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Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: a multilevel causal analysis

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Summary

1. Understanding the ecological factors driving the burden and pathogenicity of parasites is challenging. Indeed, the dynamics of host–parasite interactions is driven by factors organized across nested hierarchical levels (e.g. hosts, localities), and indirect effects are expected owing to interactions between levels.

2. In this study, we combined Bayesian multilevel models, path analyses and a model selection procedure to account for these complexities and to decipher the relative effects of host- and environment-related factors on the burden and the pathogenicity of an ectoparasite (*Tracheliastes polycolpus*) on its fish host (*Leuciscus leuciscus*). We also tested the year-to-year consistency of the relationships linking these factors to the burden and the pathogenic effects of *T. polycolpus*.

3. We found significant relationships between the parasite burden and host-related factors: body length and age were positively related to parasite burden and heterozygous hosts displayed a higher parasite burden. In contrast, both host- and environment-related factors were linked to pathogenic effects. Pathogenicity was correlated negatively with host body length and positively with age; this illustrates that some factors (e.g. body length) showed inverse relationships with parasite burden and pathogenicity. Pathogenic effects were stronger in cooler upstream sites and where host density was lower. Path analyses revealed that these relationships between environment-related factors and pathogenic effects were direct and were not indirect relationships mediated by the host characteristics. Finally, we found that the strength and the shape of certain relationships were consistent across years, while they were clearly not for some others.

4. Our study illustrates that considering conjointly causal relationships among factors and the hierarchical structure of host–parasite interactions is appropriate for dissecting the complex links between hosts, parasites and their common environment.

Key-words: Bayesian statistics, fish, heterozygosity-fitness relationships, host–parasite interaction, model selection, multilevel models, path analysis, structural equation modelling

Introduction

Given the rising incidence of many infectious diseases worldwide, understanding the ecological factors that control pathogen burden and pathogenicity is of prime importance for biodiversity conservation, the economy and human health (Harvell *et al.* 2002; Lafferty 2009). In the case of human pathogens, several approaches have been developed to identify the drivers of infectious diseases and predicting their pathogenic consequences (e.g. Carabin *et al.* 2003; Lowen *et al.* 2007). However, relatively little attention has been devoted to macroparasites affecting wildlife (but see Byers *et al.* 2008; Ostfeld *et al.* 2006).

Host–parasite systems are particular biotic interactions in the sense that their dynamics is driven by intricate factors (biotic and/or abiotic factors) and processes that interact across several scales of observation. They are indeed organized within a hierarchical structure that can make the interpretations of the environmental drivers of parasite distribution more complex (Diez & Pulliam 2007; McMahon & Diez 2007). For instance, hosts are characterized by phenotypic attributes that make them more or less resistant

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(defined here as the ability to limit parasite burden, Råberg, Graham & Read 2009) to parasites. However, these phenotypic differences can be overridden because hosts that belong to the same habitat often share common environmental factors that can themselves affect parasite infection. In such cases, two phenotypically contrasted hosts from the same habitat could share a similar parasitic load as two phenotypically similar hosts from different habitats.

Basically, the distribution of a parasite is affected by factors acting at two scales of observations (or 'levels'): the host and the surrounding environment. The host level includes factors related to the life history of the host (Combes 2001). For example, parasite intensity and prevalence often increase with the age and/or size of hosts (Loot *et al.* 2002; Vaclav, Calero-Torralbo & Valera 2008). This level also includes parameters related to the immunity and the genetic diversity of hosts (Wegner *et al.* 2003; Ortego *et al.* 2007; Blanchet *et al.* 2009b). For example, Blanchet *et al.* (2009b) found that being homozygotic at two particular microsatellite loci significantly decreased the probability of a host fish being infected by a fin-feeding ectoparasite. The environmental level corresponds to the location in which hosts (and hence their parasites) are living. It includes biotic and abiotic factors such as the host density, temperature or even pollution (Marcogliese 2005; Perez-del-Olmo *et al.* 2009). Interestingly, the effects observed at this level can be direct when a given factor directly affects the fitness of a parasite (which is often the case for ectoparasites, Oorebeek & Kleindorfer 2008), or indirect when a given factor affects a host attribute that is related to its resistance to a parasite (Aaltonen, Valtonen & Jokinen 1997). For instance, Aaltonen, Valtonen & Jokinen (1997) showed that water pollution (i.e. bleached pulp and paper mill effluents) reduced the hosts' immune system (i.e. the roach, *Rutilus rutilus*) and hence decreased their resistance to parasites. Deciphering the interactions between levels that characterize wild populations is a real challenge. However, accounting for both the hierarchical structure of host–parasite systems and the interactions between levels would certainly clarify the intricate relationships linking parasites, hosts and the environment.

A reliable analysis of data from such complex systems depends on the formulation of proper statistical models (Wikle 2003). Multilevel (or hierarchical) modelling is one possible tool for dealing with hierarchically structured data (McMahon & Diez 2007). More particularly, Bayesian multilevel models (BMMs) are of special interest because their implementation is flexible and allows the incorporation of multiple sources of variability, as well as specific correlation structures in the errors of the model (Carabin *et al.* 2003). Moreover, because in Bayesian models parameters are considered random and have a distribution iteratively generated with algorithms (e.g. Markov Chain Monte Carlo), problems inherent to low sample size can easily be handled (Basañez *et al.* 2004). However, BMMs by themselves cannot account for the interactions between levels. For example, deciphering the relative roles of direct vs. indirect environmental effects cannot be done properly unless explicitly causal models, such as confirmatory path analysis, are used. Path analysis is a statistical method in which the paths between variables are relationships (expressed as equations) where the response variables are driven by predictor(s). Very recently, path analysis has been extended to multilevel models (Shipley 2009), hence enabling the relationships between several causal relationships within a multilevel framework to be disentangled.

In this study, we combined BMMs, path analyses and a model selection procedure (Johnson & Omland 2004) to tease apart the relative effects of several ecological drivers on the burden and the pathogenicity of an ectoparasite (*Tracheliastes polycolpus*, von Nordmann 1832) on its fish host (the common dace, *Leuciscus leuciscus*, Linnaeus 1758). *Tracheliastes polycolpus*, a crustacean copepod, feeds on fins (until partial or total destruction) and severely reduces its host's fitness (Blanchet *et al.* 2009a). In this study, the first objective was to distinguish between the direct effects of host-related factors (i.e. age, body length, heterozygosity at two microsatellites and the growth rate of the host before infection), the direct effects of environment-related factors (i.e. the physical and chemical environment and host density) and the indirect effects of those environment-related factors on the burden, as well as the pathogenicity of *T. polycolpus*. Few studies have considered conjointly the analysis of parasite burden and pathogenic effects. This may be mainly attributed to the difficulty of evaluating the pathogenic effects of parasites in the wild. Here, the main pathogenic effect of *T. polycolpus* (i.e. the destruction of its fins' host, Loot *et al.* 2004) can easily be measured in the field. Taking advantage of this specificity, we tested whether the burden and pathogenic effects responded to the same factors (and in similar way): an important but rarely tested hypothesis. As we were dealing with an ectoparasite, we expected that the direct effects of both environment- and host-related variables would be considerable in explaining the burden as well as the pathogenic effects of *T. polycolpus* on *L. leuciscus*. As a second objective, we sought to test for temporal consistency (i.e. year-to-year variation) on the strength and the shape of the effects of host- and environment-related variables on the burden and the pathogenicity of *T. polycolpus*. A strong temporal consistency in the strength and the shape of the relationships between predictors, parasite burden and the pathogenicity would indicate that predictions based on restricted *a priori* knowledge are possible in such a system.

Materials and methods

BIOLOGICAL MODEL

Dace is a rheophilus Cyprinid fish species inhabiting cold streams and rivers from Western and Central Europe. Dace is the common host of *T. polycolpus*, a harmful parasitic copepod of the Lernaepodidae family. Dace can experience high parasitic loads, with up to eighty *T. polycolpus* per individual have been reported in the literature (Loot *et al.* 2004). Only the adult females of *T. polycolpus* are parasitic and they anchor themselves to the host's fins where they feed on epithelial cells and mucus. By their grazing activity, the parasites cause local bacterial inflammation, and a partial to total degradation

of the host's fins (see Blanchet *et al.* 2009b; Loot *et al.* 2004 for pictures). Fin degradation caused by female *T. polycolpus* significantly decreases the growth rate of rostrum dace (Blanchet *et al.* 2009a) and might be implicated in selective fish mortality (Blanchet *et al.* 2009b).

STUDY AREA AND SAMPLING DESIGN

We focused our study on a single dace population from the river Viaur (south-western France) that we have been monitoring for several biotic and abiotic parameters since 2006. The river Viaur is 169 km long and is located in the Adour-Garonne river drainage area. To encompass the entire environmental variation existing along the upstream–downstream river gradient, dace were sampled by electrofishing at eight sites evenly distributed along the main river channel (see Grenouillet *et al.* 2008 for more details). We therefore covered the whole range distribution of dace in this river. Fish sampling was carried out in the summer (i.e. within the first 2 weeks of July) of three consecutive years (2006, 2007 and 2008) so that all the sites were sampled three times. Because of technical constraints, one of the eight sites was not sampled in 2007 (Appendix S1).

A mean number of eighteen dace (range: 4–37, see Appendix S1) were collected at each sampling site and sampling year according to their local density. All sampled dace (N_{tot} = 406) were anaesthetized and measured (total body length ± 1 mm). The total number of *T. polycolpus* anchored to the fins of the dace was recorded to calculate individual parasitic burdens (mean = 13·17 parasite per fish, range: 0–85, see Appendix S1 for more details). To evaluate the pathogenic effects of *T. polycolpus*, we measured the level of fin degradation for each fish sampled. We visually scored 0, 1, 2, 3 or 4 points if a fin was respectively 0%, 25%, 50%, 75% or 100% eroded by the parasites. The scores attributed to each fin were summed over all the fins so that we obtained a single total score of fin degradation for each fish. The maximum score attainable was 7 fins * 4 points = 28. The maximum score we actually obtained was 16 [mean = 1·29 ± 0·35 (± SE)]. In addition, a minimum of three scales were removed from each fish to estimate the age and growth rate (see Blanchet *et al.* 2009a for details on calculation). Finally, we removed a pelvic fin tissue sample (2–4 mm^2) from each fish for genetic analysis (see the section below). All dace were then released alive at their original sampling site.

MEASUREMENT OF VARIABLES

The hierarchical sampling design used here allowed us to include both host- and environmental- levels processes (McMahon & Diez 2007). Each level was characterized by a series of factors as described below. The same factors were used to explain both the variation observed for parasite burden and for pathogenic effects (i.e. fin degradation).

Host-related factors

At the host level, we selected age (in years), total body length, growth during the first year of life and the heterozygosity measured independently at two microsatellite loci. In addition, parasite burden was used as an additional factor to explain the pathogenic effects of *T. polycolpus*. In general, older individuals generally harboured more parasites (Loot *et al.* 2002; Vaclav, Calero-Torralbo & Valera 2008). This can arise because older individuals accumulate parasites during their lifetime (the 'cumulative hypothesis' Hawlena, Abramsky & Krasnov 2005; Hayward *et al.* 2009; Roulin *et al.* 2007; Vicente, Perez-Rodriguez & Gortazar 2007). Similarly, larger hosts also harbour more parasites because they offer a higher body surface for parasites to anchor on (the 'surface hypothesis', Arneberg, Skorping & Read 1998; Bandilla, Hakalahti-Siren & Valtonen 2008). These two hypotheses can be confounded because age and body size often covary, notably in fish. Furthermore, in rostrum dace, Blanchet *et al.* (2009a) have shown that hosts with a high growth rate during their first year of life (a period of their life history during which dace are not parasitized by *T. polycolpus*) were more able to limit the fin damages caused by *T. polycolpus* (i.e. to tolerate, Råberg, Graham & Read 2009). It has been proposed that hosts that accumulated enough energy before infection (i.e. the hosts that were bigger before their first winter) were more prone to allocate resources for defending against the feeding activity of the parasite (Blanchet *et al.* 2009a). The growth during the first year of life was estimated using back-calculation methods from the growth zones of scales (Blanchet *et al.* 2009a). Finally, several studies have shown that heterozygosity measured at a set of microsatellites correlated significantly with parasite load, with heterozygous hosts, in general, being more resistant (Acevedo-Whitehouse *et al.* 2006; Ortego *et al.* 2007). In the dace – *T. polycolpus* complex, among a set of fifteen loci, two microsatellites (*Lid 8* and *Rru 4*) were significantly linked to parasite burden, with – unexpectedly – hosts being homozygotes for one (or the two) loci were more resistant than heterozygotes (Blanchet *et al.* 2009b). A full description of the analyses of these loci is provided by Blanchet *et al.* (2009b).

Environment-related factors

At the environmental level, we selected physical descriptors of the sampling sites [altitude (m), slope (%), river width (m), river depth (m), water velocity ($m\ s^{-1}$) and temperature (°C)] and chemical descriptors [dissolved ammonia (NH_4-N), silicate (SiO_2), dissolved nitrites (NO_2-N), dissolved nitrates (NO_3-N) and phosphate (PO_4)]. These physical and chemical descriptors have been found to structure the composition of the upstream–downstream gradient for fish, macroinvertebrate and diatom assemblages in the river Viaur (Grenouillet *et al.* 2008). These variables could affect both the physiological condition of the hosts as well as the life history of the parasites. A detailed description of the measurement of these variables is provided in Appendix S2.

To limit colinearity between variables, we computed a principal component analysis (PCA) on all the normalized physical and chemical variables. The first two axes of this PCA, accounting for 75% of the total variation, were kept as two synthetic independent variables (see Appendix S3 for details). The first axis mostly reflected physical variables (and was therefore named the 'physical axis' while the second axis reflected mostly chemical variables (i.e. the 'chemical axis') (Appendix S3). The major exception to this pattern was the monthly coefficient of variation for the water temperature that was highly correlated with the chemical axis rather than to the physical axis. Sites with high loading values on the physical axis were sites characterized by high altitude, low annual water temperature and high slopes, which corresponded to upstream sites. Sites with high loading values on the chemical axis were characterized by high monthly variation in water temperature but low concentrations of dissolved inorganic compounds.

In addition, host density is a well-known factor affecting the population dynamic of most parasites (see the concept of the critical community size by Grenfell & Harwood 1997). We therefore measured host density at each sampling site as the number of dace captured using electric fishing per unit time. This variable was included as an additional predictor in our analyses, which lead to three independent

predictors at the environmental level (host density, physical axis and chemical axis).

STATISTICAL ANALYSES

Our first objective was to test whether the *T. polycolpus* burden and pathogenicity were influenced by the direct effects of host-related factors, the direct effects of environment-related factors and/or the indirect effects of environment-related factors. We used a three-step modelling framework to reach this goal. First, we fitted two general models (one for ectoparasite burden and the other for ectoparasite pathogenicity) that were aimed at equating the relationships between each of the two response variables and the various explanatory variables, while accounting for the hierarchical structure of the data (see Appendix S4 for a mathematical formalization). This was performed with BMMs (Gelman & Hill 2006). Secondly, according to these general BMMs, we proposed a series of competing causal models that each corresponded to the different biological hypotheses we sought to test (five hypotheses for each response variable, see Appendices S5 and S6 and section Building competing hypotheses and competing models below). This is the core of path analyses (Shipley 2000, 2009). Thirdly, we used a model selection procedure (Johnson & Omland 2004) to decipher these different competing models.

Our second objective was to test for temporal consistency (i.e. year-to-year variation) on the strength and the shape of the effects of the various explanatory variables on each of the two response variables. This was done by modifying the chosen models (in step 3) according to certain statistical specificities of BMMs (Gelman & Hill 2006).

General BMMs

Because hosts are nested within sites themselves nested within years, hosts from a same sampling site cannot be considered as statistically and biologically independent. Likewise, sampling sites from a given year cannot be considered as independent. To account for such a hierarchical structure, we fitted BMMs using the WinBUGS software (Spiegelhalter & Best 2003). Following Gelman & Hill (2006), we built general BMMs structures with intercepts varying among years and constant slope coefficients to test our first hypothesis. For both models (parasite burden and pathogenicity), the general structure is described in Appendix S4 (WinBUGS codes for these two models available upon request). We used Markov chain Monte Carlo (MCMC, Gibbs sampler) sampling methods to characterize the posterior distributions of model parameters (Spiegelhalter & Best 2003). Standardized slope coefficients were used to assess the relative importance of each of the predictors (Murray & Conner 2009).

Building competing hypotheses and competing models

We used the path analyses framework (Shipley 2000; Grace 2006) to build several causal models corresponding to the five biological hypotheses we sought to test. For each response variable, we built five different competing models (illustrated in Appendices S5 and S6 for *T. polycolpus* burden and its pathogenic effects respectively). In the first model, we hypothesized that the parasite burden and pathogenic effects were simultaneously governed by both direct and indirect effects of environment-related factors and by host-related factors [see models (a) in Appendices S5 and S6]. In the second model, we hypothesized that the parasite burden and pathogenic effects were governed by direct effects of environment-related factors and by host-related factors [see models (b) in Appendices S5 and S6]. The third model corresponded to the hypothesis that the parasite burden and pathogenic effects were governed only by direct effects of environment-related factors [see models (c) in Appendices S5 and S6]. In the fourth model, the parasite burden and pathogenic effects were governed by indirect effects of environment-related factors and by host-related factors [see models (d) in Appendices S5 and S6]. Finally, in the fifth model, we hypothesized that the parasite burden and pathogenic effects were governed by host-related factors only [see models (e) in Appendices S5 and S6]. In these models, we did not consider the possibility that hosts influence their physical/chemical environment because 'niche construction' (Odling-Smee, Laland & Feldman 1996) processes are unlikely in this fish species (mainly because of its feeding behaviour and its relatively low abundance relative to other fish species).

In a hierarchical framework, causal links involve variables of different levels. We therefore applied directional separation tests (d-*sep* tests) of path models to overcome the difficulty imposed by the hierarchical framework (Shipley 2000, 2009). D-*sep* tests are based on directed acyclic graphs (DAG) and aim to test the fit between data and the proposed model(s) by determining the dependence or independence of all pairs of variables after statistically accounting for all possible sets of other variables (Shipley 2009). Particularly, for each DAG, we listed a set of independence claims. Independence claims are pairs of variables that should be statistically independent after accounting for other variables. In DAGs, independence claims are simply defined by pairs of variables that are not linked by an arrow. The number of independence claims varies according to the number of arrows (direct or indirect effects) included in the models. These methodologies have recently been described in depth by Shipley (2009), and we summarize the principle of d-*sep* tests in Appendix S7.

In d-*sep* tests, it is expected that if data are generated according to a given causal graph, then the null probabilities of each independence claim are mutually independent; in other words, the data are well supported by the model (Shipley 2009). This property is verified by testing whether the *P*-values obtained for the set of independence claims follow a chi-square distribution with $2k$ d.f. (k being the number of claims, Shipley 2009). As proposed by Shipley (2009), we therefore calculated a *C*-value for each hypothesis (see the formula in Appendix S8). Each *C*-value was compared with a chi-squared distribution (d.f. $= 2k$), and we rejected the causal model if the *C*-value was unlikely to have occurred by chance.

Comparing competing hypotheses

An initial step in comparing the different competing models was to verify whether the data were well supported by the model according to the *C-test* presented previously. However, the same data set can be well supported by several causal models. We therefore used a model selection procedure to identify the best model(s) among the set of competing models (Johnson & Omland 2004).

As described by Grace (2006), we adapted classical information criteria [e.g. Akaike Information Criterion (AIC)] to cope with the path-modelling framework. In this framework, and for each competing model, the information criteria is a weighted sum of a measure of badness of fit (i.e. the *C*-value) and of a measure of complexity (i.e. the number of parameters to be estimated, q), with simple models that fit well receiving low scores (Grace 2006). According to Johnson & Omland (2004), information criteria must be corrected for small sample size when the number of parameters exceeds $n/40$. This was the case for all competing models tested here. We therefore used an AIC modified for path analyses and corrected for small sample size (AICc) that was calculated as follows:

$$\text{AICc} = C + 2q * [n/(n - q - 1)]$$

with C the C-value, q the number of parameters and n the sample size.

As for other information criteria, the model that showed the lowest AICc value was considered as the 'best model' (Johnson & Omland 2004). In addition, we calculated the differences in these AICc values between each model and the best model (i.e. Δ_{AICc}). A single best model cannot be assumed if the Δ_{AICc} with other competing models is not > 2 units (Johnson & Omland 2004).

Temporal consistency of the effects

We built on the model(s) selected through the model selection procedure to test whether the effects of host- and environment-related variables were, or were not, temporally consistent (in terms of strength and shape). To do so, we simply modified the general BMMs presented earlier by varying slope coefficients between years (i.e. varying intercepts and slopes models, Gelman & Hill 2006, codes available upon request). This enabled us to obtain a single slope coefficient for each variable and each year independently. For each variable, we visually compared the slope coefficient obtained for each year with the slope coefficient obtained using the varying intercepts and constant slopes models.

Results

DESCRIPTION OF THE DATA

We performed two generalized linear models in which individual parasite burden and fin degradation were the response variables, and sampling sites and sampling years were the categorical explanatory variables. The interaction terms between 'sites' and 'years' were highly significant for both models (Appendix S9). This indicated that the differences in mean parasite burden and mean fin degradation among sampling sites significantly varied among sampling years (Fig. 1a,b).

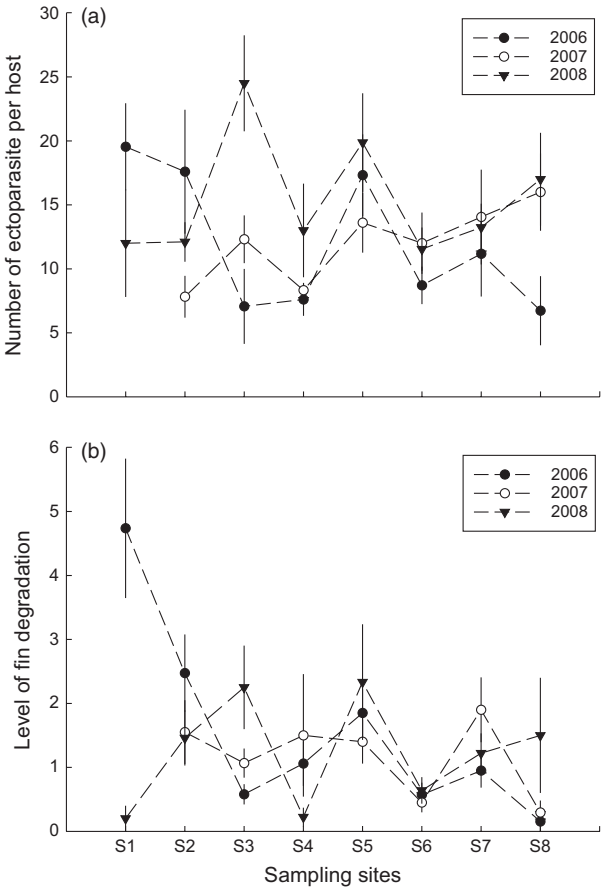

Fig. 1. (a) Patterns of parasite distribution (*Tracheliastes polycolpus*) (i.e. number of ectoparasite per host, *Leuciscus leuciscus*) among the eight sampling sites (arranged from upstream, S1, to downstream, S8) and the three sampling years (2006, 2007 and 2008) in the river Viaur. (b) Patterns of pathogenic effects imposed by *T. polycolpus* on its host *L. leuciscus* among the eight sampling sites and the three sampling years in the river Viaur. Pathogenic effects are expressed as the level of fin degradation. Bars are mean ± SE.

PATH ANALYSES AND MODEL SELECTION PROCEDURE

Concerning parasite burden, only one of the five competing models was statistically rejected (direct environment model, chi-squared statistics: $P < 0{\cdot}001$, see Table 1a). The four remaining models were well supported by the data because their C-values did not statistically differ from a chi-square distribution with $2k$ degrees of freedom (Table 1a). Among these four competing models, the 'Direct host' model had the lowest AICc value and the Δ_{AICc} were all greater than two units, meaning that this model was the single best model for fitting the data (Table 1a, see Fig. 2a for a graphical representation). In this model, the parasite burden was directly affected by variables from the host level but not by environmental variables (neither directly nor indirectly, Table 1a, Fig. 2a). All the host features except the growth rate before infection significantly affected the parasite burden (Fig. 3a). Body length had the largest impact, and larger hosts tended to have greater parasite burdens than smaller ones (Fig. 3a). Similarly, there was a significant relationship between the age of the hosts and parasite burden. Nevertheless, the strength of this association was weaker than the association between host body length and parasite burden (Fig. 3a). As expected, hosts heterozygotic at loci *Rru 4* and *Lid 8* were more infected than homozygous individuals (Fig. 3a). The effect found for *Lid 8* tended to be greater than the effect calculated for *Rru 4*. Finally, there was a weak and non-significant tendency for hosts with a high growth rate before infection to be more resistant to parasites than hosts with a low growth rate before infection (Fig. 3a).

Concerning pathogenicity, we found similarly that four of the five competing models were statistically well supported by the data (Table 1b). Among these four competing models, the 'Direct environment + Direct host' model gave the lowest AICc value and the Δ_{AICc} were all greater than two units, meaning that this model was the single best model for fitting the data (Table 1b). In this model, fin

Table 1. Statistics used to decipher between the five competing causal models used to explain (a) *Tracheliastes polycolpus* burden on its fish host *Leuciscus leuciscus* and (b) pathogenic effects (expressed as the level of fin degradation) imposed by *T. polycolpus* burden on its fish host *L. leuciscus*. Competing models with *C*-value that follows a chi-square distribution are not rejected. '*P*-value' represents the probability that *C*-value has occurred by chance given the fact that data were generated by this competing model. A model fit was assessed using a corrected Akaike Information Criteria (AICc). Models were compared using Δ_{AICc}. Causal models with lowest AICc and Δ_{AICc} lower than 2 were considered as the best models (highlighted in bold)

Causal models	*C*-value	d.d.l	*P*-value	AICc	ΔAICc
(a) Parasite burden					
Direct and indirect environment + Direct host	33·475	30	0·302	275·513	91·761
Direct environment + Direct host	57·565	54	0·344	201·388	17·637
Direct environment	195·866	64	< 0·001	299·337	115·586
Indirect environment + Direct host	40·087	36	0·293	257·382	73·631
Direct host	**64·178**	**60**	**0·332**	**183·751**	**0**
(b) Pathogenic effects (fin degradation)					
Direct and indirect environment + Direct host	36·335	32	0·273	126·715	8·727
Direct environment + Direct host	**64·060**	**58**	**0·272**	**117·988**	**0**
Direct environment	152·512	70	< 0·001	192·767	74·780
Indirect environment + Direct host	47·395	36	0·103	130·210	12·223
Direct host	77·980	64	0·112	125·010	7·023

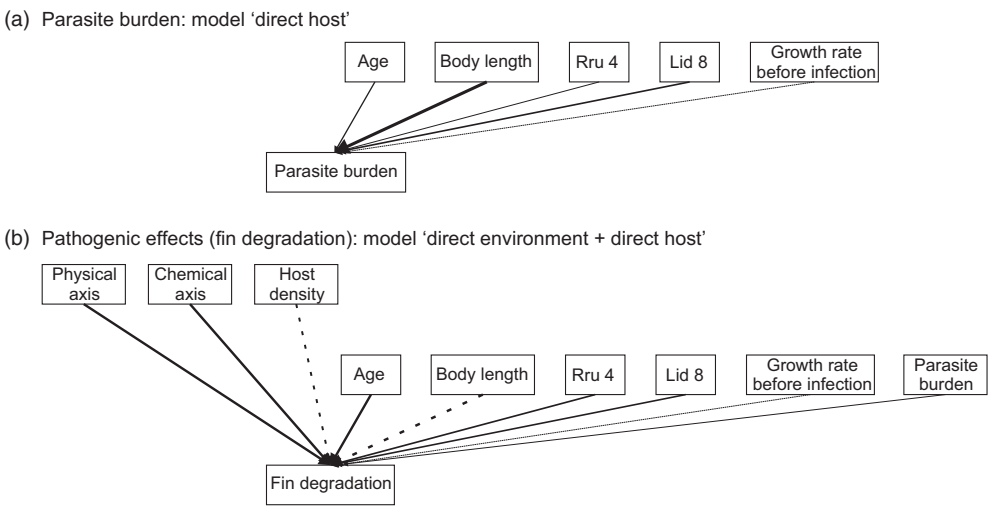

Fig. 2. Selected causal models that best explained variation in (a) *Tracheliastes polycolpus* burden and (b) pathogenic effects on its fish host, *Leuciscus leuciscus*. For parasite burden (a), the selected model illustrates that host-related factors mainly drive parasite burden in this host–parasite interaction. For pathogenic effects [i.e. fin degradation, (b)], the selected model illustrates that both host- and environment-related factors contribute to explain variation in pathogenic effects in this host–parasite interaction. 'Age' and 'Body length' are the age and the body size of the host at sampling, respectively, 'Growth rate before infection' is the host's growth rate during its first year of life (i.e. when unparasitized), 'Rru4' and 'Lid8' are the host's heterozygosity measured at loci Rru4 and Lid8, respectively. In the model concerning pathogenic effects, 'physical axis' and 'chemical axis' are synthetic variables characterizing the physical and chemical environment of sampling sites, respectively, and 'host density' is the density of *L. leuciscus* at sampling sites. In both models, the thickness of arrows is proportional to the strength of causal relationships. Arrows in dash line represent negative relationships while full arrows are positive relationships.

degradation was directly affected by variables from the host level as well as from the environmental level (Fig. 2b). We found no support for indirect environmental effects (Table 1b). At the host level, both age and body length had a strong influence on fin degradation (Fig. 3b). However, these variables had opposite effects on fin degradation, with the effect of age being positive and that of body length being negative (Fig. 3b). Parasite burden had a weak but significant positive effect on fin degradation, while the effects of the three remaining variables (growth rate before infection, heterozygosity at loci *Rru 4* and *Lid 8*) were not significant (Fig. 3b). At the environmental level, we found a significant negative effect of host density on fin degradation (fin degradation was higher for hosts living in sites where the density of dace was low), and a significant positive effect of the physical axis (Fig. 3b). This latter finding indicated that dace living in cold upstream sites showed a higher level of fin degradation.

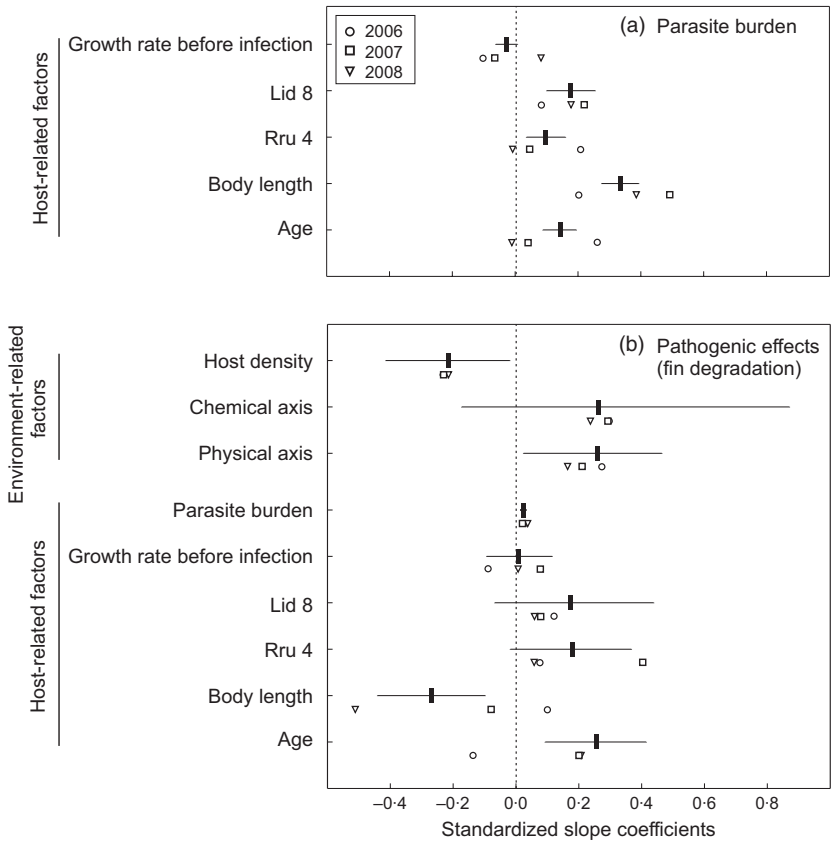

Fig. 3. Relationships between (a) host-related factors and *Tracheliastes polycolpus* burden, and (b) host-related factors, environment-related factors and pathogenic effects (expressed as the level of fin degradation) imposed by *T. polycolpus* on its fish host, *Leuciscus leuciscus*. The strength and the shape of each relationship were expressed as the standardized slope coefficient obtained from BMMs. In each plot, we represented the mean effect of each variable for all three sampling years pooled (thick vertical bars), the 95% CI for this effect across years (thin horizontal bars), as well as the effect for each sampling year independently (2006, 2007 and 2008). Confidence intervals for other values are given in Appendix S10. See Fig. 2 for variable codes.

TEMPORAL CONSISTENCY OF THE EFFECTS

Overall, the strength and the shape of the patterns of temporal consistency varied considerably according to the explanatory variable that was considered (Fig. 3a,b, Appendix S10). We highlighted three main patterns. The first pattern corresponded to variables for which both the strength and the shape of the relationship were highly consistent across years (i.e. in Fig. 3 all three points fell within the 95% CI of the 'mean' effect and they were all of the same sign). This was the case for the effect of host density, chemical axis, physical axis, *Lid 8* and parasite burden on fin degradation (Fig. 3b). For these variables, the effect size on fin degradation was highly predictable from year to year. The second pattern corresponded to variables for which the shape of the effect was consistent across years, but not the strength (i.e. in Fig. 3 all three points had the same sign but they can fall outside the 95% CI of the 'mean' effect). This was the case for the effects of *Lid 8* and body length on parasite burden (Fig. 3a, Appendix S10), as well as for the effect of *Rru 4* on fin degradation (Fig. 3b, Appendix S10). Finally, the third pattern corresponded to variables for which both the strength and the shape of the effects varied across years (i.e. in Fig. 3 the three points had different signs and at least one of them fell outside the 95% CI of the 'mean' effect). This was the case for the effects of growth rate before infection, *Rru 4* and age on parasite burden (Fig. 3a, Appendix S10), as well as for the effects of growth rate before infection, body length and age, on fin degradation (Fig. 3b, Appendix S10).

Discussion

PARASITE BURDEN

We found firm evidence that host's age and body length were positively correlated to parasite burden. Given that these variables were included simultaneously into a single model, this result adds weight to both the cumulative and the body surface hypothesis as synergistic drivers of parasite burden (Murray & Conner 2009). This would mean that (i) because of the longer residence time of older hosts, they have a higher parasitic burden than younger hosts (Thomas *et al*. 1995), and (ii) at a given age, larger hosts have a higher body surface and are more prone to attract and retain parasites (Anderson & Gordon 1982). *T. polycolpus* is a passively transmitted ectoparasite (i.e. it encounters its host according to the flow

rate), and it therefore makes sense that the probability of encounter increases with host body surface.

In addition, we confirmed the significant correlation between heterozygosity at two microsatellite loci and the burden of *T. polycolpus*. As in Blanchet *et al.* (2009b), we found that, across the three studied years, hosts that were heterozygous at either loci *Lid 8* or *Rru 4* were more parasitized than homozygous hosts. This result contrasts with most studies focusing on single-locus heterozygosity–fitness correlation (i.e. single HFCs) because, in general, heterozygosity provides a fitness advantage (e.g. Acevedo-Whitehouse *et al.* 2006; Ortego *et al.* 2007). The presence of single HFCs for microsatellite loci indicates that these loci are closely linked to functional genes (Hansson & Westerberg 2002). To test for potential association with functional genes, we used the online software BLAST (Altschul *et al.* 1990) to investigate similarities between the microsatellite flanking sequences of *Lid 8* and *Rru 4* and the genome library of zebra fish (*Danio rerio*, a cyprinid fish biological model). We found that *Rru 4* was inserted within a gene sequence coding for transcriptional enhancer factor (TEA) domain family member 1. TEA domain proteins are transcriptional factors that might be implicated in the regulation of cell proliferation (Knight *et al.* 2008) as well as in the immune response against viral and parasitic infections (Cuddapah, Cui & Zhao 2008). In the case of *Lid 8*, we found no evidence for a link between a coding gene and *Lid 8* microsatellite flanking regions. Nevertheless, this test confirms that at least *Rru 4* might be linked to an effective gene involved in immunity and that it might be subjected to the selection imposed on this gene.

Contrary to our previous findings (Blanchet *et al.* 2009a), we found no evidence for a significant relationship between host growth rate before infection and parasite burden. A major explanation for this unexpected result might be that both the strength and the shape of the relationship between the host growth rate before infection and parasite burden varied considerably between years. Indeed, this relationship turns from negative in 2006 and 2008 to positive in 2007, which might obfuscate the global relationship. Similarly, the relationship between heterozygosity at *Rru 4* and parasite burden turns from positive and significant in 2006 to non-significant in 2007 and 2008 (see Appendix S10). This temporal inconsistency in the effects of certain variables might indicate that the effect of some variables is 'environment-dependent'; i.e. there could be an unmeasured interaction between host variables and the environment. Indeed, we can speculate that in certain environments, some individual characteristics are selected for, while the same characteristics can be counter-selected in other environments. This hypothesis is supported by the findings of Lens *et al.* (2000) who found that the effect of genetic diversity on fitness traits in Taita thrush (*Turdus hellen*) was detectable in stressful environments, but not in non-stressful ones.

We found no evidence that the *T. polycolpus* burden was correlated with environmental variables (including host density), either directly or indirectly. Relationships between parasite burden and host density have already been highlighted in other host–parasite systems. For instance, in a system involving a trematode (*Cryptocotyle lingua*) and its two successive hosts (the marine snail *Littorina littorea* and birds from the Laridae family), Byers *et al.* (2008) demonstrated that the birds' density, but not the snails' density, was positively correlated with the prevalence of the trematode in snails. Similarly, Ostfeld *et al.* (2006) found that tick (*Ixodes scapularis*) abundance was affected by the density of one of the two key hosts. Intriguingly, these two studies (Ostfeld *et al.* 2006; Byers *et al.* 2008) considered parasites requiring a succession of at least two hosts, while *T. polycolpus* requires a single host to complete its life cycle. Such biological characteristic might explain why we failed to detect an effect of host density on *T. polycolpus* burden. However, with regard to the physical and chemical components of the environment, our results parallel the findings of Byers *et al.* (2008) and Ostfeld *et al.* (2006) who found no direct effects of physical and climatic variables on parasite abundance. Two non-exclusive hypotheses could explain such an absence of environmental control on the *T. polycolpus* burden. First, the *T. polycolpus* life history might be insensible to the surrounding environment. Secondly, we can hypothesize that *T. polycolpus* might be sufficiently plastic to adapt to all the environmental characteristics that we sampled across the river gradient. In other words, the environmental gradient sampled was not large enough to highlight the environmental limits of *T. polycolpus*. In that case, increasing the spatial extent of the sampling area could reveal significant relationships between environmental characteristics and the *T. polycolpus* burden.

PATHOGENIC EFFECTS

Interestingly, the host-level variables that correlated with fin degradation were not similar (either in terms of strength or shape) to those that correlated with parasite burden. For instance, as for parasite burden, age and body length were highly correlated with fin degradation. However, over the 3 years, the relationship between body length and fin degradation was negative while it was positive for parasite burden and fin degradation. This means that older individuals had a higher level of fin degradation, but that, at a given age, fin degradation was less for larger hosts. Such findings are consistent with the observations that ageing tends to decrease host tolerance (Gardner 1980) and that hosts with a better body condition are more likely to develop efficient immune responses (Christe, Moller & de Lope 1998; Moller *et al.* 1998). This indicates that the ability of individuals to tolerate parasites depends on their intrinsic characteristics (Råberg, Graham & Read 2009). However, such conclusions need to be considered with care because the effects of age and body length on fin degradation were not temporally consistent, which was the case for several other host-related variables. Indeed, for most host-related variables, the effect of the size of a predictor was rarely predictable from year to year.

In contrast, we found significant and temporally stable relationships between two of the three environmental predictors we considered and fin degradation. Indeed, every studied

year, and irrespective of the host characteristics, fin degradation was likely to be greater at cold upstream sites with a low host density. The strong and significant negative correlation between host density and fin degradation highlights a limitation of our approach. Indeed, a low host density is most likely (or at least equally likely) to be a consequence of high pathogenicity rather than as a cause, as we supposed for the models we tested. Hence, in such a specific case, causal modelling proves ineffective to attribute causation from a simple correlation (Grace 2006; Shipley 2000).

Causation was much easier to attribute considering abiotic data such as in the physical axis. Specifically, we can reasonably assume that the physical (e.g. temperature) properties of cold upstream sites strikingly affect pathogenicity in the *L. leuciscus–T. polycolpus* system. Strong pathogenic effects result either from a high parasite virulence or from a low host tolerance (Combes 2001). Parasite virulence has already been shown to vary with environmental conditions in other host–parasite systems (Laine 2008), and because environmental effects were more likely to directly affect fin degradation, we can hypothesize that the environmental conditions in cold upstream sites might increase the virulence of *T. polycolpus*. However, we cannot rule out the hypothesis that host tolerance was concomitantly lower in such sites, with environmental conditions directly affecting immunity, without affecting the host-related variables that we considered (Christe *et al.* 2000; Roulin *et al.* 2007). Distinguishing between tolerance and virulence in explaining variation in the pathogenic effects would require additional experiments and field surveys involving the measurement of the immune response in hosts.

IMPLICATIONS AND CONCLUSIONS

By combining the use of BMMs, path analyses and model selection procedures, we demonstrated that host's characteristics mainly explained the inter-site and inter-year variation observed in parasite burden. In contrast, both host and environmental characteristics explained the variation observed in the pathogenic effects. Environmental characteristic directly acted on pathogenic effects, but not through an indirect effect on host characteristics. Interestingly, some variables had opposite effects on parasite burden and pathogenicity. Finally, the strength and the shape of the effects of certain variables were consistent across years, while they were clearly not for some others.

Our results have several implications. First, because parasite burden and pathogenicity were not driven by similar predictors, our study highlights the importance of considering conjointly these two traits in future studies. Although rarely tested (but see Walker *et al.* 2010), this is an important aspect to consider because both traits can impact on the fitness of hosts (either through the investment in either resistance or tolerance, Råberg, Graham & Read 2009), and hence the eco-evolutionary dynamics of their populations. Secondly, if we assume that such a parasite has fitness consequences on its host (Blanchet *et al.* 2009a,b), the temporal inconsistency we report for several predictors (irrespectively of the parameters we considered) has important implications for understanding patterns of selection occurring in such host populations. Indeed, our results suggest that the strength and the shape of selection of host traits could vary from year to year. For instance, selection of a given host trait can be positive in a given year and neutral (or even negative) in another year. This may contribute to maintain genetic variation for underlying traits (Chaine & Lyon 2008). Finally, our results imply that predicting the burden of *T. polycolpus* in such a model and at such a spatial scale would be difficult. Indeed, in a river system, the environment is spatially structured along the upstream–downstream gradient (e.g. upstream sites are always colder than downstream sites). Hence, because the parasite burden does not seem to be driven by environmental conditions, but rather by the population structure of the hosts (and hence the movement of hosts between sites), year to year predictions would be hazardous. In contrast, pathogenic effects were mainly driven by direct environmental effects, with those effects showing a strong year-to-year stability. This result is important because pathogenic effects are often implicated in host population decrease (Walker *et al.* 2010). Being able to predict pathogenic effects according to simple environmental correlates is therefore of primary importance for conservation practices.

To conclude, there is evidence that parasites and pathogens could spread extensively as the climate gets warmer and warmer (Harvell *et al.* 2002). However, many non-climatic factors may also affect, and even overshadow, the effects of climate change on parasite spread (Lafferty 2009). The statistical approach we proposed here might contribute to distinguishing between the role of climatic and non-climatic variables on the distribution of parasites. Indeed, by considering conjointly causal relationships among factors and the hierarchical structure of host–parasite interactions, we provide a new way to dissect the complex links existing between hosts, parasites and their common environment.

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Supporting Information

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

Appendix S1. Total number of host (*Leuciscus leuciscus*) sampled [$N_{(host)}$], mean total body length (mm) of sampled host [$\pm$SD] and mean parasite burden (*Tracheliastes polycolpus*) per host [$N_{(parasite)}$] ($\pm$SD), for each sampling site (from upstream, S1, to downstream, S8) and each sampling year (2006–2008).

Appendix S2. Full description of the measurement of the environmental-related variables.

Appendix S3. Principal Component Analysis on normalized physical and chemical variables used to relate environment-related factors, *Tracheliastes polycolpus* burden and its pathogenic effects on its fish host *Leuciscus leuciscus*.

Appendix S4. Description of the two-levels hierarchical models used in this study.

Appendix S5. Graphical representation of the five path models used to disentangle the effects of host-related factors (age, size and growth rate before infection of the hosts, heterozygosity measures at microsatellites loci Rru4 and Lid8), the direct effects of environment-related factors (physical and chemical characteristics of the sampling sites, host density in the sampling sites) and the indirect effects of those environmental effects on *Tracheliastes polycolpus* burden.

Appendix S6. Graphical representation of the five path models used to disentangle the effects of host-related factors (age, size and growth rate before infection of the hosts, heterozygosity measures at microsatellites loci Rru4 and Lid8, parasite burden), the direct effects of environment-related factors (physical and chemical characteristics of the sampling sites, host density in the sampling sites) and the indirect effects of those environmental effects on pathogenic effects (fin degradation) imposed by *Tracheliastes polycolpus* on its host *Leuciscus leuciscus*.

Appendix S7. Description of d-sep tests for multilevel causal models.

Appendix S8. Formula used to calculate the *C* value for each of the five tested hypotheses.

Appendix S9. Outputs of Generalized Linear Models aiming at testing the effects of sampling sites and sampling years on the parasite burden (*Tracheliastes polycolpus*) and fin degradation.

Appendix S10. Table showing, for each sampling year (2006, 2007 and 2008) and for each best model, the standardized slope coefficients between (a) host-related factors and *Tracheliastes polycolpus* burden, and (b) host-related factors, environment-related factors and pathogenic effects (expressed as the level of fin degradation) imposed by *T. polycolpus* on its fish host, *Leuciscus leuciscus*.

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