

Phenotypic plasticity of host–parasite interactions in response to the route of infection

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Abstract

The microsporidium *Octosporea bayeri* can infect its host, the planktonic crustacean *Daphnia magna*, vertically and horizontally. The two routes differ greatly in the way the parasite leaves the harbouring host (transmission) and in the way it enters a new, susceptible host (infection). Infections resulting from each route may thus vary in the way they affect host and parasite life-histories and, subsequently, host and parasite fitness. We conducted a life-table experiment to compare *D. magna* infected with *O. bayeri* either horizontally or vertically, using three different parasite isolates. Both the infection route and the parasite isolate had significant effects on host life-history. Hosts matured at different ages depending on the parasite isolate, and at a size that varied with infection route. The frequency of host sterility and the host's lifetime reproductive success were affected by both the infection route and the parasite isolate. The infection route also affected parasite life-history. The production of parasite spores was much higher in vertically than in horizontally infected hosts. We found a trade-off between the production of spores (the parasite's horizontal fitness component) and the production of infected host offspring (the parasite's vertical fitness component). This study shows that hosts and parasites can react plastically to different routes of infection, suggesting that ecological factors that may influence the relative importance of horizontal and vertical transmission can shape the evolution of host and parasite life histories, and, consequently, the evolution of virulence.

Introduction

The effects of parasitic infection on host fitness, here referred to as virulence, vary widely between host–parasite systems, from nearly harmless (e.g. trypanosomes of bumble bees, Schmid-Hempel *et al.*, 1999) to lethal (e.g. HIV in humans, Lipsitch & Nowak, 1995), even within a single host–parasite system (e.g. temperate bacteriophage, Levin & Lenski, 1983). The existence of such variation has spurred extensive research to determine which factors influence virulence. One factor that

has received considerable attention is the degree of vertical transmission (i.e. when new hosts acquire the parasite from their parents; e.g. Fine, 1975; Yamamura, 1993; Lipsitch *et al.*, 1996; Saikkonen *et al.*, 2002). Vertical transmission restricts the success of the parasite to the fecundity of its host. On the other hand, horizontal transmission (i.e. when hosts acquire the parasite from any other host) allows new infections to offset parasite losses from host death with an increase in parasite fitness due to new infections. It has been postulated that, over evolutionary time, vertical transmission should lead to a reduction in virulence when compared to horizontal transmission (virulence as within-host growth, Frank, 1996, or reduction in host fitness, Herre, 1995). Outside the long-term evolutionary context, however, no predictions have been made on the immediate, phenotypic responses of host and parasite to the different transmission routes.

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The fitness of a parasite with vertical and horizontal transmission can be decomposed into vertical and horizontal components. The vertical fitness component (VFC) is determined by the transmission efficiency to the host's offspring and by the host's fecundity. Thus a parasite will increase its VFC by increasing the efficiency of transmission to the eggs or embryos and/or by allowing the host to have a higher reproductive success. In contrast, the horizontal fitness component (HFC) of a parasite is determined by the production of infective propagules, their infectivity, and the rate of contact with new hosts. An increase in the resources allocated to the production of infective propagules (either more or better) will thus increase the HFC. Some parasite strategies, however, may enhance the HFC but reduce host reproduction, and thus the VFC (e.g. castration or reduction of the host's lifespan). Therefore, parasites with horizontal and vertical transmission may face a trade-off between their vertical and horizontal fitness components, an idea that is supported by some empirical studies (Sweeney *et al.*, 1989; Kover & Clay, 1998; Turner *et al.*, 1998; Messenger *et al.*, 1999).

Agnew & Koella (1999) have shown that the relative contribution of the vertical and horizontal parasite fitness components may further depend on the host's life-history. Moreover, they showed that host life-history was in turn affected by the degree of exposure to the parasite, pointing to the existence of a feedback mechanism between parasite-induced changes in host life-history and the resulting changes in parasite fitness components. Many other studies support the idea that parasitism affects host life-history, either as a host adaptation (e.g. as an alternative to host resistance, Minchella, 1985), a parasite adaptation (e.g. castration, Baudoin, 1975; increased host longevity, Hurd *et al.*, 2001), an adaptation of both parasite and host (e.g. habitat choice, Karban & English-Loeb, 1997) or just as a side effect of parasitism, i.e. without a benefit for the parasite or the host (see Brown *et al.*, 2001 for a discussion). Only recently, however, has host life-history been explicitly incorporated in evolutionary models (Restif *et al.*, 2001; Gandon *et al.*, 2002).

It is important to distinguish between parasite infection (i.e. the way the parasite invades and develops in the host) and parasite transmission (i.e. the way the parasite leaves the infected host). As the parasite is growing at the expenses of the host, the effects of the infection on the host's life-history are likely to affect the parasite's transmission to a new host. In parasites with horizontal and vertical transmission, route-specific effects of the infection on host life-history may thus affect the parasite's returns for its allocation to each route, i.e. its future transmission, and thus influence the parasite's allocation between the horizontal and vertical fitness components.

To determine how the host-parasite interaction changes with respect to the infection route, we study a host-parasite system where horizontal and vertical transmission

can occur from the same infected host. We examined the plastic responses of host and parasite life-histories to experimentally produced horizontal or vertical infection, and the parasite's allocation to its vertical and horizontal fitness components. Using these data, we studied whether a trade-off exists between the horizontal and vertical parasite fitness components in this system.

Material and methods

Host-parasite system

The host

Daphnia magna Straus is a filter-feeding fresh-water cladoceran usually inhabiting eutrophic ponds. It reproduces by cyclical parthenogenesis and can be maintained by clonal reproduction under laboratory conditions. To avoid confounding effects due to genetic differences among hosts, all assayed individuals were parthenogenetic offspring produced from a single *Daphnia* several generations before the experiment. We used one clone outcrossed from two uninfected clonal lines isolated from two rock-pools near Tvärminne Field Station in Southern Finland. Unless stated otherwise, all *Daphnia* were kept in artificial medium (Klüttgen *et al.*, 1994; modified after Ebert *et al.*, 1998) at 20 °C in a dark:light cycle of 8:16 hours, and fed with the unicellular green algae *Scenedesmus* sp. During the experimental phase, all animals received equal amounts of algae and were transferred to fresh medium at equal intervals.

The parasite

Octosporaea bayeri Jírovec (1936) is a microsporidian parasite of *D. magna* common in the rock-pool populations of the islands of the Tvärminne Archipelago (Green, 1957; Ebert *et al.*, 2001). The parasite can infect its host horizontally, via spores that are released by dead hosts, and vertically, from an infected female *D. magna* to her offspring, the latter being very efficient (Vizoso *et al.*, 2005). Each infected host can potentially transmit the parasite via both routes. *O. bayeri* is easily maintained in the laboratory, as infected *D. magna* develop and reproduce. The *O. bayeri* isolates used here were obtained from three infected *D. magna* females, each collected from a rock-pool population on a different island of the archipelago. These females reproduced parthenogenetically in the lab, which resulted in three clonal populations, each infected with a different parasite isolate (referred to as Ob1, Ob2 and Ob3). In these cultures, the parasite was allowed to infect horizontally as well as vertically.

Experiment

To test how the infection route affected host and parasite life-history, we followed a cohort of *D. magna* infected

with *O. bayeri* either horizontally or vertically. We used a fully factorial design, with the two infection routes and the three parasite isolates (Ob1, Ob2 and Ob3) as factors. We added an uninfected control for descriptive purposes. Prior to this assay, we standardised the conditions of both the parasite and the host. The whole experimental procedure is depicted in Fig. 1 and described below.

First inoculation

To control the host genetic variation, we brought the three parasite isolates from infected cultures into a single host clone (Fig. 1a). For each parasite isolate, 48 uninfected three-days old *Daphnia* from a single clone were individually inoculated with a spore solution prepared by

homogenising a minimum of 15 heavily infected, dying adults from the infected cultures (Fig. 1b, 'first infection'). We used a spore-dose of 50 000 spores in 2.5 mL of artificial medium per individual, maximizing infection success for these isolates in juvenile *Daphnia* of this particular clone (Vizoso, unpublished results). Spore concentrations were estimated with a counting chamber using a phase-contrast microscope (600 \times). A further 144 three-days old *Daphnia* were inoculated with a placebo made from homogenised uninfected *Daphnia* (Fig. 1b). After 5 days, the inoculated *Daphnia* were transferred to 400 mL of medium in randomly formed groups of 24 individuals, hereafter called lines (two lines per isolate, plus six uninfected lines), which reproduced

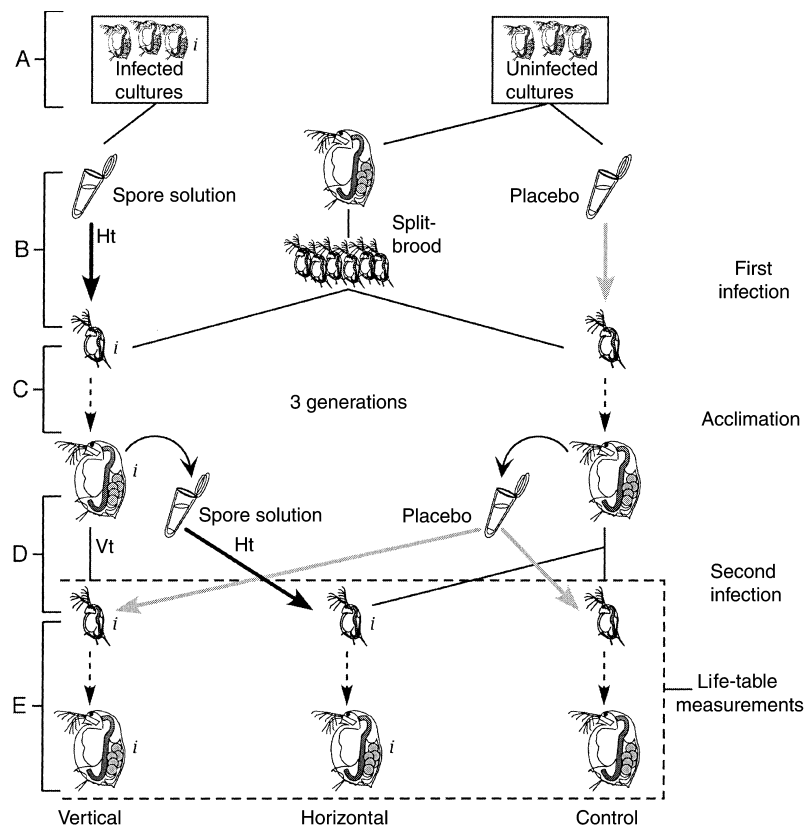


Fig. 1 Experimental design (only one isolate shown). (a) The infected and uninfected cultures were originated from single females isolated from the field, which reproduced parthenogenetically and were kept as populations in the laboratory. (b) First inoculation: the parasite isolates were transferred from their original clonal hosts to the experimental clone, thus eliminating the host's genetic diversity. A split-brood design was used to minimize maternal effects. (c) Acclimation: the parasite isolates were kept in the experimental clone for three generations.

(d) Second inoculation: the vertically infected *Daphnia* were obtained by inoculating offspring from the infected lines with a placebo prepared with adults from the uninfected lines; the horizontally infected *Daphnia* were obtained by inoculating offspring from the uninfected line with a spore solution prepared with adults from the infected lines; the controls were obtained by inoculating offspring from the uninfected line with the placebo. (e) Life-table assay: the inoculated *Daphnia* were individually followed, measuring different life-history traits until they died or reached 65 days of age, when the experiment was terminated. Thick arrows represent experimental inoculation events (black: inoculation with spores, grey: inoculation with placebo); stapled arrows represent growth of *Daphnia*; thin black lines represent offspring distribution among treatments. *i*, infected; vt, vertical transmission; ht, horizontal transmission (see text for details).

and grew during three generations (Fig. 1c, 'acclimation'). To allow for horizontal infections, dead animals were not removed.

Second inoculation

After this period of acclimation, a second inoculation was performed (Fig. 1d). A spore solution was prepared for each isolate by homogenising six heavily infected, dying adults from the respective infected lines. Spore concentrations were calculated as above. For each isolate, 48 *Daphnia* from the uninfected lines were individually inoculated with 50 000 spores in 2.5 mL of artificial medium, to produce the horizontally infected treatments. A placebo made by homogenising 18 adult *Daphnia* from the uninfected lines was administered to 48 *Daphnia* from each infected line, to produce the vertically infected treatments. A further 20 *Daphnia* from the uninfected lines received a placebo, to produce an experimental control group. All *Daphnia* were three-days old, and were inoculated individually in 24-well cell-culture plates, where treatments were fully randomized. After 6 days, a maximum of 20 randomly chosen *Daphnia* per treatment were transferred individually to jars filled with 100 mL of medium, and were followed in a life-table assay (Fig. 1e). These *Daphnia* were changed daily to new medium with a standard amount of algae until the end of the experiment.

Life-table measurements

We followed the *Daphnia* daily and individually, monitoring survival, egg production and release of offspring until they were 65 days old. At this time all infected females had ceased to reproduce, and 65% of all *Daphnia* had died. Age at maturity was defined as the day when the first eggs appeared in the brood chamber, and size at maturity was defined as the body length at that time (measured from the tip of the head to the base of the spina, under a dissecting microscope). For each clutch we counted and sexed the offspring.

Each dead *Daphnia* was frozen in 0.5 mL of medium at -20°C for spore analysis. On day 65 all the surviving *Daphnia* were frozen. To determine the number of spores per individual (spore-load), we homogenized each *Daphnia* with a plastic pestle and resuspended it in 0.5 mL of medium. We determined the concentration of spores as described above, and calculated the spore-load per host. As we were interested in the spore-load as a measure of the HFC (i.e. the allocation to future horizontal transmission), we included only those spores that appear to play a role in horizontal transmission (Vizoso & Ebert, 2004). Spore-load is thus defined as the number of spores at the time of host death or at the end of the experiment (i.e. when hosts were 65 days old) and was used as a proxy to estimate HFC. We estimated the VFC of the parasite (i.e. the allocation to future vertical transmission) as the number of offspring produced by infected *Daphnia*.

The presence of the parasite in the offspring was assessed in a random sub-sample of 62 clutches that were

kept in 100 mL jars. After 1 month, we crushed each *Daphnia* onto a glass slide and checked for spores with a light microscope (300–600 \times , phase contrast).

Individuals that did not mature or reproduce during the experiment, but that survived as long as other females which did produce three clutches, were regarded as sterile. We divided host reproduction *a posteriori* into early reproduction, comprising the first two clutches, and total reproduction, including all the offspring produced by the host until its death or the end of the experiment. We use the term reproductive success if sterile and fertile hosts are pooled together, whereas offspring production is used when sterile hosts are excluded from the analysis.

Statistical analyses

To determine the effect of infection route and parasite isolate on host life-history, we used mixed-model two-way ANOVAs with infection route (horizontal or vertical) as a fixed factor and isolate (Ob1, Ob2 and Ob3) as a random factor.

The effects of the infection route and parasite isolate on the frequency of sterility were tested with the G-test for a three-way log-linear model (Sokal & Rohlf, 1998, pp 743–755), which includes the interaction between the two factors. Then the reduction in offspring number among fertile hosts was tested with a two-way ANOVA.

We further contrasted the effect of the different treatments on offspring production with the uninfected controls using a two-tailed Dunnett's test ($\alpha = 0.05$, $k = 7$; d.f. and error square mean from a one-way ANOVA with treatments as factor; Zar, 1999, p 217). The controls were included in this experiment for descriptive purposes, as not much is still known about the effects of this parasite, and to test whether the parasite has any detrimental effect on host reproduction.

Correlations between traits (e.g. spore-load and total reproduction) were done using Spearman's correlation analyses. As the factors had a significant effect on the traits according to the two-way ANOVAs, the correlation analyses were done on the residuals of the full models.

All data used for parametric statistics were tested for normality and homogeneity of variances, and Box-Cox power transformations were done when necessary (Sokal & Rohlf, 1998, pp. 417–419). All tests were done with JMP 4 (SAS Institute Inc, 2000).

Results

Parasite life-history

We found that the infection route strongly affected the spore-load for the three *O. bayeri* isolates, with vertically infected *Daphnia* carrying up to 19 times more spores than horizontally infected ones (mean spore-load in vertical infections = $4\,093\,750 \pm 344\,098$ spores/host;

Table 1 Effects of the infection route and isolate of *O. bayeri* on different life-history traits of its host, *D. magna*. We show the results of mixed-model two-way ANOVAs with parasite infection route as fixed factor and isolate as random factor (see text for details). Bold typeface indicates *P*-values lower than 0.05. See *Materials and Methods* for details.

	Model											
	r^2	Infection route (d.f. = 1)			Isolate (d.f. = 2)			Infection route × isolate (d.f. = 2)			Error	
		MS	<i>F</i> -ratio	<i>P</i> > <i>F</i>	MS	<i>F</i> -ratio	<i>P</i> > <i>F</i>	MS	<i>F</i> -ratio	<i>P</i> > <i>F</i>	MS	<i>n</i>
Spore-load*	0.59	197253	114.8	0.008	4959.0	1.45	0.41	1713.2	0.65	0.53	2652.3	60‡
Age at maturity	0.13	4.65†	1.39	0.24	10.59†	3.2	0.048	3.9	1.2	0.32	3.3	64§
Size at maturity	0.11	0.17†	5.5	0.022	0.049†	1.6	0.21	0.027	0.9	0.41	0.031	64§
Early offspring production	0.23	43.5	2.6	0.11	52.52	3.2	0.049	47.4	3.1	0.054	15.5	64§
Total offspring production	0.26	909.4†	3.0	0.086	1928.7†	6.4	0.003	360.3	1.2	0.31	298.2	64§
Longevity	0.32	1412.8†	9.4	0.004	315.0†	2.1	0.13	383.1	2.7	0.076	140.1	51¶
Male proportion	0.23	<0.01	0.01	0.92	0.04	0.10	0.91	0.36	7.9	0.0009	0.05	64§

*Tested over the transformed data, using a Box–Cox power transformation with $\lambda = 0.266$.

†MS tested over the error SS after pooling the interaction SS (after Sokal & Rohlf, 1998, p. 337).

‡Six individuals lost in the spore-counts.

§The nine sterile individuals not included.

¶Thirteen individuals excluded due to outliving the end of the experiment.

in horizontal infections = $492\,750 \pm 246\,530$ spores/host; Fig. 2). The parasite isolate did not affect the spore-load significantly, and we found no effect of the interaction between isolate and infection route (Table 1). The 62 clutches assayed for vertical transmission contained a total of 308 *Daphnia* (182 males and 126 females), all of which were infected, for a 100% efficient vertical transmission, as previously found (Vizoso *et al.*, 2005). The effects of infection route and parasite isolate on the vertical transmission of the parasite are therefore identical to those on the host's reproduction (see below).

Host life-history

We found no overall effect of the infection route on age at maturity, although *Daphnia* infected with different isolates of *O. bayeri* matured at different ages (Fig. 3a, Table 1). Size at maturity showed a different pattern, with vertically infected hosts being significantly larger than horizontally infected ones, irrespective of their parasite isolate (Fig. 3b, Table 1). For both traits there was no significant effect of the interaction between infection route and parasite isolate. Parasite isolate had an overall significant effect on early offspring production (Fig. 3c, Table 1). The way total host reproduction was reduced varied with the infection route (Fig. 3d, including sterile hosts). All vertically infected *Daphnia* were fertile, whereas 25% of all horizontally infected *Daphnia* were sterile (G-test: 15.074, $P < 0.01$). There was no difference between isolates (G-test: 4.500, $P = 0.33$), nor was there an interaction between isolate and infection route (G-test 2.169, $P = 0.70$). Along with causing sterility, infection also caused a reduction in total offspring production (the amount of offspring produced by fertile hosts).

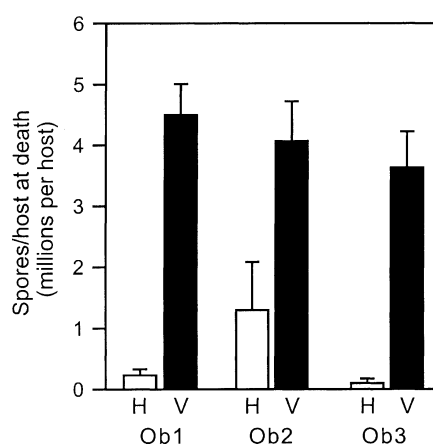


Fig. 2 Effect of infection route (H, horizontal; V, vertical) and parasite isolate (Ob1, Ob2 and Ob3) on the spore-load of *D. magna* experimentally infected with *O. bayeri*. Means and standard errors of the number of spores per host at the time of host death (or at the end of the experiment) are given.

Vertically infected hosts had on average less offspring than horizontally infected hosts, but the difference was not significant (Table 1).

About 40% of all offspring were males (Fig. 3e). This result was affected by the interaction between infection route and parasite isolate, with Ob1- and Ob3-infected hosts producing more males when infected vertically, and *vice versa* for the Ob2 hosts (Fig. 3e, Table 1). We found a strong positive correlation between total offspring production and proportion of males in this clone of *D. magna*, after correcting for treatment effects (Spearman's $\rho = 0.62$, $P < 0.01$ for infected, and $\rho = 0.44$; $P = 0.10$ for uninfected).

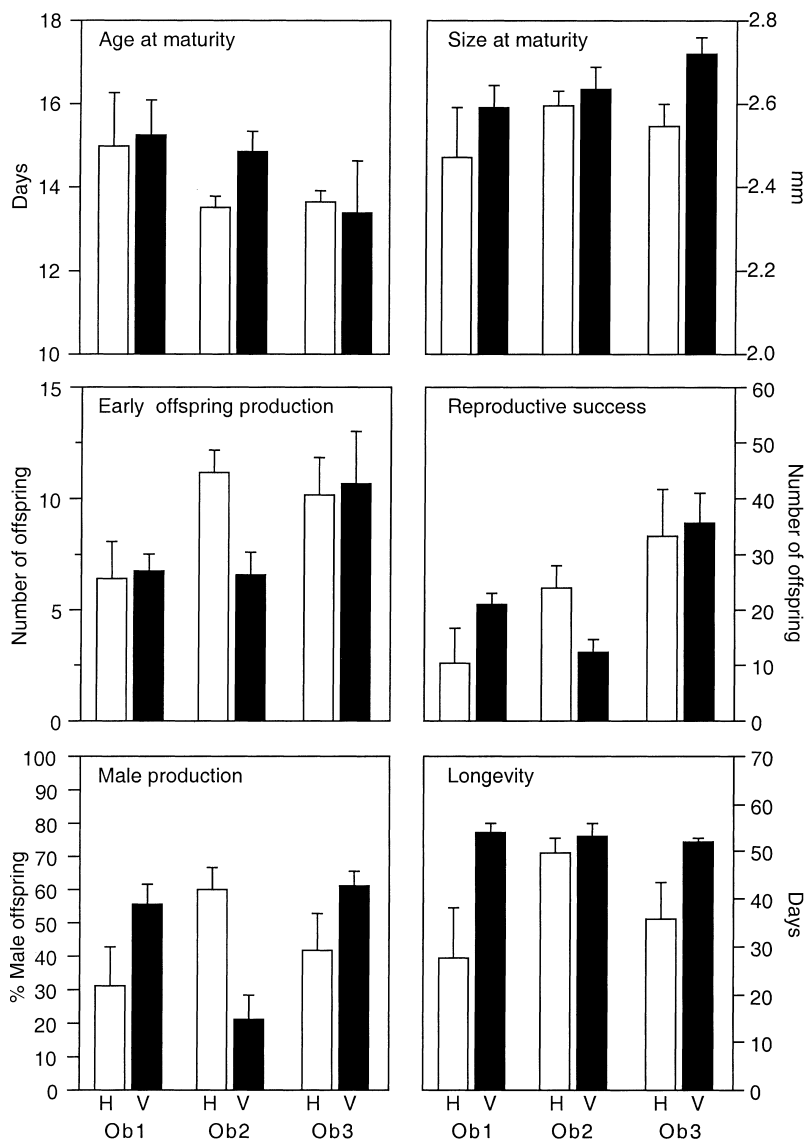


Fig. 3 Effect of infection route (H, horizontal; V, vertical) and parasite isolate (Ob1, Ob2 and Ob3) of *O. bayeri* on different life history traits of its host *D. magna*. We show the means and standard errors for each of the traits. Early reproduction is the number of offspring produced by the *Daphnia* in the first two clutches. Reproductive success is the number of offspring produced by the *Daphnia* during the experiment including sterile *Daphnia* (see text for details).

Finally, horizontally infected *Daphnia* died earlier than vertically infected ones, regardless of the parasite isolate (Fig. 3f, Table 1).

Spore-load and host life-history

Host reproductive success (including sterile hosts) diminished as spore-load increased (Table 2). This result suggests a trade-off between the parasite’s HFC (represented by the spore-load) and the VFC (represented by host reproductive success). Figure 4 plots the residuals of the VFC vs. the residuals of the HFC. The negative correlation between spore-load and host reproduction remained even when the comparison was limited to early reproduction or lifetime offspring production (excluding

sterile hosts). Spore-load also correlated with age at maturity, with hosts bearing a higher spore-load maturing later (Table 2). Hosts that survived longer had a tendency to produce more spores, but the correlation is not significant (Table 2). Further, hosts with higher spore-loads produced a significantly lower proportion of male offspring (Table 2). Finally, spore-load did not correlate significantly with size at maturity (Table 2).

Comparison with uninfected *Daphnia*

Infection with the three isolates of *O. bayeri* decreased the mean reproductive success of *D. magna* between 34% (vertically infected Ob3) and 80% (horizontally infected Ob1). Infected fertile hosts had significantly less

Table 2 Associations between *O. bayeri* spore-load and *D. magna* life-history traits. Spearman's coefficients for the correlations of the residuals from the ANOVAs reported in Table 1. The number of individuals used for the different comparisons are given in the last column (see caption of Table 1 for details). Bold typeface indicates significant *P*-values.

Trait	Spearman's ρ	<i>P</i> > ρ	<i>n</i>
Reproductive success*	-0.42	0.0009†	60
Age at maturity	0.41	0.001	58
Size at maturity	-0.19	0.16	58
Early offspring production	-0.38	0.003†	58
Total offspring production	-0.39	0.003†	58
Longevity	0.30	0.060	40
Proportion of males	-0.31	0.017	58

*Sterile individuals included.

†*P*-values after Bonferroni method (Sokal & Rohlf, 1998, p. 240).

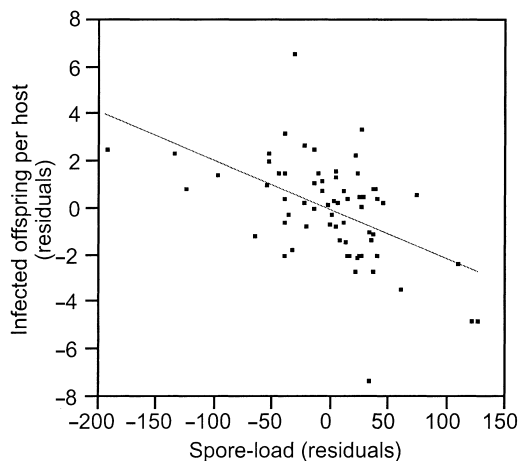


Fig. 4 Correlation between the production of offspring by *D. magna* infected with *O. bayeri* (the parasite vertical fitness component) and the spore-load of the host (the parasite horizontal fitness component). The scatter-plot of the residuals after correcting for infection route and parasite isolate (Table 1).

offspring than the controls (Dunnnett's test, two-tailed $q_{0.05,72,7} = 2.632$) in all but Ob3 groups (Ob1 horizontal: $q_{0.05,72,7} = 3.452$, $P < 0.01$; Ob1 vertical: $q_{0.05,72,7} = 5.273$, $P < 0.0001$; Ob2 horizontal: $q_{0.05,72,7} = 4.201$, $P < 0.01$; Ob2 vertical: $q_{0.05,72,7} = 6.052$, $P < 0.0001$). The effect on early reproduction was milder, from no reduction to only 42% (horizontally infected Ob1). The only significant reduction compared with the controls (Dunnnett's test, two-tailed $q_{0.05,72,7} = 2.632$) occurred in hosts vertically infected with Ob1 ($q_{0.05,72,7} = 2.852$, $P < 0.05$) and Ob2 ($q_{0.05,72,7} = 3.098$, $P < 0.05$). The infection also reduced mean longevity (weighted for those surviving beyond the end of the experiment) between 38% (horizontally infected Ob1) and 10% (vertically

infected Ob1). Although age and size at maturity differed among the treatments, age and size at maturity of uninfected *Daphnia* were both close to the total averages (uninfected *Daphnia* matured on average on day 14, when they were 2.6 mm long). Unlike other microsporidia with vertical transmission (e.g. Dunn *et al.*, 1995), infection with *O. bayeri* did not reduce the proportion of males produced by the host, which in this *D. magna* clone was of 50% when uninfected.

Discussion

Spore-load

Hosts that were infected horizontally carried a much lower spore-load than those that were infected vertically, possibly due to the differences in the process of infection between both infection routes. Microsporidia infect their hosts by harpooning the epithelial cells and discharging the polar tube, through which the infective sporoplasm is injected into the host cell (Bigliardi & Sacchi, 2001). The mechanism by which the polar tube penetrates the host cell is still unclear. But be it by piercing the host cell membrane (Frixione *et al.*, 1992, 1997; Agnew & Koella, 1999) or by a phagocytic process (Magaud *et al.*, 1997; Bigliardi & Sacchi, 2001), it definitely perturbs the host cells. When the infection occurs in the gut (as in horizontal infections of *O. bayeri*), this perturbation may cause substantial wounding of the gut epithelium, and subsequently lead to tissue destruction and/or focal infections by other pathogens. This may in turn elicit a strong immune reaction in the *Daphnia* and divert resources away from both host and parasite. Conversely, vertical infection of microsporidia usually occurs in the rather well-protected environment of the mother. Although the mechanism of vertical infection of *O. bayeri* is not fully understood, we think it happens inside the ovary (Vizoso *et al.*, 2005). In several microsporidia, the parasite enters the host oocyte through the connections with follicle cells (Terry *et al.*, 1997; Becnel & Andreadis, 1999), without harming the oocyte itself. Furthermore, the replication of the parasite during early development might be slower than host cell division (as in Dunn & Smith, 2001), so that damage to the host is kept at a minimum. Indeed, the start of spore production in vertical infections of *O. bayeri* is slower than in horizontal infections (Vizoso & Ebert, 2004). Therefore, vertically infected *Daphnia* are likely to suffer less physiological damage during the infection, and thus provide more resources for the development of the parasite. Our results indeed suggest that horizontally infected hosts were in poorer condition than vertically infected ones, as they matured at a smaller size, had a higher mortality and were frequently sterile (Fig. 3 and Table 1).

Another obvious difference between the two infection routes is the time of exposure to the parasite. Vertically

infected hosts bear the parasite longer, as infection occurs earlier, during the development of the eggs or embryo. Horizontally acquired parasites could, therefore, require a longer time to achieve the same spore production as parasites in vertically infected hosts. However, these isolates of *O. bayeri* show a saturating, density-dependent within-host growth in both vertically and horizontally infected hosts, suggesting that spore production is limited by some form of carrying capacity instead of by time (Vizoso & Ebert, 2004). Thus, even if horizontally infected hosts could live longer, they would not overcome the difference in spore loads between infection routes.

A further difference between the infection routes is that during vertical transmission host and parasite may habituate to each other. Horizontally infected hosts were not exposed to the parasite prior to the experiment, and thus were challenged with a new situation, while vertically infected hosts received the parasite from, and developed in their potentially habituated mother. For example, it is conceivable that maternal effects play a role in vertically infected *Daphnia* by increasing tolerance to the parasite. This possibility cannot be ruled out in our experimental design, and deserves further investigation.

Finally, we do not think that the 19-fold difference between the spore load of vertically and horizontally infected hosts was due to differences in the infective dose, as this would imply that horizontal infections actually had a lower spore-dose than vertical infections. The stronger detrimental effects observed in horizontally infected hosts (shorter longevity, smaller size at maturity, sterility) contradict this hypothesis, and suggest that if different, the spore dose must have been relatively higher in the horizontal infections. As discussed above, vertical transmission is likely to occur transovarially, through the germination of few spores in the ovary, with germination rates and infectivity different from those of horizontal spores. At the moment we cannot compare both doses directly, as the mechanism of vertical transmission remains unknown.

Trade-off between horizontal and vertical transmission

Our results show a significant negative correlation between the relative number of spores accumulated in the host and the relative number of offspring the host produced during its lifetime. As horizontal transmission occurs only after host death (Vizoso *et al.*, 2005), the number of spores accumulated during the host's lifetime is a measure of the parasite's allocation to horizontal transmission (our proxy for the parasite's horizontal fitness component, HFC). The number of (infected) offspring produced by the host is the fitness gain through vertical transmission (our proxy for vertical fitness component, VFC). Therefore, a negative correlation between spore-load and host lifetime reproduction rep-

resents a trade-off between the horizontal and vertical fitness of the parasite, i.e. parasites that allocated more into the production of spores gained less through vertical transmission. This trade-off may be a consequence of resource limitation, as parasites with mixed routes of transmission have to distribute the energy obtained from the host into both transmission routes.

The simple resource partitioning trade-off described here does not apply to cases where vertical transmission is indirect (i.e. parent-offspring transmission occurs externally, due to physical proximity, through the same infective agents as horizontal transmission; e.g. nematodes of mushroom-breeding flies, Jaenike, 2000; and of fig wasps, Herre, 1993). In those cases, the relative contribution of horizontal and vertical transmission to parasite fitness depends more on ecological factors (e.g. presence of offspring from multiple hosts in a patch) than on the allocation to different kinds of transmission described above. Nevertheless, the trade-off between parasite replication rate (proportional in our case to the parasite's HFC) and host reproduction (our measure of VFC) will still play a significant role in regulating the relative number of vertical infections.

Trade-offs between vertical and horizontal parasite transmission have been addressed using ecological and evolutionary approaches (Herre, 1993; Kurtti *et al.*, 1994; Mangin *et al.*, 1995; Turner *et al.*, 1998; Messenger *et al.*, 1999). Our results show that such trade-offs can also arise from physiological processes. The link between physiology and evolution is not straightforward, and requires the analysis of correlated life-history traits, epidemiology, and the ecological factors that might favour one or the other parasite fitness component in a particular environment.

The evolution of virulence and route of transmission

The trade-off between vertical and horizontal parasite fitness components may strongly influence the evolution of this host-parasite system. An increase in the relative opportunities for vertical transmission (e.g. low host density) could shift the optimal parasite strategy towards lower rates of spore production and higher rates of host reproduction. Thus, parasite fitness would increase while virulence decreases. On the other hand, if ecological conditions favour higher rates of horizontal transmission (e.g. high host density) the outcome is less clear. An important point, as stated in the introduction, is the distinction between parasite transmission and parasite infection. When considering only the transmission trade-off, one would expect that spore production increases and host fecundity decreases, producing a positive correlation between parasite fitness and virulence. However, horizontally infected hosts not only suffer more than vertically infected hosts, but also produce far fewer spores. Thus, shifting the allocation towards higher spore production and lower host fecundity will not necessarily

benefit the parasite, as the gains through horizontal transmission seem to be counterbalanced by the low spore production efficiency of subsequent horizontal infections. Moreover, as the production of spores (i.e. the allocation to horizontal transmission) is much higher in vertically than in horizontally infected hosts, vertical transmission seems to be necessary for efficient horizontal transmission. Likewise, owing to the detrimental effects on reproductive success and offspring production, the parasite could not be maintained only through vertical transmission. Our results thus suggest that the interplay of both routes of transmission is required by *O. bayeri* to successfully parasitise *D. magna*.

Spore-load and host life-history

Our results confirm that a higher within-host parasite growth reduces host fecundity. The effect was already present at the early stages of infection, as shown by the negative correlation between spore-load and early reproduction (Table 2). The host does not seem to bring reproduction forward, though, to escape the harmful effects of spore production. In fact, maturity occurred later in those hosts with higher spore-load. As delayed maturity would increase the resources available for parasite spore production at the expense of host reproduction, this life-history change could potentially increase parasite HFC at the expense of VFC. Unsurprisingly, hosts that lived longer tended to produce more spores. However, this trend was not significant. As spores accumulate during the host's lifetime (Vizoso & Ebert, 2004), we expect longer-lived hosts to accumulate more spores. Two mechanisms might have lessened this effect. On the one hand, fast-growing parasite strains might have caused hosts to die earlier, while slow-growing parasites allowed their hosts to survive longer, but accumulated a lower number of spores. Furthermore, spore production seems to reach a limit due to density dependence (e.g. by saturating the *Daphnia* body or depleting the resources, Vizoso & Ebert, 2004). Thus, parasites infecting longer-lived hosts decelerate their growth compared to parasites in shorter-lived hosts, leading to the observed weak correlation between spore-load and longevity.

Effects of the infection on host life-history

As expected, the impact of parasitism on host reproduction was stronger later in life, as the parasite exponentially increases its use of host resources to produce spores as the *Daphnia* grows older (Vizoso & Ebert, 2004). We found no evidence for an adaptive shift towards early fecundity relative to uninfected *Daphnia*, as suggested by other experiments (e.g. Minchella, 1985; Ebert *et al.*, 2004). In our experiment, however, infection occurred much earlier than host maturity. If infection occurs near maturation, hosts might be able to delay maturation as a

fecundity compensation strategy (Minchella, 1985). As the parasite requires some time to reach the host's ovaries, a shift towards early reproduction in the host could thus lead to the production of uninfected offspring (Vizoso, personal observation), increasing the possible advantages of such a compensation strategy. Infection with *O. bayeri* affected the proportion of males produced by the host, but the effect depended on the isolate and the transmission route. We did not find any hints of feminization as in other microsporidia with vertical transmission (e.g. Dunn *et al.*, 1995), possibly due to the abundant horizontal transmission available to the parasite.

The smaller size at maturity together with the shorter lifespan that we observed suggests that horizontal infection inflicts a stronger damage on the host. However, among those that reproduced, horizontally infected hosts had a higher lifetime reproduction than vertically infected hosts. A possible explanation for this seemingly contradictory observation is that parasite growth starts slower when the infection is vertical than when it is horizontal, but then accelerates more quickly as hosts mature. In such a scenario, we would expect to find that spore-loads are higher in vertically infected hosts, as we actually do. This higher spore production in vertically infected hosts would in turn use more resources from the host once they have started reproducing (as opposed to horizontally infected hosts, where the infection is likely to use more resources early in the host's life), resulting in a lower reproduction. Another possibility is that horizontally infected hosts perceive a higher virulence from the parasite, and invest more in reproduction at the expense of growth and longevity. Horizontally infected *Daphnia* also suffered from sterility. Whether this is a parasite adaptation (active castration) or a side effect of parasite infection remains unclear, as sterile *Daphnia* did not bear a higher spore-load.

In conclusion, although horizontal infection may seem less valuable for the parasite, it is indispensable from an epidemiological point of view. As infection reduced host fitness, purely vertical transmission would eventually lead to the displacement of infected lineages by uninfected hosts, and thus the parasite would be lost from the host population. Therefore, at least some horizontal transmission is needed to maintain the parasite. Our results also suggest that vertical infection may help to boost horizontal transmission (in the next parasite 'generation'). At this epidemiological level, the two transmission routes thus seem to be complementary for the parasite's transmission success, contrasting with the suggested trade-off at the level of (individual) resource allocation.

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