Genetic mapping and coccidial parasites: Past achievements and future prospects

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Coccidial parasites including *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Neospora caninum*, *Toxoplasma gondii* and the *Eimeria* species can cause severe disease of medical and veterinary importance. As many as one-third of the human population may carry *T. gondii* infection, and *Eimeria* are thought to cost the global poultry production industry in excess of US$2 billion per annum. Despite their significance, effective vaccines are scarce and have been confined to the veterinary field. As sequencing and genotyping technologies continue to develop, genetic mapping remains a valuable tool for the identification of genes that underlie phenotypic traits of interest and the assembly of contiguous genome sequences. For the coccidian, cross-fertilization still requires *in vivo* infection, a feature of their life cycle which limits the use of genetic mapping strategies. Importantly, the development of population-based approaches has now removed the need to isolate clonal lines for genetic mapping of selectable traits, complementing the classical clone-based techniques. To date, four coccidial species, representing three genera, have been investigated using genetic mapping. In this review we will discuss recent progress with these species and examine the prospects for future initiatives.

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1. Introduction

Protozoan apicomplexan parasites impact globally on human and animal health, frequently causing suffering, ill health, death and severe economic loss. Within the phylum Apicomplexa many of the most devastating parasites have been positioned in the subclass Coccidia and are referred to as 'coccidial' parasites (figure 1). Examples including *Toxoplasma gondii*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* can cause severe human disease. Indeed, as much as a third of the global human population has been predicted to carry a *Toxoplasma* infection (Montoya and Liesenfeld 2004) and the *Cryptosporidium* and *Cyclospora* species are widely considered to be pathogens of emerging importance (Morens *et al.* 2004). Similarly, parasites including the *Eimeria* species, *Isospora* species and *Neospora caninum* can have a dramatic impact on economic animal production and animal welfare, as well as human poverty (Perry *et al.* 2002; Beck *et al.* 2009).

Extensive efforts have been made to develop strategies to control the coccidial parasites over the last century (Beach and Corl 1925; Williams *et al.* 1978; Blake *et al.* 2011a), but, disappointingly, progress has been limited. Innate drug susceptibility varies from negligible to complete (e.g. the *Cryptosporidium* and *Eimeria* species respectively; Chapman 1997; Mead 2002), but where effective drugs have been identified, resistance frequently develops quickly and rapidly becomes widespread (Chapman 1997). Successful vaccines are scarce and have been confined to the veterinary field to date (Williams *et al.* 1999; Innes *et al.* 2009). In response to our need to understand and ultimately control the coccidial parasites, a wide range of research strands have been pursued. In the field of genetics the relative ease with which reverse genetic strategies may be deployed has elevated *T. gondii* to model organism status, although the obligate requirement for *in vivo* passage has hindered application to most of the coccidia (Clark *et al.* 2008). Classical forward genetics strategies face similar practical hindrances,

Keywords. Coccidia; *Cryptosporidium*; *Eimeria*; genetic mapping; *Toxoplasma gondii*

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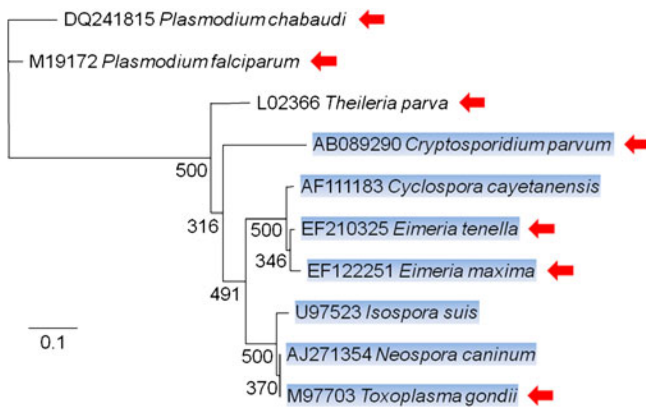

Figure 1. Phylogenetic tree illustrating the evolutionary relationship between selected apicomplexan parasites. Parasites placed within the subclass Coccidia are highlighted in blue boxes. Species for which genetic maps have been published, or whose recombination parameters have been defined, are indicated with red arrows. Maximum likelihood phylogeny constructed using phyML with substitution model TN93 and 500 bootstrap replications (Guindon *et al.* 2010).

although a small number have been undertaken with coccidial parasites.

Prior to the appearance of molecular biology, classical genetics was largely based on the use of genetic linkage to map the inheritance of specific phenotypes, where a departure from the anticipated Mendelian ratio informed upon genotype (Sturtevant 1913). Genetic mapping remains a valuable tool in studies with unsequenced, unfinished and fully sequenced genomes. Applications with coccidial parasites have included identification of genetic loci associated with developmental rate, resistance to chemotherapy, susceptibility to immune killing, virulence and as a resource for future strain-specific quantitative trait locus mapping (Shirley and Harvey 2000; Su *et al.* 2002; Boyle *et al.* 2008; Blake *et al.* 2011a). Fortuitously, coccidial parasites are haploid throughout much of their life cycles, indicating the absence of a heterozygote effect at these times. Nonetheless, all genera investigated have been found to be capable of a diploid sexual life cycle stage, during which segregation and recombination does occur (Sibley *et al.* 1992; Shirley and Harvey 2000; Tanriverdi *et al.* 2007). For many the sexual life cycle stage appears to be obligatory, although parasites such as *N. caninum* and *T. gondii* feature alternative reproductive strategies (Shirley and Harvey 2000; Su *et al.* 2002). To date, four coccidial species, representing three genera, have been investigated using genetic mapping (figure 1). The past findings and future prospects for genetic mapping with these and other coccidial parasites are reviewed here. Physical genome mapping has also been undertaken for several coccidial parasites, although these studies are not described in detail here.

1.1 *Toxoplasma gondii*

The highly successful protozoan parasite *T. gondii* has a number of robust animal models and is very amenable to genetic manipulation, making it an ideal model organism for studying the genetics of apicomplexan parasites (Sibley *et al.* 2002). The subpopulation structure of *T. gondii* differs according to geographic location, exhibiting a largely clonal structure in Europe and North America although isolates from South America are more diverse and genetically distinct (Howe and Sibley 1995; Ajzenberg *et al.* 2004; Dubey *et al.* 2008). Many South American isolates exhibit low linkage disequilibrium, indicating that these parasites have undergone frequent sexual recombination unlike the European and North American isolates (Ajzenberg *et al.* 2004).

The life cycle of *T. gondii* is typical of the heteroxenous coccidia. Sexual reproduction occurs only in the definitive host, the cat, but asexual replication can occur in a wide range of warm-blooded vertebrate hosts including mice and humans (Dubey and Frenkel 1976). Following sexual development the oocysts are shed into the environment where they contaminate food and water and infect a definitive or intermediate host. Once shed the oocysts undergo meiosis in the environment (Dubey and Frenkel 1976). The life cycle of *T. gondii* has two features that may support the persistence of a largely clonal population structure in much of the world. Firstly, sexual development and self-fertilization can take place only in the cat to yield infectious oocysts. Simultaneous infection with different *T. gondii* strains occurs relatively infrequently in cats; thus, the opportunities for genetic recombination events are limited (Dubey *et al.* 2004). Secondly, *T. gondii* oocysts can infect intermediate hosts directly via the oral route, which is unusual among the Apicomplexa as it facilitates transmission without a sexual stage (Cornelissen and Overdulve 1985).

Experimental genetic mapping approaches have exploited the ability of *T. gondii* to undergo cross-fertilization in the cat using co-infection with tissue cysts from two separate parasite strains. Initial studies revealed that clones from the type III lineage could cross and self-fertilize at relatively similar frequencies, and genetic crosses have also been undertaken with types II and III as well as types I and III (Sibley *et al.* 1992; Su *et al.* 2003). The first cross reported used the PLK and CEP strains to establish a genetic linkage map for *T. gondii* (Sibley *et al.* 1992). The map was based upon the inheritance patterns of 64 Restriction Fragment Length Polymorphism (RFLP) DNA markers that defined 11 different chromosomes (linkage groups). Preliminary linkage assignments were provided for genetic loci associated with resistance to sinefungin and adenine arabinoside on chromosomes IX and V, respectively (Sibley *et al.* 1992). However, the resolution of the original map was low and

limited by fragmented sequence information and no corresponding physical map (Khan *et al.* 2005).

The first high-resolution genetic map for *T. gondii* was developed by Khan *et al.* (2005) to facilitate forward genetic analysis. A total of 14 linkage groups, representing the 14 chromosomes, were identified comprising a total genetic size of approximately 592 cM (Khan *et al.* 2005). Several unusual features of the *T. gondii* genome were revealed during the construction of the high resolution genetic map. Firstly, high frequencies of closely adjacent apparent double crossover events were observed, possibly representing gene conversions (Khan *et al.* 2005). Such a high frequency of closely spaced double crossovers is important for mapping phenotypes by linkage analysis; however, the mechanisms by which these occurred has not been determined (i.e. true double crossovers versus gene conversions). Crossovers, but not non-reciprocal conversions, can be used to rigorously delimitate intervals spanning quantitative trait loci (QTLs) (Khan *et al.* 2005). The current density of markers is therefore likely to underestimate the number of conversions if single marker events do represent gene conversions. Increasing the density of markers on the genetic map will enhance our ability to map complex traits that differ between the three clonal lineages. Secondly, large regions of genetic homogeneity were found among archetypal clonal lineages, indicating that relatively few outbreeding events had occurred since their recent origin (Khan *et al.* 2005). Another unusual feature of the *T. gondii* genome was that strain-specific SNPs were distributed asymmetrically, with several chromosomes exhibiting surprisingly homogenous haplotypes. On some chromosomes strain-specific SNPs occurred within restricted regions. Additionally, the identification of regions with limited polymorphism interspersed with regions that showed mixed patterns of SNPs now bears comparison with the segmental structure described for the first chromosome sequenced from the *Eimeria tenella* genome (Khan *et al.* 2005; Ling *et al.* 2007). The underlying cause behind the differing patterns of SNPs is unclear, but they indicate that little recombination has occurred in large regions of the genome when compared with the ancestral *T. gondii* SNP pattern (Su *et al.* 2003). Interestingly, in experimental crosses the behaviour of the non-homogenous regions did not differ from the rest of the genome (Khan *et al.* 2005). Future investigation of *T. gondii* should aim to resolve these SNP patterns, especially as a global investigation of the distribution of SNPs across the genome detected a similar pattern (Khan *et al.* 2005).

Linkage analyses have proved effective in identifying loci associated with several drug resistances, despite the unusual features of the genome, and provide a framework for analysis of complex traits in the Apicomplexa (Khan *et al.* 2005). Genetic mapping has also been used with *T. gondii* to identify QTLs for complex phenotypes such as virulence (Su *et al.* 2002). In mice, for example, type I *T. gondii* is frequently lethal, while types II and III are less virulent (Sibley and Boothroyd 1992; Su *et al.* 2002). Following a genetic cross between a highly virulent type I strain (GT-1) and a type III strain with a much lower virulence level (CTG), a panel of recombinant progeny were produced and analysed, identifying several QTLs associated with acute virulence. Two loci conserved within type I strains were found to account for 60% (QTL chromosome VII ~50% and QTL chromosome IV ~10%) of the virulence phenotype, indicating that discrete genes common to the type I lineage strains control virulence in mice (Su *et al.* 2002). Expanding studies to include genetically distinct strains from outside Europe and North America is likely to provide opportunities for more targeted genetic mapping and QTL analysis, providing a powerful tool for analysis of the molecular basis of complex traits such as virulence in toxoplasmosis.

1.2 *Eimeria*

The term ‘coccidiosis’ can be used to describe disease arising from infection with any of the coccidia, although it is more commonly used to refer to disease caused by infection with *Eimeria* species parasites. Eimerian coccidiosis can affect all livestock species, most notably poultry, in a host-specific manner (Shirley *et al.* 2005). For example, seven *Eimeria* species are known to be capable of causing disease in the domestic chicken and at least seven species can infect the domestic turkey, but none can productively replicate in both hosts (Taylor *et al.* 2007). The identification of *Eimeria* species capable of infecting birds, mammals, amphibians, reptiles or fish suggests the occurrence of hundreds, or probably thousands, of *Eimeria* species (Taylor *et al.* 2007; Jirku *et al.* 2009; Gibson-Kueh *et al.* 2011).

Eimerian parasites have an obligate direct faecal/oral life cycle and most can cause enteric disease. Those that infect the chicken can be divided into two subgroups based upon disease symptoms. Infection with *Eimeria necatrix*, *E. tenella* and *E. brunetti* can result in haemorrhagic coccidiosis, characterised by haemorrhage of the intestinal wall most frequently associated with the early asexual phases of replication, high morbidity and high mortality. In contrast, infection with *E. acervulina*, *E. maxima*, *E. mitis* and *E. praecox* can result in malabsorptive coccidiosis, commonly associated with a lesser degree of intestinal haemorrhage. More significantly the later sexual stages of the eimerian life cycle impose an economic and welfare cost associated with poor nutrient uptake in malabsorptive coccidiosis, although morbidity and mortality can be high in severe cases. While the global impact of coccidiosis is difficult to define, estimates suggest an annual cost to the global poultry production industry in excess of US$2 billion (Shirley *et al.* 2005). Current control

primarily relies upon prophylactic chemotherapy, although drug resistance is widespread. Vaccination by infection with one or more live wild-type or attenuated parasite line is effective, but relatively expensive and uptake has been limited to the breeder and layer sectors (Shirley *et al.* 2005). Novel cost-effective control strategies are urgently required.

A requirement for *in vivo* passage has long proven to be a limiting factor for genetic studies with *Eimeria* species parasites in the absence of an effective *in vitro* system (Clark *et al.* 2008; Yan *et al.* 2009). While the production of a genetic cross is straightforward using simultaneous infection of a single chicken, the subsequent isolation of a panel of clones is technically demanding and resource intensive, relying on *in vivo* passage of a single sporocyst, the clonal unit (figure 2; Shirley and Harvey 2000). Thus, separation of hybrid progeny parasites for use in a genetic mapping panel from pure parental-type progeny (arising from cross- and self-fertilization respectively) has been an essential step to enrich for informative progeny. Enrichment has been achieved by including a double selective step during a second *in vivo* passage. During the selection phase, parent-specific deleterious selection is imposed such that progeny of either pure parental-type are severely inhibited or completely prevented from replicating, while hybrid progeny lacking the loci that confer susceptibility replicate successfully (figure 2). Potential selective barriers include anticoccidial drug resistance, escape from immune killing or early life cycle completion [precocious development, the method of attenuation used during creation of the majority of live attenuated anticoccidial vaccines (Shirley and Harvey 2000)]. The genetic map constructed for *E. tenella* used resistance to the drug arprinocid and the ability to reproduce

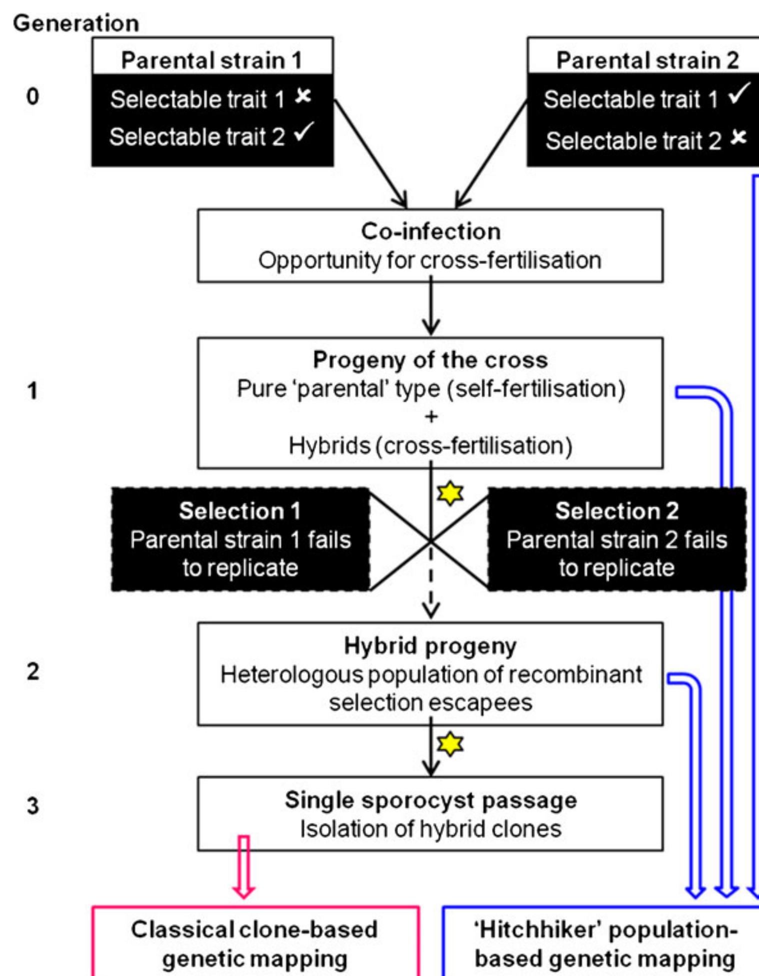

Figure 2. Strategies applied for use in genetic mapping studies with *Eimeria* species parasites. A flow-diagram representing the processes required for a classical clone-based strategy, as used for *Eimeria tenella* and *E. maxima* (Shirley and Harvey 2000; Blake *et al.* 2011b), and the population-based strategy developed and used with *E. maxima* (Blake *et al.* 2011a). Parasite generation number at each stage of the process and the occurrence of meiotic division (yellow stars) is indicated.

~18 h early to selectively isolate only hybrid progeny (Shirley and Harvey 2000). The genetic map constructed for *E. maxima* used resistance to the drug robenidine and the ability to escape strain-specific immune killing (Blake *et al.* 2011a). Importantly, selection for all of these traits occurred *in vivo*, prior to the meiotic division which takes place in the environmental oocyst stage (figure 2). Thus, it was necessary to defer imposition of the double selective barrier to a second *in vivo* passage, allowing a second meiotic opportunity and requiring the calculated recombination parameters to be presented as a range assuming an even or uneven crossover occurrence (table 1; Blake *et al.* 2011a).

Two genetic maps have been constructed for *Eimeria* species parasites, both based primarily on markers generated using Amplified Fragment Length Polymorphism (AFLP; (Shirley and Harvey 2000; Blake *et al.* 2011b)). In total 443 and 647 polymorphic DNA markers were incorporated into maps for *E. tenella* and *E. maxima* respectively, although the total map sizes differed considerably (653 and 2883.9 cM respectively). While the difference was in part explained by the larger number of genetic markers and meiotic opportunities afforded to the *E. maxima* mapping panel before cloning, the map unit per meiotic generation for this parasite was still approximately two to five times smaller than calculated for *E. tenella* (table 1). While it is clear that recombination rate varies across a genome, including both hot and cold spots of recombination, such polymorphism between closely related species was unexpected (Blake *et al.* 2011b; Katzer *et al.* 2011). Importantly, the first chromosome to be sequenced from the *E. tenella* genome revealed unusual segmentation characterized by large repeat-rich and repeat-poor regions (Ling *et al.* 2007). RFLP analyses indicated a higher recombination rate within the repeat-rich regions. While a similar structure has been described from the first large-scale genomic analyses reported for *E. maxima*, the less defined and more widespread repeat distribution may indicate a greater opportunity for recombination (Blake *et al.* 2011b).

Consideration of the populations of parasites prepared during construction of the *E. tenella* genetic map prompted a hypothesis that genetic loci under deleterious selection might be mapped using a novel hitchhiker-like mapping strategy (Blake *et al.* 2004). Comparable studies with the malarial parasite *Plasmodium chabaudi* supported the notion (Martinelli *et al.* 2005a). Thus, using the strain-specific selectable traits escape from (i) immune killing and (ii) robenidine chemotherapy, a panel of selected and unselected hybrid parasite populations were produced as described in figure 2 (Blake *et al.* 2011a). Using a set of 1122 AFLP markers found to be polymorphic between the *E. maxima* Houghton and Weybridge strains, six loci representing ~0.77% of the genome were mapped. Subsequent studies have identified two immunoprotective antigens as priority anticoccidial vaccine candidates (Blake *et al.* 2011a).

At present *E. tenella* and *E. maxima* are the only *Eimeria* species represented by genetic maps. *Eimeria* species that infect hosts other than the chicken remain under-resourced with even simple sequence data scarce. The time and resources required suggest that it is unlikely any further maps will be constructed in the near future. Nonetheless, as sequencing and genotyping technologies progress the need for genetic maps remains high as invaluable resources for forward genetics studies and the assembly of new genome sequences.

1.3 *Cryptosporidium*

The genus *Cryptosporidium* has appeared relatively cryptic in recent years. Consideration of the parasite's life cycle and morphology supported identification as a coccidian, although following molecular characterization it is now widely recognized to be more closely related to the gregarines

Table 1. Summary of genetic maps and recombination parameters published for apicomplexan parasites

Species	Estimated genome size (Mb)*	Number of chromosomes	Recombination map unit per meiosis (Kb cM^{-1})	Reference
Theileria parva	8.31	4	4.6	Katzer *et al.* 2011
Cryptosporidium parvum	**9.10**	**8**	**10–56**	**Tanriverdi *et al.* 2007**
Plasmodium chabaudi	18.8	14	13.7	Martinelli *et al.* 2005b
Plasmodium falciparum	23.26	14	17	Su *et al.* 1999
Eimeria tenella	**55**	**14**	**264**	**Shirley and Harvey 2000**
Eimeria maxima	**55**	**14**	**60–120**	**Blake *et al.* 2011b**
Toxoplasma gondii	**63**	**14**	**104**	**Khan *et al.* 2005**

*Data obtained from EUPathDB (*http://eupathdb.org*).

The coccidial parasites are shown in bold. Comparison using linear regression demonstrated a significant association between estimated genome size and recombination map unit (0.902, $p<0.01$; using the lower figures where a range has been reported) with smaller genomes featuring relatively higher rates of recombination.

(Carreno *et al.* 1999). Within the genus, parasites initially identified as distinct sub-specific genotypes have now each been accorded species status. Thus, the 'human' and 'cattle' *C. parvum* genotypes 1 and 2 are now recognized as *Cryptosporidium hominis* and *C. parvum*, respectively (Hashim *et al.* 2006). While both species are capable of causing severe human disease, *C. parvum* also effectively parasitises many other mammals including cattle and is widely considered to be an emerging zoonotic pathogen (Morens *et al.* 2004). Cryptosporidiosis, the disease caused by *C. hominis* and *C. parvum*, results in severe but usually self-limiting diarrhoea in immunocompetent individuals but can be life-threatening to the immunocompromised. Treatment is usually focused on reducing the impact of the symptoms while the infection runs its course in the absence of effective therapeutic or prophylactic chemotherapy (Abrahamsen *et al.* 2004).

As described for the *Eimeria* species, *Cryptosporidium* parasites can only be cultured *in vitro* at a very low efficiency (Karanis and Aldeyarbi 2011), indicating a requirement for *in vivo* passage in cross-fertilization studies. A further complication has been a lack of selectable traits which might be used to selectively isolate hybrid progeny of a genetic cross (Tanriverdi *et al.* 2007). The first evidence of genetic recombination in *C. parvum* was reported in 2002 using a gamma interferon knockout (IFN-γ; GKO) mouse system (Feng *et al.* 2002). Using the genetically distinct MD and UG, *C. parvum* isolates with a panel of seven informative microsatellite markers plus the polymorphic β-tubulin intron, Feng and colleagues were able to demonstrate the inheritance of hybrid haplotypes by two recombinant progeny lines (Feng *et al.* 2000, 2002). Subsequently, the same group developed their studies using the MD and TU114 isolates and a larger panel of 40 genotyping loci including single nucleotide polymorphism (SNP), mini- and microsatellite markers (Tanriverdi *et al.* 2007). Following an initial cross in chemically immunosuppressed C57BL/6 mice 16 hybrid clones were eventually selected by limiting dilution or single oocyst dosing during serial passage in GKO mice. Between four and seven generations were required to isolate each putative clonal line, with clonality inferred by the presence of a single parental allele at each of the genotyping loci. Genotypic characterisation provided a haplotype for each clone.

Building on their haplotype data, Tanriverdi and colleagues reported an average genome wide recombination rate of between 10 and 56 kbcM^{-1} for *C. parvum* (table 1). As for the *Eimeria* species a recombination rate range was required since multiple generations, and thus multiple opportunities for meiotic recombination, preceded the isolation of clonal lines (Tanriverdi *et al.* 2007). Nonetheless, the relatively high recombination rate described for *C. parvum* supports reports of high rates of recombination within many protozoan genomes. Interestingly, statistical comparison of estimated genome size and rate of recombination reveals a significant negative correlation among those apicomplexan parasites for which data is available (table 1; Pearson correlation 0.902, $p<0.01$). Since eukaryotic genome size is frequently only proportionately related to chromosome number (i.e. larger genomes are often split across larger, rather than more numerous chromosomes), this finding was not unexpected (Meznar *et al.* 2010). However, conservation of the negative relationship between genome size and recombination rate for the five species for whom data is available and whose karyotypes include 14 chromosomes hints at a more profound relationship. Possible explanations include a minimum requirement for genetic recombination per round of replication to generate genetic diversity or weaken linkage between immune relevant genetic loci, mechanisms by which a parasite population can rapidly evolve and evade immune-mediated killing.

2. Other coccidia

The life cycles of all known coccidial parasites appear to include a sexual phase, although for several such as *T. gondii*, this is not obligatory. The opportunity for cross-fertilization does provide scope for the use of classical genetic mapping, but the restricted host range and lack of *in vitro* alternatives during sexual replication imposes a practical limitation for many. For the parasites described here, this obstacle has been overcome, but for the majority the technical or economic difficulties of working with the sexual stages has discouraged mapping. Within the genus *Eimeria* five species that infect the chicken remain to be investigated. Other *Eimeria* species that infect turkeys, cattle or goats including *E. meleagrimitis*, *E. bovis* and *E. ninakohlyakimovae* can cause severe suffering and economic loss, but practical issues related to working with these hosts have proven to be limiting. Beyond the *Eimeria* the most likely candidates to be subjected to genetic mapping include the *Isospora* species such as *Isospora suis* [now widely considered to be part of the *Sarcocystidae*, not the *Eimeriidae*, but still coccidial parasites (Elsheikha *et al.* 2005)] and *N. caninum*, although progress in the near future is unlikely. Exceptions include the *Cryptosporidium* species, widely identified as important emerging pathogens (Morens *et al.* 2004). Additional parasites of interest include the *Cyclospora* and *Sarcocystis* species, but again supporting sexual replication will be a major hindrance.

3. Future prospects

Continuing developments in sequencing and genotyping technologies promise to revolutionize the analysis of genetic crosses. Next-generation sequencing already supports the

rapid and cost-effective re-sequencing of multiple genomes while medium- and high-throughput genotyping strategies based on single nucleotide polymorphisms, copy number variation or other genomic features allows detailed genome-wide characterization. Application of these technologies to genetic mapping can improve linkage group resolution, accurately identify points of crossover and fine-map loci of interest. Comparison with physical maps and genome sequences can identify hot and cold spots of genetic recombination with relevance to studies of genome structure and evolution. Strategies supporting the creation of physical genome maps include restriction mapping (Putignani *et al.* 1999), HAPPY mapping (first applied to a coccidian in the landmark whole genome map for *C. parvum*; Piper *et al.* 1998), fluorescent *in situ* hybridization and more recently optical mapping. Free access to multiple annotated sequence resources through publicly available databases such as EuPathDB and GeneDB (*http://eupathdb.org* and *http://www.genedb.org*) now provides invaluable support for genotyping studies, prompting a demand for associated bioinformatics development. Freely available software such as Map Manager QTX and MapMaker have been widely used in genetic mapping and linkage studies and remain effective tools for use with medium-throughput genotyping tools (Lander *et al.* 1987; Manly *et al.* 2001). Nonetheless, classical genetic mapping strategies still require the production of (i) a mapping panel – a process which requires the production of a genetic cross and (ii) the selection of progeny clones. The development of a population-based mapping strategy suitable for use with deleterious selectable traits of the coccidia has removed the second limiting factor for many phenotypic traits, but the requirement for sexual reproduction and cross-fertilization persists. Until *in vitro* culture of coccidial parasites including the sexual life cycle stages becomes a reality it is unlikely that many other coccidial genomes will be classically mapped. Nonetheless, the burgeoning significance of both the One Health agenda and global food security concerns point to increased interest in coccidial parasite genetics, promoting further development and application of population-based mapping strategies.

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