JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12125

Microsatellite and single-nucleotide polymorphisms indicate recurrent transitions to asexuality in a microsporidian parasite

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Keywords:

asexual reproduction;
mode of transmission;
population genetic structure.

Abstract

Assessing the mode of reproduction of microparasites remains a difficult task because direct evidence for sexual processes is often absent and the biological covariates of sex and asex are poorly known. Species with geographically divergent modes of reproduction offer the possibility to explore some of these covariates, for example, the influence of life-history traits, mode of transmission and life-cycle complexity. Here, we present a phylogeographical study of a microsporidian parasite, which allows us to relate population genetic structure and mode of reproduction to its geographically diverged life histories. We show that in microsporidians from the genus *Hamiltosporidium*, that use the cladoceran *Daphnia* as host, an epidemic population structure has evolved, most probably since the last Ice Age. We partially sequenced three housekeeping genes (alpha tubulin, beta tubulin and hsp70) and genotyped seven microsatellite loci in 51 *Hamiltosporidium* isolates sampled within Europe and the Middle East. We found two phylogenetically related asexual parasite lines, one each from Fennoscandia and Israel, which share the unique ability of being transmitted both vertically and horizontally from *Daphnia* to *Daphnia*. The sexual forms cannot transmit horizontally among *Daphnia*, but presumably have a complex life cycle with a second host species. In spite of the similarities between the two asexual lineages, a clustering analysis based on microsatellite polymorphisms shows that asexual Fennoscandian parasites do not share ancestry with any other *Hamiltosporidium* that we have sampled. Moreover, allele sequence divergence at the hsp70 locus is twice as large in Fennoscandian than in Israeli parasites. Our results indicate that asexual reproduction evolved twice independently, first in Fennoscandian and more recently in the Israeli parasites. We conclude that the independent origin of asexuality in these two populations is associated with the altered parasite mode of transmission and the underlying dynamics of host populations.

Introduction

Assessment of the genetic population structure of parasites is a powerful tool for studying the epidemiology and evolution of parasitic organisms (Tibayrenc & Ayala, 2002, 2012). Processes which cannot be observed directly, such as cryptic sexual reproduction (see Heitman, 2010) and low rates of a second mode of transmission (Wendte *et al.*, 2010), can leave clear traces in the genetic population structure and thus help to understand the evolution and epidemiology of infectious agents (Burt *et al.*, 1996; Alby & Bennett, 2010). A trait of particular concern is the mode of reproduction, as it is key for understanding the evolution of parasites. For example, populations of the asexual rust fungus *Melampsora lini* in Australia are geographically separated from those that alternate between sexual and asexual modes of reproduction (Barrett *et al.*, 2008b). In this system, both the parasite and its host, the wild flax *Linum*

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marginale, locally diverge in their modes of reproduction, that is, the obligately asexual fungus is found in selfing host populations, to which it is locally adapted, whereas flax populations carrying the facultatively sexual pathogen are mainly outcrossing, with larger diversity in pathogen infectivity and host resistance (Nemri *et al.*, 2012). Contrasting with the scenario observed in Australia, some regions of France show local coexistence of sexual and asexual lineages of *M. larici-populina,* a related rust species (Xhaard *et al.*, 2011).

It is noteworthy that a number of pathogenic organisms show 'epidemic' population structures, that is, recurrent transitions to asexuality from an otherwise sexual or outcrossing population (MacLeod *et al.*, 2001). Outbreaks of highly successful parasite genotypes are often associated with asexual reproduction (Sibley & Ajioka, 2008; Carriconde *et al.*, 2011), suggesting that asexual forms may outcompete their sexual relatives under certain ecological conditions. The degree to which asexual reproduction influences the diversity within and between populations depends on several other life-history traits. Asexual reproduction generally decreases the amount of genetic diversity within populations; its effect on the divergence between populations is associated with dispersal, and whether or not asexuality is obligate (Barrett *et al.*, 2008a). Because many parasites lack free-living stages, dispersal is usually mediated by the host. For example, tick populations of the black-legged kittiwake (*Rissa tridactyla*) show patterns of genetic structure with isolation by distance, whereas tick populations of the Atlantic puffin (*Fratercula arctica*) are only weakly structured at a large geographical scale (McCoy *et al.*, 2003). These differences are largely explained by differences in mobility and breeding characteristics of the host species (Blouin *et al.*, 1995; McCoy *et al.*, 2003).

Different processes of both the host and its parasite populations may dominate in different systems. Therefore, it is suggested that consideration of life history and spatial structure are central to understanding dynamics of host–parasite interactions (Burdon & Thrall, 2008). Parasite transmission modes are believed to play a key role in determining parasites population genetic structuring, by influencing disease dynamics, persistence and by mixing genotypes within one host. Besides vertical and horizontal transmission among hosts of the same species, horizontal transmission to other species, as part of a complex life cycle, may affect the distribution of genetic diversity in space and time (Barrett *et al.*, 2008a; Xhaard *et al.*, 2011). The ability to horizontally transmit between different host species may decrease parasite population differentiation on the one hand, but increase within-population diversity on the other (Barrett *et al.*, 2008a). Allogenic trematodes that are transmitted between freshwater and terrestrial hosts show less population structuring than autogenous trematodes transmitted only between freshwater hosts (Criscione & Blouin, 2004). Horizontal transmission involving multiple hosts may also reduce inbreeding. For example, it has been hypothesized that it is advantageous for trematodes to keep a second intermediate host in their life cycle, and by this avoid inbreeding, rather than going directly from snail to definitive host (Rauch *et al.*, 2005). In this system, the second intermediate host 'collects' different cercarial clones over time, and the different parasite genotypes engage in sexual reproduction after a definitive host ingests the second intermediate host (Rauch *et al.*, 2005). Nonetheless, reducing the number of hosts within a parasite's life cycle can be ecologically advantageous, as for example, in situations where one host is very rare or absent (Poulin & Cribb, 2002) or when parasites face trade-offs during adaptation to different hosts (Ebert, 1998; Davies *et al.*, 2001).

The complexity of life cycles may not only affect rates of mixing and thus influence inbreeding but it may also influence the mode of reproduction of a parasite. In parasites with complex life cycles, distinct modes of reproduction are often specific to different hosts in the life cycle, with asexual reproduction in one host and sexual reproduction in another host species (Cox, 2009). Thus, in cases where a second host is not an obligate part of the life cycle, the availability of a second host necessary to complete the sexual cycle can dramatically influence the amount of population diversity. For example, studies on allozyme polymorphisms showed higher allele richness in sexual populations of the wheat stem rust *Puccinia graminis,* where the second host (*Berberis vulgaris*) is present, than in regions where sexual reproduction had ceased about 50 years back with the eradication of the alternate host (Burdon & Roelfs, 1985). In summary, the population genetic structure of parasites results from a complex interplay between different life-history traits, including mode of reproduction, mode of transmission and life-cycle complexity (Criscione *et al.*, 2005).

Microsporidia are obligate intracellular parasites of a wide range of vertebrate and invertebrate species. Because they vary strongly in their life history (Dunn & Smith, 2001), microsporidians are good models for investigating how parasite transmission, mode of reproduction and life-cycle complexity influence population genetic structure. Furthermore, population genetic patterns may be used to understand when and under which circumstances these distinct life-history strategies evolve. Microsporidia have shown independent transitions to asexuality within major clades, suggested to have been accompanied by the loss of a second host, that is, by a switch from an indirect to a direct life cycle (Ironside, 2007). Members of the phylum Microspora have vertical, horizontal or mixed-mode transmission cycles, and sometimes transmission varies even among closely related species (Dunn & Smith, 2001; Smith, 2009). The extreme diversity of microsporidian life

cycles provides the unique opportunity to perform comparisons between closely related species with different modes of transmission and reproduction. However, there are no polymorphic genetic markers available outside the realm of microsporidia that infect humans (Xiao *et al.*, 2001; Feng *et al.*, 2011), a major difficulty in assessing their genetic structure and epidemiology (Mathis *et al.*, 2005). As microsporidian infections in humans are considered to be opportunistic (Mathis *et al.*, 2005), they may not represent a naturally evolved situation, giving a distorted picture of microsporidian population structure and diversity. The accumulation of genomic data from several microsporidia species allows us now to engage in population genetic studies with highly polymorphic markers.

We have recently shown that two microsporidian parasites of the cladoceran *Daphnia* differ in their modes of reproduction (Haag *et al.*, 2013). *Hamiltosporidium tvaerminnensis* (formerly called *Octosporea bayeri*) that is only found in Fennoscandia, reproduces asexually and its sister species *H. magnivora* reproduces sexually. Previous studies have shown that *H. tvaerminnensis* transmits both vertically from mother to offspring and horizontally from *Daphnia* to *Daphnia* (Mangin *et al.*, 1995; Vizoso & Ebert, 2004), whereas *H. magnivora* is only vertically transmitted in *Daphnia*, and supposedly requires a second, yet unidentified, host (Mangin *et al.*, 1995). In our previous study (Haag *et al.*, 2013), based on samples from the north and north-west of Europe, we speculated that sexual reproduction occurs in the alternate host of *H. magnivora*, being therefore linked to its mode of transmission (Fig. 1). Since then, we obtained samples of *D. magna* individuals infected with *Hamiltosporidium* spp. from other localities across Europe and the Middle East. For the two largest samples obtained (Israel and Iran), experiments showed that, besides vertical transmission, horizontal transmission among *D. magna* is possible in the Israeli population (Goren & Ben-Ami, 2013), but not in the Iranian population (Elham Sheikh-Jabbari, unpublished results). Using newly developed microsatellite markers, and single-nucleotide polymorphisms, we test the hypothesis that horizontal transmission among *Daphnia* is associated with asexual reproduction in *Hamiltosporidium* natural population.

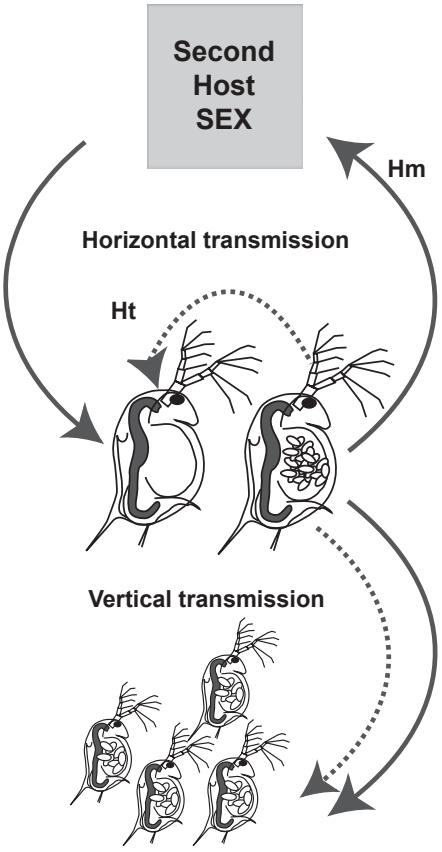

Fig. 1 Transmission modes in *Hamiltosporidium* spp. *H. tvaerminnensis* (Ht) is both horizontally and vertically transmitted among *Daphnia*, whereas *H. magnivora* (Hm) is supposed to require an alternate host for horizontal transmission. We test the hypothesis that this second host is required for sexual reproduction.

Materials and methods

Obtaining Hamiltosporidium samples

Populations of *Hamiltosporidium* sp are found in Europe and near Asia. *H. tvaerminensis* is found in *D. magna* inhabiting rock pools of the Skerry Islands in the Baltic Sea, where approximately 50% of *D. magna* populations are infected (Ebert *et al.*, 2001). In previous studies, this parasite was named *Octosporea bayeri*, but has since been classified in the newly created genus *Hamiltosporidium* due to its particular morphological and ecological features (Haag *et al.*, 2011). *H. magnivora* had previously been included in the genus *Flabelliforma*, and is more generalist than *H. tvaerminnensis:* it is also found in *D. pulex* and *D. longispina*; its geographical distribution is wider, having been found in Central and Eastern Europe, where prevalence in *Daphnia* populations seems to be low. In two *D. magna* populations of Belgium, for instance, it was shown that the maximal prevalence of *H. magnivora* is around 6% (Decaestecker *et al.*, 2005). In contrast, *H. tvaerminnensis* frequently reaches 100% prevalence in Baltic rock pool populations (Lass & Ebert, 2006; Lass *et al.*, 2011).

Fifty-one *Hamiltosporidium*-infected *D. magna* individuals (parasite isolates) were obtained from Sweden, Finland, Germany, Belgium, Southern France, Italy, Israel and Iran (Table S1). All samples were used for DNA extraction and population genetic analyses. Because the parasite was rare in several *Daphnia* populations that have been surveyed, three types of parasite samples have been used: (i) infected adult *D. magna* obtained from natural populations; (ii) infected adult hosts obtained by

hatching ephippia collected from natural *D. magna* populations; (iii) Experimentally infected *D. magna* individuals. Experimental infections were obtained by exposing young *D. magna* to sediments collected from a pond and suspended in water. We have made experimental infections using *D. magna* clones native to the pond from where the sediments were collected.

Generating Hamiltosporidium genetic markers

DNA of individual *Hamiltosporidium*-infected *Daphnia* was purified using the DNeasy blood and tissue kit (Qiagen) and used as template for PCR. SNP detection was performed by sequencing the amplicons of three nuclear markers, that is, alpha tubulin, beta tubulin and hsp70, as previously described (Haag *et al.*, 2013). These three genes were selected for SNP analyses because they have shown fixed heterozygosity in *H. tvaerminnensis*, but not in *H. magnivora*. In addition, we used the *H. tvaerminnensis* genome (GenBank ACSZ00000000) to search for short tandem repeats using the SciRoKo software (Kofler *et al.*, 2007). Seven microsatellite loci, that showed a good performance in PCR experiments, were chosen for fragment analyses (Table S2). Briefly, each *Hamiltosporidium*-specific microsatellite locus was amplified using 50 ng of template DNA, 10 pmol of the reverse primer, 1 pmol of the forward primer (containing a M13F probe 5′ tail, see Table 1), 9 pmol of the fluorescently labelled (6-carboxy-fluo-rescine, FAM) M13F primer, 200 μM dNTPs, 1 U *Taq* DNA Polymerase (Sigma), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin in a reaction volume of 10 μL. The PCR profile consisted of an initial denaturation step at 95 °C for 2 min, over 35 amplification cycles (annealing at 50 °C for 30 s, extension at 72 °C for 30 s, denaturation at 95 °C for 30 s) and a final extension at 72 °C for 10 min. Fragments were size separated and analysed using an AB3130 capillary sequencer (Life Technologies Corporation, Carlsbad, CA, USA).

Analyses of Hamiltosporidium genetic structure and history

Two data sets were generated for population genetic analyses: one alignment with 49 concatenated SNPs of alpha tubulin, beta tubulin and hsp70 genes, and the combined genotypes of seven microsatellite loci (see Tables S2 and S3). Because microsatellites evolve much faster than SNPs, and because a large proportion of SNPs in our study are linked, whereas microsatellite loci are most likely segregating independently, the two types of data were analysed separately. Observed and expected heterozygosities for SNPs and microsatellites were calculated using Arlequin version 3 (Laurent Excoffier *et al.*, 2007). The 49-SNPs data set was imported into Network version 4 (available at http://www.fluxus-engineering.com/). Heterozygous sites were resolved using the 'replace ambiguous sites' tool of the software that replaces any ambiguity by the most frequent character state at the respective site of the alignment. The resolved alignment was then used to produce a median-joining network (Bandelt *et al.*, 1995), with default parameters and weighting transversions twice as much as transitions.

Hamiltosporidium allele phylogeny was also inferred from hsp70 sequences (867 bp) obtained as described above. An alignment of these amplicons containing 25 heterozygous SNPs was phased using the 'open unphase' tool implemented in DnaSP version 5 (Librado & Rozas, 2009). Isolates from Finland (OER-3-3 and SML-28), Belgium (Fm-K1-1 and Fm-T1-2) and Israel (H6 and IPE) had their phases confirmed by cloning the respective amplicons into plasmid vectors as previously described (Haag *et al.*, 2013). To evaluate allele sequence divergence, the genetic distance between hsp70 alleles was calculated for each *Hamiltosporidium* isolate using Mega 5 (Tamura *et al.*, 2011). Genetic distances were grouped (see below) and compared by the Wilcoxon rank sum test using JMP 9 (SAS Institute Inc., Cary, NC, USA). A Neighbour-Joining allele phylogeny was inferred using the Kimura two-parameter model and tested using a bootstrap of 1000 replicates.

Microsatellite data of all seven loci were used to infer population genetic structure using the program Structure version 2.3.1 (Pritchard *et al.*, 2000). The number of clusters (K) of highest likelihood was inferred using the Structure Harvester software (available at http://taylor0.biology.ucla.edu/structureHarvester/#). We tested K values ranging from 1 to 6, and simulations were repeated 10 times for each value of K. The probability of inclusion of each individual in one of the inferred clusters (Q) was estimated using the admixture model with default parameters, a MCMC of 100 000 replications and a burnin of 10 000 steps.

Both SNP and microsatellite data sets were used for analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992), at different levels and models of hierarchical subdivision. A 'population' is here referred to as the isolates sampled from a particular geographical location: Fennoscandia (Skerry Islands of Finland and Sweden, $n = 24$), Germany ($n = 2$), Belgium ($n = 5$), France ($n = 2$), Italy ($n = 1$), Iran ($n = 8$), Israel ($n = 5$).

Table 1 Observed (Ho) and expected (He) heterozygosities in the four largest *Hamiltosporidium* samples analysed in our study. Shaded areas indicate samples with fixed heterozygosity.

		SNPs		Microsatellites	
	n	Ho	He	Ho	He
Fennoscandia	23	.93	0.48	0	0.09
Israel	5	.91	0.52	1.00	0.56
Belgium	5	∪.35	0.36	0.29	0.46
Iran	8	0.30	0.31	0.52	0.52

Populations were grouped according to two different models: (i) reproduction mode; or (ii) coancestry (see Fig. 2a). The 'reproduction mode' model separates populations into asexual, that display fixed heterozygosity, and sexual, showing random assortment of alleles. The 'coancestry' model (as inferred by Structure) separates populations in other two groups, that is, those obtained from Fennoscandia separated from all remaining samples. Individuals belonging to these distinct coancestry clusters significantly differ in their genetic composition at microsatellite loci (see below).

Daphnia mitochondrial polymorphisms

Hosts were haplotyped by using partial sequences (675 bp) of the mitochondrial cytochrome oxidase 1 gene (cox1) as marker. General primers designed for cladocerans (Folmer *et al.*, 1994) were used in PCR reactions performed in a final volume of 50 μL containing 50 ng of template DNA, 20 pmol of each primer, 200 μM dNTPs, 1 U *Taq* DNA Polymerase (Sigma), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin. The PCR profile consisted of a 'touch down' procedure with an initial denaturation step at 95 °C for 5 min, over 10 amplification cycles in which the annealing temperature decreased 1 °C every cycle, starting from 50 °C for 30 s, extension at 72 °C for 60 s, denaturation at 95 °C for 30 s, followed by 15 more cycles with annealing temperature of 40 °C and a final extension at 72 °C for 5 min. Amplicons were purified and sequenced directly (Microsynth, Balgach, Switzerland). Sequences were analysed for quality, aligned using CodonCode Aligner version 4 (CodonCode Corporation, Centerville, MA, USA) and the alignment of 40 sequences containing reeds of good quality were used to build a network based on 37 SNPs, as described above for the *Hamiltosporidium* data.

Results

We found 49 variable sites (SNPs) in alpha tubulin, beta tubulin and hsp70 genes among 51 *Hamiltosporidium* isolates obtained from Europe, Israel and Iran (Table S3). In addition, we genotyped the same isolates for seven microsatellite loci (Table S2). The North European samples (*H. tvaerminnensis*) were monomorphic for all but one locus (ms2863) that showed an alternative allele in a single isolate (SEG-9-15; see Table S4). The other samples showed variable levels of polymorphism at different loci, the most variable being ms3091 (AAT motif), with eight alleles, and the least being ms756 (AATA motif), which was monomorphic. In the most variable microsatellite locus, we did not find any shared allele between the northern and the remaining samples.

The majority of hosts analysed in our study harboured single *Hamiltosporidium* infections, except three *Daphnia* clones from Iran that seemed to contain mixed genotypes, as more than two alleles have been found for some microsatellite loci (see Table S4). These isolates also showed double peaks of different heights on sequencing chromatograms (not shown), an indication that distinct genotypes were present in unequal proportions in these host individuals. By cloning PCR products from a reasonable number of isolates, we have previously shown that equivalent sequencing peak heights are good indicators of true heterozygosity, as opposed to mixed infections (Haag *et al.*, 2013). To avoid ambiguity, we excluded these isolates from the SNP and microsatellite data sets in the further analyses presented here.

Geographic structuring of nucleotide and microsatellite polymorphisms

The *Hamiltosporidium* samples analysed in our study showed fixed heterozygosity in two geographical regions: Fennoscandia and Israel (Table 1). *H. tvaerminnensis* (Fennoscandia sample) showed fixed heterozygosity only at SNPs (and almost complete monomorphism at the microsatellite markers), whereas the Israeli samples showed fixed heterozygosity for both SNP and microsatellite markers. Israeli and Fennoscandian *Hamiltosporidium* parasites are phylogenetically related and have similar modes of transmission and reproduction, sharing alpha tubulin, beta tubulin and hsp70 alleles. Single-nucleotide polymorphisms clearly distinguish asexual from putatively sexual populations (Fig. 2b). In contrast, microsatellite data separate our samples in two clusters with different assignment of populations ($K = 2$, Fig. 2c). One cluster including the Fennoscandian populations that we previously regarded as a separate species, *H. tvaerminnensis* (Haag *et al.*, 2011), and another cluster with all remaining samples, including the asexual parasites from Israel.

To confirm that distinct models of population structure best explain the distribution of genetic diversity in each data set, we performed AMOVA on SNP and microsatellite data at distinct hierarchical levels considering two alternative models of *Hamiltosporidium* population structure. The model that best explains our SNP data is one that separates asexual (Fennoscandia and Israel) from putatively sexual populations (Germany, Belgium, France, Iran and Italy), ($F_{CT} = 0.188$, $P = 0.00098$, see Table 2). Nevertheless, according to this model, most variation remains within populations (54.73%), and by adding the individual level (a single host individual) to the analysis (not shown), 83.32% of the variation is found within individuals, reflecting considerable amounts of heterozygosity. The genetic structure that best fits our microsatellite data is based on coancestry (Table 2; $F_{CT} = 0.448$, $P < 0.00001$), separating *H. tvaerminnensis* (Fennoscandian population) from the remaining samples (Fig. 2c). According to this model,

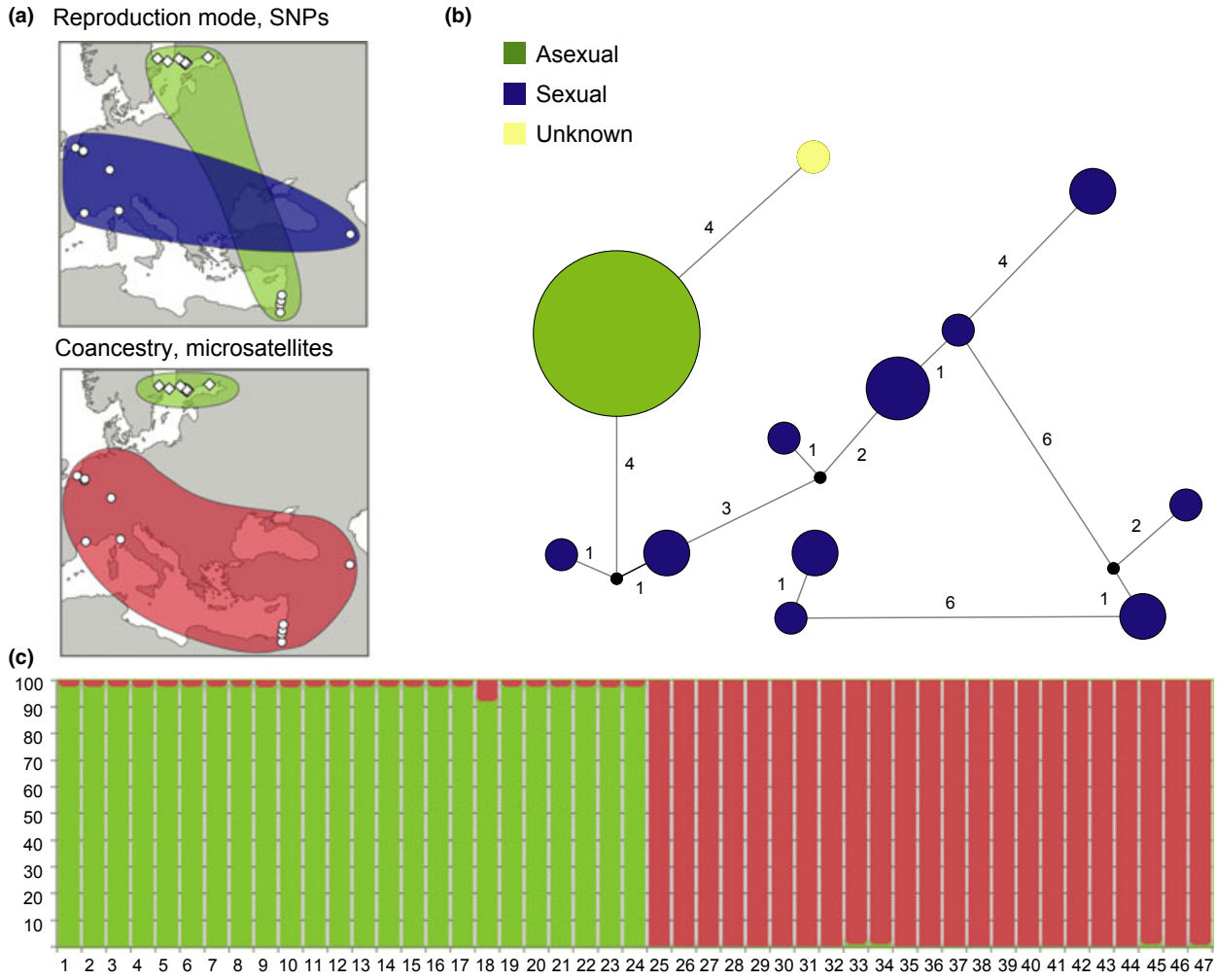

Fig. 2 Analyses of population genetic structure of *Hamiltosporidium* spp. (a) Geographical distribution of *Hamiltosporidium* samples, plotted on two models of genetic structure. Circles represent isolates sampled from the same location, diamonds represent groups of islands located in close proximity, which have been pooled for illustration (see Table S1 for the geographical coordinates of each location). The two alternate groupings refer to the models of genetic structure inferred from SNP data or from microsatellite data. (b) Genotype network based on *Hamiltosporidium* SNP data. Circle sizes are proportional to their frequency and are coloured according to the parasite mode of reproduction. Numbers on each branch represent the number of evolutionary steps (nucleotide substitutions). (c) Individual *Hamiltosporidium* isolates (1–24, from Fennoscandia; 25–47 from the remaining regions; refer to Table S4 for details) are clustered using an admixture model based on microsatellite data; the population structure with highest likelihood ($K = 2$) is shown. *Y*-axis indicates the probability of population assignment to each cluster.

44.83% of the total microsatellite allelic diversity that has been sampled is found between Fennoscandia and the remaining isolates. By adding the individual level to the analyses (not shown), 26.63% of the total variation is found within individuals.

Phylogenetic analysis

We further assessed the phylogenetic history of *Hamiltosporidium* by phasing the hsp70 alleles in the 47 isolates that constituted single infections, generating two alleles for each isolate. For the Fennoscandian and the Israeli parasites, we confirmed the phases by molecular cloning. A phylogeny of all hsp70 alleles is shown on Fig. 3. There are two major clades in the allele tree, and those that have suffered clonal expansion in the asexual Fennoscandian and Israeli populations are part of the same clade (see Fig. 3a). Nonetheless, the phasing process unravelled genetic differences between these two asexual parasites. The majority of the hsp70 genotypes of isolates from Western Europe contain alleles from clade 1, whereas the alleles from clade 2

Table 2 Analysis of molecular variance (AMOVA) of single-nucleotide and microsatellite polymorphisms based on the two models of population structure shown in Fig. 2a. Shaded areas indicate the population structures that fit each data set best.

		Coancestry			Reproduction mode		
		% Var	Fixation index		% Var	Fixation index	
SNPs	Among groups	−1.89	F_{CT}	−0.019 N	18.78	F_{CT}	0.188*
	Among populations within groups	43.49	F_{SC}	0.427*	26.49	F_{SC}	0.326*
	Within populations	58.39	F_{ST}	0.416*	54.73	F_{ST}	0.453*
Microsatellites	Among groups	44.83	F_{CT}	0.448*	14.72	F_{CT}	0.147 NS
	Among populations within groups	28.42	F_{SC}	0.515*	55.99	F_{SC}	0.656*
	Within populations	26.75	F_{ST}	0.732*	28.29	F_{ST}	0.707*

*$P < 0.01$.
NS, not significant.

are mostly found in Central and Northern Europe as well as in the Middle East. All asexual Israeli parasites are heterozygous for an allele found in Italy (IsR-1) and another found in a single Fennoscandian isolate (FHS181-2). Furthermore, the divergence between alleles within *H. tvaerminnensis* isolates is significantly larger than within the Israeli and all remaining isolates (Fig. 3b). In contrast with the disjunct geographical distribution of clade 1 and clade 2 hsp70 alleles from the parasite (Fig. 3c), there is no clear geographical pattern of distribution for the host mitochondrial haplotypes. We found 37 variable sites in our cox1 data sequences from 40 *D. magna* individuals that belong to 14 distinct haplotypes. One of those haplotypes is by far the most abundant and geographically widespread (see Fig. S1).

Discussion

We have sampled *Hamiltosporidium* parasites from different regions in Europe and the Middle East, and genotyped them for SNP and microsatellite markers. Samples from both Fennoscandia and Israel showed fixed heterozygosity. This was known for the Fennoscandian population (*H. tvaerminnensis*), and was interpreted as being a result of obligate asexuality (Haag *et al.*, 2013). In accordance with the predictions of our hypothesis (Fig. 1), the *Hamiltosporidium* parasites from these two geographical regions are unique for being vertically and horizontally transmitted among *Daphnia* hosts (Vizoso & Ebert, 2004; Goren & Ben-Ami, 2013). Isolates from the remaining localities show variable combinations of SNPs and microsatellite alleles, suggesting that polymorphisms had been shuffled via sexual reproduction. In pathogenic species of fungi that undergo varying degrees of sexual reproduction, it was found that sexual populations show larger diversity of virulence phenotypes, as well as higher mutability of avirulence genes, implying a higher coevolutionary potential of sexual as compared with asexual pathogens (Barrett *et al.*, 2008a; Daverdin *et al.*, 2012). Nonetheless, sex has been recently lost in several lineages of red fungi (basidiomycetes; Coelho *et al.*, 2011), and certain microsporidia are believed to have lost sexual reproduction via the switch to a direct mode of transmission (Ironside, 2007). Asexual reproduction has the advantage of allowing the preservation of adaptive genome configurations, but it apparently does not persist for long periods of evolutionary time, that is, phylogenetic lineages of asexual pathogens are frequent, and most of them have a recent origin (Coelho *et al.*, 2011; Sun & Heitman, 2011).

Population genetics and recurrent asexuality

The two types of population genetic data that we have analysed in our study (SNPs and microsatellites) revealed apparently different patterns of genetic structuring. At the fast-evolving microsatellite markers, we found a high degree of differentiation between the Fennoscandian and all remaining *Hamiltosporidium* populations. In contrast, at the level of slowly evolving nucleotide polymorphisms, we found a high similarity between Israeli and Fennoscandian parasites. Slowly evolving genetic markers reveal deeper phylogenetic relationships, whereas fast-evolving markers allow inferences of recent population dynamics and divergence (Avise, 2000; Hewitt, 2001). The similarity observed between the Fennoscandian and Israeli populations for the slowly evolving markers was also found in phylogeographical studies based mtDNA polymorphisms of the host of our parasite, *D. magna* (De Gelas & De Meester, 2005). Accordingly, relatedness between Israelian and Fennoscandian parasites could result from parasite specialization or from shared history of parasite and host. However, mapping parasite mode of reproduction on the *D. magna* network obtained with our cox1 data reveals that asexual *Hamiltosporidium* parasites are found in a variety of distantly related host haplotypes (Fig. S1).

We believe that the asexual genotypes found in these two populations have originated independently. All Israeli parasites are heterozygous for hsp70 alleles belonging to clade 2, that is, for one allele that is

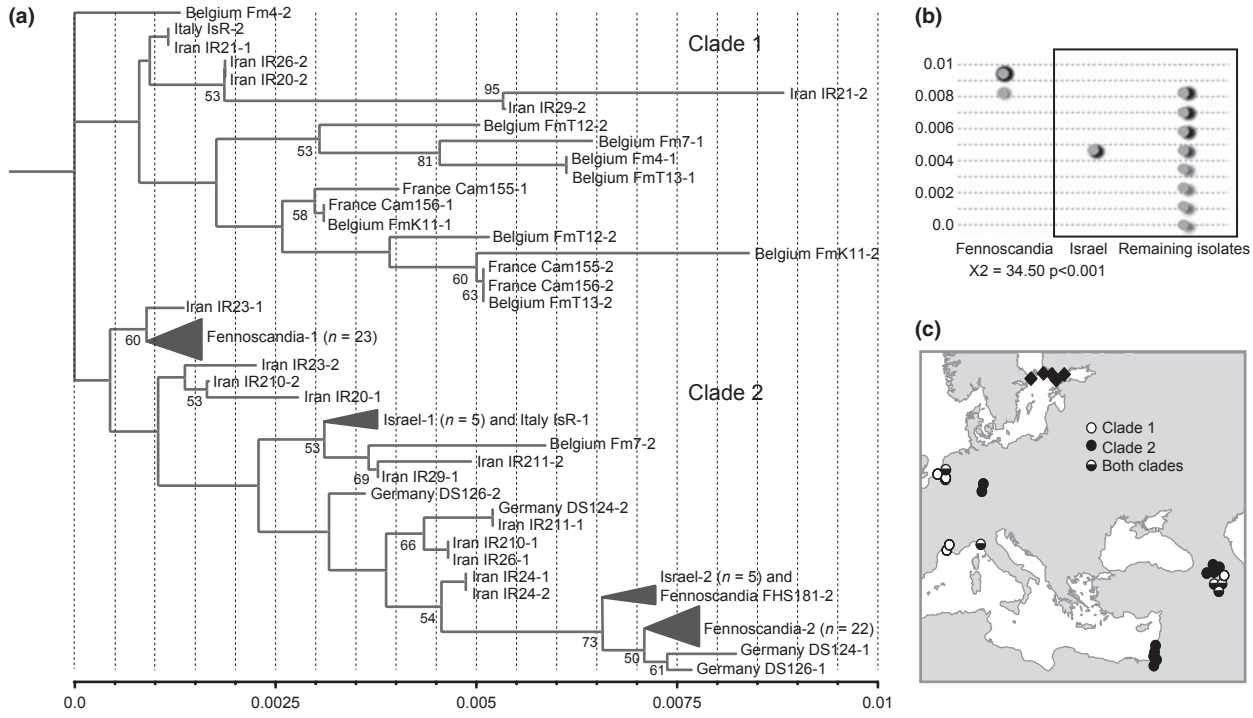

Fig. 3 *Hamiltosporidium* allele sequence divergence at the hsp70 locus. (a) Neighbour-joining tree of hsp70 phased alleles that separate in two major clades. Alleles are labelled with the corresponding isolate code and geographical origin (see Table S1 for details), followed by numbers 1 or 2. In Fennoscandia and Israel, asexual reproduction leads to the expansion of identical alleles within populations (triangles; n indicates the number of times an allele is found in the respective location). Bootstrap values above 50% are displayed, and the scale represents the amount of genetic change along the tree branches. (b) Genetic distance between the two alleles of each isolate. Isolates were separated in three groups: Fennoscandia, Israel and the remaining samples. The shade intensity of plots is proportional to the number of isolates. The result for the Wilcoxon rank sum test is shown on the bottom; groups within a square differ significantly from Fennoscandia. (c) Geographical distribution of *Hamiltosporidium* hsp70 genotypes, separating alleles in each of the two clades shown in (b).

shared with a single isolate from Fennoscandia and another that is shared with the Italian isolate. Moreover, allele sequence divergence at the hsp70 locus is twice as large in the Israeli than in the Fennoscandian genotype. If allele sequence divergence is caused by the lack of meiotic recombination, it should be proportional to the time since sexual reproduction has been lost (Meselson effect; Judson & Normark, 1996). Our results together suggest that asexuality is recurrent in *Hamiltosporidium*, having originated more recently in the Israeli population than in *H. tvaerminnensis*, from Fennoscandia. The conclusion of independent origin of asexuality would explain (i) the fixed heterozygosity of different SNPs in populations from these two geographical regions, (ii) the different patterns of microsatellite diversity (monomorphism in Fennoscandia and fixed heterozygosity in Israel) and (iii) the shared microsatellite ancestry of sexual populations from Western Europe and Iran with asexual parasites from Israel.

Recurrent evolution of asexual parasite lineages had already been described for African trypanosomes, that seem to show an 'epidemic population structure' (MacLeod *et al.*, 2001) as originally proposed for pathogenic bacteria (Maynard-Smith *et al.*, 1993). Under this model of population structure, highly successful genotypes regularly arise and expand from an otherwise panmitic population. An epidemic genetic structure would be expected for a pathogen during an epidemic, in the ecological sense (Maynard-Smith *et al.*, 1993), but is not necessarily restricted to that ecological scenario, as it could be stable in time and/or space, that is, the ecological definition of 'epidemic' is different from the genetic definition (MacLeod *et al.*, 2001). In African trypanosomes, the epidemic clones seem to originate each time the parasite becomes infective to humans (MacLeod *et al.*, 2001). In *Hamiltosporidium*, the Fennoscandian and Israeli epidemic clones seem to have originated at different times and are linked by their ability to transmit both horizontally and vertically among *D. magna*.

The transition from sexual to asexual reproduction in plants and animals is frequently associated with a major change in geographical distribution, often biasing asexual lineages towards environments that were severely affected by the glacial cycles of the Pleistocene (Kearney, 2005). The Scandinavian peninsula was entirely

covered by ice until about 12 000 years ago (Harff *et al.*, 2011) and the distribution of wet and arid land in Israel suffered major changes during late Pleistocene, due to the influence of strong Mediterranean winds (Enzel *et al.*, 2008). Such biases in the geographical distribution of asexuals might be a consequence of selection for the demographic effects of asexuality as asexuals have a two-fold advantage in their reproductive output (Maynard-Smith, 1978), and may thus colonize empty habitats more efficiently; geographical biases could also reflect a hybrid advantage as many asexual lineages result from hybridization (Kearney, 2005). On the other hand, multiple and recent transitions to asexual reproduction in different taxa suggest that asexuality might be contagious, and have a simple genetic basis (Paland *et al.*, 2005; Sandrock & Vorburger, 2011). A hybrid origin cannot be ruled out for the Israeli *Hamiltosporidium* population as it shows fixed heterozygosity at two microsatellite loci, and the hsp70 locus shows fixed heterozygosity for alleles found respectively in Italy and Fennoscandia. Nevertheless, if the mode of reproduction in *Hamiltosporidium* has a simple genetic basis, it could either have resulted from a contagious process, or from independent colonization of empty habitats by asexual genotypes proceeding from distinct Pleistocene refugia. Similarly, the genetic structure of two species of anther smut pathogens (*Microbotryum lychnis-dioicae* and *M. silenes-dioicae*) in Europe is largely explained by migrations from separate glacial refugia localized in the Mediterranean region (Vercken *et al.*, 2010).

Genetic diversity and the interplay of demographic history, modes of reproduction and transmission

All known populations of *Hamiltosporidium* are transmitted vertically to asexual and sexual host eggs, with high efficiency. In contrast, populations differ in the way by which the parasite is horizontally transmitted. *Hamiltosporidium* probably requires a second host to complete the sexual cycle (Mangin *et al.*, 1995), and the spores that might be used for horizontal transmission to that host are not infective to *Daphnia*. The asexual forms of *Hamiltosporidium* produce environmental spores that allow direct transmission among *Daphnia*. The ability of being transmitted both horizontally and vertically is probably key for the persistence of *H. tvaerminnensis* in the *D. magna* metapopulation system of the Skerry islands (Vizoso *et al.*, 2005), where local extinction rates of the *Daphnia* host have been estimated to reach 20% per year (Pajunen & Pajunen, 2003). Our data on slowly evolving markers show a close phylogenetic relatedness between the Fennoscandian and Israeli *Hamiltosporidium* populations, and both seem to produce an environmental spore type that can be transmitted horizontally among *Daphnia*. All Israeli isolates that we have studied were sampled in temporary rain pools, suggesting a habitat with similar host metapopulation dynamics. Certain habitat-specific factors might have selected for parasite genotypes that are capable of horizontal transmission. Without the need of a second host in the life cycle, the parasite might have been able to spread to new habitats and reach locally very high prevalence (Ebert *et al.*, 2007). This advantage came at the cost of losing meiotic recombination.

It is possible that the clonal nature of these microsporidians is primarily associated with the capacity of horizontal transmission and the underlying host population dynamics, rather than a physiological incapacity of sexual reproduction. For those sexual populations where mode of transmission was investigated in detail, horizontal transmission among *Daphnia* was not possible (Elham Sheikh-Jabbari and Karen L. Haag, unpublished results). Provided that the asexual parasite lineages retain their ability of being transmitted horizontally to both the second host and to other *Daphnia*, asexuality could result simply from the absence of the second host, but without loss of the ability to mate. As the second host is not known, we cannot test this hypothesis. Similarly, pathogenic fungi have been shown to skip their sexual phase by switching to a direct mode of transmission in the absence of a suitable alternate host (Burdon & Roelfs, 1985). It is not always easy to disentangle the causes of variation in parasite life-history traits, as they often result from the interaction between host and parasite genotypes. In the microsporidian *Edhazardia aedis* that infects mosquitos (*Aedes aegypti*), horizontal transmission is enhanced in hosts selected for late pupation (Agnew & Koella, 1999; Koella & Agnew, 1999). On the other hand, life-history traits of the rust fungus *Puccinia coronata*, a pathogen of oats (*Avena sativa*), were shown to be differentially affected by the genotypes of pathogen and host, whereas the fungus genotype has a stronger effect on the early stage of infection efficiency, host genotypes most strongly affect the later pathogen life-history traits (Bruns *et al.*, 2012). We did not find any association between *Hamiltosporidium* modes of transmission and the host cox1 haplotypes, suggesting that it is primarily a parasite-determined trait. If parasite populations would harbour genetic variation for infectivity to *Daphnia* or to the alternate host, they would expect to show polymorphism in their modes of reproduction, as is seen for some rust fungi (Xhaard *et al.*, 2011). However, we did not find *Hamitosporidium* genotypes from the same population showing different modes of transmission. The amount of genetic diversity for life-history traits may vary across species, but transitions from sexual to asexual reproduction via the switch from an indirect to a direct mode of transmission have been reported for other microsporidian clades (Ironside, 2007).

Distinct modes of transmission may lead to different parasite population structuring (Criscione & Blouin, 2004; Wolinska *et al.*, 2011), and parasites that rely on a larger number of hosts to complete their life cycle

may harbour more genetic diversity (Rauch *et al.*, 2005). This is because horizontal transmission among host species with distinct habits creates more opportunities for parasite admixture and gene flow. This is the first time that we find *D. magna* individuals harbouring *Hamiltosporidium* mixed infections (Iranian population). Mixed infections with different genotypes have been suggested for other microsporidians, for example, *Enterocytozoon bieneusi* in humans (Akinbo *et al.*, 2012) and swine (Reetz *et al.*, 2009), and *Ordospora colligata* in *Daphnia* (Wolinska *et al.*, 2009). However, as these studies were based on rRNA markers that are usually duplicated within single genomes, mixed infections could have been confounded with diversity among paralogues of a single parasite clone (see, for instance, O'Mahony *et al.*, 2007). It was suggested that a direct life cycle would lead to a higher differentiation among populations (Wolinska *et al.*, 2011). Indeed, *Enterocytozoon bieneusi*, a microsporidian with direct life cycle, shows highly structured populations in the human host, with no recombination (Li *et al.*, 2012). However, its epidemic nature that leads to highly divergent populations is rather a consequence of obligate asexuality. We show that asexual reproduction in *Hamiltosporidium* is associated with its mode of transmission, which in turn seems to be correlated with the host population dynamics. Our study illustrates how the amount and spatial distribution of genetic diversity in parasite populations is influenced by interplay among life-history traits that may evolve in response to the environment.

Acknowledgments

Thanks to Dita Vizoso for her help with the illustrations. Special thanks to Jürgen Hottinger, Jason Andras and Christoph Haag for providing us *Hamiltosporidium*-infected *Daphnia*, and to Urs Michael Stiefel for his help with the PCR, cloning and sequencing. This study was supported by the Swiss National Science Foundation.

References

Agnew, P. & Koella, J.C. 1999. Life history interactions with environmental conditions in a host–parasite relationship and the parasite's mode of transmission. *Evol. Ecol.* **13**: 67–91.

Akinbo, F.O., Okaka, C.E., Omoregie, R., Dearen, T., Leon, E.T. & Xiao, L. 2012. Molecular epidemiologic characterization of *Enterocytozoon bieneusi* in HIV-infected persons in Benin City, Nigeria. *Am. J. Trop. Med. Hyg.* **86**: 441–445.

Alby, K. & Bennett, R. 2010. Sexual reproduction in the *Candida* clade: cryptic cycles, diverse mechanisms, and alternative functions. *Cell. Mol. Life Sci.* **67**: 3275–3285.

Avise, J.C. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press, Harvard.

Bandelt, H.J., Forster, P., Sykes, B.C. & Richards, M.B. 1995. Mitochondrial portraits of human populations using median networks. *Genetics* **141**: 743–753.

Barrett, L.G., Thrall, P.H., Burdon, J.J. & Linde, C.C. 2008a. Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends Ecol. Evol.* **23**: 678–685.

Barrett, L.G., Thrall, P.H., Burdon, J.J., Nicotra, A.B. & Linde, C.C. 2008b. Population structure and diversity in sexual and asexual populations of the pathogenic fungus *Melampsora lini*. *Mol. Ecol.* **17**: 3401–3415.

Blouin, M.S., Yowell, C.A., Courtney, C.H. & Dame, J.B. 1995. Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* **141**: 1007–1014.

Bruns, E., Carson, M. & May, G. 2012. Pathogen and host genotype differently affect pathogen fitness through their effects on different life-history stages. *BMC Evol. Biol.* **12**: 135.

Burdon, J.J. & Roelfs, A.P. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. *Phytopathology* **75**: 1068–1073.

Burdon, J.J. & Thrall, P.H. 2008. Pathogen evolution across the agro-ecological interface: implications for disease management. *Evol. Appl.* **1**: 57–65.

Burt, A., Carter, D.A., Koenig, G.L., White, T.J. & Taylor, J.W. 1996. Molecular markers reveal cryptic sex in the human pathogen Coccidioides immitis. *PNAS* **93**: 770–773.

Carriconde, F., Gilgado, F., Arthur, I., Ellis, D., Malik, R., Van de Wiele, N. *et al.* 2011. Clonality and α-a recombination in the Australian *Cryptococcus gattii* VGII population - an emerging outbreak in Australia. *PLoS ONE* **6**: e16936.

Coelho, M., Gonçalves, P. & Sampaio, J. 2011. Evidence for maintenance of sex determinants but not of sexual stages in red yeasts, a group of early diverged basidiomycetes. *BMC Evol. Biol.* **11**: 249.

Cox, F.E.G. 2009. *Modern Parasitology: A Textbook of Parasitology*. Blackwell Science, Oxford.

Criscione, C.D. & Blouin, M.S. 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. *Evolution* **58**: 198–202.

Criscione, C.D., Poulin, R. & Blouin, M.S. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Mol. Ecol.* **14**: 2247–2257.

Daverdin, G., Rouxel, T., Gout, L., Aubertot, J.-N., Fudal, I., Meyer, M. *et al.* 2012. Genome structure and reproductive behaviour influence the evolutionary potential of a fungal phytopathogen. *PLoS Pathog.* **8**: e1003020.

Davies, C.M., Webster, J.P. & Woolhous, M.E. 2001. Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite, *Proc. Biol. Sci.* **268**: 251–257.

De Gelas, K. & De Meester, L. 2005. Phylogeography of *Daphnia magna* in Europe. *Mol. Ecol.* **14**: 753–764.

Decaestecker, E., Declerck, S., De Meester, L. & Ebert, D. 2005. Ecological implications of parasites in natural *Daphnia* populations. *Oecologia* **144**: 382–390.

Dunn, A.M. & Smith, J.E. 2001. Microsporidian life cycles and diversity: the relationship between virulence and transmission. *Microbes Infect.* **3**: 381–388.

Ebert, D. 1998. Experimental evolution of parasites. *Science* **282**: 1432–1436.

Ebert, D., Hottinger, J.W. & Pajunen, V.I. 2001. Temporal and spatial dynamics of parasite richness in a *Daphnia* metapopulation. *Ecology* **82**: 3417–3434.

Ebert, D., Altermatt, F. & Lass, S. 2007. A short term benefit for outcrossing in a *Daphnia* metapopulation in relation to parasitism. *J. R. Soc. Interface* **4**: 777–785.

Enzel, Y., Amit, R., Dayan, U., Crouvi, O., Kahana, R., Ziv, B. *et al.* 2008. The climatic and physiographic controls of the eastern Mediterranean over the late Pleistocene climates in the southern Levant and its neighboring deserts. *Global Planet. Change* **60**: 165–192.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Excoffier, L., Laval, G. & Schneider, S. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform.* **2005**: 47–50.

Feng, Y., Li, N., Dearen, T., Lobo, M.L., Matos, O., Cama, V. *et al.* 2011. Development of a multilocus sequence typing tool for high-resolution genotyping of *Enterocytozoon bieneusi*. *Appl. Environ. Microbiol.* **77**: 4822–4828.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotechnol.* **3**: 294–299.

Goren, L. & Ben-Ami, F. 2013. Ecological correlates between cladocerans and their endoparasites from permanent and rain pools: patterns in community composition and diversity. *Hydrobiologia* **701**: 13–23.

Haag, K.L., Larsson, J.I.R., Refardt, D. & Ebert, D. 2011. Cytological and molecular description of *Hamiltosporidium tvaerminnensis* gen. et sp. nov., a microsporidian parasite of *Daphnia magna*, and establishment of *Hamiltosporidium magnivora* comb. nov. *Parasitology* **138**: 447–462.

Haag, K.L., Traunecker, E. & Ebert, D. 2013. Single nucleotide polymorphisms of two closely related microsporidian parasites suggest a clonal population expansion after the last glaciation. *Mol. Ecol.* **22**: 314–326.

Harff, J., Endler, R., Emelyanov, E., Kotov, S., Leipe, T., Moros, M. *et al.* 2011. Late Quaternary climate variations reflected in Baltic Sea sediments. In: *The Baltic Sea Basin* (J. Harff, S. Björck, P. Hoth, eds), pp. 99–132. Springer, Berlin.

Heitman, J. 2010. Evolution of eukaryotic microbial pathogens via covert sexual reproduction. *Cell Host Microbe* **8**: 86–99.

Hewitt, G.M. 2001. Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Mol. Ecol.* **10**: 537–549.

Ironside, J.E. 2007. Multiple losses of sex within a single genus of Microsporidia. *BMC Evol. Biol.* **7**: 48.

Judson, O.P. & Normark, B.B. 1996. Ancient asexual scandals. *Trends Ecol. Evol.* **11**: 41–46.

Kearney, M. 2005. Hybridization, glaciation and geographical parthenogenesis. *Trends Ecol. Evol.* **20**: 495–502.

Koella, J.C. & Agnew, P. 1999. A correlated response of a parasite's virulence and life cycle to selection on its host's life history. *J. Evol. Biol.* **12**: 70–79.

Kofler, R., Schlötterer, C. & Lelley, T. 2007. SciRoKo: a new tool for whole genome microsatellite search and investigation. *Bioinformatics* **23**: 1683–1685.

Lass, S. & Ebert, D. 2006. Apparent seasonality of parasite dynamics: analysis of cyclic prevalence patterns. *Proc. Biol. Sci.* **273**: 199–206.

Lass, S., Hottinger, J., Fabbro, T. & Ebert, D. 2011. Converging seasonal prevalence dynamics in experimental epidemics. *BMC Ecol.* **11**: 14.

Li, W., Cama, V., Feng, Y., Gilman, R.H., Bern, C., Zhang, X. *et al.* 2012. Population genetic analysis of *Enterocytozoon bieneusi* in humans. *Int. J. Parasitol.* **42**: 287–293.

Librado, P. & Rozas, J. 2009. DnaSP V5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.

MacLeod, A., Tait, A. & Turner, C.M. 2001. The population genetics of *Trypanosoma brucei* and the origin of human infectivity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**: 1035–1044.

Mangin, K.L., Lipsitch, M. & Ebert, D. 1995. Virulence and transmission modes of two microsporidia in *Daphnia magna*. *Parasitology* **111**: 133–142.

Mathis, A., Weber, R. & Deplazes, P. 2005. Zoonotic potential of the Microsporidia. *Clin. Microbiol. Rev.* **18**: 423–445.

Maynard-Smith, J. 1978. *The Evolution of Sex*. Cambridge University Press, Cambridge.

Maynard-Smith, J., Smith, N.H., O'Rourke, M. & Spratt, B.G. 1993. How clonal are Bacteria? *Proc. Natl Acad. Sci. USA* **90**: 4384–4388.

McCoy, K.D., Boulinier, T., Tirard, C. & Michalakis, Y. 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution* **57**: 288–296.

Nemri, A., Barrett, L.G., Laine, A.-L., Burdon, J.J. & Thrall, P.H. 2012. Population processes at multiple spatial scales maintain diversity and adaptation in the *Linum marginale* - *Melampsora lini* association. *PLoS ONE* **7**: e41366.

O'Mahony, E.M., Tay, W.T. & Paxton, R.J. 2007. Multiple rRNA variants in a single spore of the microsporidian *Nosema bombi*. *J. Eukaryot. Microbiol.* **54**: 103–109.

Pajunen, V.I. & Pajunen, I. 2003. Long-term dynamics in rock pool *Daphnia* metapopulations. *Ecography* **26**: 731–738.

Paland, S., Colbourne, J.K. & Lynch, M. 2005. Evolutionary history of contagious asexuality in *Daphnia pulex*. *Evolution* **59**: 800–813.

Poulin, R. & Cribb, T.H. 2002. Trematode life cycles: short is sweet? *Trends Parasitol.* **18**: 176–183.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rauch, G., Kalbe, M. & Reusch, T.B.H. 2005. How a complex life cycle can improve a parasite's sex life. *J. Evol. Biol.* **18**: 1069–1075.

Reetz, J., Nöckler, K., Reckinger, S., Vargas, M.M., Weiske, W. & Broglia, A. 2009. Identification of *Encephalitozoon cuniculi* genotype III and two novel genotypes of *Enterocytozoon bieneusi* in swine. *Parasitol. Int.* **58**: 285–292.

Sandrock, C. & Vorburger, C. 2011. Single-locus recessive inheritance of asexual reproduction in a parasitoid wasp. *Curr. Biol.* **21**: 433–437.

Sibley, L.D. & Ajioka, J.W. 2008. Population structure of *Toxoplasma gondii*: clonal expansion driven by infrequent recombination and selective sweeps. *Annu. Rev. Microbiol.* **62**: 329–351.

Smith, J.E. 2009. The ecology and evolution of microsporidian parasites. *Parasitology* **136**: 1901–1914.

Sun, S. & Heitman, J. 2011. Is sex necessary? *BMC Biol.* **9**: 56.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance,

and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739.

Tibayrenc, M. & Ayala, F.J. 2002. The clonal theory of parasitic protozoa: 12 years on. *Trends Parasitol.* **18**: 405–410.

Tibayrenc, M. & Ayala, F.J. 2012. Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proc. Natl. Acad. Sci. USA* **109**: E3305–E3313.

Vercken, E., Fontaine, M.C., Gladieux, P., Hood, M.E., Jonot, O. & Giraud, T. 2010. Glacial refugia in pathogens: European genetic structure of anther smut pathogens on *Silene latifolia* and *Silene dioica*. *PLoS Pathog.* **6**: e1001229.

Vizoso, D.B. & Ebert, D. 2004. Within-host dynamics of a microsporidium with horizontal and vertical transmission: *Octosporea bayeri* in *Daphnia magna*. *Parasitology* **128**: 31–38.

Vizoso, D.B., Lass, S. & Ebert, D. 2005. Different mechanisms of transmission of the microsporidium *Octosporea bayeri*: a cocktail of solutions for the problem of parasite permanence. *Parasitology* **130**: 501–509.

Wendte, J.M., Miller, M.A., Lambourn, D.M., Magargal, S.L., Jessup, D.A. & Grigg, M.E. 2010. Self-Mating in the definitive host potentiates clonal outbreaks of the apicomplexan parasites *Sarcocystis neurona* and *Toxoplasma gondii*. *PLoS Genet.* **6**: e1001261.

Wolinska, J., Giessler, S. & Koerner, H. 2009. Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. *Appl. Environ. Microbiol.* **75**: 7051–7059.

Wolinska, J., Spaak, P., Koerner, H., Petrusek, A., Seda, J. & Giessler, S. 2011. Transmission mode affects the population genetic structure of *Daphnia* parasites. *J. Evol. Biol.* **24**: 265–273.

Xhaard, C., Fabre, B., Andrieux, A., Gladieux, P., Barrès, B., Frey, P. *et al.* 2011. The genetic structure of the plant pathogenic fungus *Melampsora larici-populina* on its wild host is extensively impacted by host domestication. *Mol. Ecol.* **20**: 2739–2755.

Xiao, L., Li, L., Visvesvara, G.S., Moura, H., Didier, E.S. & Lal, A.A. 2001. Genotyping *Encephalitozoon cuniculi* by multilocus analyses of genes with repetitive sequences. *J. Clin. Microbiol.* **39**: 2248–2253.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Haplotype networks based on *Daphnia magna* cox 1 gene.

Table S1 List of *Hamiltosporidium* isolates analyzed in the present study, with their respective location, origin and geographic coordinates.

Table S2 *Hamiltosporidium* microsatellite loci, primer sequences, allele size and number.

Table S3 *Hamiltosporidium* single nucleotide polymorphisms found in alpha tubulin, beta tubulin and hsp70 genes.

Table S4 Hamiltosporidium genotypes for seven microsatellite loci. Loci numbers refer to the genomic contig containing each microsatellite, and allele numbers indicate the size of PCR products. Iranian isolates shown on orange background represent mixed infections.

Table S5 Daphnia magna single nucleotide polymorphisms found in the mitochondrial cox1 gene.

Received 2 December 2012; accepted 7 January 2013