisascaris schroederi* slightly lagged behind that of its giant panda host. In particular, the authors suggest the sharp N_e decline of roundworms in the last 10,000 years could reflect the human-induced N_e decline in giant pandas in this time frame.

Surveys of candidate drug-resistant loci and scans for loci under selection

In parasitology, the identification of loci under selection has largely centred on the origin and genetic basis for drug resistance (see Gilleard, 2006; Gilleard & Beech, 2007; Doyle & Cotton, 2019 for reviews). Prior to NGS, population genetic methods were used to determine if parasite candidate genes were under selection where candidate genes were sometimes identified in non-parasitic species such as *Caenorhabditis elegans* or suspected based on possible drug mode of action. As described in Gilleard (2006), the candidate gene approach 'involves making an "educated guess" as to which genes might be involved in resistance and then conducting experimental work to test the hypothesis.' Doyle & Cotton (2019) note, however, that few candidate genes have shown associations with resistance phenotypes in the field. The high genetic diversity commonly found in many nematodes along with high among-species life history diversity has likely led to the evolution of multiple independent mechanisms for drug resistance in helminth parasites (Doyle & Cotton, 2019). The main exception to where a candidate locus has shown association to resistance in pre-NGS studies is with the β-tubulin isotype 1 gene among livestock nematodes. In particular, three non-synonymous substitutions at three codons can be found to accompany benzimidazole treatment failure (reviewed in Doyle & Cotton, 2019).

Deep amplicon NGS approaches (similar to the parabiome approach) have been applied to survey the frequencies of the possible β-tubulin isotype 1, resistant alleles among several nematode species (table 3). Compared to traditional polymerase chain reaction, the deep amplicon methods enable high sample throughput and in certain instances, can be applied to multiple nematode species simultaneously (Avramenko *et al.*, 2019; Chihi *et al.*, 2022). Studies on human and horse roundworms (*A. lumbroides* and *Parascaris equorum*, respectively) did not find any resistant alleles at the three candidate codons of β-tubulin despite use of benzimidazoles (Tydén *et al.*, 2014; Roose *et al.*, 2021). In contrast, several studies reported the resistant-associated SNP F200Y in the β-tubulin gene of various trichostrongylid nematodes collected from sheep or cattle (Ali *et al.*, 2019; Avramenko *et al.*, 2019; Avramenko *et al.*, 2020; Melville *et al.*, 2020). Interestingly, phylogenetic analysis of β-tubulin haplotype by Ali *et al.* (2019) revealed that the F200Y allele arose independently multiple times in samples of *H. contortus* from Pakistan, but only once among samples of *H. placei*. Although the deep amplicon surveys of β-tubulin isotype 1 have enabled detection of resistant alleles at low frequency and have identified other possible resistant alleles,

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no formal tests of selection or drug-resistant association have been conducted in these studies.

Two studies on *S. mansoni* (table 3) also conducted targeted drug-resistant loci surveys, but in Chevalier *et al.* (2019) and Le Clec'h *et al.* (2021b), the candidate loci were identified *a priori* via linkage mapping (Valentim *et al.*, 2013) or a laboratory-based association analysis (Le Clec'h *et al.*, 2021a). In addition, these two studies used exome capture rather than a targeted amplicon approach to examine the loci of interest. Exome capture provides extended haplotype information beyond the small genetic regions obtained from deep amplicon sequencing. In Chevalier *et al.* (2019), samples of *S. mansoni* from the Middle East, Africa, South America and the Caribbean were surveyed for variants of the sulphotransferase gene *SmSULT-OR*, the locus that was identified in mapping studies of oxamniquine resistance (Valentim *et al.*, 2013; Chevalier *et al.*, 2014). The mapped variant associated with oxamniquine resistance, p.E142del, along with six other likely resistance variants were found in African samples. Moreover, they identified an identical haplotype block between a Caribbean and West African sample, suggesting a common origin of p.E142del that predates the use of oxamniquine. These results support the origin of drug-resistance stemming from standing genetic variation rather than a *de novo* origin (Chevalier *et al.*, 2014). In Le Clec'h *et al.* (2021b) a transient receptor potential channel (TRPM) on chromosome 3 was associated with praziquantel resistance in a laboratory-based assay. Interestingly, this TRPM was shown to be activated by nano-molar quantities of praziquantel, suggesting it is a likely target for praziquantel (Park *et al.*, 2021). A subsequent field survey of the TRPM alleles by Le Clec'h *et al.* (2021b), though, only found a single possible resistance allele in a heterozygous state across 122 individuals from Africa, South America and the Middle East.

While targeted approaches of candidate loci have their role, a primary limitation is the ability to identify novel genes involved in resistance (Doyle & Cotton, 2019). In contrast, various methods now exist to scan genome data for signatures of selection (Ahrens *et al.*, 2018; Luikart *et al.*, 2018; Bourgeois & Warren, 2021). Such methods may rely on within population samples (e.g. scanning for regions of reduced nucleotide diversity or various EHH statistics) or outlier analyses of population differentiation (e.g. absolute differentiation (F_{ST})). The latter are often conducted between two or more samples that differ in phenotype (e.g. drug resistance). Inference of selection is reinforced with association-based analyses that aim to detect relationships between environmental variables and genetic variants. Although such genome scans are useful to identify regions under selection, there are caveats regarding both genome scans and association tests (see Barrett & Hoekstra, 2011; Cruickshank & Hahn, 2014; Luikart *et al.*, 2018; Doyle & Cotton, 2019). Importantly, not accounting for population structure among samples can lead to false associations. In addition, differentiation scans between two phenotypically characterized groups (e.g. drug-resistant and susceptible) assume that the cause of differentiation is related to the driver of the phenotype (e.g. drug selection pressure). However, and especially if the two samples are confounded with different geographical origins, additional selection pressures may be driving differentiation (Barrett & Hoekstra, 2011; Doyle & Cotton, 2019). Also, differentiation tests based on F_{ST} can be affected by linked selection reducing diversity in areas of low recombination (Cruickshank & Hahn, 2014).

Several helminth studies have used genome-wide approaches to scan for loci under selection with an emphasis on detecting selection from drug pressure (table 4). For example, Berger *et al.* (2021) conducted within population tests for *S. mansoni* from Ugandan districts Mayuge and Tororo that had long and short histories of praziquantel treatment, respectively. While multiple genome regions in Mayuge showed evidence of selection consistent with higher drug pressure, only a few possible impactful variants could be functionally annotated and these were not found directly under the peak signals of selection. The TRPM locus identified by Le Clec'h *et al.* (2021b) also did not show evidence of selection. One region on chromosome 3, though, showed evidence of selection from multiple tests and annotation indicated ion exchange proteins, which have been implicated in blockage of praziquantel uptake (Kohn *et al.*, 2001; Valle *et al.*, 2003). Global studies using genome scans in *H. contortus* have revealed both within-sample and between-sample signatures of selection around the β-tubulin isotype 1 locus, consistent with extensive use of benzimidazoles (Sallé *et al.*, 2019; Doyle *et al.*, 2020). In addition, differentiation analyses between isolates that differ in ivermectin resistance revealed elevated F_{ST} along a segment of chromosome 5 (Sallé *et al.*, 2019; Doyle *et al.*, 2020), a region identified in mapping studies (Doyle *et al.*, 2019a, 2022b). On a Swedish sheep farm with suspected ivermectin treatment failure, a pre-treatment and post-treatment comparison of nucleotide diversity also implicated this same region of chromosome 5 to be involved in ivermectin resistance (Baltrušis *et al.*, 2022).

Even with most studies focusing on selection from drug pressures, a few studies have found signatures of selection for genes that are likely not under anthelmintic pressure (table 4). For example, analyses from schistosome hybrids have identified loci that may be under selection from host immune systems (Platt *et al.*, 2019; Landeryou *et al.*, 2022). Choi *et al.* (2016) propose that some of the highly differentiated loci between forest and savanna populations of *O. volvulus* may be involved in blackfly vector specificity. In Sallé *et al.* (2019) outlier differentiation tests between populations with arid vs. wetter climates and/or association tests with climate variables identified potential loci involved in drought stress or the dauer (developmental arrest) pathway in *H. contortus*. The authors call for a better understanding of genetic links between traits such as hypobiosis (arrested larval development in the host) and unfavourable environmental conditions. In a study on the filarial nematode *W. bancrofti*, Small *et al.* (2019) found evidence of selection at a locus annotated as providing resistance to acetylcholinesterase inhibitors. Such inhibitors are a common mode of action in pesticides used to control vectors. Thus, the use of pesticides on the mosquito host of *W. bancrofti* may be resulting in inadvertent selection on the parasite.

Linkage mapping

Linkage mapping uses experimental crosses along with genotypical and phenotypical characterization of parents and their offspring to identify co-segregation between genetic markers and quantifiable phenotypes (quantitative trait loci (QTL)) (Broman *et al.*, 2003; Falconer & Mackay, 2009). Genetic cross data have been used to determine the mode of inheritance of genetic markers or phenotypes in parasitic helminths (Le Jambre & Royal, 1977; Le Jambre *et al.*, 1979; Habe *et al.*, 1985; Cioli *et al.*, 1992; Hunt *et al.*, 2010; Detwiler & Criscione, 2011; Redman *et al.*, 2012). However, parasitic helminth linkage maps are relatively recent, with the first maps generated by targeted genotyping of microsatellites or other nuclear markers (Criscione *et al.*, 2009;

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Table 3. Targeted drug-resistant loci surveys.

Species	Study objective	Brief summary
Ascaris lumbricoides and *Ascaris suum* [1]	Deep amplicon sequencing of β-tubulin from samples collected in Ethiopia, Tanzania (human hosts) and Belgium (pig hosts). Primers designed around two paralogs: type A and type B (author labelled)	Type A was a single copy locus, but type B primers may have had additional off-target amplification of other paralogous loci. All variation for type A was found in an intron; type B amplicon sequence variants had non-synonymous single nucleotide polymorphisms (SNPs), but not clear if represents paralogs. No previously known benzimidazole resistance associated SNPs were detected for type A or type B.
Ancylostoma caninum [2]	Deep amplicon sequencing of β-tubulin isotype-1 based on samples collected from dogs in Georgia and Florida, United States	Resistance associated SNP, F167Y, was found at high frequencies (47.4–99.7%) in samples collected from dogs with persistent hookworm infections after treatment with anthelmintics. F167Y was found at much lower frequencies (0.0–8.8%) in samples collected from dogs with no history of anthelmintic treatment. Another common resistance associated SNP, F200Y, was not found in any sample
Haemonchous contortus and *Haemonchous placei* [3]	Deep amplicon sequencing of β-tubulin Isotype-1 among samples collected from nine cattle and three buffalo across six abattoirs in Punjab province of Pakistan (a region with low levels of drug use)	Among *Haemonchus contortus* samples, the benzimidazole-resistant SNP, F200Y, had within-host frequencies of 7–57% in four of the six abattoirs. Among *Haemonchus placei* samples, SNP F200Y had 0.4–5% within-host frequencies from four of six abattoirs. Phylogenetic analysis of β-tubulin haplotype showed that F200Y arose independently multiple times in *H. contortus*, but only once in *H. placei*
Nematodirus battus [4]	Pyrosequencing and deep amplicon sequencing of β-tubulin isotype-1 across samples from sheep farms in the United Kingdom	Benzimidazole-resistance SNP F200Y found in 12–27% of the farms depending on sequencing method. F200Y allele frequency was low, 2.2%, within farms and genotypes at this site were not in Hardy–Weinberg across all samples. The latter may indicate that the locus is under selection, but analysis is confounded with geography. The resistant SNP F167Y was found on a few farms (within location frequency 1.2%) for the first time in this nematode species
Parascaris equorum [5]	Deep amplicon sequencing of β-tubulin isotypes 1 and 2 for benzimidazole-resistance SNPs from a Swedish farm and two United States isolates from Kentucky	Did not find any SNPs in codons 167, 198 or 200 of β-tubulin isotype one or two genes. Authors state that the result was unexpected given the increased use of benzimidazole for treatment of *Parascaris*
Schistosoma mansoni [6]	Sequenced exomes to screen variants around a sulphotransferase gene (*SmSULT-OR*) associated with oxamniquine resistance. Samples from South America, West Africa, East Africa, Middle East and Caribbean. Non-synonymous to synonymous divergence or polymorphism ratio tests conducted to test for selection on variants	85 *SmSULT-OR* mutations found across Old World samples. Four of these, including the p.E142del mutation that confers resistance, were found in New World samples. Molecular evolution selection tests were not significant. Thermodynamic modelling and *in vitro* assays identified six independent resistance mutations; these variants had frequencies of 4.3 to 14.9% in Old World samples. Haplotypes containing the resistant variant p.E142del were very similar from Puerto Rico and Niger samples, suggesting a common origin that predates use of oxamniquine in the New World. The authors emphasize that there is standing variation for oxamniquine resistance across the Old World
S. mansoni [7]	Association test between praziquantel susceptible and resistant strains to identify possible drug-resistant loci under selection. The drug susceptibility phenotype was measured via lactate production in response to exposure to praziquantel. Subsequently screened natural populations for candidate drug-resistant locus	Association test identified a transient receptor potential channel (TRPM) on chromosome 3. A series of experiments indicated recessive inheritance and functionally validated this gene's role in resistance (e.g. blocking the gene enhanced praziquantel resistance). Exome capture of genome from African, South American and Middle Eastern field samples found one sample out of 122 with a TRPM nonsense mutation in a heterozygous genotype. Hence, the resistance allele was rare (<1%) and the resistance phenotype would not be manifested
Trichostrongylid nematodes [8]	Validation of deep amplicon sequencing in comparison to pyrosequencing. Genotyped β-tubulin isotype-1 from samples collected from sheep farms in the United Kingdom	Agreement between deep amplicon sequencing and pyrosequencing. Drug-resistant allele, F200Y, commonly found in samples from *Teladorsagia circumcincta* (65.52–67.53%), *H. contortus* (42.08–43.12%) and *Teladorsagia colubriformis* (53.57–61.67%). The resistance allele, F167Y, found at high frequencies in *H. contortus* (41.08–48.99%), but was at low frequencies or absent in other species. The resistance allele frequencies in lambs and ewes had high levels of agreement. Authors hypothesized that species variation in third larval stage emergence timing may influence drug-resistance selection pressure and thus, explain variation of drug-resistant allele frequencies among some of the nematode species
Trichostrongylid nematodes [9]	Deep amplicon sequencing of β-tubulin istotype-1 variants from North and South American cattle populations and bison from Canada	Benzimidazole resistance-associated mutations occurred at low frequencies among trichostrongylid species in cattle and bison in North America. *Haemonchus contortus* from cattle in Brazil had a high frequency (48.78–90.22%) of the drug-resistant associated SNP F200Y. In the drug-resistance associated codons 167, 198 and 200, they found new SNPs, which the authors hypothesized may lead to benzimidazole resistance

[1] Roose *et al.* (2021); [2] Jimenez Castro *et al.* (2019); [3] Ali *et al.* (2019); [4] Melville *et al.* (2020); [5] Tydén *et al.* (2014); [6] Chevalier *et al.* (2019); [7] Le Clec'h *et al.* (2021b); [8] Avramenko *et al.* (2019); [9] Avramenko *et al.* (2020).

Table 4. Genome-wide scans for loci under selection.

Species	Method for detecting selection	Brief summary
Baylisascaris schroederi [1]	Integrated haplotype score (iHS) and nucleotide diversity assessed within nematode samples of captive and wild panda populations, cross-population extended haplotype homozygosity (XP-EHH) tested between samples	Four genes associated with drug resistance (based on previous studies) showed evidence of positive selection in captive, but not wild population samples. Authors state that this result is consistent with the anthelmintic treatment of captive pandas compared to wild pandas
Dirofilaria immitis [2]	Fisher's exact test to pre-screen for differentiating single nucleotide polymorphisms (SNPs) between sample pools of macrocyclic lactone susceptible and reduced efficacy	With a set of 186 differentiating SNPs, Chi-square test for genotype frequency differences between susceptible and resistance individual microfilariae found 158 significant. Authors suggest that these SNPs could serve as potential markers for macrocyclic lactone resistance
Haemonchus contortus [3]	Absolute differentiation (F_{ST}) between ivermectin-resistant (England) and susceptible (Australia) strains	General genetic differentiation screen for outlier loci. Based on functional annotation descriptions, seven genes with outlier SNPs hypothesized to be targets of ivermectin
H. contortus [4]	Tested for selective sweeps based on allele frequency spectrum in samples from China, United Kingdom and Australia	Evidence of selective sweeps only in United Kingdom and Australian samples. Provided descriptions of functional annotations of genes in these regions
H. contortus [5]	Broad world sampling. Assessed Tajima's D and nucleotide diversity around β-tubulin isotype 1 locus. Pairwise F_{ST} and cross-population composite likelihood ratio test (XP-CLR) between ivermectin-resistant and susceptible isolates. Pairwise F_{ST} between samples that differ in climate conditions along with association tests between SNPs and environmental variables	Three population samples showed evidence of selective sweeps in region surrounding β-tubulin isotype 1 consistent with benzimidazole resistance. General genetic differentiation screen and functional annotation descriptions identified some loci that may have a role in ivermectin resistance. Genetic differentiation screens between populations from arid vs. wetter climates and association tests with climate variables identified loci with associations to environmental conditions. Functional annotation of these regions revealed loci in dauer pathway or loci related to stress tolerance
H. contortus [6]	Pairwise F_{ST} and Fisher's exact test between ivermectin-resistant (China and United Kingdom) and susceptible (China and Australia isolates) strains. Assessed among-strain variance of Tajima's D	General genetic differentiation screen for outlier loci. After functional annotation, RNAi assay conducted on two candidate genes. Cytochrome P450 gene had increased sensitivity to ivermectin when blocked; authors hypothesized that this gene detoxifies xenobiotics
H. contortus [7]	Broad world sampling; F_{ST} among samples	General genetic differentiation screen for outlier loci confirmed results of Sallé *et al.* (2019) for evidence of selection in region surrounding β-tubulin isotype 1. Also found elevated F_{ST} in a region on chromosome 5 that was found associated with ivermectin resistance in a linkage mapping study (Doyle *et al.,* 2019a)
H. contortus [8]	F_{ST} and Fisher's exact test between ivermectin pre-treatment and post-treatment groups collected from a Swedish sheep farm with suspected treatment failure. Tajima's D and nucleotide diversity calculated within treatment groups	No overall genetic differentiation, and no difference in nucleotide diversity or Tajima's D between treatments. General genetic differentiation screen did not reveal any compelling candidate loci. But increased nucleotide diversity found in post-treatment group on a region of chromosome 5; a region previously found associated with ivermectin resistance in a linkage mapping study (Doyle *et al.,* 2019a). Increased diversity hypothesized to be driven by increase in low frequency variants conferring resistance
H. contortus [9]	Pairwise F_{ST} between two benzimidazole treated and two untreated sheep farms in Pakistan. Examined expected heterozygosity and linkage disequilibrium (LD) within populations. Deep amplicon sequencing of isotype-1 and isotype-2 β-tubulin to estimated frequency of resistant-associated SNPs	Genome-wide SNPs showed low differentiation between all sampled populations. Region on chromosome 1 containing the β-tubulin isotype-1 gene had elevated F_{ST} in comparisons of treated vs. untreated. Reduced expected heterozygosity and high LD in same region in treated populations only. A region on chromosome 2, containing the β-tubulin isotype-2 locus, had reduced expected heterozygosity, but no elevated LD. Detected the resistance-associated SNP, F200Y, in high frequencies (92.5–100%) from treated populations treated in low frequencies (2.5–12.5%) from untreated populations. Resistance-associated SNPs were not detected in β-tubulin isotype-2 with the exception of one sample, which had F200Y in a heterozygous state
Onchocerca volvulus [10]	F_{ST} between West Africa and Ecuador samples and forest vs. savanna samples within West Africa	General genetic differentiation screen for outlier loci found outlier regions containing 300 annotated genes. Prior transcriptome work showed these genes to have higher transcript abundance than other genes in microfilarial stage vs. other stages. Authors hypothesized that some loci possibly involved in chemosensation under divergent selection in relation to blackfly vector specificity

O. volvulus [11]	F_{ST} between pooled ivermectin good responder (GR) and sub-optimal responder (SOR) samples within both Ghana and Cameroon	General genetic differentiation screen found outliers clustered in multiple discrete genome regions. But only one significant SNP site overlapped in both the GR vs. SOR comparisons of Ghana and Cameroon. Provided descriptions of functional annotations of genes in these regions. No known ivermectin SOR genes were found to be significant in comparisons
Schistosoma haematobium [12]	BayeScan (software that tests drift vs. selection as cause of allele frequency differences) and XP-EHH tested between Zanzibari and Nigerien populations, which do not and do show evidence of hybridization with *Schistosoma bovis*, respectively.	Regions of directional selection identified between Zanzibari and Nigerien populations. Strongest signal was on a region of chromosome 4, which had the highest frequency of introgressed alleles from *S. bovis*. Nucleotide diversity also reduced in this region in Nigerien sample. This region has an invadolysin gene, which the authors speculate may be involved in tissue penetration or immune evasion
Schistosoma mansoni [13]	Ten *S. mansoni* individuals from Africa or Guadeloupe. d_N/d_S between *S. mansoni* and *S. rodhaini*; Hudson–Kreitman–Aguade (HKA) and McDonald–Kreitman (MK) tests within *S. mansoni* samples	Many annotated coding loci show positive or purifying selection via d_N/d_S tests. Likewise, many genes showed evidence of balancing or directional selection from HKA or MK tests. Based on functional annotation descriptions and gene ontology enrichments, authors suggest that some of these genes may facilitate adaptation to human hosts
S. mansoni [14]	Compared samples from Ugandan districts Mayuge and Tororo (long and short histories of praziquantel treatment, respectively). Assessed iHS and nucleotide diversity within districts and XP-EHH and F_{ST} between. Also, evaluated impact of a single treatment round via F_{ST} and association tests	General screen for outlier loci showed evidence of selection in several regions. But, after functional annotation, possible impactful variants for drug resistance were at low frequencies and most were not directly under the selection signal peak. No genetic differentiation between praziquantel pre-treatment and post-treatment. No convincing variant association with reduction in egg number phenotype
S. mansoni [15]	XP-EHH between praziquantel pre-treatment and post-treatment populations around Lake Victoria, as well as between annual and quarterly treated populations. Conducted association test between SNP genotypes and egg reduction rate phenotype	General genetic differentiation screen and functional annotation descriptions identified some loci that may have a role in reduced susceptibility to praziquantel. No association was found between genetic variants and egg reduction rate
S. mansoni [16]	Tested for selective sweeps based on allele frequency spectrum and haplotype homozygosity. Conducted simulations to estimate range of values expected under neutrality	Analyses identified five chromosomal regions in Brazil, three in Niger and three in Senegal with evidence of selection. Two separate instances of shared regions were recovered (one region between Brazil and Senegal populations and one region between Niger and Senegal populations). Identified several housekeeping genes in Brazilian samples in regions with evidence of selection and suggest that selection is being driven by adaptation to the newly colonized environment
Schistosoma haematobium and *Schistosoma guineensis* [17]	Genomic cline analysis of *S. haematobium* and *S. guineensis* to identify loci with extreme introgression patterns	41.7% (10/24) of pre-selected, tegumental antigen loci had excess ancestry from one of the parental lines whereas only 16.5% (167/1009) autosomal loci had excess ancestry. Authors hypothesized that such antigen loci could be under selection from mammalian host immune system
Trichuris trichiura [18]	Pairwise F_{ST} between human samples from China, Uganda and Honduras. F_{ST} between Ugandan human samples and two nematodes from captive baboons	Outliers found in general genetic differentiation screens, but there were few enriched terms after gene ontology analysis except in the Ugandan human vs. baboon samples. No evidence for selection in or around codons for the β-tubulin gene, which is associated with resistance to benzimidazole
Wuchereria bancrofti [19]	Molecular evolution analyses based on DH (a combination of Tajima's D and Fay and Wu's H), HKA and MK tests from Papua New Guinea samples	Found evidence of regions consistent with directional or balancing selection. Provided descriptions of functional annotations of genes in these regions
W. bancrofti [20]	The statistic hapFLK (tests differences in haplotype frequencies accounting for hierarchical structure) tested with samples from Haiti, Mali, Papua New Guinea and Kenya	General scan for outlier loci identified 18 possible regions under selection. Provided descriptions of functional annotations of genes in these regions

[1] Han *et al.* (2022); [2] Bourguinat *et al.* (2015); [3] Luo *et al.* (2017); [4] Khan *et al.* (2019); [5] Sallé *et al.* (2019); [6] Khan *et al.* (2020); [7] Doyle *et al.* (2020); [8] Baltrušis *et al.* (2022); [9] Wit *et al.* (2022); [10] Choi *et al.* (2016); [11] Doyle *et al.* (2017); [12] Platt *et al.* (2019); [13] Crellen *et al.* (2016); [14] Berger *et al.* (2021); [15] Vianney *et al.* (2022); [16] Platt *et al.* (2022); [17] Landeryou *et al.* (2022); [18] Doyle *et al.* (2022a); [19] Small *et al.* (2016); [20] Small *et al.* (2019).

Nemetschke *et al.,* 2010). The advent of NGS has enabled rapid, cost-effective, and genome-wide linkage and QTL mapping (Bailey-Wilson & Wilson, 2011; Jaganathan *et al.,* 2020). Since 2013, NGS has been utilized in linkage and QTL mapping studies in the trematode *S. mansoni* and the nematodes *H. contortus* and *Teladorsagia circumcincta* (table 5).

Anderson *et al.* (2018) provided a detailed review of linkage and QTL mapping of oxamniquine resistance in *S. mansoni* based on the studies of Valentim *et al.* (2013) and Chevalier *et al.* (2014). Here, we just highlight that *S. mansoni* is amenable to standard F2 design because crosses between parental worms are facilitated by asexual reproduction in snails. Hence, many individuals of the same clone (i.e. a single female or male parent genotype) can be crossed to many individuals of another clone. Downstream phenotyping is possible on individuals but pooling large numbers of individuals to conduct bulk segregant analysis (BSA) is also feasible. The latter approach genotypes two pooled groups either with different phenotypes or where one pool is subjected to a selective pressure to identify regions enriched for alleles from one of the parents. Variations on BSA have been termed extreme QTL (X-QTL) or linkage group selection (Michelmore *et al.,* 1991; Culleton *et al.*, 2005; Ehrenreich *et al.*, 2009). In *S. mansoni*, both individual phenotyping (Valentim *et al.,* 2013) and X-QTL (Chevalier *et al.,* 2014) approaches combined with whole genome or exome data from NGS enabled fine mapping of a sulphotransferase encoding gene involved in oxamniquine resistance.

Anderson *et al.* (2018) also discussed other epidemiologically relevant traits, such as host specificity, virulence and cercarial shedding that could be mapped in *S. mansoni*. Recently, Le Clec'h *et al.* (2021a) performed a QTL analysis of cercarial shedding, an important transmission trait, and snail-host virulence (using laccase-like activity and haemoglobin rate in the haemolymph as a proxy) by performing reciprocal crosses of high and low cercarial shedding individuals. Whole genome data from the F0 parents and exome data from the F1 and F2 generations revealed five QTLs that explained 28.56% of the variance in cercarial production. While there was good support for the polygenic inheritance of cercarial production, no significant QTLs were found for the snail haemolymph phenotypes. To our knowledge, this study is the only non-drug-resistant phenotype to be mapped in a helminth.

In contrast to linkage mapping in *S. mansoni*, mapping studies in livestock nematodes have been challenging due to high levels of within strain diversity that make creating inbred lines difficult, the need for surgical transfer to stage crosses and the need for assays to phenotype individuals for drug resistance (Gilleard, 2006; Gilleard & Redman, 2016). For example, pairing a single male and single female in host may not yield a successful mating (contrast with the many individuals of a clone in *S. mansoni*). Consequently, various other crossing strategies have been employed to create inbred lines (Sargison *et al.,* 2018), estimate recombination rates, or to map traits (table 5). For example, Doyle *et al.* (2018) generated a recombination map in *H. contortus* with a pseudo-testcross, which enables recombination estimates among loci that are heterozygous in a mother and that segregate 1:1 in her F1 offspring. SNP variants were called from Illumina reads mapped to a reference genome and a map of recombination rates across chromosomes was generated by overlaying the physical and linkage maps.

Choi *et al.* (2017) and Doyle *et al.* (2019a) mapped drug-resistant loci in *T. circumcincta* and *H. contortus,* respectively, using introgression-mapping approaches (table 5). There are variations on the method, but in brief, introgression mapping for drug resistance entails crossing parental lines that are drug-resistant and susceptible. The F1 is backcrossed to the susceptible line and offspring are subjected to drug exposure within hosts. Backcrossing to the susceptible line as well as subsequent drug exposure is repeated to create a largely 'susceptible' genetic background that is enriched for the drug-resistant allele (s) at one or more loci. Enriched regions are identified by testing for regions of differentiation (often inferred by F_{ST}) between pools of individuals from the susceptible line compared to the introgressed–backcrossed line or other downstream comparisons based on BSA. Using a multi-drug-resistant line of *T. circumcincta*, Choi *et al.* (2017) found putative resistance genes for the drugs oxfendazole, levamisole and ivermectin. However, Choi *et al.* (2017) stated that the fragmented genome of assembly of *T. circumcincta* meant the number of differentiated regions could not be accurately estimated and precluded assessment of the number of independent loci involved in drug resistance. Indeed, Doyle *et al.* (2019) demonstrated the benefits of a contiguous-assembled genome in mapping. With the chromosome-level assembly of *H. contortus*, they found a single QTL for ivermectin drug resistance on chromosome 5. In contrast, when using older fragmented assemblies, signals of selection were dispersed across multiple scaffolds. The latter would have led to an incorrect conclusion of multiple loci being involved in resistance (Doyle *et al.,* 2019a). Recently, X-QTL has been used to map drug resistance in *H. contortus* (Niciura *et al.,* 2019; Doyle *et al.*, 2022b). When compared to introgression mapping, X-QTL is faster, less expensive and requires fewer hosts and crossing cycles; these advantages make X-QTL a promising method for future QTL mapping studies among helminths.

Concluding remarks

Genomic approaches have enabled parasitologists to delve deeper into helminth population biology and evolution. For example, NGS facilitated efficient methodologies to classify parasite community richness and changes in community composition (table 1, Online supplementary table S1). Population genomic methods enabled improved inferences on the timing of hybridization/introgression events among schistosomes (e.g. Platt *et al.*, 2019; Berger *et al.*, 2022), as well as provided a means to monitor the genetic impacts of helminth control measures including surveillance of drug-resistant alleles (tables 2 and 3), or the use of reservoir hosts (Durrant *et al.*, 2020). Genomic tests of selection have not only verified known loci but have also led to the discovery of novel candidate loci conferring adaptation to drug resistance (table 4). In addition, selection has been detected in novel regions that may influence parasite host specificity, development, or immune evasion traits (Choi *et al.*, 2016; Platt *et al.*, 2019; Sallé *et al.*, 2019).

Although there have been advancements, there remain hurdles in the application of and inferences drawn from NGS and consequently, population genomics of parasites. A main limitation is the limited amount of quality DNA template per individual due to the inherent small size of many helminth life cycle stages. One common approach has been to utilize pooled samples (e.g. Bourguinat *et al.*, 2015; Doyle *et al.*, 2017; Khan *et al.*, 2019), but this limits many of the advantages of using single specimen-based data. For example, analysis of individuals enables use of ROH to estimate inbreeding (Ceballos *et al.*, 2018), easier

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Table 5. Linkage mapping.

Parasite species	Method	Brief summary
Haemonchus contortus [1]	Pseudo-testcross	Generated a map of recombination rates across chromosomes. Kinship analysis identified polyandry with at least eight males mating with a single female. Triploid offspring also found
H. contortus [2]	Introgression mapping, bulk segregant analysis	The same, single quantitative trait loci (QTL) on chromosome 5 identified between ivermectin-resistant lines of two geographical origins when compared to a susceptible line. Differentiated region did not contain previously suspected candidate loci. Demonstrated importance of a contiguous assembly in mapping traits
H. contortus [3]	Reciprocal F2 genetic crosses using multiple individuals, extreme QTL (X-QTL) of F3 generation	Allele frequency differences between F3 pools of monepantel pre-treatment and post-treatment pools mapped to three previous candidate genes for monepantel resistance. Method was faster, cheaper and required fewer sheep hosts than introgression mapping methods in Choi *et al.* (2017) and Doyle *et al*. (2019)
H. contortus [4]	F2 genetic cross with X-QTL (pre-sampling and post-sampling using fenbendazole, levamisole, or ivermectin) in F3 generation. With ivermectin only, another X-QTL in F4 generation	Single QTL for fenbendazole resistance on chromosome 1 near β-tubulin isotype 1; found increase in F200Y SNP post-treatment. Levamisole resistance associated with acetylcholine receptors in two major QTLs on chromosomes 4 and 5. Same QTL on chromosome 5 for ivermectin resistance identified as in mapping study of Doyle *et al*. (2019), but with higher resolution suggesting the gene *cky*-1. Transcription assays of *cky*-1 show overexpression in ivermectin-resistant vs. sensitive strains. Two less prominent QTLs associated with ivermectin resistance were also identified: one on chromosome 5 and one on chromosome 2, both of which corresponded to previously identified candidate genes. Only the main QTL on chromosome 5 was consistently identified across replicates in the additional X-QTL of the F4 generation
Schistosoma mansoni [5]	F2 genetic cross, phenotyped individuals	Single QTL for oxamniquine resistance found on chromosome 6. RNA interference and biochemical complementation identified a sulphotransferase gene. Different loss-of-function alleles found in the laboratory strain vs. a field sample (see also Chevalier *et al.*, 2016)
S. mansoni [6]	F2 genetic cross, X-QTL	Validation of X-QTL as a means to map oxamniquine resistance in *S. mansoni*. The region on chromosome 6 that contains the sulphotransferase gene identified in Valentim *et al.* (2013) was enriched for alleles from the drug-resistant parent
S. mansoni [7]	Reciprocal F2 genetic crosses, phenotyped individuals	Cercarial shedding was found to be polygenic with five QTLs that explained 28.56% of variance in the phenotype. Analysis of shedding over time revealed that the five QTLs predominated at different times, suggesting that different parasite genes affect cercarial production across the duration of snail infection. No significant QTLs were found for snail virulence (as measured by laccase-like activity and haemoglobin rate in the haemolymph)
Teladorsagia circumcincta [8]	Introgression mapping	Found candidate-resistant genes to oxfendazole, levamisole and ivermectin based on genome-wide absolute differentiation comparison of susceptible line to introgressed line

[1] Doyle *et al.* (2018); [2] Doyle *et al*. (2019); [3] Niciura *et al.* (2019); [4] Doyle *et al.* (2022b); [5] Valentim *et al.* (2013); [6] Chevalier *et al.* (2014); [7] Le Clec'h *et al.* (2021a); [8] Choi *et al.* (2017).

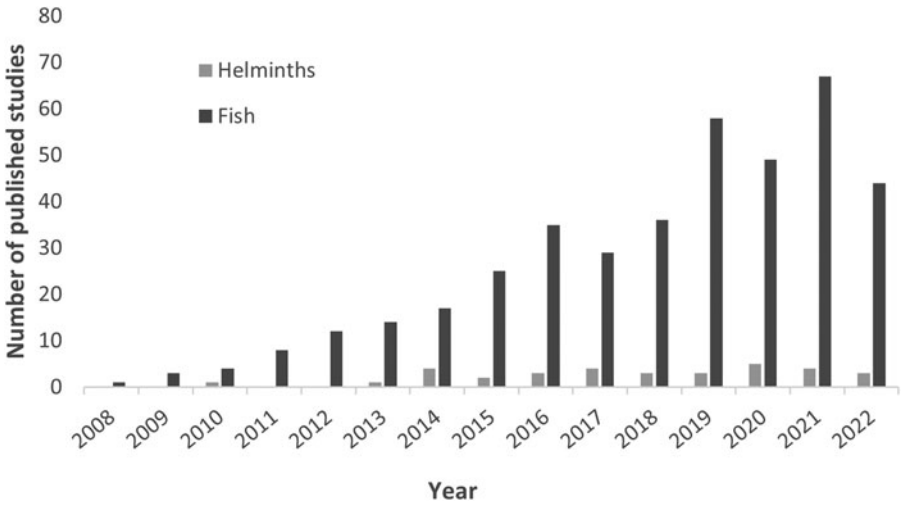

Fig. 1. Comparison of the number of population genomic studies per year between helminths and fish. We conducted a Web of Science search where the search term [('population genomics' or 'population genomic')] was searched with [fish] and then searched with [(helminth* or trematod* or nematod* or cestod* or monogene* or fluke* or tapeworm* or roundworm*)]. In both, the date range was from 1 January 2005 to 31 December 2022 and type of study was restricted to 'article, review article, and book chapter'. In the parasite search, we excluded studies including *Caenorhabditis*. Extreme caution is advised in strictly interpreting results as we recognize there are caveats in the search (e.g. plant nematodes may be included or studies in this review did not end up in the search). As such, we regard the analysis as a simple heuristic means to reflect overall trends in the field. See Online supplementary table S1.

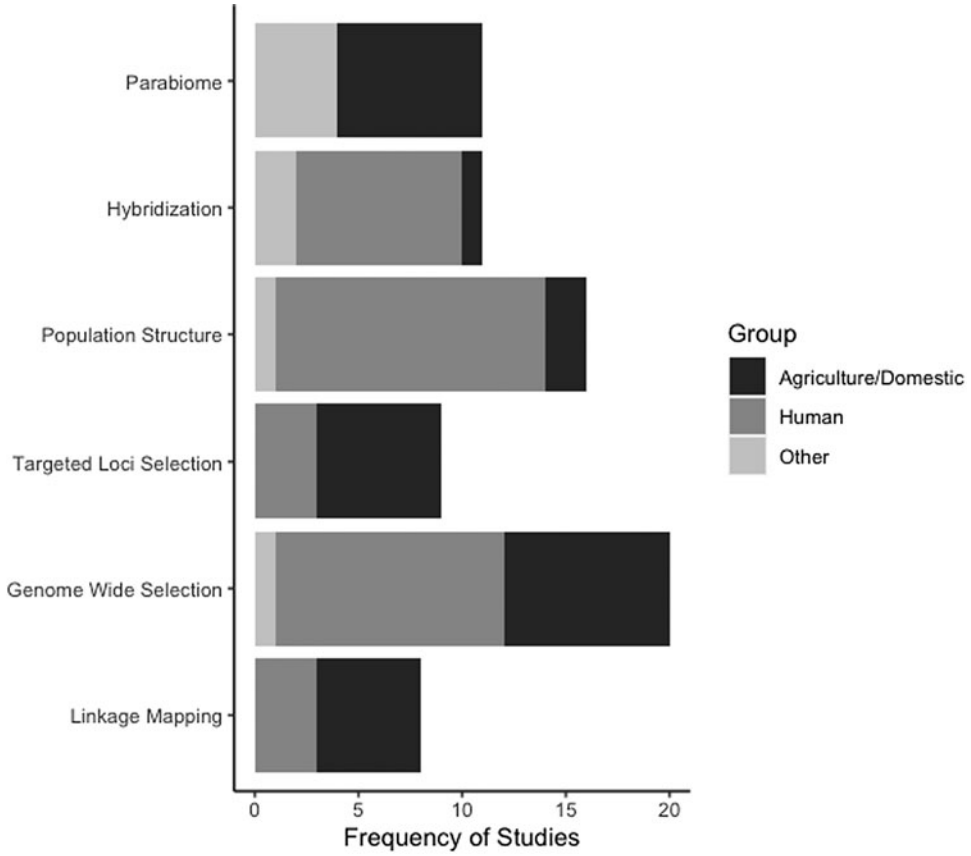

Fig. 2. Frequencies of studies examining helminth parasites in either domesticated animals, humans, or other hosts from each topical section of the review (S2). Host groups were determined based on the focal host group in the study. A study could be included under more than one topical section. See Online supplementary table S2.

assessment of sequencing error vs. a true rare variant (Anand *et al.*, 2016) and the use of relatedness measures to examine local scale transmission (e.g. Shortt *et al.*, 2021). Moreover, unequal contributions of genetic material per individual in a pooled sample can create allele frequency bias and thus, downstream summary statistic (e.g. F_{ST}) bias if sample sizes are small (Schlötterer *et al.*, 2014; Hivert *et al.*, 2018). Haplotype construction and inferences using linkage disequilibrium are also hindered with short-read pooled data (Schlötterer *et al.*, 2014). Doyle *et al.* (2019b) explored different extraction methods on individual helminth eggs/larvae, but DNA quantification was 'inconsistent and largely unsuccessful'. Other studies have used whole genome amplification to increase template in helminth eggs/larvae (Shortt *et al.*, 2017; Le Clec'h *et al.*, 2018; Platt *et al.*, 2019); however, the method leads to fragmented DNA. Low yield or fragmented DNA precludes use of third generation sequencing

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(TGS), that is, long-read data, of individuals. An advantage of TGS is the creation of highly contiguous sequences that can span long repetitive regions (Amarasinghe *et al.*, 2020). As such, structural variation (e.g. inversions, large insertions or deletions) can be assessed and phased haplotypes can be constructed, enabling analyses with ROH and linkage disequilibrium patterns (Wit & Gilleard, 2017; Ceballos *et al.*, 2018).

Inferences from helminth population genomics have also been limited in scope. Although genome scans have identified regions under selection, the context of what is driving the selection is often ambiguous. Studies often rely on *post hoc* annotations to explain why there might be selection in that genomic region. Even when there are strong signals of selection in regions with assumed relation to drug resistance, only a handful of studies followed up these findings with functional validation (Khan *et al.*, 2020; Le Clec'h *et al.*, 2021b). In general, with few exceptions (e.g. Sallé *et al.*, 2019), there has been little attempt to correlate environmental variables or historical geological features with helminth genomic variation. The latter type of studies often requires large sample sizes, which understandably might be difficult to achieve in many helminth systems due to technical constraints in collecting specimens.

In general, the application of NGS and population genomics has yet to reach its realized potential in parasitology. Figure 1 illustrates a heuristic comparison (see fig. 1 legend for methods, Online supplementary table S1) of the frequency of 'population genomic' studies per year between a common vertebrate group ('fish') and helminth parasites. Upon the introduction of NGS in 2005, the number of studies among fish has shown a continued gradual increase since 2008 (a total of 402 studies in all years analysed). However, the number of studies among helminths is largely non-existent prior to 2014 with the number of studies per year remaining rather stagnant since 2014 (a total of 33 studies in all years analyzed). The current trend of population genomics in parasitology mirrors historical trends in the application of allozyme and microsatellite markers to parasite population genetics where there is an approximate ten-year lag compared to studies on 'fish' (compare fig. 1 to fig. 1 in Criscione, 2016, which provides a parallel heuristic analysis based on the use of microsatellite markers).

In addition to the slow integration of NGS and population genomics in parasitology, the taxonomic breadth of the studies available for our review is very limited and is biased to parasites of humans and/or domestic animals (fig. 2, Online supplementary table S2). Furthermore, the vast majority of the studies we reviewed are centred on drug resistance, despite the many other aspects of parasite biology that could potentially be addressed using genomic data. Helminth parasites are incredibly species rich (estimates range from 100,000–350,000 species, though 85%–95% are unknown; Carlson *et al.*, 2020), inhabit diverse ecosystems, and display a myriad of life histories, life cycles and host use. As such, there are numerous opportunities to use population genomics to elucidate unknown helminth biology and genetic diversity. For example, there are many gaps in our knowledge about parasite life cycles where described species are known from only a single stage (Blasco-Costa & Poulin, 2017). The expansion of parabiome-like approaches could prove very useful in both elucidating host use and cryptic parasite species. In addition, the diversity of helminths themselves can be used to address the consequential challenge of linking comparative population genomic patterns to species' life history and ecology (Glémin *et al.*, 2019). For instance, there are also various life cycle patterns, mating systems (e.g. selfing vs. outcrossing), or modes of reproduction (asexual vs. sexual) among helminths (e.g. Detwiler *et al.*, 2017; Kasl *et al.*, 2018; Criscione *et al.*, 2022) that enable among-species population genomic comparisons. Unfortunately, such comparisons cannot be drawn from the existing studies we reviewed. For example, most of the parasites reviewed here, barring schistosomes and filarial nematodes, have direct life cycles. Certainly, future fruitful avenues will be to use population genomics to explore how life cycle complexity may shape population structure or to ascertain if loci under selection might be influencing complex life cycle evolution itself. In conclusion, we emphasize that there is great potential for population genomics to elucidate helminth population biology and evolution as well as the potential for helminths to contribute to our understanding of broader ecological and evolutionary concepts. In doing so, we reiterate the arguments made by Carlson *et al.* (2020) to increase resources (e.g. funding, taxonomic and classical parasitology training) for expanding our knowledge base about helminth biodiversity. As such, we hope that future trends in helminth population genomics reflect the taxonomic and life history breadth displayed by these parasites.

Supplementary material. To view supplementary material for this article, please visit 10.1017/S0022149X23000123.

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Conflicts of interest. None.

Ethical standards. Not applicable.

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