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Swimming against the current: genetic structure, host mobility and the drift paradox in trematode parasites

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Abstract

Life-cycle characteristics and habitat processes can potentially interact to determine gene flow and genetic structuring of parasitic species. In this comparative study, we analysed the genetic structure of two freshwater trematode species with different life histories using cytochrome *c* oxidase I gene (COI) sequences and examined the effect of a unidirectional river current on their genetic diversity at 10 sites along the river. We found moderate genetic structure consistent with an isolation-by-distance pattern among subpopulations of *Coitocaecum parvum* but not in *Stegodexamene anguillae*. These contrasting parasite population structures were consistent with the relative dispersal abilities of their most mobile hosts (i.e. their definitive hosts). Genetic diversity decreased, as a likely consequence of unidirectional river flow, with increasing distance upstream in *C. parvum*, which utilizes a definitive host with only restricted mobility. The absence of such a pattern in *S. anguillae* suggests that unidirectional river flow affects parasite species differently depending on the dispersal abilities of their most mobile host. In conclusion, genetic structure, genetic diversity loss and drift are stronger in parasites whose most mobile hosts have low dispersal abilities and small home ranges. An additional prediction can be made for parasites under unidirectional drift: those parasites that stay longer in their benthic intermediate host or have more than one benthic intermediate hosts would have relatively high local recruitment and hence increased retention of upstream genetic diversity.

Keywords: freshwater, genetic diversity loss, linear ecosystems, population genetic structure, trematodes

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Introduction

Dispersal among populations is essential for maintaining genetic connectivity across a species' geographical range. To a large extent, gene flow depends on the dispersal abilities of a species, as well as on landscape features such as the presence of barriers to dispersal. For parasitic organisms, gene flow and the extent of genetic structure among populations are crucial for several key evolutionary processes, ranging from local adaptation to speciation (Gandon & Michalakis 2002; Criscione *et al.* 2005; Huyse *et al.* 2005; Thompson 2005). Given the small size and limited intrinsic mobility of the infective stages of parasites, gene flow depends mostly on the potential of hosts to disperse their parasites between geographically isolated populations. In the case of parasites with complex life cycles (i.e. involving multiple hosts and transmission challenges), population structure should be determined by the vagility of the most mobile host species (see Prugnolle *et al.* 2005). In freshwater ecosystems, there is some evidence that parasites using hosts capable of moving from river to river, such as birds, show reduced genetic structuring among populations compared to those constrained to aquatic hosts only (Criscione & Blouin 2004; Louhi *et al.* 2010). Nonetheless, our understanding of genetic structuring in parasitic species is still very limited, in particular with respect to the influence of life-cycle characteristics and transmission routes.

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In addition to the potential effects of life history on gene flow, habitat features can also shape the population connectivity of parasite species. In rivers, for instance, the drift paradox predicts that the continuous unidirectional dispersal of organisms driven by the water current (from upstream to downstream) may only be partially countered by active recolonization of upstream sites (Müller 1954, 1982). Rivers are dendritic habitats that theoretically might be best approximated by the linear stepping-stone model in which immigrants are exchanged only between neighbours in a one-dimensional chain of subpopulations (Kimura & Weirs 1964). Unidirectional water flow promoting dispersal of organisms along downstream corridors sets the conditions for asymmetric migration and population sizes (e.g. Fraser *et al.* 2004), with upstream populations prone to experience local extinctions and population bottlenecks (Hänfling & Weetman 2006). If gene dispersal is also predominantly unidirectional downstream, then levels of gene diversity should correlate with stream position (Ritland 1989). For example, alleles found upstream may represent only a subset of those present at downstream sites, with genetic diversity of populations decreasing from downstream to upstream (e.g. Gornall *et al.* 1998; Werle 2005; Hänfling & Weetman 2006; Barson *et al.* 2009; Pollux *et al.* 2009). Moreover, regional geomorphological characteristics are likely to affect the levels of genetic connectivity among distinct river drainages (Waters *et al.* 2001; Burridge *et al.* 2006). Therefore, both life-cycle characteristics and habitat processes can potentially interact to determine gene flow and genetic structuring of parasite species.

No previous study has investigated the impact of unidirectional river flow on parasites that completely rely on their hosts for recolonization of upstream sites. Here, we conducted a comparative study of two common parasites of freshwater fish in New Zealand, *Coitocaecum parvum* (Trematoda: Opecoelidae) and *Stegodexamene anguillae* (Trematoda: Lepocreadiidae). These two trematode species complete their entire life cycles in aquatic systems and both have three hosts in their life cycle. In the life cycle of *C. parvum* (Fig. 1a), eggs produced by adult worms inside the gut of upland bully (*Gobiomorphus breviceps*) are released in host faeces. Free-living miracidia hatch, swim and penetrate the mud snails (*Potamopyrgus antipodarum*) in which intramolluscan stages develop and reproduce asexually, ultimately producing cercariae that emerge from the snail to find and penetrate amphipods where they encyst as metacercariae. Later, amphipods harbouring metacercariae are ingested by the upland bully, with the fish serving as definitive host in which sexual reproduction takes place. By contrast, adults of *S. anguillae* parasitize native eels (New Zealand longfin eel, *Anguilla dieffenbachii*, in the studied river). The eggs of this trematode are released in eel faeces, with hatching miracidia then infecting mud snails (*P. antipodarum*), where intramolluscan stages develop and reproduce asexually. Cercariae of *S. anguillae* emerging from snails subsequently locate and infect upland bullies (*G. breviceps*), where they encyst as metacercariae, awaiting predation by eels, their definitive host (Fig. 1b).

As outlined above, these two trematode species share two hosts in their life cycle, the upland bully and the freshwater mud snail. Other than that, they have fundamentally different life-cycle pathways, *C. parvum* involving two invertebrate hosts and *S. anguillae* with two vertebrate hosts. While downstream dispersal of infective trematode stages (free-living larvae and metacercariae infecting amphipods) might be passively driven by river current alone, upstream recolonization probably requires active fish dispersal. The most

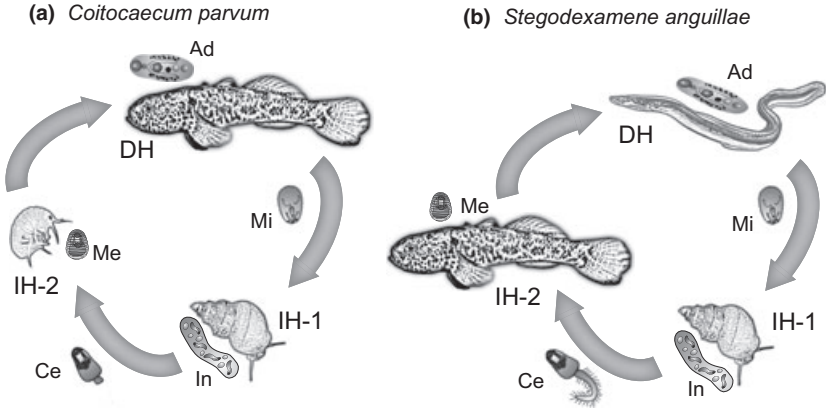

Fig. 1 Schematic life cycle of (a) *Coitocaecum parvum* and (b) *Stegodexamene anguillae*. DH, definitive host; IH-1, first intermediate host; IH-2, second intermediate host; Ad, adult trematode infecting definitive host; Mi, free-living miracidium; In, intramolluscan stages of the trematode infecting first intermediate host; Ce, free-living cercariae; Me, metacercariae infecting second intermediate host.

mobile host of *C. parvum*, the upland bully (*G. breviceps*), is a relatively sedentary fish species, with each territorial male typically confined to a particular rock and its immediate surroundings (McDowall 1990). In contrast, the most mobile host of *S. anguillae*, the longfin eel (*A. dieffenbachii*), actively migrates 10–100-km upstream from the sea early in its freshwater life (McDowall 1990). In addition, older eels have an average home range of 10 m (in very productive streams, Jellyman & Sykes 2003), with larger ranges observed in large braided rivers and low-productivity streams (D. J. Jellyman, personal communication); eels can also disperse at least 350-m upstream when attracted by odour (Jellyman & Graynoth 2005). Differences in the life-cycle routes of these two parasites, together with the contrasting mobility and home range characteristics of their hosts, make this a perfect host–parasite system to study the impact of such features on the genetic makeup of parasite populations.

In this study, we aim to test the following predictions: (i) *C. parvum* should show stronger population structure than *S. anguillae*, because the latter's most mobile host has higher dispersal abilities; (ii) parasite species genetic diversity should decrease with increasing distance upstream, as a consequence of the unidirectional river flow; (iii) the degree of loss in upstream genetic diversity should reflect differences in the transmission routes of the parasite, in particular the host's ability to disperse.

Materials and methods

Study area, fish sampling and parasite collection

The Manuherikia River is a tributary of the Clutha River on the South Island, New Zealand. We sampled the lower section of the Manuherikia River (Fig. 2), along a ~70-km stretch downstream from Falls Dam. This section of the river includes two gorges that we used to distinguish three regions: 'downstream' including sampling sites 1–3, 'midstream' including sites 4–5 and 'upstream' including sites 6–10. In August 2010, we sampled upland bully fish larger than 4 cm standard length at 10 sites along the river (3–18 specimens per site) numbered sequentially from downstream to upstream (Fig 2, see Table 1 for coordinates). Geographic distances among sampling sites were measured as river distances. Fish were captured by electrofishing, euthanized by spinal cord severing and frozen at −20 °C. Fish were subsequently thawed and dissected, and parasites recovered according to standardized protocol. Parasites were identified and immediately

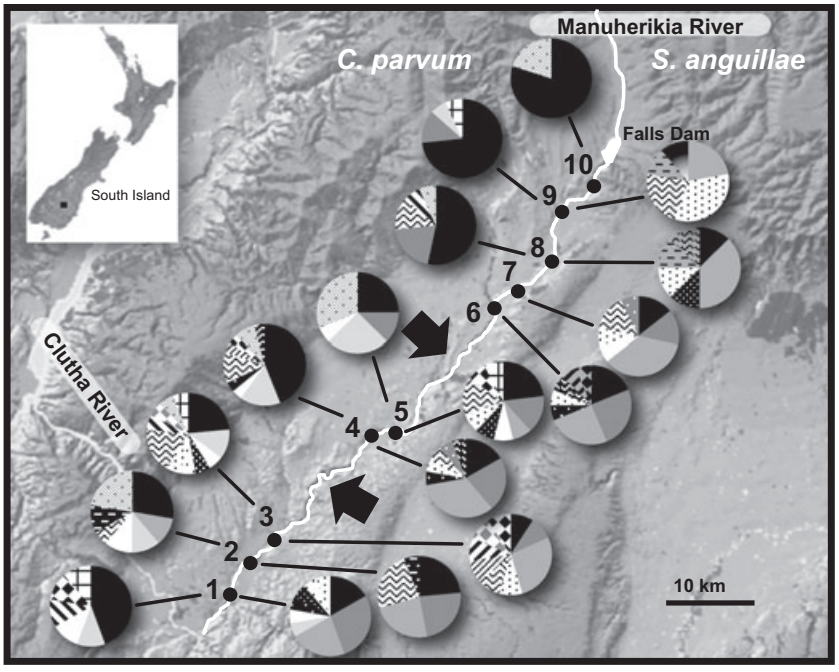

Fig. 2 Studied section of the Manuherikia river, and junction with the Clutha river, in South Island (New Zealand), showing the 10 sampling sites numbered sequentially from downstream to upstream. Arrows indicate the two gorges in this section of the river that delineate the three regions distinguished here (downstream, midstream and upstream). Pie charts on the map indicate the relative proportions of haplotypes at each site (see total number of samples per site in Table 1) for the two trematode species, *Coitocaecum parvum* and *Stegodexamene anguillae*.

Table 1 Population genetics summary statistics for *Coitocaecum parvum* and *Stegodexamene anguillae* at each site and totals for the river

	Coordinates (New Zealand grid)	*n* fish	*n* specimens	N_h	N_p	*h*	π	Tajima's *D*	Fu's Fs
C. parvum sampling sites									
10	E2261833; N5584198	6	5	2	2	0.4 ± 0.237	0.00102 ± 0.00061	−0.97256	1.04
9	E2260810; N5583727	9	15	4	8	0.467 ± 0.148	0.00151 ± 0.00069	−1.91494	0.034
8	E2260015; N5579132	8	15	5	4	0.695 ± 0.109	0.00132 ± 0.00032	−0.52537	−1.453
5	E2245196; N5563056	6	16	5	8	0.808 ± 0.053	0.00398 ± 0.00049	1.04287	1.535
4	E2243168; N5562780	3	25	9	11	0.783 ± 0.073	0.00347 ± 0.00058	−0.23775	−1.487
3	E2233922; N5552927	2	17	11	13	0.926 ± 0.045	0.00501 ± 0.00066	0.06548	−3.55
2	E2231811; N5550908	2	18	7	11	0.869 ± 0.047	0.00453 ± 0.00069	0.38563	0.246
1	E2229788; N5547804	3	9	6	10	0.833 ± 0.127	0.00363 ± 0.00096	−0.66449	−1.298
Total for the river			120	18	19	0.785 ± 0.033	0.00345 ± 0.00029	−0.67674	−4.473
S. anguillae sampling sites									
9	E2260810; N5583727	4	9	5	10	0.861 ± 0.087	0.00617 ± 0.0082	0.82741	0.871
8	E2260015; N5579132	4	9	7	11	0.917 ± 0.092	0.00609 ± 0.00074	0.26763	−1.607
7	E2256614; N5576034	2	13	7	23	0.846 ± 0.085	0.00997 ± 0.00256	−0.2399	1.19
6	E2254679; N5574220	5	16	8	22	0.875 ± 0.053	0.01343 ± 0.00099	1.70848	2.04
5	E2245196; N5563056	5	14	9	26	0.923 ± 0.05	0.0128 ± 0.00186	0.42333	0.26
4	E2243168; N5562780	3	17	8	22	0.846 ± 0.066	0.01127 ± 0.00188	0.86062	1.712
3	E2233922; N5552927	2	11	9	24	0.945 ± 0.066	0.01171 ± 0.00233	0.01351	−1.363
2	E2231811; N5550908	2	18	6	22	0.843 ± 0.04	0.0111 ± 0.00191	0.85702	4.268
1	E2229788; N5547804	2	17	8	24	0.86 ± 0.055	0.01274 ± 0.00142	1.04162	2.151
Total for the river			124	23	35	0.863 ± 0.016	0.01126 ± 0.00066	0.65775	0.058

Sampling sites refer to Fig. 2.
n fish, number of fish from which trematodes were recovered; *n* specimens, number of specimens of each trematode species analysed; N_h, number of different haplotypes observed; N_p, number of polymorphic sites observed; *h*, haplotype diversity ± standard deviation; π, nucleotide diversity ± standard deviation; Tajima's *D* test statistic (Tajima 1989) and Fu's Fs test statistic (Fu 1997).

fixed in 99° molecular grade ethanol for later molecular analysis.

DNA extraction, amplification, sequencing and alignment

DNA extractions consisted of placing individual parasites into 1.5-mL tubes in 600 μL of 5% chelex containing 0.1 mg/mL proteinase K, incubating at 60 °C overnight, boiling at 90 °C for 8 min and centrifuging at 15 000 ***g*** for 10 min. We amplified partial cytochrome *c* oxidase I gene (COI) and 16S ribosomal RNA genes using primers JB3 (forward 5′-TTTTTTGGGCATC CTGAGGTTTAT-3′; Bowles *et al.* 1993) and Plag16S-COIdR (reverse 5′-TCGGGGTCTTTCCGTCT-3′) (newly designed). Both markers are mitochondrial, linked and might not be neutral but still offer sufficient variability to test our hypotheses. Polymerase chain reaction (PCR) amplifications were performed with 25-μL reactions containing 2.5 μL of extraction supernatant, 1× PCR buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl at pH 8.8), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.5 mM each primer and 0.7 unit BIOTAQ™ DNA polymerase (Bioline Ltd). The following thermocycling profile was used for amplification: denaturation of DNA (95 °C for 3 min); 38 cycles of amplification (94 °C for 40 s, 49 °C for 30 s and 72 °C for 1 min); and 4 min extension hold at 72 °C. PCR products were purified using PCR Product Pre-Sequencing Kit™ (Affymetrix/USB Corporation). PCR primers were used for sequencing, and PCR amplicons were cycle-sequenced from both strands using ABI BigDye™ Terminator v3.1 Ready Sequencing Kit, alcohol-precipitated and run on an ABI 3730xl automated sequencer. Contiguous sequences were assembled and edited using Sequencher™ (GeneCodes Corp. version 5).

Newly obtained sequences were about 1500 bp long and included partial COI, tRNA-Thr and partial 16SrRNA genes. Partial 16S rDNA sequences were not used further as they were too conserved at the geographical scale of this study. The tRNA-Thr sequence was also excluded. COI fragments were aligned using MUSCLE implemented in MEGA 4.0 (Tamura *et al.* 2007).

Statistical analysis

The number of unique haplotypes, number of polymorphic sites, haplotype and nucleotide diversity (*h* and π) were calculated for each species at each sampling site and over all sites (i.e. all individuals treated as one

sample) with DnaSP version 5.10 (Librado & Rozas 2009). Tajima's *D* (Tajima 1989) and Fu's Fs (Fu 1997) statistics were calculated to assess the consistency of observed genetic variation with a neutral model of evolution at each sampling site and over all sites combined for each species with 1000 permutations, using Arlequin version 3.11 (Excoffier *et al.* 2005). Significant deviations from neutrality can be caused by selection, but also by demographic fluctuations. Fu's Fs statistic in particular is highly sensitive to demographic expansions, which produce large negative values (Fu 1997). Genetic population structure was examined for each species at two levels using hierarchical analysis of molecular variance as implemented in Arlequin. Genetic structure (Φ_{ST} estimate) was investigated among all samples by treating samples from sites 1 to 10 as separate subpopulations. Genetic structure (Φ_{CT} estimate) was also examined among three regions separated by two gorges, 'downstream', 'midstream' and 'upstream', and among sites within these regions (Φ_{SC} estimate). Additionally, the fixation index (Φ_{ST}) was calculated for pairwise comparisons between all collection sites. The corrected Akaike information criterion (AICc) of jModelTest version 0.1.1 (Posada & Crandall 1998) was used to select the most appropriate model of sequence evolution for each species that corresponded to TPM1uf + I for *C. parvum* and HKY + I for *S. anguillae*. However, these models are not implemented in Arlequin; therefore, the Tamura & Nei (1993) model of sequence evolution, Arlequin's closest available approximation of the models listed earlier, was instead used for calculations of Φ_{ST}. Significance of genetic structure was determined via 10 000 permutations (Excoffier *et al.* 1992).

A statistically parsimonious haplotype network was constructed using TCS version 1.21 (Clement *et al.* 2000) for each trematode species. Isolation by distance was tested by using the relationship between genetic ($\Phi_{ST}/1-\Phi_{ST}$; Rousset 1997) and geographic distance along the river among all sites for each species. A Mantel test (Mantel 1967) was run using 1000 randomizations as implemented in the Isolation by Distance Web Service version 3.16 (Jensen *et al.* 2005). To test whether the genetic diversity of parasite populations decreases from downstream to upstream sites, multiple linear regression analyses between both haplotype and nucleotide diversity and distance from the river mouth (considered as the junction of the Manuherikia river with the Clutha river) were carried out separately for each species. In addition, the number of parasites sequenced at each site was included as an additional predictor variable to take into account the variation that could be explained by having unequal number of specimens sequenced per site.

Results

DNA sequences comprising 781 and 702 bp unambiguously aligned positions of the COI gene were analysed for 120 individuals of *C. parvum* and 124 individuals of *S. anguillae*. Sequences for both species overlapped; but *C. parvum* sequences included 70 additional bases in the 5′ end and had a conserved insertion of 9 bp at the 3′ end of the COI. Sequences for *C. parvum* showed 19 polymorphic sites segregating 18 different haplotypes, whereas those for *S. anguillae* sequences contained 35 polymorphic sites segregating 23 haplotypes (Table 1; Genbank accession numbers JN244793–JN244833). Relative frequency of haplotypes recovered from each sampling site is represented as pie charts in Fig. 2. *Coitocaecum parvum* showed lower haplotype and nucleotide diversity upstream (sites 8–10) than downstream (Table 1). In contrast, haplotype diversity for *S. anguillae* remained roughly constant along the river; though, nucleotide diversity was reduced by ~50% at the two most upstream sites (however, the sample sizes were smaller at those sites) (Table 1). In general, larger sample sizes of fish in some sites were necessary because of low infection

Table 2 Two-level hierarchical analysis of molecular variance for each trematode species

Species	Geographic analysis	Source of variation	d.f.	Percentage of variation	Fixation indices	*P*-value
Coitocaecum parvum	Overall	Among sites	7	2.39	$\Phi_{ST} = 0.02388$	0.121
	Sites 1–10	Within sites	112	97.61		
	Among three regions	Among regions	2	6.3	$\Phi_{CT} = 0.06397$	0.034
	Sites 1–10	Among sites within regions	5	−2.52	$\Phi_{SC} = -0.02685$	0.789
		Within sites	112	96.22		
Stegodexamene anguillae	Overall	Among sites	8	1.72	$\Phi_{ST} = 0.01719$	0.218
	Sites 1–9	Within sites	115	98.28		
	Among three regions	Among regions	2	−2.88	$\Phi_{CT} = -0.02879$	0.895
	Sites 1–9	Among sites within regions	6	3.87	$\Phi_{SC} = 0.03764$	0.094
		Within sites	115	99.01		

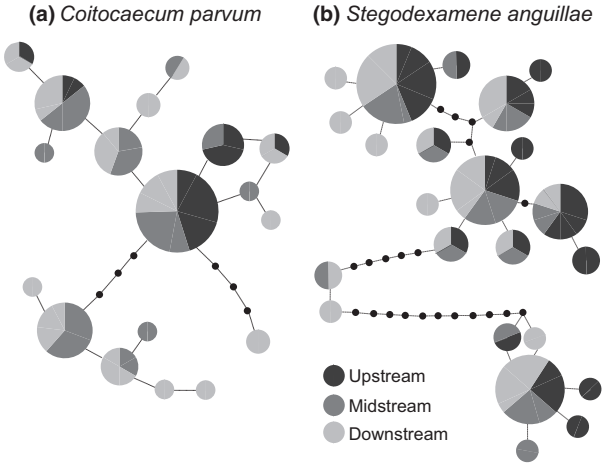

Fig. 3 Statistically parsimonious haplotype networks for (a) *Coitocaecum parvum* and (b) *Stegodexamene anguillae*. Each circle represents a haplotype and circle area represents haplotype frequency. Small black circles represent inferred haplotypes not observed in the data; all connections represent a single mutational step. The proportion of each haplotype recovered from each of the three regions of the river is represented by different shades.

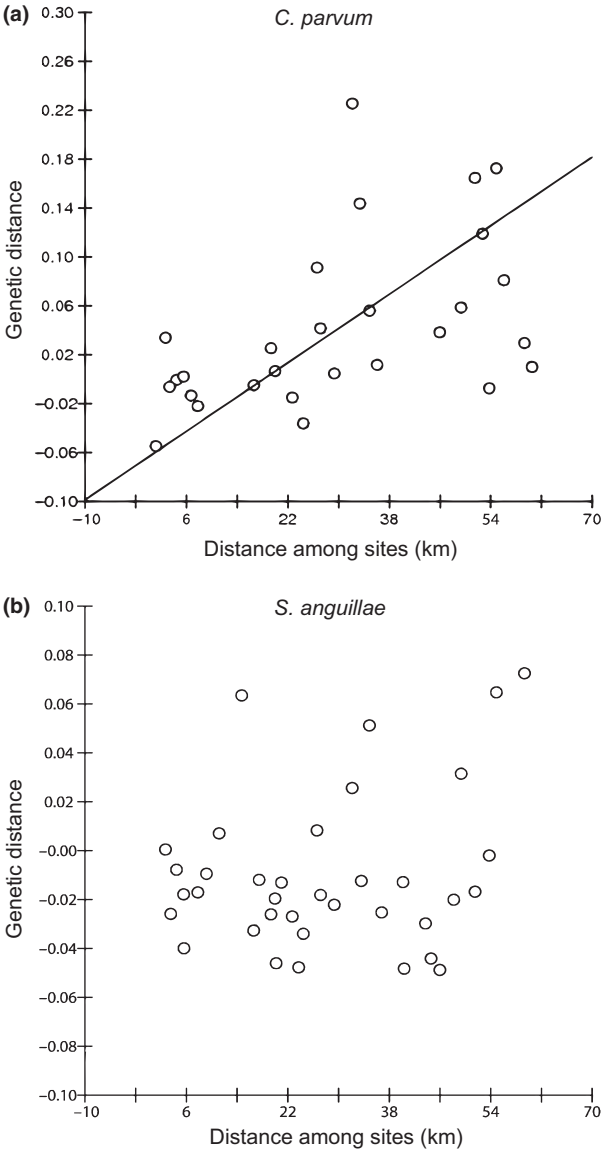

Fig. 4 Isolation-by-distance analysis. Relationship between pairwise geographical distances and genetic distances ($\Phi_{ST}/1-\Phi_{ST}$) between sites, for the two trematode species, (a) *Coitocaecum parvum* and (b) *Stegodexamene anguillae*.

intensity of parasites in fishes at those sites (Table 1). Neutrality tests yielded significant results only for *C. parvum* at site 9 (Tajima's $D = -1.914$; $P < 0.05$) and site 3 (Fu's Fs = −3.55; $P < 0.05$), suggesting that substantial demographic shifts may have occurred at these particular sites. Although Fu's Fs statistics yielded a negative value at the river level (all locations combined), this was not significant (Table 1).

Hierarchical AMOVA analysis revealed significant regional genetic differentiation for *C. parvum* ($P < 0.05$), with 6.3% of genetic variance explained by 'upstream, 'midstream' and 'downstream' region groupings. By contrast, samples of *S. anguillae* did not show any genetic differentiation among populations or regions (see Table 2). Pairwise Φ_{ST} comparisons among sites for *C. parvum* resulted in five of 28 significant ($P < 0.05$) ones, whereas in inter-region analyses two of three comparisons were significant (Φ_{ST} for 'downstream' was significantly different from 'midstream', and Φ_{ST} for 'midstream' was significantly different from 'upstream'). Pairwise Φ_{ST} among sites for *S. anguillae* showed five of 36 significant comparisons, whereas among regions none was significant (Table S1, Supporting information).

The parsimonious haplotype network for *C. parvum* revealed a common haplotype, occurring at a frequency of approximately 0.50 in the upstream region (Fig. 3a). Three additional haplotypes (frequencies 0.14, 0.13 and 0.09) were detected commonly at 'downstream' and 'midstream' regions but were absent at the 'upstream' region. All haplotypes were connected by three or fewer mutations, with only one reticulation detected. 'Upstream' *C. parvum* were only represented in one haplogroup, typically separated from one another by single mutations (Fig. 3a), whereas 'midstream and downstream' ones were distributed throughout the network. The haplotypes of *S. anguillae* could not be joined into a single network at the 95% connection limit but were connected at the 90% limit (Fig 3b). Three common haplotypes were identified, each of them showing a star-like structure with four to five satellite haplotypes. These distinct *S. anguillae* haplogroups were

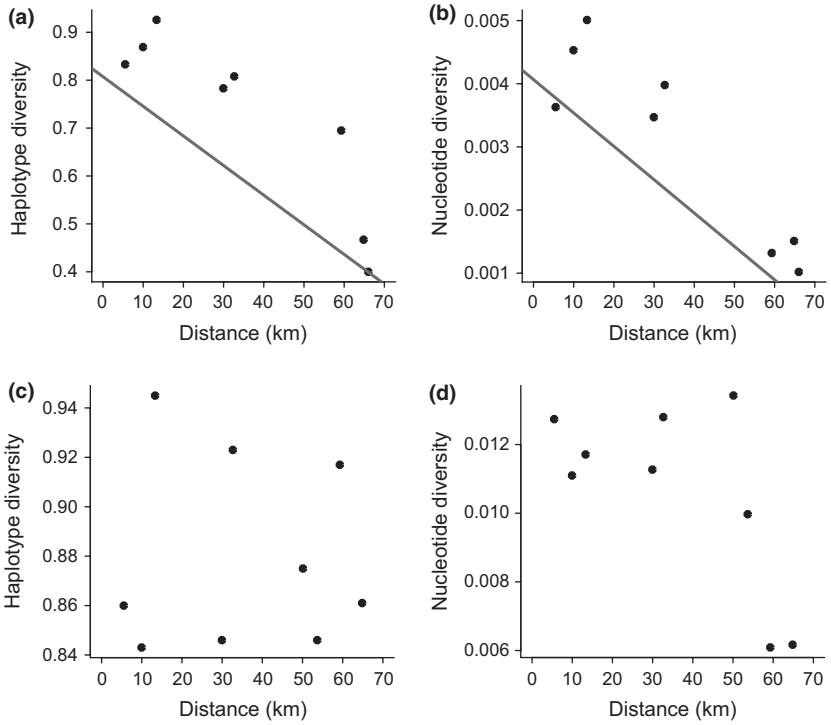

Fig. 5 Regression analyses between (a) *Coitocaecum parvum* haplotype diversity and distance from river junction; (b) *C. parvum* nucleotide diversity and distance from the river junction; (c) *Stegodexamene anguillae* haplotype diversity and distance from river junction; (d) *S. anguillae* nucleotide diversity and distance from river junction. Note: lines shown are derived from multiple regression (see materials and methods section).

divergent from one another by up to 12 mutations, with each haplogroup distributed widely across 'downstream', 'midstream' and 'upstream' regions.

Mantel tests revealed a significant correlation between genetic and geographic distances for *C. parvum* ($R^2 = 0.242$; $P = 0.025$; $\beta = 3.505 \times 10^{-3}$) but not for *S. anguillae* ($R^2 = 0.059$; $P = 0.079$; $\beta = 1.934 \times 10^{-3}$) (Fig. 4). Multiple regression analyses for *C. parvum* (Fig. 5) revealed a significant relationship between haplotype diversity ($R^2 = 0.869$; $P = 0.006$) and both distance from the river mouth (junction between the Manuherikia and the Clutha rivers) and the number of specimens. Of the two independent variables, distance showed a strong negative association ($\beta = -6.18 \times 10^{-6}$; $P = 0.005$; Fig. 5a) with haplotype diversity but the number of specimens did not ($\beta = 8.86 \times 10^{-3}$; $P = 0.162$). Nucleotide diversity also showed a significant relationship ($R^2 = 0.889$; $P = 0.004$) with distance ($\beta = -5.28 \times 10^{-8}$; $P = 0.003$; Fig. 5b) and number of specimens ($\beta = 5.68 \times 10^{-5}$; $P = 0.217$), but only distance was significantly associated with the nucleotide diversity. The results hold when using the exact same overlapping region of the COI for *C. parvum* (711 bp) as for *S. anguillae* (Tables S2 and S3, Supporting information). Multiple regression analyses for *S. anguillae* (Fig. 5c,d) showed no overall relationship ($R^2 = 0.501$; $P = 0.124$) between haplotype diversity and distance and number of specimens. In this case, distance was not found associated with haplotype diversity ($\beta = -1.04 \times 10^{-6}$; $P = 0.155$; Fig. 5c), whereas the number of specimens showed a marginally significant negative association ($\beta = -1.01 \times 10^{-2}$; $P = 0.050$) with haplotype diversity. Multiple regression analysis between nucleotide diversity and our two independent variables revealed a marginally significant relationship ($R^2 = 0.626$; $P = 0.053$); however, neither of the two independent variables showed a significant association with nucleotide diversity (distance: $\beta = -3.39 \times 10^{-8}$; $P = 0.419$; Fig. 5d; number of specimens: $\beta = 4.57 \times 10^{-4}$; $P = 0.119$). Therefore, increasing distance upstream is associated with decreased genetic diversity of *C. parvum* but not in the case of *S. anguillae*.

Discussion

Influence of the definitive host' ability to disperse the parasites on the population genetic structure of the parasite

In complex parasite life cycles that include multiple host species, parasite dispersal and genetic structure should be constrained by the host with the highest dispersal rate (Jarne & Théron 2001; Prugnolle *et al.* 2005).

Our study contrasted two trematodes restricted to the aquatic environment that share two of the three hosts in their life cycle. For each trematode, the fish definitive host represents the most mobile host in the cycle, i.e. the one presumably most responsible for trematode gene flow and genetic structure among populations. The distinct patterns of genetic differentiation detected in *C. parvum versus S. anguillae* are consistent with expectations based on the contrasting dispersal abilities of their most mobile hosts (upland bully for *C. parvum* and longfin eel for *S. anguillae*). Specifically, the pattern of isolation by distance in *C. parvum* indicates that dispersal occurs typically on small scales, congruent with the bully's sedentary habits (McDowall 1990) and smaller home range in comparison with longfin eels. By contrast, the lack of spatial genetic structure observed in *S. anguillae* is consistent with the relatively high dispersal capability (Jellyman & Graynoth 2005) of its most mobile host. The use of more variable markers, such as microsatellites or single-nucleotide polymorphisms, might possibly show genetic structure in both species. Nevertheless, we expect it would still be stronger for *C. parvum* than for *S. anguillae* as a consequence of the different dispersal abilities of their hosts.

While river current alone might be sufficient to drive downstream dispersal of infective trematode stages, upstream gene flow presumably requires active fish dispersal. Given the moderate genetic structure observed among regions (~21.6 km apart) and the isolation-by-distance pattern detected for *C. parvum*, upstream recolonization appears to be limited in this species. Indeed, upstream dispersal of *C. parvum* might only occur via migration of juvenile fish prior to their establishment of territories (Hopkins 1970). Conversely, *S. anguillae* showed homogeneity through all scales and no isolation by distance, suggesting that the eel host is able to disperse this parasite upstream on a regular basis [at least within its home range of ≥10 m (Jellyman & Sykes 2003) or occasional explorations of up to 350 m (Jellyman & Graynoth 2005)]. Previous studies have demonstrated the influence of host dispersal ability on the genetic makeup and distribution of parasites infecting fish, mammals and birds (Blouin *et al.* 1995; Criscione & Blouin 2004; Prugnolle *et al.* 2005; Louhi *et al.* 2010). We have taken this comparative approach a step further, corroborating previous findings and providing the first evidence in parasites restricted exclusively to aquatic environments.

Additional factors potentially influencing population genetic structure

The differences in genetic structure between the two parasites could also be influenced by the effective population size (N_e) of the two species; however, using a single mitochondrial marker, we could not accurately measure N_e. There are no data available on the abundance of neither bullies nor eels in the section of the Manuherikia river we studied. In addition, calculation of intensity of infection as an approximation of relative parasite N_e would require a larger sample of hosts for the measure to be accurate and representative (Poulin & Morand 2004) because of the aggregated distribution of parasites (Poulin 2007) and the generally low prevalence of endoparasites (Poulin 1998). As mentioned in the results, we required larger fish samples in some sites because of the low infection intensity of parasites detected in fishes at those sites (Table 1). It is likely that in addition to the differences in dispersal ability of the hosts (i.e. relative dispersal of the parasite), populations of the two parasite species also differ in N_e at some sites. Such differences in N_e might also contribute to the observed population genetic structures.

Furthermore, genetic diversity and population genetic structure of *S. anguillae* could be influenced by historical factors. The eel definitive host is known to occasionally migrate among streams so it might occasionally transport *S. anguillae* among them. Low levels of gene flow among streams could lead to somewhat divergent clades within a location (see Irwin 2002). Thus, the among-stream dynamics of eels might influence genetic diversity of *S. anguillae* in the studied river. Alternatively, divergence between *S. anguillae* haplogroups could be due to isolated populations (maybe cryptic species) that might have now come back into contact, increasing genetic diversity of *S. anguillae*. However, current data do not allow us to confirm any of the aforementioned possibilities at this time.

Influence of the unidirectional water flow (promoting the drift of organisms) on the genetic diversity of parasites

At the scale of our study (~70 km), it seems the strong decrease in genetic diversity observed for *C. parvum* is most likely an effect of continuous unidirectional river flow, biasing both migration and population sizes, resulting in a loss of genetic diversity upstream (Ritland 1989). This result supports previous findings of asymmetric dispersal in freshwater systems subject to unidirectional flow (see for revision on plants Pollux *et al.* 2009 and for theoretical work on animals, mostly insects, Pachepsky *et al.* 2005) and both asymmetric dispersal and effective populations sizes (Fraser *et al.* 2004; Hänfling & Weetman 2006). In other cases where such asymmetry in dispersal has not been detected, the dispersal ability of the organisms in question (e.g. freshwater insects with flying adults) may be sufficient to

counter the effects of unidirectional river current (Müller 1954, 1982; Waters 1972). Broadly, such upstream dispersal may resolve the drift paradox (Pachepsky *et al.* 2005) and allow persistence of organisms upstream. Using simulation modelling, Anholt (1995) concluded that any type of dispersal with an upstream component (not only flight or wind dispersal) could lead to population persistence. In the case of the trematodes studied, their entire life cycle takes place in the aquatic environment, and their most mobile hosts appear to play the equivalent role of the adult flying insect or wind in achieving upstream movement. On one hand, the lower dispersal abilities of *C. parvum*'s definitive host (upland bully) cannot entirely compensate with upstream movement the drift of *C. parvum* haplotypes being continuously washed away. Consequently, we detected a decline in genetic diversity from upstream to downstream sites, where haplotype diversity accumulates as consequence of the continuous influx of haplotypes from upstream. As remarked by Pachepsky *et al.* (2005), propagation speed and persistence are strongly related. Reduced upstream recolonization by upland bully and *C. parvum* increases the chances of both host and parasite being washed away by the unidirectional water flow, reducing their population sizes (e.g. Fraser *et al.* 2004) and becoming temporarily extinct at upstream sites [or at least, prone to experience population bottlenecks (see Hänfling & Weetman 2006)]. On the other hand, the definitive host of *S. anguillae* (eels) disperses the parasite upstream more effectively so that, at the small scale of our study, we observed no significant loss of genetic diversity as a consequence of unidirectional river flow. However, both parasites showed a loss of nucleotide diversity upstream (despite it was not significant for *S. anguillae*). We could speculate that low infection intensity upstream could promote self-fertilization of the parasites (i.e. both species are hermaphrodites), which could potentially lead to a loss of nucleotide diversity. In general, we can propose that the drift of parasites, genetic structuring and genetic diversity loss will be more pronounced in parasite species whose most mobile hosts have low dispersal abilities and small home ranges.

Potential solutions to the drift paradox from the parasites' perspective

Additional compensatory movements upstream proposed for other systems to resolve the drift paradox created by river flow can also apply to parasite persistence in rivers. Waters (1972) suggested that the paradox would be resolved if insect larvae resided mainly on the benthos and only the surplus above local carrying capacity would drift downstream. Trematodes have a benthic intermediate host, i.e. snails, in which the infection can last from months to the host's lifespan (years) (Esch & Fernandez 1994; Curtis 1995; Soldánová & Kostadinova 2011). Therefore, following Waters' hypothesis applied to trematodes, we might expect that those trematodes that can stay for longer in their benthic intermediate host and those that have more than one benthic intermediate host would have higher resilience against being washed away, higher population sizes upstream and also that their upstream genetic diversity would be maintained for longer. Other parasites with indirect life cycles such as myxozoans, some nematodes (e.g. Dioctophymatoidea) and cestodes also use benthic intermediate hosts like annelids (e.g. Wolf & Markiw 1984; Courtney & Christensen 1987; Anderson 2000). Thus, the same hypothesis could apply to other parasitic groups as well. Several authors have also suggested the presence of refugia in the stream (Lancaster & Hildrew 1993a,b; Winterbottom *et al.* 1997a,b; Lancaster 2000) and the effect of variability in the direction of stream flow (e.g. turbulence or estuarine river subject to tides) as resolutions for the paradox. Although these two factors have not been thoroughly investigated, they could also apply to parasites. As an extension to our study, the contribution of smaller streams to the main river species' genetic diversity would also be interesting to assess, as it represents a more complex model with variability in stream flow and the possibility for refugia. Furthermore, using this information in simulation modelling studies could be appropriate for epidemiological studies in freshwater aquaculture and studies on parasite invasions with stages in running waters as it could improve management measures.

Conclusions

The patterns of genetic differentiation found in this comparative study were consistent with the expectations based on the contrasting dispersal abilities of the most mobile hosts of trematodes restricted to aquatic environments, supporting previous evidence from other systems. As predicted, genetic diversity decreased with increasing distance upstream, as a consequence of the unidirectional river flow, in a trematode species utilizing a definitive host with restricted mobility. Overall, following our initial prediction, loss of genetic diversity affects parasite species differently depending on the dispersal abilities of their most mobile host, at least at the geographic scale of our study.

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Data accessibility

DNA sequences: Genbank accessions JN244793–JN244833.

Data deposited at Dryad: Haplotypes found at each site linked to Genbank accession numbers, DRYAD entry doi:10.5061/dryad.s781c7kq.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Pairwise analyses of molecular variance estimations.

Table S2 Population genetics summary statistics for *Coitocaecum parvum* (COI sequence length = 711 bp; overlapping completely with COI-sequenced fragment of *Stegodexamene anguillae*) at each site and totals for the river.

Table S3 Multiple regression analyses results using the exact same overlapping region for *C. parvum* (711 bp) as for *S. anguillae* (data from Table S2, Supporting Information).

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