Oecologia
DOI 10.1007/s00442-008-1272-y

POPULATION ECOLOGY - ORIGINAL PAPER

Why do parasitized hosts look different? Resolving the "chicken-egg" dilemma

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Received: 23 April 2008 / Accepted: 16 December 2008

Abstract Phenotypic differences between infected and non-infected hosts are often assumed to be the consequence of parasite infection. However, pre-existing differences in hosts' phenotypes may promote differential susceptibility to infection. The phenotypic variability observed within the host population may therefore be a cause rather than a consequence of infection. In this study, we aimed at disentangling the causes and the consequences of parasite infection by calculating the value of a phenotypic trait (i.e., the growth rate) of the hosts both before and after infection occurred. That procedure was applied to two natural systems of host–parasite interactions. In the first system, the infection level of an ectoparasite (*Tracheliastes polycolpus*) decreases the growth rate of its fish host (the rostrum dace, *Leuciscus leuciscus*). Reciprocally, this same phenotypic trait before infection modulated the future level of host sensitivity to the direct pathogenic effect of the parasite, namely the level of fin degradation. In the second model, causes and consequences linked the growth rate of the fish host (the rainbow smelt, *Osmerus mordax*) and the level of endoparasite infection (*Proteocephalus tetrastomus*). Indeed, the host's growth rate before infection determined the number of parasites later in life, and the parasite biovolume then decreased the host's growth rate of heavily infected hosts. We demonstrated that reciprocal effects between host phenotypes and parasite infection can occur simultaneously in the wild, and that the observed variation in the host phenotype population was not necessarily a

Communicated by Steven Kohler.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-008-1272-y) contains supplementary material, which is available to authorized users.

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Published online: 03 February 2009

consequence of parasite infection. Disentangling the causality of host–parasite interactions should contribute substantially to evaluating the role of parasites in ecological and evolutionary processes.

Keywords Causal links · Pathogenic effects · Reciprocal effects · Growth rate · Parasite · Behavior · Path analysis · Model selection · Susceptibility

Introduction

Phenotypic differences in morphology, behavior and physiology between individuals that are associated with parasite infection status have been reported for a wide range of host and parasite taxa (see Combes 1991; Moore and Gotelli 1990; Thomas et al. 2005 for reviews). These infection-associated differences are traditionally categorized into four kinds of mutually exclusive phenomena; three refer to consequences of the infection and one to a cause. The first hypothesis states that phenotypic changes can be non-adaptive and accidental side effects due to the infection. The second hypothesis suggests that phenotypic changes can be host adaptations aimed at reducing the detrimental fitness consequences of infection. Alternatively, the third hypothesis states that host phenotypic changes following infection are adaptive tactics of the parasite to maximize its fitness (Minchella 1985; Hurd 2001). Finally, the phenotypic variability observed within the host population may be a cause rather than a consequence of the infection (Poulin 1998; Barber et al. 2000). Pre-existing differences in the host phenotype would then promote differential susceptibility to infection. Despite a vast literature on these issues (see Thomas et al. 2005), it is still often problematic to establish causality between host phenotype and infection status, especially in naturally infected organisms.

Assessing causality links between host phenotype and parasite infection is comparable to the classical "chicken-egg" dilemma. In the present context, the question is formulated as follows: does the phenotype of the host determine the level of infection, or does the level of infection determine the host phenotype? Currently, most studies assume that observed variation in the host phenotype population is a consequence of parasite infection (e.g., Lello et al. 2005; Holmstad et al. 2006; Wood et al. 2007). Otherwise, the possibility that the host phenotype can cause different patterns of parasite infection is reduced by experimentally infecting the hosts or by removing parasites from infected hosts (e.g., Albon et al. 2002; Barber and Svensson 2003; Seivwright et al. 2005; Schultz et al. 2006; Blair and Webster 2007). Very few tests have yet been developed to explore the possibility that both causes and consequences may intervene, having reciprocal effects in underlying the relationship between parasite infection and phenotypic variation within the host population (but see Barber 2005; Bourque et al. 2006). Such kinds of reciprocal effects between hosts and parasites have been widely considered for understanding the evolution of epidemiological characteristics (e.g., genes involved in host resistance or parasite virulence; reviewed by Lambrechts et al. 2006). In this case, an evolving genotype-by-genotype interaction is assumed in most mathematical and empirical tests (e.g., matching alleles models or gene-for-gene models; Combes 1998; Lambrechts et al. 2006). However, this approach has to our knowledge never been applied to phenotypic traits implied in the encounter probability between hosts and parasites (Combes 1998). The possibility of reciprocal effects of host's traits linked to the encounter rate and parasite infection seems highly probable given that genetic parameters and other intrinsic parameters, such as age, sex or hormonal levels of the host, have been found to influence infection probabilities (Paterson et al. 1998; Poulin 1998; Poulin et al. 2000; Krasnov et al. 2005; Seivwright et al. 2005).

Resolving the "chicken-egg" dilemma in this case requires knowing three parameters: (1) the level of individual parasite infection I_0 at a moment t_0 of the host life history, (2) the individual phenotypic value P_0 at t_0 (i.e., during infection) and (3) the individual phenotypic value P_{0-1} at t_{0-1}, namely, before the infection occurred. If parasites alter the host phenotype, one would expect a significant relationship between P_0 and I_0, but no relationship between P_{0-1} and I_0. Alternatively, if phenotypic differences lead to differential susceptibility to infection, one would expect a significant relationship between P_{0-1} and I_0. In the latter case, a relationship between P_0 and I_0 is also expected, but this relationship would be the "ghost" of a causal relationship between P_{0-1} and I_0. If host phenotypes reflect both the causes and consequences of parasitic infection, disentangling the relative influence of each becomes far more problematical. Testing these predictions is highly feasible in laboratory settings for which both the phenotype of the hosts and the infection rate can be controlled. However, in some animals, such as fish, these three parameters can be directly extracted from field sampling (i.e., using solid structures such as scales for evaluating a phenotypic trait such as the growth rate both before and after infection), therefore creating a form of "uncontrolled" experiment and hence offering an opportunity for testing the "chicken-egg" dilemma in a natural setting.

In this paper we attempted to identify the relative importance of causes and consequences in shaping the relationship between host phenotype and parasite infection from two host–parasite systems collected in natural ecosystems. The first interaction involved a fin-feeding ectoparasite (*Tracheliastes polycolpus*) and its fish host (the rostrum

dace, *L. leuciscus burdigalensis*). The second interaction involved an endoparasite (the cestode, *Proteocephalus tetrastomus*) occurring in the intestine of its fish host (the rainbow smelt, *Osmerus mordax*). For both models, we back-calculated the growth of the hosts before parasite infection occurred and following the initial infection. Using statistical computing, we explored the relationships between growth rate before and after infection and the level of parasite infection to disentangle the causes and the consequences of parasite infection.

Study systems and specific hypotheses

The fin-feeding ectoparasite

The copepod *T. polycolpus* is a monoxene (i.e., a single host species in its life history) ectoparasite occurring in wild fish populations and in freshwater aquaculture (Walker et al. 2006). *T. polycolpus* is highly specific to the rostrum dace. In this species, only the female is parasitic and is anchored to host fins to feed on the epithelial cells and mucus of the host. This grazing activity characteristically damages the fins, leading to their destruction (i.e., a direct pathogenic effect, see Loot et al. 2004). In fish, fins are used for stability, agility and propulsion of the body during locomotion and thus play a central role in food capture (Schrank and Webb 1998; Lauder and Drucker 2002). We thus hypothesized that infection by *T. polycolpus*, by inducing the degradation of fins, should contribute to decreasing the growth rate of rostrum dace (i.e., an indirect pathogenic effect). If a significant negative correlation between parasite infection and the growth rate of rostrum dace is observed, this could be interpreted as a consequence of parasite infection only if the host growth rate before the infection failed to predict the future infection level.

The intestinal endoparasite

The cestode *P. tetrastomus* is a species-specific parasite of smelt (Osmeridae). The life cycle of *P. tetrastomus* is not known, but it has been suggested that planktonic copepods serve as intermediate hosts of the parasite before entering the intestine of Osmerid fishes (Scholz et al. 2004). Here, we re-analysed published data of a field survey held in the Saint Lawrence middle estuary (Quebec, Canada) that aimed at evaluating the effect of *P. tetrastomus* on the survival of the early life-history stages of rainbow smelt (Bourque et al. 2006). Infection appears to be limited to the larval and juvenile stages of the host species (Bourque et al. 2006). Previous work suggested that fish that survived the infection by *P. tetrastomus* were on average larger than the uninfected survivors both prior to and after the infection (Bourque et al. 2006). Furthermore, Bourque et al. (2006) suggested that infection decreases the growth rate of rainbow smelt heavily infected by *P. tetrastomus*, while the growth rate of weakly infected fish did not seem to be affected by the parasite. We re-analyzed this data to specifically establish the causal relationships between parasite infection and the growth rate of heavily and weakly infected rainbow smelt, respectively. As in the case of rostrum dace, the negative correlation between the level of parasite infection and the growth rate of heavily infected smelt could be interpreted as a consequence of parasite infection only if the host growth rate before the infection failed to predict the future infection level.

Materials and methods

The fin-feeding ectoparasite

Sampling strategy

Adult and juvenile rostrum dace (from 1- to 10 age-year-old fish) were sampled by electric fishing in the Viaur river (France, 44°15′N, 2°33′E) in June 2003 ($n = 48$ dace) and 2005 ($n = 103$ dace) (between 20 and 25 June each year). Each fish was anesthetized, measured (± 1 mm), the total number of parasites on fins and body surface counted, and the extent of fin degradation caused by the parasites visually scored. On average, the prevalence was 94% for the 2 sampling years, and intensity varied between 1 and 64 parasites per fish (13.68 ± 13.64, mean $\pm$ SD). To evaluate fin degradation, we scored 0, 1, 2, 3 or 4 points if a fin was 0, 25, 50, 75 or 100% eroded by the parasites, i.e., a score of two points means that approximately 50% of the area of the fin was eaten by the parasites (see Fig. 3 in Loot et al. (2004) for a picture of a heavily damaged rostrum dace). 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 same observer (G.L.) scored the fin degradation for all 151 dace. In order to calculate the growth rate and the age of each fish, five to six scales were sampled from the left flank above the lateral line. All of these fish were released alive at their sampling site.

Growth evaluation and analyses

In fish, age and size-at-age are currently measured by counting and measuring the annual growth zones that form the scales (these zones are called “annuli” and are formed during winter, when the growth is low (Francis 1990). After preparation (see Loot et al. 2002 for details), the scales were placed between two microscope slides and photographed with a camera connected to an image analysis

system (Leica Microsystems MZ 16A). Several measurements were then made with the ImageTool free software (http://ddsdx.uthscsa.edu/dig/itdesc.html). First, we measured the total radius of each scale in order to model the relationships between the total body length of the fish at the sampling date (TL) and the total scale radius (TR) for all dace specimens. The best fit for modeling the relationship between the total length (TL) and the total scale radius (TR) was given by a power function: $TL = 2.5318 \times TR^{0.9144}$ ($r^2 = 0.9268$, $P < 0.001$). We then used the non-linear back-calculation technique of Monastyrsky (1930, cited in Loot et al. 2002), which allows the use of a set of measurements of marks (R_i) present on one fish individual at one time i (expressed in year) to infer its length (L_i) at the time of formation of each mark (Francis 1990). Back-calculation of fish lengths was: $L_i = TL \times (R_i/TR)^{0.9144}$, with L_i being the fish body length at age i. This formula was used to back-calculate the body length of dace during their last winter of life before sampling and the body length of dace during their first winter of life. First, because the dace were sampled at the same period in both sampling years, we subtracted the body length at the sampling date minus the back-calculated body length during their last winter to evaluate the individual growth rate during the period of parasite infection (the last 6 months). Our field surveys and a laboratory experiment suggested that *T. polycolpus* remains anchored to the host for several months before laying eggs and dying (G. Loot et al., unpublished data). Second, because dace are not parasitized by *T. polycolpus* before their first winter of life (prevalence = 0%, $n = 36$, G. Loot and S. Blanchet, unpublished data), the body length of dace during their first winter was used as a surrogate of the growth rate of the hosts before infection by the parasite.

We combined path analyses (Shipley 2000) and a model selection procedure (Johnson and Omland 2004) to explore causal relationships among variables in this host–parasite interaction and particularly to disentangle the causes and consequences of parasite infection on host growth rate. 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). The response variables in one equation may form predictors in others, thereby forming sequences of causal relationships (Shipley 2000). Model selection procedure is used to compare a particular set of a priori hypotheses, each expressed by a model (see Johnson and Omland 2004). Here we compared 15 different competing models. Models were based on the different hypotheses that can govern relationships among parasite infection, fin degradation and host growth rate (illustrated in Supplementary Figure 1). Each model has in common the inclusion of four response variables (i.e., growth rate before infection, growth rate during infection, parasite number and fin degradation) and one co-variable (host age; Fig. 1). In all the 15 models, host age was included as a co-variable to control for a possible effect of age on host growth rate during infection (older fish grow more slowly, Matthews 1998), on parasite number and fin degradation (older fish are more prone to accumulate parasites, Combes 1998; Fig. 1). We also assumed in all models that fin degradation was positively influenced by parasite number (see Fig. 1). In the first three models, we hypothesized that host growth rate before infection might be a cause of parasite infection and/or fin degradation. We regrouped these models (models M1 to M3, Supplementary Figure 1) in the category "Host phenotype as a cause." In three other models, we hypothesized that parasite number and/or fin degradation might affect the growth rate of the host during infection. These three models (models M4 to M6, Supplementary Figure 1) were hereafter regrouped in the category "Host phenotype as a consequence." Finally, we built nine models that integrated the possibility that growth before infection affects parasite number and/or fin degradation, which in turn affects the growth rate during infection. These nine models (models M7 to M15, Supplementary Figure 1) were hereafter regrouped in the category "Host phenotype as a cause and a consequence." In all of these models, the growth rate and the age of individual were log-transformed to obtain linearity of the relationship. Path analyses were performed using AMOS 5 (Arbuckle 2003).

To compare these models, we first judged if the covariance structure of each model did not differ from that of the data (maximum likelihood χ^2 statistics were used for such a

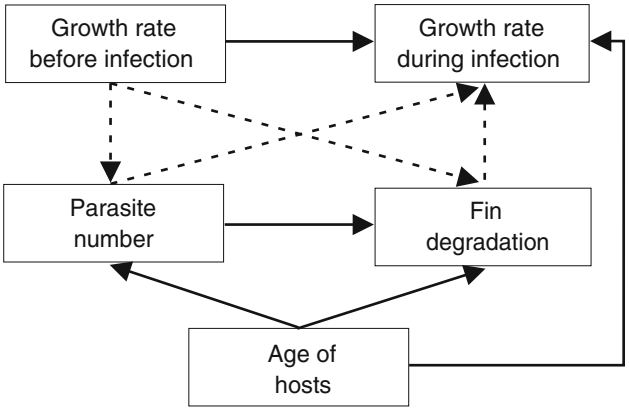


Fig. 1 Graphical representation of the possible pathways linking the growth rate of the host, the rostrum dace (*Leuciscus leuciscus*), before and during the infection by the ectoparasite, *Tracheliastes polycolpus*, with the age of the host, the number of ectoparasites and the fin degradation induced by the ectoparasite. According to these possible pathways, we built 15 models for testing hypotheses about causes and consequences in this host–parasite interaction (see Supplementary Figure 1). In these 15 models, the *continuous arrows* were kept constant and each model differs according to the presence or absence of the *dotted arrows* (see Supplementary Figure 1)

purpose; a non-significant χ^2 identifies a good fit between predicted and observed covariance matrices). 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 and Omland 2004). We then calculated the differences in these AIC values between each model and the best model (i.e., Δ_{AIC}). A single best model cannot be assumed if the Δ_{AIC} with other competing models is not greater than two units (Johnson and Omland 2004). Such a result would indicate that several models are well supported by the data. Finally, we calculated the "Akaike weight" (W_i), which can be interpreted as the probability that a given model i is the best model for the observed data, given the candidate set of models (see Johnson and Omland 2004 for calculation of W_i).

Each year of sampling (2003 and 2005) was fitted independently to the whole set of models to test for the temporal stability of the relationships. A model selection procedure was done for each year independently.

The intestinal endoparasite

Sampling strategy

Details on study area and sampling can be found in Bourque et al. (2006). Briefly, 1-m plankton nets (500-µm mesh size) were used to catch rainbow smelt larvae at a single station in the St. Lawrence middle estuary on 12 July 2001 ($n = 75$). At this period, the prevalence of the *P. tetrastomus* was 74%, and average intensity was 2.08 ± 1.54 (mean $\pm$ SD). Each fish was preserved in 95% ethanol. At the laboratory, each fish was measured and then dissected to count the number of parasites in the digestive tract (if present). Parasites were also measured (length, width and height) using an ocular micrometer to calculate the relative biovolume [i.e., B_p = (Biovolume of the parasite/Biovolume of the fish host) $\times$ 100] they occupied in the intestinal tract of the fish (the formula is described in Bourque et al. 2006). As both the number of parasites and the space they occupy in the host can affect host growth rate, these two measures were considered in our analyses (Bourque et al. 2006). All of these fish hatched approximately 70 days before the sampling date. As these fish were too small for scale reading, otoliths were used to back-calculate the size of the fish at a given day (otoliths are ear stones for which daily growth increments are visible, Campana and Jones 1992). Thus, to calculate the individual growth rate, otolith structures were extracted from each fish (see Bourque et al. 2006 for details).

Growth evaluation and analyses

Based on the method developed by Sirois et al. (1998) and detailed in Bourque et al. (2006), we back-calculated the body length of rainbow smelt at day 1, 45 and 70 after hatching. Growth rate before infection was calculated as the difference in body length at day 45 minus the body length at day 1. Indeed, a weekly survey demonstrated that day 45 corresponded to the appearance of the parasite in this system (Bourque et al. 2006). Finally, we subtracted the body length at day 70 minus the back-calculated body length at day 45 to evaluate the individual growth rate during the period of parasite infection. These measures of body length were log transformed for growth calculations.

According to Bourque et al. (2006), the sampled fish were divided into three groups: uninfected (no parasite), weakly infected ($0\% < B_p < 0.17\%$) and heavily infected ($B_p > 0.17\%$). To disentangle causes and consequences in such an example, the whole dataset was divided into two sub-datasets: the uninfected and the weakly infected fish, and the uninfected and the heavily infected fish. We did this because preliminary analyses indicated that treating the dataset as a whole blurred some important distinctions between heavily and weekly infected fish. In this case study, we also used path analyses to test several hypotheses that differed in their causal links (Shipley 2000). Here, the relative simplicity of the system facilitates the development of only three competing hypotheses (i.e., three competing models) that were tested for the two sub-datasets independently. In these three models, a latent variable (i.e., a variable that is not directly observed but rather mathematically inferred from others measured variables, Shipley 2000) called "Infection level" was inferred according to two indicators variables, namely parasite number and parasite biovolume. The unstandardized regression weight (i.e., the contribution of each indicator variable on the latent variable) was set to 1 for each indicator variable (Shipley 2000), and we assumed that the parasite biovolume was dependent upon the number of parasites harbored by a host. The first hypothesis supposed that growth variability before infection was the cause of the differential level of infection in the host population (category "Host phenotype as a cause"). In the second hypothesis, we supposed that the growth variability observed during infection was a consequence of parasite infection (category "Host phenotype as a consequence"). Finally, in the third hypothesis we considered that the growth rate before infection determined the future level of parasite infection, and in turn parasite infection affected the growth rate of rainbow smelt during infection. This third model therefore integrated both alternative interpretations of parasite infection (category "Host phenotype as a cause and a consequence"). These three competing models were compared using a model selection procedure as described above.

Results

The fin-feeding ectoparasite

All models we tested but two (M9 and M13, χ^2 statistics: $P < 0.05$ in 2003, see Table 1) were interpretable as the covariance structure of those models did not significantly differ from that of the data (χ^2 statistics; all $P > 0.05$, Table 1). In both years, the model demonstrating the lowest AIC value included both causes and consequences of parasite infection (model M10, see Table 1 and Supplementary Figure 1 for a graphical description of this model). According to this model, the growth rate during the infection period was affected by the number of parasites, but not by the level of fin degradation (Table 1). According to the regression weight (results not shown) and a graphical representation (Fig. 2), the effect of parasite number on growth rate during infection was negative in both sampling years (Fig. 2). This model further suggests that the growth rate of dace before infection occurred affected the level of fin degradation, but not the number of parasites harbored by a host (Table 1). As illustrated in Fig. 3, in both years, the fin degradation was lower when the host exhibited a higher growth rate during their first year of life (i.e., before infection occurred).

It is worth noting that this model cannot be considered as the single best model for fitting the data. Indeed, four and five other models in 2003 and 2005, respectively, have a Δ_{AIC} lower than 2, signifying that these models are also well supported by the data (Table 1). There was a very strong temporal stability in this host–parasite system since the same five models were judged as being well supported by the data during both sampling years (models M2, M3, M10, M11 and M14, see Table 1, Supplementary Figure 1). Very importantly, none of these five models was from the category "Host phenotype as a consequence" (Table 1), meaning that models including only causal relationships from the parasite to the host growth rate were among the worst for supporting the data.

The intestinal endoparasite

The six models we tested were interpretable as the covariance structure of the models did not significantly differ from that of the data (χ^2 statistics; all $P > 0.05$; see Fig. 4a–f). The selected model was different according to the level

Table 1 Summary description of the 15 competing models built to disentangle causal relationships between the growth rate (G in the table) of rostrum dace (*Leuciscus leuciscus*) before and during infection by the ectoparasite, *Tracheliastes polycolpus*, the number of ectoparasite on each host and the level of fin degradation induced by the ectoparasite (see also Supplementary Figure 1)

Model	G before infection → parasite number	G before infection → fins degradation	Parasite number → G during infection	Fins degradation → G during infection	Model category	Year 2003			Year 2005		
						AIC	Δ_{AIC}	W_i	AIC	Δ_{AIC}	W_i
M1	X				Cause	30.75*	6.22	1.09	28.95*	3.16	3.29
M2		X			Cause	**24.79***	**0.26**	**21.40**	**25.85***	**0.06**	**15.51**
M3	X	X			Cause	**26.31***	**1.78**	**10.01**	**26.81***	**1.02**	**9.60**
M4			X		Consequence	28.98*	4.45	2.63	27.96*	2.17	5.40
M5				X	Consequence	31.19*	6.66	0.87	29.15*	3.36	2.98
M6			X	X	Consequence	30.96*	6.43	0.98	29.15*	3.36	2.98
M7	X		X		Cause/consequence	30.49*	5.96	1.24	28.96*	3.17	3.28
M8		X		X	Cause/consequence	26.74*	2.21	8.07	**27.00***	**1.21**	**8.73**
M9	X			X	Cause/consequence	32.69$^{n.s.}$	8.16	0.41	30.16*	4.37	1.80
M10		X	X		Cause/consequence	**24.53***	**0.00**	**24.37**	**25.79***	**0.00**	**15.99**
M11	X	X	X		Cause/consequence	**26.05***	**1.52**	**11.40**	**26.82***	**1.03**	**9.55**
M12	X	X		X	Cause/consequence	28.25*	3.72	3.79	28.01*	2.22	5.27
M13	X		X	X	Cause/consequence	32.47$^{n.s.}$	7.94	0.46	30.16*	4.37	1.80
M14		X	X	X	Cause/consequence	**26.51***	**1.98**	**9.05**	**27.04***	**1.25**	**8.56**
M15	X	X	X	X	Cause/consequence	28.03*	3.50	4.23	28.01*	2.22	5.27

Models M1 to M3 included pathway(s) suggesting that host growth rate before infection was a cause of parasite number and/or fin degradation. Models M4 to M6 included pathway(s) suggesting that host growth rate during infection was a consequence of parasite number and/or fin degradation. Models M7 to M15 included pathways suggesting that host growth rate before and/or during infection was both a cause and a consequence of parasite number and/or fin degradation. The best models for fitting the data have the lowest Akaike information criteria (AIC) values, a Δ_{AIC} lower than 2 and higher *Wi* probabilities (the Akaike weight). Models providing a good fit between predicted and observed covariance matrices are indicated by a asterisk (χ^2 statistic; $p > 0.05$), while those that did not fit the data are indicated by n.s. (χ^2 statistic; $p < 0.05$). Each sampling year (2003 and 2005) was analyzed independently. The best models are highlighted in bold

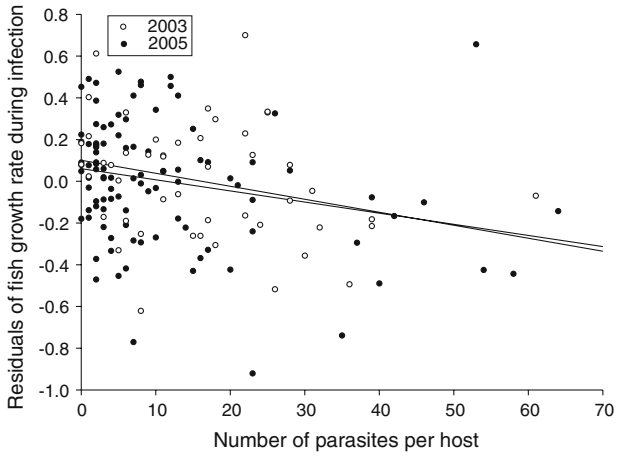


Fig. 2 Linear relationship between the growth rate of rostrum dace (*Leuciscus leuciscus*) during infection by the ectoparasite, *Trachelias-tes polycolpus*, and the number of ectoparasites harbored by each individual in 2003 (*open circle*) and 2005 (*dark filled circle*). The growth rate of rostrum dace is qualified as the residuals of a linear regression linking the growth rate of rostrum dace during infection with the growth rate before infection and the age of the individual

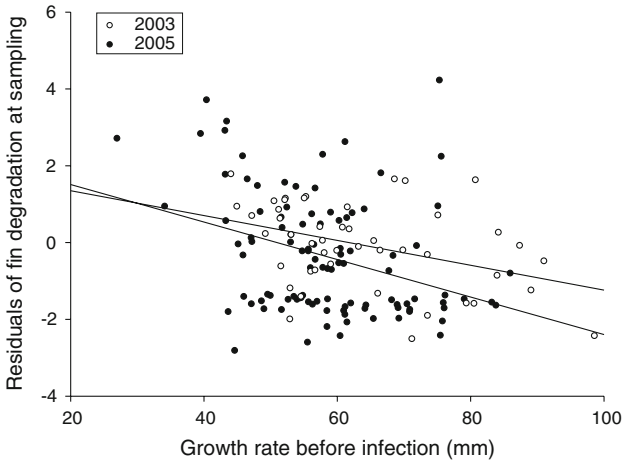


Fig. 3 Linear relationship between the level of fin degradation of rostrum dace (*Leuciscus leuciscus*) induced by the ectoparasite, *Tracheliastes polycolpus*, and the growth rate of rostrum dace before infection occurred in 2003 (*open circle*) and 2005 (dark filled circle). The level of fin degradation of rostrum dace is qualified as the residuals of a linear regression linking the level of fin degradation with the number of parasites and the body length of the individual at sampling

of host infection (weakly or heavily infected hosts). Indeed, concerning weakly infected hosts, the best model based on the AIC values was the one including a causal link from the host phenotype before infection to the level of parasite infection (see Fig. 4a, weakly infected host). This model was the single best model for supporting the data since Δ_{AIC}s were higher than 2 when AIC values were compared with the two other models (results not detailed but see Fig. 4). This model highlighted a positive relationship between the growth rate of the host before infection and the infection level (Fig. 4a). Concerning the heavily infected hosts, the best model in term of AIC values was the one including both causes and consequences in the host–parasite interaction (Fig. 4f, heavily infected host). Again, Δ_{AIC}s were higher than 2 when AIC values were compared to the two other models, indicating that it was the single best model for supporting the data (results not detailed, but see Fig. 4). In this model, the growth rate before infection positively determined the future infection level of the host. In turn, the infection level had a significant negative impact on the host growth rate. The best selected models based on AIC values had the highest probabilities of being the best for fitting the observed data (i.e., the Akaike weight W_i, Fig. 4a, f).

Pooling all the fish in a single dataset (i.e., pooling non-infected, weakly infected and highly infected fish) led to models that were all interpretable (χ^2 statistics; all $P > 0.05$, results not shown) and to the similar conclusion that the model including only consequences was the worst for the observed data (host phenotype as a consequence: $W_i = 17.78\%$; host phenotype as a cause: $W_i = 53.85\%$; host phenotype as a cause and a consequence: $W_i = 28.37\%$, results not shown). However, treating our data in this way blurred the differential effect of the parasite on weakly and highly infected fish (see above).

Discussion

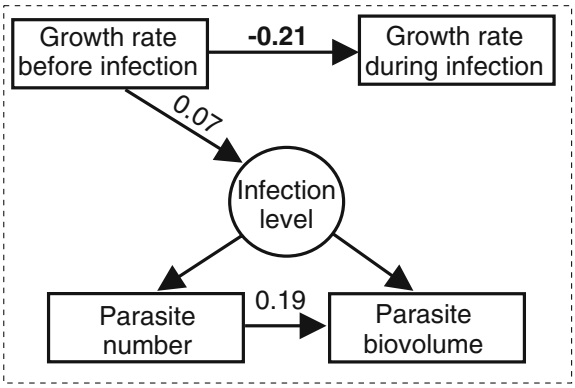
Does the phenotype of the host determine the level of infection, or does the level of infection determine the host phenotype? Although this distinction is of great importance for determining the level of implication of parasites in ecological and evolutionary processes, disentangling the causes and the consequences of host-parasite interactions is often viewed as a very difficult task to resolve (Poulin 1998; Arnott et al. 2000; Barber et al. 2000). In fact, such a dilemma is generally ignored by authors; thus, the hypothesis that phenotypic characteristics existing in the host population prior to infection determines the future level of infection is generally not considered.

Here, we used a new approach in an attempt to disentangle the chicken-egg dilemma in host-parasite interaction. This approach was applied to two host-parasite systems that differed in terms of life cycle of the parasite. In these examples, we highlighted complex and reciprocal interactions between a morphological trait of the host (the growth rate) and infective settings of the parasite.

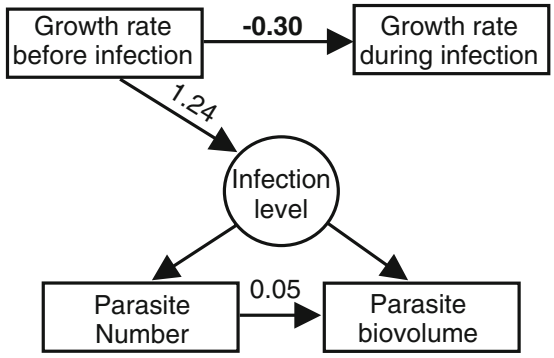
In the first example, we found evidence for a negative relationship between the levels of infection of the fin-feeding ectoparasite and the growth rate of its host, the rostrum dace. Such a relationship was expected since it has been documented in nature for several animal species

Host phenotype as a cause

(a) $Chi^2_{(2)} = 1.23$; p = 0.54; AIC = 17.23; W*i* = 62.14%

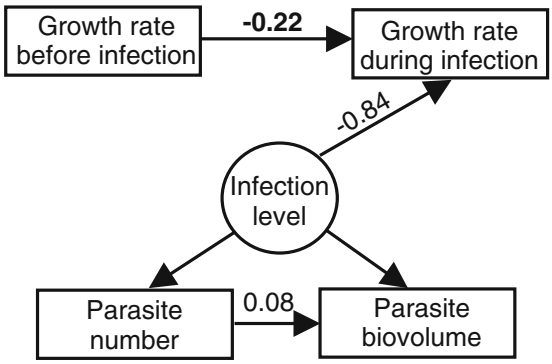


(b) $Chi^2_{(2)} = 5.56$; p = 0.06; AIC = 21.56; W*i* = 11.93%

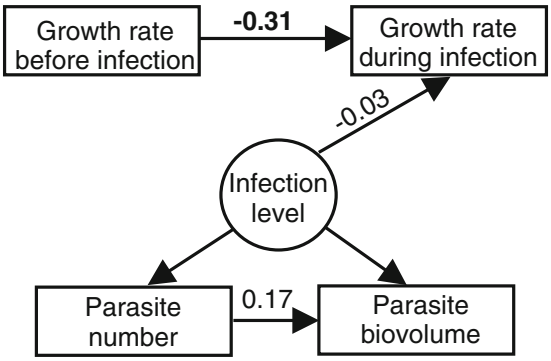


Host phenotype as a consequence

(c) $Chi^2_{(2)} = 3.58$; p = 0.17; AIC = 19.58; W*i* = 19.16%

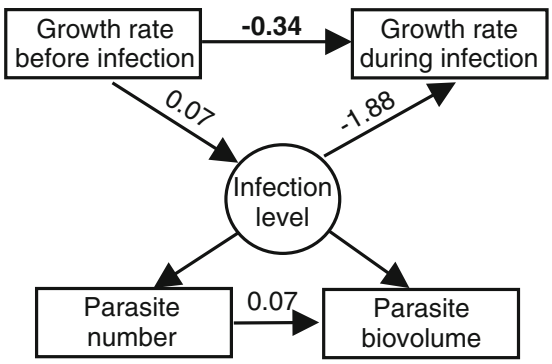


(d) $Chi^2_{(2)} = 4.52$; p = 0.10; AIC = 20.52; W*i* = 20.00%

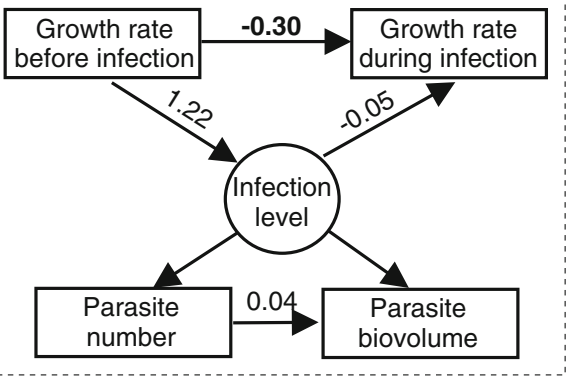


Host phenotype as a cause and a consequence

(e) $Chi^2_{(1)} = 1.63$; p = 0.20; AIC = 19.63; W*i* = 18.69%



(f) $Chi^2_{(1)} = 0.09$; p = 0.77; AIC = 18.09; W*i* = 67.97%



Weakly infected host

Heavily infected host

(Saino et al. 1998; Bize et al. 2003; Nilsson 2003; Finley and Forrester 2003). However, by using a model selection approach, we showed that it was hazardous to conclude that host growth rate was purely a consequence of parasite infection without considering more complex possibilities. In addition, we found weak support for the hypothesis that growth depression resulted from the degradation of the fins (see Introduction). On the contrary, we found that the level

◀ **Fig. 4** Path analyses for weakly infected (*left column*) or heavily infected (*right column*) hosts examining the causal relationships between the growth rate of rainbow smelt (*Osmerus mordax*) and the level of infection by the endoparasite, *Proteocephalus tetrastomus*. A latent variable (i.e., a variable that is not directly but rather mathematically inferred from others measured variables) called "Infection level" was inferred according to two indicator variables, number of parasites and parasite biovolume. The regression weights for these two indicator variables were a priori set to 1. **a**, **b** Models assume that growth variability (both before and after infection) was the cause of the differential level of infection in the host population. **c**, **d** Models assume that the growth variability observed during infection was a consequence of parasite infection. Finally, **e**, **f** models assume both causes and consequences in this host-parasite interaction. *Single-pointed arrows* indicate causal relationships, and their unstandardized regression weights are indicated above the *arrows*. Significant ($P < 0.05$) path coefficients are in *bold*. For a given model, a non-significant χ^2 statistic indicates a good fit between predicted and observed covariance matrices. For each case (weakly and heavily infected hosts), the best model (framed in a *dashed-line box*; **a** and **f**) for fitting the data has the lowest Akaike information criteria (AIC) value and the higher *Wi* probability (the Akaike weight)

of fin degradation was negatively influenced by the growth of the host before the infection occurred, even after controlling for the number of parasites. This result indicated that the direct pathogenic effect of *T. polycolpus* (i.e., the fin degradation) was (in part) determined by the phenotype of the host earlier in its life. While the mechanism explaining such a relationship is still unknown, one may hypothesize 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. It is worth noting that interpreting causal relationships in this host-parasite interaction requires caution. The five models were well supported by the data, indicating that no single interpretation is possible and that further detailed studies are needed to clearly understand such a host-parasite interaction. However, none of these five "best" models identified growth rate during infection as a pure consequence of parasite infection. Hence, only considering that the parasite affects the phenotype of the host (i.e., the traditional approach) would lead to an erroneous interpretation of host-parasite interactions. To summarize this example, a possible pathway of interactions in this biological model is that the level of parasitic infection caused a significant change in the host's phenotype (i.e., growth), and this same trait (earlier in life) modulated the direct pathogenic effect of the parasite (i.e., fin degradation).

The second example involved a cestode endoparasite that colonizes the intestine of an estuarine fish, the rainbow smelt. We found two different patterns according to the level of parasite infection in the rainbow smelt. First, for fish that were weakly infected, we found little evidence that the parasite altered the host growth rate. In the selected model, the phenotype of the host determined the infection level of the host (i.e., the number of parasites and also the biovolume they occupied in the host). Thus, the host phenotypic variance is best considered as a cause of parasite infection rather than a consequence. Secondly, for fish that were heavily infected, we found evidence that both causes and consequences dictated the relationship between the host phenotype and the level of parasite infection. Indeed, here we highlighted that the growth rate of the host before infection positively affected the future infection level of hosts, and subsequently the infection level negatively affected the host growth rate, which can classically be interpreted as a pathogenic effect (Poulin 1998). Overall, this suggests that both causes and consequences occurred in this system, but the consequence of parasite infection (i.e., decrease in the host's growth rate) was only detected on hosts that were heavily infected.

The positive relationship between the growth rate before infection and the infection level we highlighted in both models may be explained by the fact that fish growing faster have a higher consumption rate than others, and therefore should have a higher foraging rate and hence a higher probability of ingesting infected copepods (Combes 1998; Hutchings et al. 2002; Hall et al. 2007). An alternative non-exclusive explanation could be that fish with lower growth rates cannot tolerate infection and suffer higher levels of mortality. Thus, larger fish prior to the infection may be more capable of mounting an immune response to resist the infection or simply be more capable of tolerating an infection (but if infected by too many parasites, they suffer a growth decrease). This latter hypothesis agrees with the size-selective mortality reported in Bourque et al. (2006).

The exact interactions linking *P. tetrastomus* and rainbow smelt are, of course, probably more complex than those presented here, and more experimental and observational studies are needed. Indeed, even if the two selected models had a high probability of being the best for the observed data (>60%), it is important to note that some causal relationships were not significant at the 0.05 level. However, our conclusions validate the previous observations made by Bourque et al. (2006) and more importantly confirm that considering both cause and consequence can provide new insight into understanding the dynamics of host–parasite relationships.

These two examples highlight that model selection procedures allowed testing several competing hypotheses, which are at times difficult to disentangle. This illustrates that potential interactions between host phenotype and parasite infection are complex and that, even with well-designed controlled experiments, they may be difficult to resolve. However, both examples revealed that reciprocal effects can occur within a host-parasite relationship and also that host phenotype variability within the host population should not be solely seen as a consequence of parasite

infection. We used fish as subjects because back-calculating age and growth is easily feasible by using scales or others structures, such as otoliths. However, such reciprocal effects should also be observed in other taxa. For species in which back-calculation is difficult to achieve, techniques such as capture-marking-recapture may help to identify reciprocal effects in the field.

To conclude, our results stress the importance of considering that reciprocal interactions between the host phenotype (e.g., growth, morphology or behavior) and parasite infection can occur simultaneously, and that changes in host phenotype can be a cause and/or a consequence of parasite infection. Host-parasite interactions are often compared to prey-predator interactions (Combes 1998). There is no doubt that some individuals in a population have phenotypic traits that make them more susceptible to be preyed upon by a predator (e.g., those being bolder or more colored, for instance, Olendorf et al. 2006; Bell and Sih 2007). Similarly, it seems evident that some phenotypic traits make some individuals more susceptible to parasitic infections. Currently, the differential susceptibility between hosts and parasite is rarely considered, principally because most studies on the effects of parasites on host phenotype experimentally infect hosts using individual exposure to parasites (e.g., Barber and Svensson 2003; Blair and Webster 2007). Such a design should be altered to exploit any natural pre-existing differences in the susceptibility of hosts and for disentangling causes and consequences in host-parasite interactions (Poulin 1998; Barber et al. 2000). Disentangling the causes and consequences of host–parasite interactions should provide new insights into the role of parasites in ecological processes, such as community assembly and ecosystem functioning, and also on understanding co-evolutionary processes (Marcogliese and Cone 1997; Hatcher et al. 2006; Lambrechts et al. 2006; Wood et al. 2007).

Acknowledgements We sincerely thank the many field assistants that helped in sampling fish. We also thank P. Heeb for stimulating discussions, S. Brosse and two anonymous referees for constructive comments on the manuscript and S. Kohler for statistical advice.

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