

# Patterns and processes of alternative host use in a generalist parasite: insights from a natural host–parasite interaction

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## Summary

1. Host specificity is a major, yet poorly understood, property of parasites. Generalist parasites generally exploit a principal host species and a series of alternative host species. Understanding patterns of alternative host use may help predict phylogenetic diversification of parasite lineages and the emergence of infectious diseases. However, the ecological and evolutionary determinants underpinning alternative host use remain elusive.

2. Here, we investigated, in the wild, patterns of alternative host use in a freshwater ectoparasite copepod (*Tracheliastes polycolpus*), and we tested several *a priori* hypotheses regarding determinants underlying these patterns. We specifically answer two related questions: (i) why do parasites use alternative host species? and (ii) why do parasites preferentially use one particular alternative host species rather than another?

3. We first showed that *T. polycolpus* was able to use five alternative host species in addition to its principal host species, the dace (*Leuciscus burdigalensis*). Using causal analyses, we demonstrated that, overall, the rate of alternative host use was higher when parasite burden on the principal host was higher, providing support for the 'parasite density' hypothesis. Then, phylogenetic regressions revealed that the specific use of these alternative host species does not occur randomly, but according to the 'ecological similarity' hypothesis: parasites preferentially use host species that are ecologically close to the principal host species, irrespective of the phylogenetic distance and of the alternative host density. The fitness of parasites on alternative host species was similar to that of parasites on the principal host species, except for the smallest body-sized alternative host species for which parasite fitness was lower. Finally, using microsatellite markers, we demonstrated that this differential host use did not lead to genetically isolated parasite populations.

4. Our study suggests that encounter rate may be a key factor in predicting patterns of alternative host use and unravels intriguing questions about the contribution of phenotypic plasticity to the use of a large host spectrum by a generalist parasite.

**Key-words:** co-evolution, ectoparasite, freshwater fishes, host specificity, path analyses

## Introduction

Host specificity is defined as the extent to which a parasite is restricted in the range of host species it is able to infect at a given developmental stage in its life cycle (Combes

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2001). At one extreme, highly specific parasites infect a single host species (e.g. fish monogenean ectoparasites; Šimková *et al.* 2006; Šimková & Morand 2008), and at the other extreme, generalist parasites are able to establish populations on several host species (e.g. the hen flea *Ceratophyllus gallinae* is able to infect up to eighty host species; Tripet & Richner 1997). From an evolutionary perspective, a classic question is how selection has favoured such a myriad of life-history strategies in parasites and pathogens, and how this can lead to speciation (Poulin & Keeney 2008). From an ecological perspective, host specificity is a key element for predicting the ability of non-native parasites to infect novel (native) hosts (Poulin 1992; Douda *et al.* 2012) and for understanding important mechanisms such as dilution effects (Johnson & Thieltges 2010; Keesing *et al.* 2010; Renwick & Lambin 2013).

In generalist parasites, the host species supporting the majority of individuals in a parasite population is traditionally considered the principal host species, whereas other host species are considered alternative host species (Poulin & Mouillot 2004). Patterns of alternative host use have been described for many parasite and pathogen groups, including viruses (Parrish *et al.* 2008; Benmayor *et al.* 2009), avian malaria parasites (Ricklefs, Fallon & Bermingham 2004), lice (Johnson, Adams & Clayton 2002), monogenean worm parasites of fish (Desdevises *et al.* 2002) and beetle nematodes (Mayer, Herrmann & Sommer 2009). Although patterns of alternative host use have been extensively described, the ecological determinants underpinning such patterns are difficult to isolate and have to date rarely been investigated. For instance, for a given parasite species, patterns of alternative host use can drastically vary over spatial and temporal scales, and why a parasite species can be highly specific in a given environment and highly generalist in another environment is yet to be clarified.

The first key question about alternative host use concerns the proximal causes: what are the factors that will motivate a parasite to use alternative host species rather than a single principal host? According to conceptual and theoretical works, causes of alternative host use encompass modifications regarding either the parasite or the host. Concerning macroparasites, changes in parasite density may be a key factor explaining alternative host use (i.e. the 'parasite density' hypothesis, Combes 2001). Indeed, high parasite density on the principal host generally increases the number of infective propagules in the surrounding area and hence the probability of accidental encounter (and hence use) of alternative host species (Poulin 1998). Additionally, a high parasite density increases overcrowding and within-host competition for limited resources, which may also favour alternative host use (Emelianov 2007). From a host point of view, changes in the principal host density may also motivate the use of alternative host (the 'principal host density' hypothesis). Notably, facing a decrease in the density of the principal host, parasites may colonize new host species to hedge their bets against the

risk of co-extinction by reducing their dependence on a single resource (Bush & Kennedy 1994; Koh *et al.* 2004).

The second key question concerns the characteristics of the alternative host species being used by a generalist parasite: what makes an alternative host species more suitable than another one? Concerning parasites with direct life cycles, three hypotheses could help explain patterns of alternative host use. First, the 'host ecological similarity' hypothesis states that a parasite is more likely to use alternative host species that is ecologically closely related to the principal host species (Timms & Read 1999; Cooper *et al.* 2012). Indeed, host species that share resources (e.g. habitat or diet) will be more prone to encounter the same species of parasites and hence to be infected accidentally (Poulin 1998; Combes 2001). Second, the 'host phylogenetic similarity' hypothesis states that a parasite's potential host range is determined by physiological and immunological characteristics of the hosts (Combes 2001; Khokhlova *et al.* 2012). Because of the genetic basis of these characteristics, the use of a particular alternative host species is likely to depend on the phylogenetic distance between the alternative host species and the principal host species (assuming phylogenetic conservatism for physiological and immunological characteristics, Krasnov *et al.* 2004; Khokhlova *et al.* 2012). These first two hypotheses are likely to interact whether ecologically similar species are also phylogenetically closely related (i.e. functional traits are phylogenetically conserved, Blomberg, Garland & Ives 2003). Finally, the 'alternative host availability' hypothesis states that the density of suitable hosts required for parasite persistence represents an important determinant of alternative host use (Grenfell & Harwood 1997; Perlman & Jaenike 2003; Poulin & Mouillot 2004). In general, abundant species will be more prone to encounter the parasite and to support viable parasite populations (Lively & Dybdahl 2000; Loot *et al.* 2006). Ultimately, a related question concerns the fitness of parasites on alternative host use (Khokhlova *et al.* 2012); is the fitness of parasites similar among alternative host species? Although this question is central to better understanding of the ecological and evolutionary implications of alternative host use, it has rarely been addressed for wild populations.

In this study, we focused on an interaction involving a crustacean ectoparasite, *Tracheliastes polycolpus* (Nordmann 1832), and its fish host, the dace *Leuciscus burdigalensis* (Fryer 1982; Loot *et al.* 2004). The direct life cycle of this parasite makes it a perfect model for studying patterns of alternative host use. Briefly, only females are parasitic, attaching to fins after having been fecundated by dwarf free-living males (Fryer 1982). During this parasitic phase (which is between 1–2 months), females feed on the epithelial cells and mucus, inflicting local lesions and the partial or total destruction of fins (Loot *et al.* 2004). Following this phase, females lay eggs in the water-column. This parasite has several fitness consequences for dace, such as reduced feeding and growth rate (Blanchet *et al.* 2009). Dace are a widespread freshwater fish belonging to the

Cyprinidae family. In Western Europe (notably in French watersheds), it is the principal host of *T. polycolpus* (G.L. Loot & S.B. Blanchet, unpublished data), although this parasite has been recorded on other fish species [*Leuciscus idus* (Galicka & Penczak 1989; Sobiecka, Jukiewicz & Piasiecki 2004), *Leuciscus walecki* (Yamaguti 1940), *Leuciscus cephalus* (Barzegar & Jalali 2009) and *Phoxinus phoxinus* (Tuffery 1967)]. Such observations suggest that this parasite species is weakly specific and may hence be capable of using alternative hosts.

The goal of this study was to explore the ecological determinants driving patterns of alternative host use in natural ecosystems. Using an extensive survey of *T. polycolpus* in two natural systems, we first described and quantified the use of alternative host species by *T. polycolpus*. Second, we determined the proximal factors motivating the use of alternative host species. According to the 'parasite density' and the 'principal host density' hypotheses, we predicted that the use of alternative host species would be higher in sites where mean parasite burden on the dace is high and where dace density is low. Third, we isolated the host characteristics that make one alternative host species better than another. According to the 'host ecological similarity' and the 'host phylogenetic similarity' hypotheses, we expected *T. polycolpus* to preferentially use fish species that are ecologically and/or phylogenetically closer to the principal host, *L. burdigalensis*. In addition, we predicted that *T. polycolpus* would use alternative host species with a high local relative density (the 'alternative host availability' hypothesis). Then, we tested whether parasite fitness (parasite body size, presence of egg-clutches and egg numbers) differed between parasites found on the principal host and those found on alternative host species. Finally, patterns of alternative host use can be the outcome of a 'true' generalist parasite, but can also be the result of a specific parasite that has switched to new alternative hosts. Host switching is defined as an effective transfer from a principal to a new host species (Charleston & Robertson 2002; Clayton *et al.* 2003) and is expected to lead to parasite divergence between alternative and the principal host species (and ultimately speciation, Borghuis *et al.* 2009). In contrast, such a divergence is not expected for a generalist parasite (in this case, a single genotype infects several alternative host species). Hence, to test whether the use of alternative host species likely resulted from host switching events or from a generalist parasite, we used microsatellite markers to assess genetic differentiation between parasites belonging to the different host species.

## Materials and methods

### STUDIED AREA AND FISH SAMPLING

Fish sampling was performed during July 2008 in two rivers located in south-western France in the Garonne River drainage: the Célé River and the Viaur River. These two rivers were chosen because of their close geographical proximity and their similarities in terms of hydrological and chemical characteristics (G.L. Loot & S.B.

Blanchet, unpublished data). Respectively, the Célé and the Viaur Rivers are 136 km and 169 km long, their drainage areas cover 1350 km<sup>2</sup> and 1530 km<sup>2</sup> and their annual mean flow ranges from 7–30 m<sup>3</sup> s<sup>-1</sup> and 8–25 m<sup>3</sup> s<sup>-1</sup>.

Both rivers sustain similar fish assemblages composed of about 15–20 fish species (including non-native species, personal data). In this study, we focused on the ten most abundant fish species that were common to the two rivers, as the others were too rare to be sampled effectively. Eight of these species belong to the Cyprinidae family: the dace (*Leuciscus burdigalensis*, that is, the principal host of *T. polycolpus* in French river systems, Blanchet and Loot unpublished data), the bleak (*Alburnus alburnus*), the barbel (*Barbus barbus*), the toxostoma (*Parachondrostoma toxostoma*), the gudgeon (*Gobio gobio*), the chub (*Squalius cephalus*), the Eurasian minnow (*Phoxinus phoxinus*) and the roach (*Rutilus rutilus*). The other two species were the stone loach (*Barbatula barbatula*) and the brown trout (*Salmo trutta*), which belong, respectively, to the Balitoridae and Salmonidae families.

Targeted fish species were collected using electric-fishing at eight (Viaur River) and seven (Célé River) sampling sites. Sampling sites were evenly distributed along the upstream-downstream environmental gradient of each river to reflect all environmental conditions available in these rivers (see Blanchet *et al.* 2010 for details on sampling sites). We used a DEKA 7000, generating 200–500 V with an intensity range of 1–3 A to capture fish. At each site, we estimated the density (nb. ind./100 m<sup>2</sup>) of each fish species following a standardized single-pass electric-fishing method (Bohlin *et al.* 1989; Lobon-Cervia & Utrilla 1993). A total of 4252 individuals, comprising the ten targeted species, were collected (see Table S1 in Supporting Information for details). All fish specimens were anaesthetized and measured to the nearest millimetre (total body length).

### PARASITE SAMPLING

The presence/absence, as well as the total number, of *T. polycolpus* (parasite burden) was noted for each fish. Parasitism was expressed at the fish species level by calculating prevalence and mean intensity at the scale of the sampling site (Bush *et al.* 2001). Parasite prevalence is the ratio between the number of infected individuals and the total number of sampled individuals. Parasite mean intensity is the mean number of parasites per infected host. Specimens of *T. polycolpus* were removed from each parasitized fish species and individually stored in a 1.5 ml Eppendorf® containing 70% alcohol. All fish specimens were released alive at their site of origin.

### ESTIMATING AND COMPARING THE RATE OF PRINCIPAL AND ALTERNATIVE HOST USE

We used generalized linear mixed models (GLMMs) to quantify and compare the use of the principal and alternative host species by *T. polycolpus*. We built two models in which *T. polycolpus* prevalence and mean intensity, respectively, was the response variables, while host species identity and river identity were the predictors. The two-way interaction term between species identity and river identity was tested when possible (i.e. for the 'prevalence' model but not for the 'intensity' model, see Results section). For both response variables, we used the site value for each species as the replicate unit. We accounted for pseudo-replication (species within site) by including site identity as a random variable (Bolker *et al.* 2009).

### UNDERSTANDING CAUSAL FACTORS UNDERPINNING THE USE OF ALTERNATIVE HOST SPECIES

To identify proximal causes of alternative host use, we used model selection procedures that we applied to path analyses (Shipley

2000; Johnson & Omland 2004; Cardon *et al.* 2011). We specifically sought to decipher between the ‘principal host density’ hypothesis (with host density being expressed as the number of dace per 100 m<sup>2</sup>) and the ‘parasite density’ hypothesis (with parasite density being expressed as the intensity of *T. polycolpus* per dace individual). In addition, because we pooled the two rivers into a single analysis, we tested the ‘River identity’ hypothesis, which sought to test whether the rate of alternative host use differed between rivers, irrespective of the two other hypotheses. Each competing hypothesis was expressed as a causal model, and seven different competing models were built according to the three hypotheses cited (Fig. 1). The first three models corresponded to single-hypothesis models [i.e. one model for each of the three hypotheses, see models (a–c) in Fig. 1]. Three other models corresponded to models that simultaneously considered two of the three hypotheses as possible drivers of alternative host use [see models (d–f) in Fig. 1]. Finally, the last model considers that all three hypotheses simultaneously drive alternative host use [see model (g) in Fig. 1]. In all models, we constrained host and parasite densities to co-vary with river identity, so that their effects were tested independently of the river identity and *vice versa* (see Fig. 1). The sampling site was the replicate unit in these analyses ( $n = 15$ ), and the rate of alternative host use was expressed as the proportion of alternative hosts (i.e. all individuals of all fish species combined except *L. burdigalensis*) that were found to be parasitized in a given site. It is worth noting that we considered only alternative host species that were found to be present in all sampling sites. By doing so, we avoid biasing results by including species that were particularly prone to be parasitized (i.e. with a relatively high prevalence) but that were present in some sites only.

To compare these models, we first judged whether the covariance structure of each model did not differ from that of the data. Maximum likelihood chi-square statistics were used for this purpose; a nonsignificant chi-square identifies a good fit between predicted and observed covariance matrices (Grace 2006). We then used Akaike Information Criteria (AIC) values to rank these competing models, with the model that displayed the lowest AIC value being considered as the ‘best model’ (Johnson & Omland 2004). We calculated the differences in these AIC values between each model and the best model (i.e.  $\Delta AIC$ ). A single best model cannot be assumed if the  $\Delta AIC$  with other competing models is not greater than 2 units (Johnson & Omland 2004). Such a result would indicate that several models are well supported by the data.

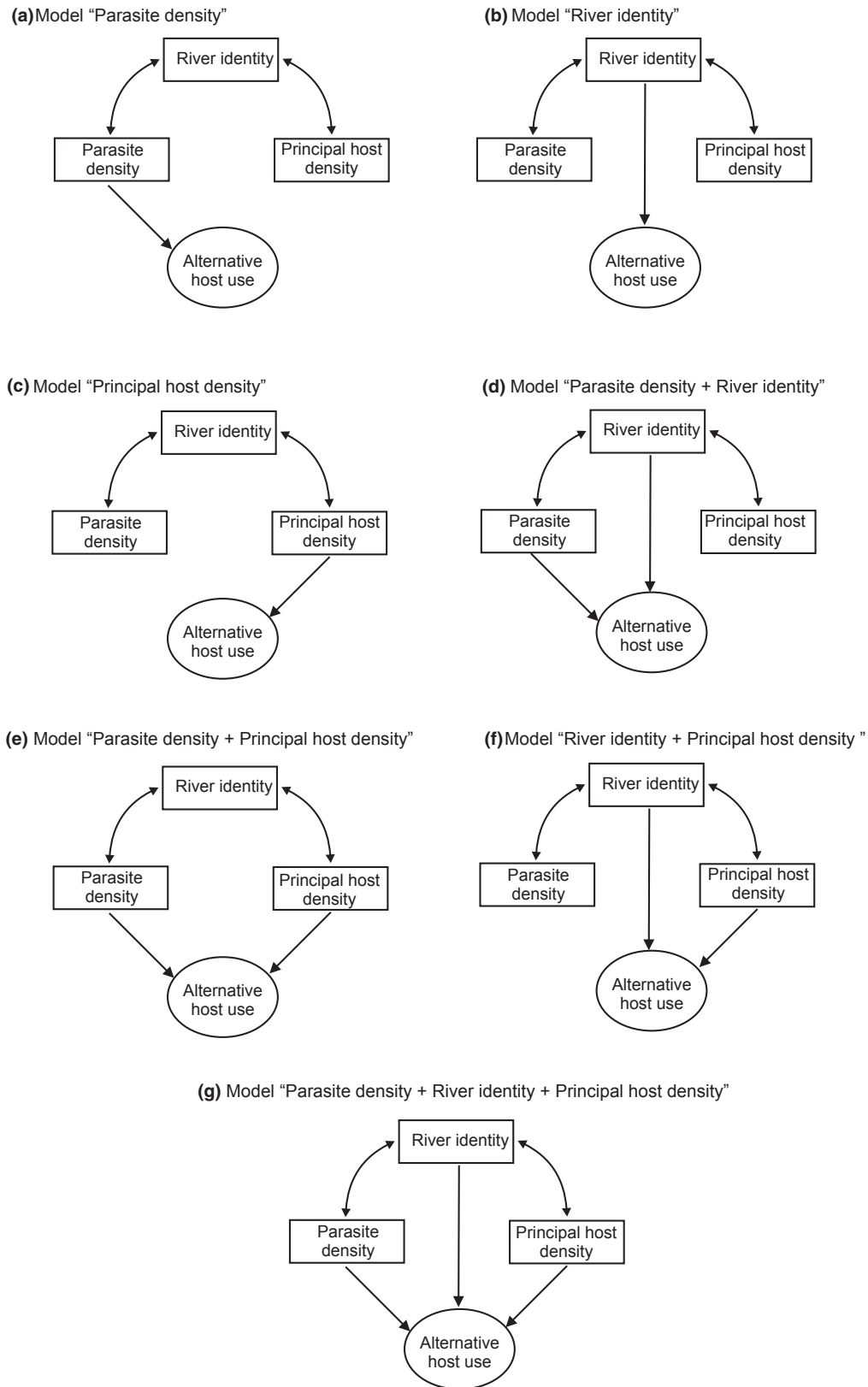
#### UNDERSTANDING CAUSAL FACTORS UNDERPINNING THE CHOICE OF AN ALTERNATIVE HOST SPECIES

Our third aim was to test which of the ‘host phylogenetic similarity’, ‘host ecological similarity’ and ‘alternative host availability’ hypotheses best explained why *T. polycolpus* is more prone to use a particular alternative host species rather than another. Each of these hypotheses was expressed as a single metric. First, for the ‘host phylogenetic similarity’ hypothesis, we constructed a tree describing the phylogenetic relationships between the ten targeted fish species (see Fig. S1). This tree was derived from a larger tree including 34 native freshwater fish from France (Blanchet *et al.* unpublished). For each fish species, partial DNA sequences of two mitochondrial genes (cytochrome b and cytochrome oxidase 1) and of one ribosomal gene (16S) were obtained from GenBank. For each gene, sequence alignments were performed under BioEdit, using the alignment software ClustalW (Thompson, Higgins & Gibson 1994). Alignments were verified with the naked-eye and manually corrected if necessary. The best model of evolution for each gene was calculated using the software PAUP\*4.0b10 (Swofford 1993) and selected using the

software MODELTEST (through AIC values, Posada & Buckley 2004). We used Maximum Likelihood methods (as implemented in PhyML) to build a phylogenetic tree that accounted for the model of evolution of each gene (Felsenstein 1983; Guindon & Gascuel 2003). Bayesian posterior probabilities were used to assess the support of inferred branching. The reconstructed phylogeny was congruent with Cyprinids phylogeny published using larger species pools (Gaubert, Denys & Oberdorff 2009). We used this tree to calculate the phylogenetic distance (i.e. an index of phylogenetic similarity) between each alternative host and the principal host (*L. burdigalensis*). Second, for the ‘host ecological similarity’ hypothesis, we built a matrix of ecological similarity among the ten targeted fish species. This ecological similarity matrix was based on variables related to the microhabitat use and to the diet habits of each fish species. Variables related to the microhabitat use were the preferred water velocity (m.s<sup>-1</sup>), habitat depth (m) and substrate roughness (mm). Variables related to the diet habits were the feeding depth of each species (water-column, bottom or surface feeder) and the trophic status of each species (omnivorous, predator, insectivorous or herbivorous). For each variable, the data were taken from multiple literature sources (Mann 1996; Lamouroux *et al.* 1999; Keith & Allardi 2001; Oberdorff *et al.* 2002). We used this information to calculate a distance matrix (measured as the Gower similarity index as it includes both categorical and continuous variables, Gower 1971) providing an ecological index of similarity among fish species. We used this matrix to calculate the ecological similarity between each alternative host and the principal host species (*L. burdigalensis*). Third, we used the mean (relative) density of each alternative fish species as a metric to test the ‘alternative host availability’ hypothesis. In the following analyses, all sampling sites were pooled and the species identity is the replicate unit.

In a first analysis, we sought to quantify the level of phylogenetic signal (i.e. the tendency for related species to resemble each other) for each of the following traits: *T. polycolpus* prevalence, alternative host density and ecological similarity to *L. burdigalensis* (with *L. burdigalensis* being set to 0). We did not consider *T. polycolpus* intensity in this analysis as sample size (i.e. the number of species harbouring at least one parasite) would have been too low for statistical treatments. We used the standardized Blomberg’s K (Blomberg, Garland & Ives 2003) to quantify phylogenetic signal; a K less than one implies that relatives resemble each other less than expected under Brownian motion evolution (i.e. the stochastic expectation of trait evolution along a tree), and a K greater than one implies that close relatives are more similar than expected under Brownian motion evolution (Blomberg, Garland & Ives 2003). We tested for a significant phylogenetic signal by applying the method of independent contrasts as proposed in Blomberg, Garland & Ives (2003). In this method, standardized phylogenetically independent contrasts (PICs) are measured and the associated variance is used as an index of phylogenetic signal. The observed index is statistically compared against null (or random) expectations by permuting the data across the tips (Blomberg, Garland & Ives 2003). Data from the two rivers were averaged and pooled within species into a single analysis. The presence of significant phylogenetic signals for *T. polycolpus* prevalence would indicate an important role of phylogeny in explaining patterns of host use.

In a second analysis, we computed a GLMM that tested for all three hypotheses at the same time; *T. polycolpus* prevalence at the species level was the dependent variable and relative host densities, phylogenetic similarity and ecological similarity between each alternative host species and *L. burdigalensis* were the predictors. In addition, we included ‘river identity’ as a categorical predictor, and we computed all two-way interactions including river identity to test for slope differences between rivers. Because we calculated two prevalence values per species (i.e. one value per river), we



**Fig. 1.** Graphical representation of the seven path models used to disentangle causal relationships between *Tracheliastes polycolpus* alternative host use, the density of the principal host (*Leuciscus burdigalensis*, ‘principal host density’ hypothesis), the parasite density on the principal host (‘parasite density’ hypothesis) and the identity of the river (the Viaur or Célé river, ‘river identity’ hypothesis). Each model sought to test a specific hypothesis: models (a) to (c) tested for each hypothesis individually, models (d) to (f) tested two hypotheses at a time and model (g) tested all three hypotheses at a time. Double-headed arrows indicate covariation between variables, whereas the single-arrow indicates a causal link.

included 'species identity' as the random variable. *L. burdigalensis* was not included to avoid biasing results.

#### EVALUATING PARASITE FITNESS ON ALTERNATIVE HOSTS

Our fourth aim was to test whether parasites could reproduce on alternative hosts and more generally whether the fitness of parasites differs between the principal and alternative host species and among host species. This was performed by comparing fitness parameters of parasites (body size, presence of egg-clutches and mean number of eggs) among host species. Parasites were collected in the Viaur River only, as sample size would have been too low in the Célé River (notably for alternative host species). At the laboratory, a picture of each parasite was taken with a digital camera. The camera was fixed on an L-shaped bracket to keep the same axis of view for all parasites, and a metric ruler was placed at the base to provide a baseline scale. The same focal length was kept to avoid any picture distortion. We used ImageTool software (free download at <http://compdent.uthscsa.edu/dig/itdesc.html>) to measure body size of all parasites. We noted whether females carried egg-clutches, and we counted the number of eggs in one of the two egg-clutches at 10× magnification. We then computed GLMs to compare the mean body size of *T. polycolpus* (Gaussian error-term), the presence of egg-clutches (binomial error-term) and the number of eggs in each egg-clutch (poisson error-term) among fish species. Because parasites were sampled on sampling sites that differed environmentally (notably in term of water temperature), we included 'site identity' as a categorical factor in all GLMs.

#### EVALUATING GENETIC DIFFERENTIATION BETWEEN PRINCIPAL AND ALTERNATIVE HOSTS

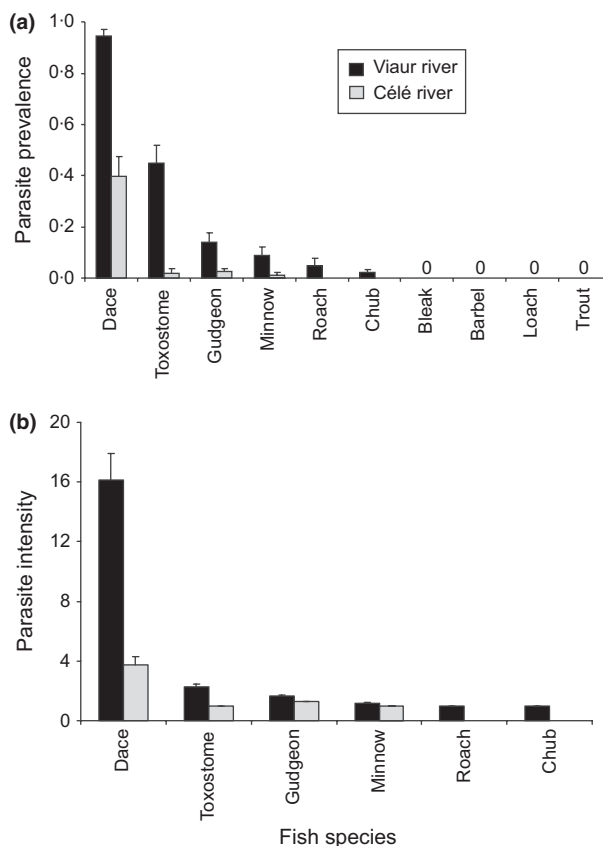
We used eight microsatellite markers to compare patterns of genetic diversity and genetic differentiation among parasites sampled on four fish species (parasite intensity and prevalence were too low on other fish species for valuable statistical analyses): dace ( $n = 79$  parasites spread over six sites), toxostome ( $n = 18$  parasites spread over four sites), gudgeon ( $n = 30$  parasites spread over six sites) and minnow ( $n = 16$  parasites spread over six sites). Microsatellite loci were specifically designed for *T. polycolpus* using throughput sequencing methods (Loot and Blanchet, unpublished protocol) and amplified using Polymerase Chain Reactions (see Appendix S1 for PCR recipes, primer sequences are available upon request). Amplified fragments were then separated on an ABI PRISM™ 3730 automated capillary sequencer (Applied Biosystems, Foster City, California, USA). Allelic sizes were then scored using GENEMAPPER™ v.4.0 (Applied Biosystems). For each parasite group (a parasite group by host species), locus-by-locus heterozygosity (observed and expected) as well as Fis estimates was calculated using GENETIX version 4.05.2 (Belkhir *et al.* 2002). Departure from the Hardy–Weinberg equilibrium (HWE) was tested using GENEPOP version 3.4 (Raymond & Rousset 1995). Two measures of genetic diversity were computed for each parasite group. Allelic richness (AR), corrected for the minimum sampling size ( $n = 16$ ), was computed using FSTAT version 2.9.3.2 (Goudet 1995), and expected heterozygosity ( $H_e$ ) was computed using GENETIX version 4.05.2 (Belkhir *et al.* 2002). These two measures of genetic diversity were compared among parasite groups using analyses of variance (ANOVAS). We then investigated the genetic divergence among the four parasite groups by estimating the  $F_{st}$  between all pairs of groups using the program FSTAT version 2.9.3.2 (Goudet 1995). The significance of each pairwise comparison was assessed using permutation-based tests (the level of significance was adjusted following Bonferroni corrections as follows:  $\alpha = 0.05/6 = 0.008$ ).

## Results

#### PATTERNS AND RATE OF ALTERNATIVE HOST USED BY *T. POLYCOLPUS*

Among the ten studied fish species, we found that six of them (four in the Célé River and six in the Viaur River) were infected by *T. polycolpus*; namely the dace, the toxostome, the gudgeon, the chub, the minnow and the roach (Fig. 2a). Accordingly, *T. polycolpus* prevalence varied significantly among species (Table 1a), with prevalence being particularly high for dace and toxostome (Fig. 2a). We also found that *T. polycolpus* prevalence significantly varied between rivers, with prevalence being higher in the Viaur River (Table 1a, Fig. 2a). However, this pattern does not hold true for all species as we found a significant interaction between 'river identity' and 'species identity'. This might indicate for instance that prevalence was particularly high for toxostome in the Viaur River, but not in the Célé River (Fig. 2a).

Concerning *T. polycolpus* intensity, we similarly found significant differences among species and between rivers (Table 1b). *Tracheliastes polycolpus* intensity was higher in



**Fig. 2.** Barplots representing (a) the mean parasite prevalence and (b) the mean parasite intensity on ten potential fish host species sampled in the Viaur (black bars) and Célé (grey bars) rivers. Parasite intensity is represented only for infected fish species. Error bars are standard errors across the sites sampled.

**Table 1.** Outputs of generalized linear mixed models testing the effects of river identity (i.e. two rivers) and species identity (i.e. ten potential fish host species) on (a) the prevalence of *Tracheliastes polycolpus* and (b) the *T. polycolpus* intensity on potential fish hosts. The interaction term between river identity and species identity was not computed for the parasite intensity model because all combinations were not represented. "d.f." is for degree of freedom

	d.f.	F-value	P-value
(a) <i>T. polycolpus</i> prevalence			
River identity	1, 15	22.494	<0.001
Species identity	9, 84	96.840	<0.001
River identity*Species identity	9, 84	17.744	<0.001
(b) <i>T. polycolpus</i> intensity			
River identity	1, 15	5.465	0.033
Species identity	5, 28	15.941	<0.001

the Viaur River where it was particularly high for dace (intensity was up to 16 individuals per host for dace, whereas it was between 1 and 2 individuals for all other species, Fig. 2b).

#### CAUSAL FACTORS UNDERPINNING THE USE OF ALTERNATIVE HOST SPECIES BY *T. POLYCOLPUS*

All tested models except one ('principal host density', Table 2, Fig. 1c) were interpretable, as the covariance structure of these models did not significantly differ from that of the data ( $\chi^2$  statistics; all  $P > 0.05$ , Table 2). The model demonstrating the lowest AIC value was the model including the 'parasite density' hypothesis only (see Table 2 and Fig. 1a for a graphical description of this model). According to this model, the standardized regression weight between parasite density on dace and prevalence on alternative hosts species was positive and highly significant

**Table 2.** Summary of the statistics used to decipher between the seven competing models used to explain rate of alternative host use. Competing model with *C*-value that follows a chi-square distribution are not rejected ( $P > 0.05$ ). *P*-value represents the probability that *C*-value has occurred by chance given the fact that data were generated by this competing model. Models are compared using Akaike Information Criterion (AIC) and  $\Delta$ AIC. Causal models with lowest AIC and  $\Delta$ AIC lower than 2 are the best models for fitting data. The best models are highlighted in bold

Causal model	<i>C</i> -value	d.f.	<i>P</i> -value	AIC	$\Delta$ AIC
Parasite density	1.66	3	0.645	15.66	0
River identity	4.55	3	0.208	18.55	2.884
Principal host density	8.71	3	0.030	22.71	7.044
Parasite density + River identity	1.35	2	0.510	17.35	1.684
Principal host density + Parasite density	1.66	2	0.440	17.68	2.014
Principal host density + River identity	4.43	2	0.110	20.44	4.774
Principal host density + Parasite density + River identity	1.32	1	0.250	19.32	3.654

( $r = 0.663$ ,  $n = 15$ ,  $P = 0.001$ ), hence validating predictions about the 'parasite density' hypothesis. When this correlation was measured for each river separately, we found a positive and significant correlation for the Célé River ( $r = 0.927$ ,  $n = 7$ ,  $P = 0.007$ ) while the correlation was still positive, but weaker and not significant for the Viaur River ( $r = 0.494$ ,  $n = 8$ ,  $P = 0.249$ ). It is worth noting that this model cannot be considered as the single best model for fitting the data. Indeed, a second model ('parasite density + river identity', Fig. 1d for illustration) has a  $\Delta$ AIC lower than 2, indicating that this model is also well supported by the data (Table 2). This second model further suggests that, all things being equal, alternative host use tends to be higher in the Viaur than in the Célé River.

#### CAUSAL FACTORS UNDERPINNING THE CHOICE OF AN ALTERNATIVE HOST

None of the three traits we tested demonstrated phylogenetic signal that significantly departed from Brownian motion evolution (Table 3). There was therefore no clear phylogenetic conservatism for any of these traits. This result is not in accordance with the 'phylogenetic similarity' hypothesis.

According to the GLMMs, we found a highly significant and positive relationship between *T. polycolpus* prevalence and the index of ecological similarity (Table 4). In addition, the interaction term between the index of ecological similarity and river identity was not significant, indicating that the slope of this relationship did not differ between rivers (Table 4). This relationship indicates that high values of ecological similarity (i.e. a species that is ecologically close to *L. burdigalensis*) are significantly associated with higher *T. polycolpus* prevalence, hence providing support for the 'host ecological similarity' hypothesis. Neither of the other parameters was significant, even when interaction terms were removed from the analysis (not shown), which leads little support for the two other hypotheses.

#### PARASITE FITNESS ON ALTERNATIVE HOSTS

*Tracheliastes polycolpus* fitness traits were analysed for four fish species only: dace, toxostome, gudgeon and minnow. Among these species, toxostome are the most ecologically close to the principal host (similar body size and feeding habits), whereas minnow are the most ecologically

**Table 3.** Test of phylogenetic signal for parasite prevalence, host ecological similarity and alternative host density. No significant phylogenetic signals were detected

Trait	K	PICobs	PICrand	<i>P</i> -value
Parasite prevalence	1.384	0.315	0.504	0.127
Host ecological similarity	0.587	1.994	18.362	0.488
Alternative host density	0.558	1633.081	1964.627	0.267

**Table 4.** Outputs of generalized linear mixed models (GLMMs) aiming at testing the effects of river identity (two rivers), alternative host availability (measured as alternative host densities), host phylogenetic similarities (measured as the phylogenetic distance between each alternative host species and the principal host species, *Leuciscus burdigalensis*) and host ecological similarities (measured as the similarity between each alternative host species and the principal host species, *Leuciscus burdigalensis*), on the level of *Tracheliastes polycolpus* alternative host use

	d.f.	F-value	P-value
River identity	1, 2	4.854	0.159
Alternative host density	1, 2	1.259	0.379
Host phylogenetic similarity	1, 7	1.512	0.344
Host ecological similarity	1, 7	14.161	<b>0.007</b>
Alternative host density*River identity	1, 2	0.669	0.499
Host phylogenetic similarity* River identity	1, 2	1.956	0.297
Host ecological similarity* River identity	1, 2	16.014	0.057

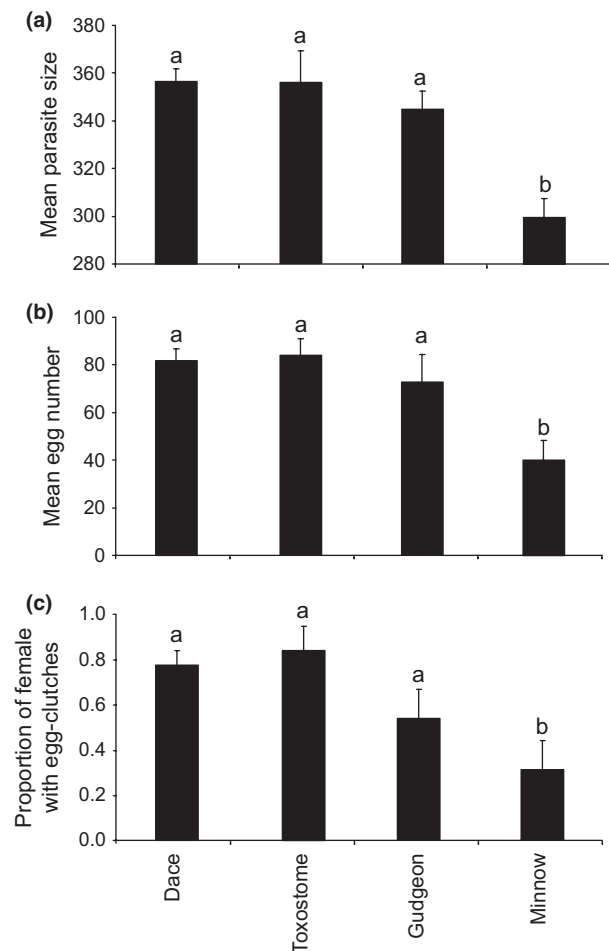
**Table 5.** Outputs of generalized linear models testing the effects of host species identity and sampling site identity on (a) the body size, (b) the number of eggs and (c) the presence of egg-clutch of the ectoparasite *Tracheliastes polycolpus*

	d.f.	F-value	P-value
(a) <i>T. polycolpus</i> body size			
Species identity	3, 76	9.217	<0.001
Site identity	3, 71	0.792	0.559
(b) Eggs number			
Species identity	3, 50	3.409	0.025
Site identity	3, 45	0.647	0.665
(c) Presence of egg-clutch			
Species identity	3, 76	NA	0.007
Site identity	3, 71	NA	0.469

distant (smaller body size and drift feeder, while dace are bottom feeders). For the three traits we measured (parasite size, egg number and the presence of egg-clutches), we found significant differences between fish species (Table 5). Particularly, mean parasite size was significantly smaller, eggs were significantly less numerous, and egg-clutches were significantly less present in minnows than in other fish species (Fig. 3). It is worth noting that this fish species also has the lowest parasite prevalence and intensity among these four fish species (Fig. 2) and is also the smallest fish species.

#### GENETIC DIFFERENTIATION BETWEEN PARASITES ON PRINCIPAL AND ALTERNATIVE HOST SPECIES

We detected no significant deviation from HWE for any loci and parasite groups. Neither did we detect significant linkage disequilibrium among loci. Patterns of genetic diversity were similar among parasites from different host fish species (ANOVAS, all *P*-values > 0.58). There was no



**Fig. 3.** Barplots representing fitness traits of *Tracheliastes polycolpus* on four fish host species sampled in the Viaur river. Bars are (a) the mean parasite body size, (b) the mean egg number per egg-clutch and (c) the proportion of female parasites with egg-clutches. Error bars are standard errors. Bars with the same letters are statistically identical (contrast tests,  $P > 0.05$ ).

**Table 6.** Table showing the genetic differentiation between *Tracheliastes polycolpus* specimens anchored in four host fish species: the dace (the principal host species), the toxostome, the gudgeon and the minnow. The upper diagonal shows the *F*<sub>st</sub> values (a measure of genetic differentiation) calculated using eight microsatellites markers. The lower diagonal shows the *P*-values for these *F*<sub>st</sub> ( $\alpha = 0.05/6 = 0.008$  after a Bonferroni correction)

	Dace	Toxostome	Gudgeon	Minnow
Dace	–	0.018	0.000	0.002
Toxostome	0.043	–	0.006	0.034
Gudgeon	0.421	0.229	–	0.006
Minnow	0.374	0.014	0.252	–

significant genetic differentiation among parasites from the four host fish species (Table 6).

#### Discussion

We found that *T. polycolpus* was able to use alternative host species, with the rate of alternative host use varying



across rivers and sampling sites. Overall, our results show that the rate at which *T. polycolpus* uses alternative host species increases with the density of *T. polycolpus* on the principal host, providing strong support for the ‘parasite density’ hypothesis. This observation is congruent with an experimental finding in another system demonstrating that the use of alternative host species increases with the density of the parasitoid wasp *Nasonia regina* (Cornell & Pimentel 1978). This positive relationship between parasite intensity and alternative host use may be due either to a ‘passive’ mechanism whereby increasing propagules increases the encounter rate between potential host species and the infective stage of the parasite or to an ‘active’ mechanism whereby parasites maximize their fitness using alternative host species for reducing intraspecific competition on the principal host species (Poulin 1998; Emelianov 2007). Although it is likely that these two mechanisms act simultaneously, we previously demonstrated that *T. polycolpus* fitness (i.e. body size and egg number) is positively correlated with parasite intensity (Loot *et al.* 2011), indicating that intraspecific competition may not be a major issue in *T. polycolpus*. Interestingly, we further found that the positive relationship between parasite intensity and the rate of alternative host use was stronger and steeper in the Célé River than in the Viaur River, the former river sustaining a lower parasite burden. Parasite burden observed in the Viaur River is extremely high (Loot & Blanchet, unpublished data), and this may indicate that the rate of alternative host use reaches a threshold at high parasite density. This result would indicate that environmental conditions may indirectly affect the rate of alternative host use, notably when particular conditions favour the life cycle of *T. polycolpus*. Additional direct environmental effects cannot be ruled out as, all else being equal, we found evidence that the rate of alternative host use was higher in the Viaur River (see the second best model in Table 2). Further empirical and experimental studies are required to better understand the direct and indirect links between environmental conditions and alternative host use. In contrast, we found weak support for the ‘principal host density’ hypothesis (Bush & Kennedy 1994; Koh *et al.* 2004). Overall, this leads us to conclude that parasite intensity on the principal host was the main factor underpinning differential rates of alternative host use.

Our results also suggest that the use of alternative hosts is not random, but, on the contrary, is favoured towards targeted host species. Among the nine potential alternative host species we investigated, five of them have been found to be infected by *T. polycolpus*, which confirms previous studies (Yamaguti 1940; Tuffery 1967; Galicka & Penczak 1989; Sobecka, Jukiewicz & Piasecki 2004; Barzegar & Jalali 2009). Although these five fish species belong to the Cyprinids family, we failed to detect a significant phylogenetic conservatism regarding the use of a particular alternative host species, which is congruent with other findings in host–parasite interactions (Poulin 2005; Grim *et al.* 2011; but see Krasnov *et al.* 2004; Khokhlova *et al.* 2012).

This may be explained by (i) a lack of phylogenetic conservatism for physiological, immunological or life-history traits involved in the resistance of host to parasite (Poulin 2005) or (ii) the reduced taxonomic scale at which we investigated the strength of phylogenetic conservatism (the wider the scale investigated, the higher the chance to detect a significant phylogenetic signal, Poulin, Krasnov & Mouillot 2011). Encounter rate between a given host species and a parasite (i.e. specific encounter rate) has been proposed as an important mechanism in explaining patterns of alternative host use in host communities (Combes 2001; Detwiler & Minchella 2009; Cooper *et al.* 2012). Specific encounter rate can increase if host availability increases (Detwiler & Minchella 2009), and/or if alternative host species live in close ecological contact with the principal host species (i.e. the main reservoir of parasites, Cooper *et al.* 2012). Here, there was no significant effect of the density of alternative host species on the success of alternative host use, hence providing little support for the ‘alternative host availability’ hypothesis (see also Teder, Tammaru & Pedmanson 1999 for a similar experimental result). Rather, our results support the hypothesis that ecological similarity between alternative and principal hosts increases the use of a particular alternative host species (the ‘host ecological similarity’ hypothesis), and hence reinforce recent findings (Cooper *et al.* 2012). It is, however, noteworthy that the relationships between ecological similarity and alternative host use were overall relatively weak. This indicates that other potential traits explaining the actual use of a particular alternative host species may have been missed, and future studies should consider host immunological and behavioural resistance (Clayton *et al.* 2003; Bush & Clayton 2006; Bush 2009) as well as physiological incompatibilities (Bush, Sohn & Clayton 2006; Grim *et al.* 2011).

For two of the alternative host species (toxostome and gudgeon), we found that parasite fitness traits (body size, number of eggs and proportion of females with egg-clutches) were similar between principal and alternative host species. However, all fitness traits were severely depressed when *T. polycolpus* was sampled on the minnow. This result is consistent with a recent experimental study demonstrating that fitness greatly varies among alternative host species, with fitness tending to be higher when parasites infected alternative host species closely related to the principal host species (Khokhlova *et al.* 2012). Here, given the inferred phylogenetic relationships between host species (Fig. S1), phylogenetic distance between principal and alternative host species was probably not the cause of the decreased fitness observed in parasites infecting minnows. Rather, this result provides additional support to the growing view that host body size may strongly constrain the success of alternative host use (Bush & Clayton 2006; Bush 2009; Detwiler & Minchella 2009). Indeed, the minnow is the smallest (i.e. max length = 10 cm, mean length = 5 cm) of the three alternative host species we investigated for parasite fitness and is hence the species

with the greatest body size divergence from the principal host. It is possible that energy provided by such small-bodied species is not sufficient for *T. polycolpus* to adequately develop eggs and/or that the feeding and anchoring apparatus (i.e. the maxilla and the bulla respectively) used by *T. polycolpus* does not allow optimal grazing of fins of small-bodied species (Clayton & Johnson 2003; Bush & Clayton 2006; Ziętara *et al.* 2007).

Using highly polymorphic microsatellite markers, we failed to detect significant population divergences among parasite specimens sampled from the alternative host species. This result lends little support to the hypothesis that the use of alternative host species by *T. polycolpus* results from switching events (*sensu stricto*), but rather suggests that *T. polycolpus* is a generalist parasite with significant mixing among parasite specimens from different host species. This conclusion has to be considered with care as it is possible that (i) host switching is too recent to generate divergences among these parasite ‘populations’ and/or (ii) that our genetic markers are not powerful enough to detect subtle differentiation among populations. Regarding the latter, a regional scale analysis of the genetic diversity of *T. polycolpus* (sampled on dace only) revealed (with the same set of markers) a spatial structure with significant divergences between populations isolated by ~100 km within the same river basin (Blanchet & Loot, unpublished). This indicates that our markers may have enough power to detect population structure in this species and that geographical isolation might be more important than habitat (i.e. host species) isolation in driving *T. polycolpus* population divergence. Irrespective of the exact mechanism explaining our patterns, our results indicate that interbreeding may still occur at a rate impeding genetic divergence, which would indicate that phenotypic plasticity may play a role in the use of alternative host species.

To conclude, the use of alternative host species (i.e. host specificity) has long fascinated evolutionary ecologists (Poulin 1998; Combes 2001; Kuris *et al.* 2007) as it is related to the evolution of life-history strategies in parasites, and because it questions the mechanisms enabling a parasite to be, or not to be, a generalist (Combes 2001; Bush & Clayton 2006; Grim *et al.* 2011). However, few studies have dealt with the ecological determinants of alternative host use in natural populations. Our study is one of the few highlighting the ecological factors (parasite density and ecological similarity) at the forefront of a major eco-evolutionary process. In addition, our results are of prime importance for fundamental and conservation issues, as the ability of parasites and pathogens to use alternative host species may result in the emergence of new diseases and is hence of great concern to human health and the management of wild and domesticated populations of animals. For instance, understanding factors and processes driving alternative host use may help to better predict the potential effects of non-native parasites, notably if parasites use alternative host species as a ‘bridgehead’ for reaching new sites of invasion. Finally, we show that the

fitness of parasites remains remarkably high in most, although not all, alternative host species. In such a circumstance, one may ask why we observed variation in the rate of alternative host use. Indeed, if the fitness of the parasite is similar between principal and alternative host species, we might predict that parasites should always use alternative host species at the same rate across environmental conditions and even that parasites should not have a preference for a principal host species (dace in our case). An element of the response may be that encounter rate may not only be a key factor in predicting the use of alternative host species (our result), but also in predicting which species will be the principal host species (Cooper *et al.* 2012). Similarly, the high fitness observed on alternative host species suggests an important role of phenotypic plasticity in favouring alternative host use and hence for explaining the specialist-generalist continuum in parasites. These are in our opinion two interesting perspectives for future studies.

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## Supporting Information

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

**Table S1.** Sampling size for each potential host species.

**Fig. S1.** Phylogenetic tree for the potential host species.

**Appendix S1.** Description of the microsatellites recipes.