Male-biased sex-ratio distortion caused by *Octosporea bayeri*, a vertically and horizontally-transmitted parasite of *Daphnia magna*

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Abstract

Female-biased sex-ratio distortion is often observed in hosts infected with vertically-transmitted microsporidian parasites. This bias is assumed to benefit the spread of the parasite, because male offspring usually do not transmit the parasite further. The present study reports on sex-ratio distortion in a host–parasite system with both horizontal and vertical parasite transmission: the microsporidium *Octosporea bayeri* and its host, the planktonic cladoceran *Daphnia magna*. In laboratory and field experiments, we found an overall higher proportion of male offspring in infected than in uninfected hosts. In young males, there was no parasite effect on sperm production, but, later in life, infected males produced significantly less sperm than uninfected controls. This shows that infected males are fertile. As males are unlikely to transmit the parasite vertically, an increase in male production could be advantageous to the host during phases of sexual reproduction, because infected mothers may obtain uninfected grandchildren through their sons. Life-table experiments showed that, overall, sons harboured more parasite spores than their sisters, although they reached a smaller body size and died earlier. Male production may thus be beneficial for the parasite when horizontal transmission has a large pay-off as males may contribute more effectively to parasite spread than females.

1. Introduction

The mode of parasite transmission has been suggested to be a critical determinant of host–parasite dynamics (Bull, 1994; Kover and Clay, 1998; Dunn and Smith, 2001). Horizontal transmission, on the one hand, occurs between related or unrelated hosts of the same or different generations. Vertical transmission, on the other hand, takes place only within a host lineage, where parasites pass vertically from parent to offspring, usually from mothers to their offspring. Both transmission routes can impose constraints on the parasite reproductive rate (Frank, 1996; Galvani, 2003). Horizontally-transmitted parasites depend on the rate of contact between infective propagules and new hosts and thus usually do not benefit directly from altering host reproduction. The evolution of vertical transmission has been postulated as a parasite adaptation during periods of low host density or host diapause (Fine, 1975). However, exclusively vertically-transmitted parasites that harm their hosts will be lost from the population unless they increase the amount or quality of female offspring (Fine, 1975; Bull, 1994). Thus, a shift from horizontal to vertical transmission is expected to select for reduced virulence (Herre, 1993, 1995; Frank, 1996) and exclusively vertically-transmitted parasites are generally assumed to harm their hosts less than horizontally-transmitted ones (Smith and Dunn, 1991). Parasites with both routes of transmission may have

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a conflict of resource allocation between investments in horizontal versus vertical transmission (Lipsitch et al., 1996; Vizoso and Ebert, 2005). In parasites with horizontal and vertical transmission it is difficult to predict equilibrium virulence and host-life-history adaptation (Ebert and Herre, 1996).

Vertical transmission is usually maternal (Becnel and Andreadis, 1999; Dunn and Smith, 2001). In species with vertical transmission only, there is strong selection on the parasite to bias the sex-ratio in favour of females, because male offspring are a dead end for parasite transmission. Parasite strategies to increase vertical transmission include male killing and feminisation of male hosts (Kellen et al., 1965; Hurst, 1991; Smith and Dunn, 1991; Hurst, 1993; Hurst and Majerus, 1993; Ironside et al., 2003a), both of which are common among vertically-transmitted microsporidia (Caubet et al., 2000; Ironside et al., 2003a; Terry et al., 2004).

Despite of the advantages of killing or feminizing males in the case of exclusively vertical transmission (Ironside et al., 2003b), a parasite transmitted vertically as well as horizontally may benefit from infecting males. Although male hosts do not transmit parasites vertically (a rare exception is the *Drosophila* Sigma Virus, e.g. Altizer and Augustine, 1997), they can transmit the parasite horizontally and all available host resources may be invested in horizontal transmission. In this scenario, selection may favour parasites with increased spore-production in males, possibly at the cost of increased virulence (Dunn and Smith, 2001).

In microsporidia, a group of obligate, intracellular parasites, which are considered to be a sister group of the fungi (Hirt et al., 1999; Baldauf, 2003; Terry et al., 2004), vertical and horizontal transmission are often found within the same species. *Octosporea bayeri* is a microsporidium with horizontal and vertical transmission that parasitises the cladoceran, *Daphnia magna*. *Daphnia magna* is a cyclic parthenogen, that is, it reproduces asexually and sexually. The production of males is environmentally determined. Previous studies suggest that *O. bayeri* can affect the sex-ratio of its host's offspring (Vizoso and Ebert, 2005). Certain genotypes increased the proportion of male offspring when infected, while one genotype had a decreased ratio of males to females. This study was, however, conducted with hosts and parasites isolated from different populations and combined in the laboratory. Thus, these combinations did not reflect a co-evolved state.

Male-biased sex-ratio distortion could be advantageous to the parasite (increased horizontal transmission) or to the host (offspring of infected sons, but not daughters, are more likely to be parasite-free). In this study, we test for sex-ratio distortion in *D. magna* that has co-evolved in natural populations with *O. bayeri* in an individual life-history experiment in the laboratory and in a population experiment in the field. In order to investigate the different roles of male and female hosts for horizontal parasite transmission, we compare parasite spore-production and virulence.

In addition, we attempt to elucidate which antagonist may benefit from sex-ratio distortion: the host and/or the parasite.

2. Materials and methods

2.1. Host–parasite system

Daphnia magna is a filter-feeding, fresh-water cladoceran usually found in eutrophic shallow ponds. It reproduces by cyclic parthenogenesis. Under favourable conditions such as during the warmer season it mainly reproduces asexually. Sex is environmentally determined and sons and daughters from the same mother are genetically identical. When environmental conditions deteriorate (mainly as a consequence of increased density) more sons are produced and females produce resting eggs, which require fertilization. Resting eggs can survive harsh conditions such as drought and frost (Ebert et al., 2001, 2007). Via passive migration of resting eggs, colonisation of unoccupied habitats as well as immigration into existing populations occurs in the metapopulation system the *Daphnia* used for this study (Ranta, 1979; Pajunen and Pajunen, 2003). When environmental conditions improve, females hatch from resting eggs and start asexual reproduction. Being sexually produced, these females are founders of genetically unique clones.

In nature, *D. magna* is frequently infected with a large number of ecto- and endoparasites (Green, 1974; Ebert et al., 2001, 2005). The microsporidium *O. bayeri* is an obligate intracellular parasite, infecting the fat cells and ovaries of its host (Jirovec, 1936). *Octosporea bayeri* can infect its host horizontally, via water-borne spores released only after host death, and vertically from infected females to their offspring (Vizoso et al., 2005). *Octosporea bayeri* is a very common parasite of *Daphnia* in the rock pool metapopulation of Tvärminne Archipelago in southwest Finland (Ebert et al., 2001; Lass and Ebert, 2006), and this high prevalence may be due to its ability to transmit itself horizontally as well as vertically (Vizoso et al., 2005).

The host clones and parasite isolates used in this study originate from different rock pool populations near the Tvärminne Field Station in southwest Finland. The rock pools in this Archipelago are known to form a very dynamic metapopulation system of *Daphnia* where passive migration of resting eggs ensures colonisation of unoccupied habitats as well as immigration into existing populations (Ranta, 1979; Pajunen and Pajunen, 2003). Migration is suggested to have an important effect on the host–parasite interactions (Altermatt et al., 2007).

Host–parasite combinations used for Experiments 1, 3 and 4 were collected 3 months before the experiments, whereas those used in Experiment 2 were collected 1 year prior to the experiment. Experiment 5 was performed outdoors in the Tvärminne Archipelago in Finland with host–parasite combinations sampled close by. *Daphnia magna* and *O. bayeri* were sampled by isolating infected females

from different rock pools shortly after the *Daphnia* had hatched from resting eggs in spring. Thus, these host–parasite combinations are of natural origin. In the laboratory the infected animals were kept separately and under constant conditions that yield asexual reproduction. The *D. magna* clones from the Finish rock pool populations have a high propensity to produce male offspring. Even under favourable conditions it is common to observe 30–50% male offspring. This potential for sexuality may be an adaptation to the inherent instability of the rock pool habitats caused by frequent summer droughts or sudden invasion of brackish water from the Baltic Sea.

All *Daphnia* in the laboratory were kept in an artificial medium (Klüttgen et al., 1994 modified after Lass and Ebert, 2006) at 20 °C with 16 h days, and fed with the unicellular green algae *Scenedesmus* spp. All animals received equal amounts of algae and were transferred weekly to fresh medium. The infected combinations were used for Experiments 2 and 3. Individual females from each of the sympatric host–parasite populations were cured, following the procedure described in Zbinden et al. (2005). We were able to cure 13 clones. Replicate populations of these 13 cured clones were re-infected. Thus all lines experienced the same curing procedure. Of these 13 clones, we were able to re-infect seven with the parasite isolate from this particular clone. These seven re-infected lines and their uninfected counterparts were kept under laboratory conditions for several generations and used for Experiments 1 and 4.

2.2. Experiment 1: host-offspring sex-ratio, fecundity and survival

To determine how infection affects host-offspring, fecundity and survival in different host–parasite combinations, we performed a life-table experiment to compare survival and reproduction of uninfected and infected males and females. For each of the seven uninfected clones and the corresponding seven infected clones, 13 replicates per sex were used, for a total of 364 animals (7 clones × 2 sexes (females and males) × 2 treatments (uninfected and infected) × 13 replicates). Newborns (<24 h old) were individually transferred to 100 ml jars, filled with 80 ml of medium. The jars were paired; next to each female was a male of the same treatment group and clone. The medium was changed simultaneously, twice a week until females reproduced; thereafter it was changed on the day the female reproduced, with a maximum interval of 7 days. *Daphnia* were fed daily, the first 4 days with 1×10^6 algal cells, the following 10 days with 2×10^6 algal cells and thereafter with 3×10^6 algal cells. Sex-ratio was determined in the offspring of all females ($n = 182$), at 50× magnification using a stereo microscope. Survival of males and females ($n = 364$) was monitored three times per week until females produced their first clutch, and daily afterwards. Six females and three males were lost due to handling errors. For the sex-ratio analysis females without offspring were not included

(19 (10%)). The experiment continued until all animals had died.

To determine whether infection affected the sex-ratio of host offspring, we compared the proportion of males produced by infected versus uninfected females in an analysis of variance (ANOVA) with infection as the fixed factor and clone as a random factor. To investigate whether infection affected fecundity (number of offspring) of *D. magna* females, an ANOVA with infection as fixed factor and clone as random factor was performed. To determine whether infection affected resting egg (ephippia) production, an ANOVA with fixed factor for infection and clone as random factor was performed. For these analyses, the data were arcsine transformed to normalise the distribution of the data. To test whether infection and sex affected host longevity, the survival curves were analysed with a parametric survival fit, an Effect Likelihood Ratio Test (SAS, Institute, 2003. JMP 5.1 Inc., Cary, NC, USA). Results are presented as the mean ± standard error.

2.3. Experiment 2: spore dynamics in males and females

To investigate whether infected males and females differ in the number of parasite spores produced (spore-load), we arbitrarily chose two sympatric host–parasite combinations. Thirty-five newborn females and males per combination were individually transferred to 100 ml jars (a total of 140 *Daphnia*). The animals were transferred to fresh medium twice a week, and fed 2×10^6 algal cells per day per animal. Every 10th day, five males and five females from each host–parasite combination were randomly chosen and sacrificed to quantify spore-load. By day 55 all males had either been sampled or had died prior to sampling and the experiment was concluded. In total, males and females were sampled six times. At sampling, we measured *Daphnia* body length (from the top of the head to the base of the spina) under a stereo microscope (50× magnification). To determine the spore-load, the animals were homogenised with a plastic pestle in a standard volume of water and spores were quantified using a Thoma counting chamber and phase-contrast microscopy (400× magnification). Forty-four of 140 animals (31%) died before the day on which they were designated for sacrifice and thus were excluded from the analysis.

2.4. Experiment 3: host–parasite combination effect on spore-load

In order to determine whether the differences in spore-load in males and females were spurious, Experiment 2 was expanded to include 10 more sympatric host–parasite combinations. However, here spore-loads were determined only at day 20 and 30 after host birth. For each date and sex, we had 10 replicates ($n = 400$).

Sixty-six of 400 animals (16%) died before their designated day for sacrifice and thus had to be excluded from the analysis. To test whether males and females differed

in the amounts of spores produced over time in experiments 2 and 3, and if this depended on the host–parasite combination, we compared the spore-loads using an analysis of co-variance (ANCOVA) with sex as fixed factor, host–parasite combination as random factor, and time as a covariate. Spore-load was cube root transformed. To determine whether body volume affected spore-load, the volume of each animal was calculated as: volume = $0.2418 \times \text{length}^{2.593}$ (Pulkkinen and Ebert, 2004) and a further analysis for spore-load was done after correcting spore-load for volume (spore-load divided by volume).

2.5. Experiment 4: sperm production

To determine whether infection affects male sperm production, and if spore-production correlates with sperm production, sperm- and spore-load of infected and uninfected males of four randomly chosen sympatric host–parasite combinations were counted at days 12, 19 and 26 after host birth. A total of 480 juvenile male *Daphnia* (<48 h) were individually transferred to 100 ml jars. The animals were transferred to fresh medium once a week and fed three times a week, the first week with 2×10^6 algal cells, the following week with 3×10^6 algal cells, and with 5×10^6 algal cells thereafter. On the day of each sampling date, 20 males of each host–parasite combination and treatment (either infected or uninfected) were randomly chosen and sacrificed to determine sperm- and spore-load. To measure sperm- and spore-load the animals were homogenised with a plastic pestle in a standard volume of Ringer solution (1 L water, 6 g NaCl, 0,075 g KCl, 0.1 g CaCl2, 0.1 g NaHCO3) and spores and sperm were quantified using a Thoma counting chamber and phase-contrast microscopy (400× magnification).

To investigate whether infection affects sperm production over time, and if this depends on the host–parasite combination, sperm counts were compared in uninfected and infected males using an ANCOVA with infection as fixed factor, host–parasite combination as random factor and time as covariate. Additionally, for the time × infection interaction, a contrast-analysis was performed. Sperm counts were square root transformed. All analyses were performed using JMP 5.1 (SAS, Institute, 2003. JMP 5.1 Inc., Cary, NC, USA). $P < 0.05$ was considered significant.

2.6. Experiment 5: population sex-ratio

Contrary to the other experiments, this experiment was performed under field conditions in the Tvärminne Archipelago in southwest Finland. To investigate the effect of infection status and of initial population density on sex-specific parasitism in *D. magna* populations, a replicated population experiment was performed. In early May 2007, more than 300 adult, egg-bearing *D. magna* females were collected in one natural population and brought to the laboratory. Each female was genetically unique, having

hatched from a sexually produced resting egg. They were kept under optimal conditions in the laboratory and released asexual offspring within 1–2 days. Clutch size under spring conditions is generally large (>25). The infection status of each isofemale line was determined by inspecting the squeezed mother for spores of *O. bayeri* using a phase-contrast microscope (400× magnification). The newborns were thus classified as infected or uninfected and used in the field experiment 3–4 days after hatching. Experimental populations were set up using four different densities (2.5, 5, 10, 40 *Daphnia* L^{-1}) with half of the juveniles infected and the other half uninfected, and the same proportion of offspring of eight uninfected and eight infected isofemale lines per experimental population. This set of four densities was repeated 12 times, using different isofemale lines each time (12×4 densities). The populations were placed in plastic buckets filled with 6 L of filtered (20 µm mesh size) natural pool water next to natural rock pools. Eleven days after the start of the experiment, all females were checked for the presence of asexual clutches. The reproductive status of females was checked non-invasively, as clutch presence in the brood pouch can be scored by eye. Twenty-eight days after the commencement of the experiment, random samples from each bucket were brought to the laboratory. From each replicate population, approximately 50 individuals were kept for at least 10 days under laboratory conditions to ensure sufficient parasite development. Then, all individuals were inspected for infection and gender under the phase-contrast microscope (400× magnification). Adults belonging to the initial population were not included in the prevalence estimates, only the offspring generation.

The field experiment on offspring sex-ratio was analysed with a logistic regression to test for the effect of initial population density and offspring gender on prevalence. A generalised linear model (specified with a quasibinomial error distribution), which accounts for the different sample sizes in sons and daughters and the binomial character of the infection data, was applied. The analyses were performed in R (R Development Core Team (2006). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org) using the libraries Mass, Lme4, Matrix, Hmisc and base.

3. Results

3.1. Experiment 1: host-offspring sex-ratio, fecundity and survival

The number of offspring was significantly higher for uninfected females (63.81 ± 4.03) than for infected females (28.67 ± 3.67) (Fig. 1A; ANOVA; infection: $F_{1,145} = 9.13$, $P = 0.02$; Clone: $F_{6,145} = 2.0377$, $P = 0.204$; infection × clone: $F_{6,145} = 7.87$, $P < 0.0001$). The generally low fecundities are a consequence of the low food level, which was chosen to avoid overfeeding of the males. Infected
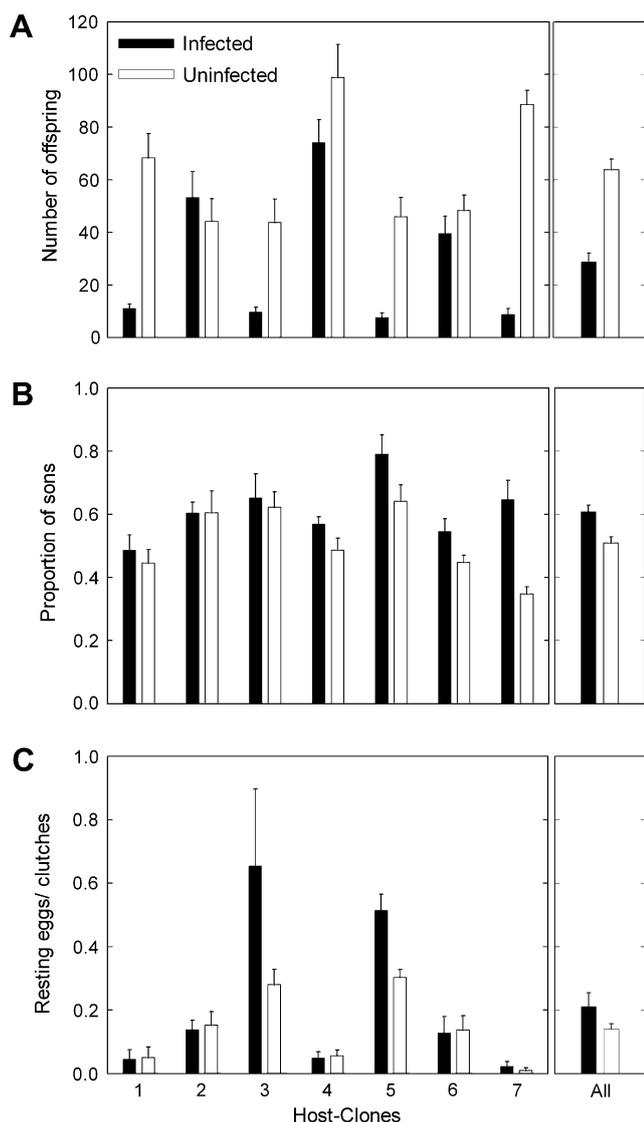
Fig. 1. Number of offspring (life-time fecundity) (A), proportion of sons (B) and proportion of resting eggs (C) in a life-table experiment with females from seven different *Daphnia magna* clones either uninfected (white bars) or infected (black bars) with their sympatric *Octosporea bayeri* parasite. Bars represent the standard error.

females produced about 10% more sons (0.61 ± 0.02) than uninfected females (0.51 ± 0.02) (Fig. 1B; ANOVA; infection: $F_{1,145} = 6.64$, $P = 0.04$; host–parasite combination: $F_{6,145} = 7.17$, $P = 0.02$; infection × host–parasite combination: $F_{6,145} = 2.26$, $P = 0.04$. This shift towards more male offspring persisted throughout the lifetime of the mothers (Fig. 2). In contrast, the production of resting eggs (which requires males for fertilisation) was not affected by parasite infection, but differed strongly among host–parasite combinations (Fig. 1C; clone: $F_{6,145} = 22.7$, $P = 0.0007$; infection: $F_{1,145} = 1.609$, $P = 0.2511$; clone × infection: $F_{6,145} = 1.3516$, $P = 0.24$). We could not find any correlation between fecundity reduction in females and the sex-ratio of their offspring ($P = 0.15$). However, more host–parasite combinations may be necessary to confirm the absence of such a correlation.
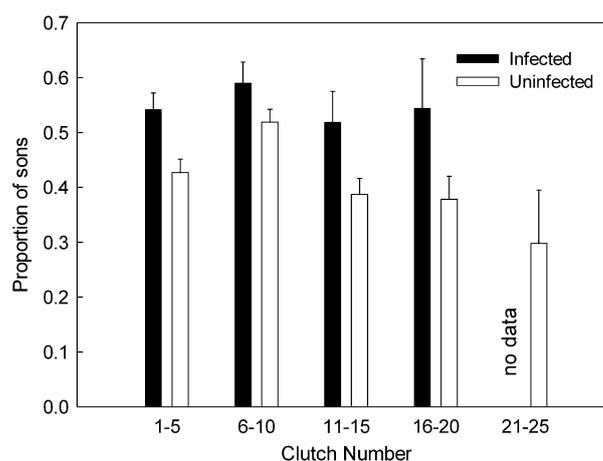

Fig. 2. Proportion of sons in asexual offspring of seven clones of *Daphnia magna* females either uninfected (white bars) or infected (black bars) with their sympatric microsporidian parasite *Octosporea bayeri*. Means across five successive clutches are shown. Bars represent the standard error.

Infected animals had a significantly shorter life-span than uninfected ones (Fig. 3; Effect Likelihood Ratio Test; Infection: $\chi^2_1 = 24.41$, $P < 0.001$; sex: $\chi^2_1 = 157.77$, $P < 0.001$; infection × sex: $\chi^2_1 = 0.94$, $P = 0.64$), and males lived for a significantly shorter time than females. There was no significant interaction between infection and sex (Fig. 3).

3.2. Experiment 2: spore dynamics in males and females

Daphnia magna males infected with *O. bayeri* had a significantly higher spore-load over time compared to females (Table 1, Fig. 4). Body volume was significantly smaller in males than females (Wilcoxon signed Rank test, $P < 0.0001$; Fig. 4), and thus the difference in spore-load increased when corrected for body volume (Table 1 and Fig. 4A and B).
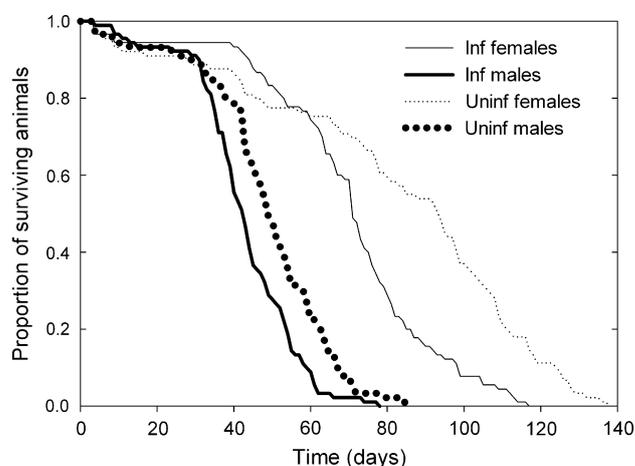

Fig. 3. Survival of male (bold line) and female (fine line) *Daphnia magna* either uninfected (dotted line) or infected (solid line) with their sympatric *Octosporea bayeri* parasite.

Table 1
Analysis of co-variance testing whether *Daphnia magna* males and females infected with the microsporidian parasite *Octosporea bayeri* differ in their spore-load at different time points

Effect	df	ms	*F* ratio	*P*
Spores/individual				
Host–parasite combination	1	7,231	17.90	0.0001
Sex	1	2,281	5.70	0.018
Time	1	354,034	898.98	<0.0001
Host–parasite combination × time	1	7510	19.10	<0.0001
Error	91	393.8		
Spores/volume				
Sex	1	14,150	73.41	<0.0001
Host–parasite combination	1	2339	11.62	0.001
Time (covariable)	1	144,034	742.28	<0.0001
Sex × time	1	5,334	27.67	<0.0001
Host–parasite combination × time	1	2,270	11.78	<0.001
Error	90	192.7		

The response variable spore count was either calculated per individual (spores/individual) or divided by the body volume of the animal (spores/volume). A cube-root transformation of the response variable spore count was done to obtain a normal distribution of the data. The models were simplified by excluding non-significant interactions.
df, degrees of freedom; ms, mean squares.

3.3. Experiment 3: host–parasite combination effect on spore-load

The results of Experiment 3 confirmed that spore-load is mostly higher in males than in females (Table 2). In nine out of 10 host–parasite combinations males had a higher spore-load than females (Fig. 5), even when body volume was not taken into account.

3.4. Experiment 4: sperm production

Our analysis of sperm counts did not reveal a significant effect of infection status, but revealed a significant time × infection interaction (Fig. 6 and Table 3). A contrast analysis showed that the significance of the time x infection interaction resulted from older males producing less sperm when infected (Table 4).

3.5. Experiment 5: population sex-ratio

The proportion of females carrying eggs in their brood chamber declined as initial density increased (linear regression of proportion of egg carrying females (arc-sine square root transformed) against initial density ($\log_{10}$): $r^2 = 0.26$, $t = 4.2$, $P = 0.0001$) indicating that the density treatment worked as expected. At all four densities, prevalence of *O. bayeri* was significantly higher among male offspring than among female offspring, and did not significantly depend on initial population density (logistic regression; sex: $t = 5.35$, $P < 0.0001$; $\log_{10}$ of initial density: $t = 1.2$, $P = 0.23$; Fig. 7). As each population started with 50% infected and 50% uninfected females and horizontal transmission can be excluded, the higher proportion of infected male offspring is likely to be a consequence of infected females producing proportionally more sons than uninfected females. To compare the observed prevalence with the data on sex-ratios presented in Fig. 1, we calculated the percentage of males among infected *Daphnia* ($49 \pm 3\%$) and among uninfected *Daphnia* ($28 \pm 2\%$); Proportion test: $\chi^2 = 81$, df = 1, $P < 0.0001$.

4. Discussion

Our results show a shift towards male-biased sex ratios among *D. magna* offspring infected with a horizontally and vertically-transmitted microsporidian parasite. This effect can be seen from the individual culture experiments and is suggested from the field experiment (Figs. 1 and 7). Male-biased sex-ratio distortion was independent of host age (Fig. 2) and initial host population density (Fig. 7), but varied across host and parasite genetic combinations. We are aware of only two studies that suggest male-biased sex-ratio distortion induced by a parasite (i.e. phenotypic external masculinisation in female isopods infected with a cytoplasmic virus (Juchault et al., 1991), and acceleration of the transition to male in sequentially hermaphroditic fish infected with a myxozoan (Schärer and Vizoso, 2003)), while feminisation has been shown for numerous host–parasite systems (e.g. Dunn et al., 1993; Jiggins et al., 1998, 2001; Terry et al., 2004).

Daphnia are known to start sexual reproduction, including male production, under crowded conditions or when food becomes limited (Hobaek and Larsson, 1990; Kleiven et al., 1992). Thus, one could speculate that the observed increase in the proportion of males in infected hosts could potentially represent a general response to stress caused by the infection. However, we did not find a relationship between the effect of the parasite on the fecundity of the mother and its effect on male production (Fig. 1). Females with fewer offspring did not produce more males, indicating that the observed male bias does not simply reflect a response to stress. However, more host–parasite combinations may be necessary to confirm the absence of such a correlation. In addition, male production, but not ephippia production, was increased in association with *O. bayeri* infections (Fig. 1). Male-bias was also independent of the initial population density in the population experiment (Fig. 7). These results suggest that increased male production is not simply an effect of poor host condition. Further, this interpretation is consistent with the observation that other, purely horizontally-transmitted parasites that certainly cause stress to their hosts do not bias host offspring sex-ratio (D. Ebert, unpublished data).

In a system with exclusively vertically-transmitted parasites, female-biased sex-ratio distortion increases parasite transmission and thus benefits the parasite but not the host. In our system, where both vertical and horizontal transmission are possible, male-biased sex-ratio distortion could be beneficial for the host and/or the parasite. For parasite-induced changes in host traits, it is notoriously difficult to
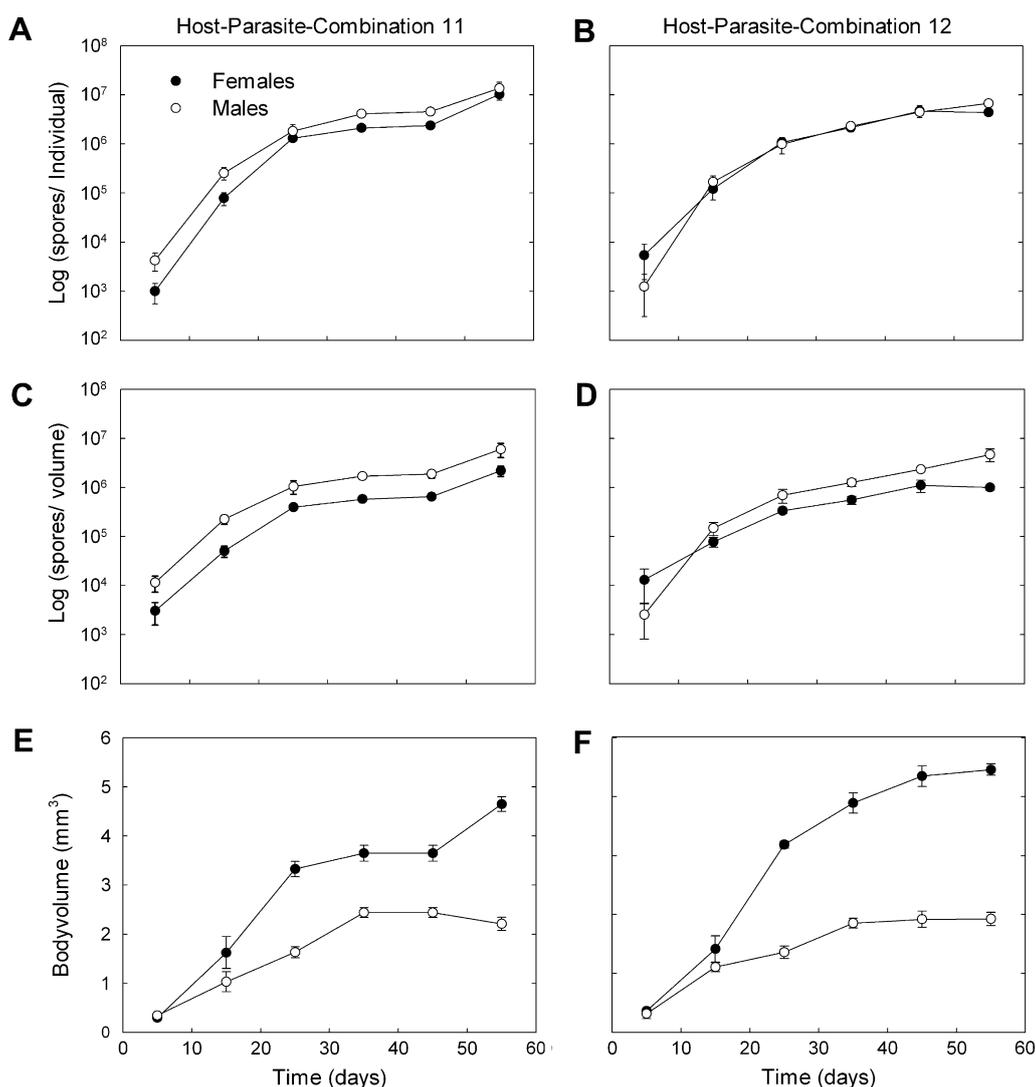
Fig. 4. Spore dynamics (A–D) and body volume (E and F) of male (empty circles) and female (black circles) *Daphnia magna* of two different sympatric *Daphnia* (host)–*Octosporea bayer* (parasite) combinations (A, C, E and B, D, F, respectively). Spore counts and volume calculations were performed every tenth day. The spore counts of the animals were either by individual (A and B) or the spores were divided by host's body volume (C and D). Note that spore counts at day 5 suffer from large stochastic variation, as the absolute number of spores in the sampling volume (counting chamber) at this age was very low. Bars represent the standard error.

Table 2
Analysis of co-variance testing whether *Daphnia magna* males and females infected with the microsporidian parasite *Octosporea bayeri* differ in their spore-load

Effect	df	ms	F ratio	P
Spores/individual				
Sex	1	20,220	76.43	<0.0001
Host–parasite combination	9	5,038	17.70	<0.0001
Time	1	95,939	463.45	<0.0001
Sex × host–parasite combination	9	274.5	1.29	0.24
Sex × time	1	2.319	0.01	0.93
Host–parasite combination × time	9	601.4	1.72	0.22
Sex × host–parasite combination × time	9	348.9	1.69	0.09
Error	294	207.0		

The response variable spore count was cube-root transformed to achieve a normal distribution of the data.
df, degrees of freedom; ms, mean squares.

determine whether the change is beneficial for the host, the parasite, none or both (Dawkins, 1982). Furthermore, there may be a conflict of interest and the observed trait value may be a product of antagonistic co-evolution (Dawkins and Krebs, 1979). Following, we try to lay out the conditions under which a male-biased sex-ratio is favourable to the host versus the parasite.

Spore-production was, on average, higher in males than in females. This effect became even stronger when taking the smaller size of the males into account. We discuss two non-exclusive explanations of this finding. The first is based on a resource allocation trade-off. Vertical and horizontal transmission rates are negatively correlated in the *O. bayeri*–*D. magna* system, suggesting a trade-off between resource allocation to vertical and horizontal transmission (Vizoso and Ebert, 2005). In males, the parasite cannot invest in vertical transmission. Thus, it pays for the parasite
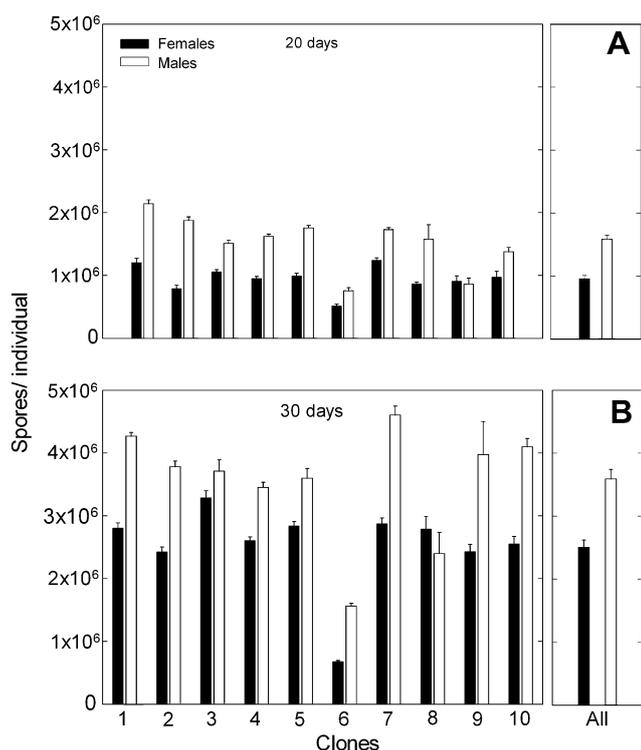
Fig. 5. Number of spores per host individual in female (black bars) and male (white bars) *Daphnia magna* infected with the microsporidium *Octosporea bayeri*, measured after 20 (A) and 30 days (B). The small panels on the right show the mean overall host–parasite combinations. Bars represent the standard error.
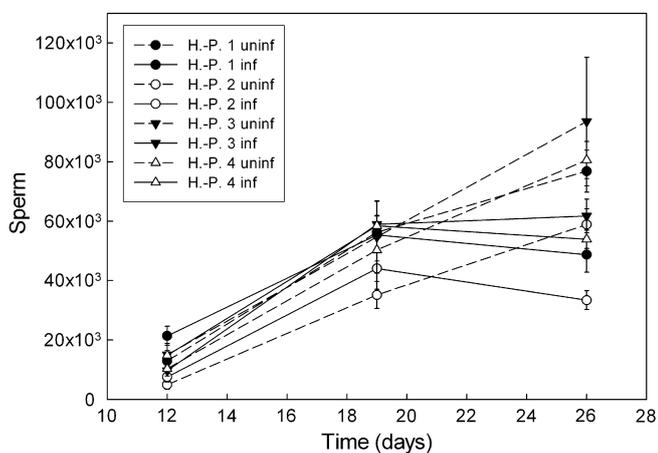

Fig. 6. Sperm production of *Daphnia magna* males either uninfected (dashed line) or infected (solid line) with their sympatric *Octosporea bayeri* microsporidian parasite. The experiment was performed using four different host–parasite (H–P) combinations (1, 2, 3, 4). Sperm were counted after 12, 19 and 26 days. Bars present the standard error.

Table 3

Analysis of co-variance testing whether *Daphnia magna* males from four different host–parasite combinations either uninfected or infected with their sympatric microsporidian *Octosporea bayeri* parasite differed in their sperm load

Effect	df	ms	F ratio	P
Sperm/individual				
Infection	1	574.04	0.1210	0.7281
Host–parasite combination (random)	3	67,112.4	12.18	0.006
Time	2	964,935	175.1	<0.001
Time × host–parasite combination	6	5,510.82	1.16	0.33
Infection × time	2	49,189.4	10.37	<0.001
Error	445	4,745		

df, degrees of freedom; ms, mean squares.

Table 4

Contrast analysis for effect of infection at three time points. The degrees of freedom (df) for the numerator and denominator were 1 and 6.004 for all contrasts, respectively

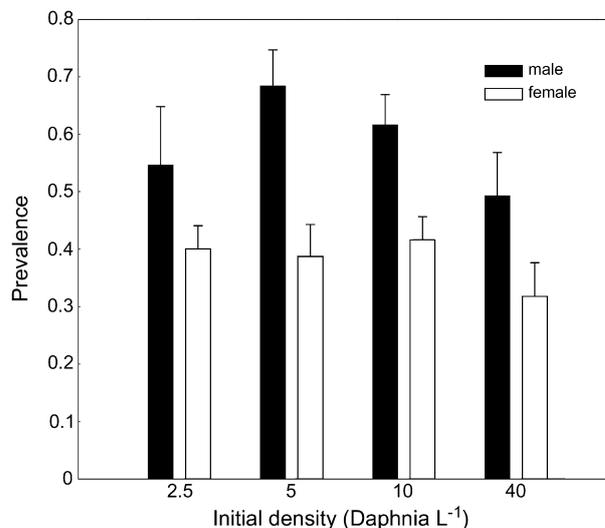| Effect	SS	F ratio	P
Contrasts			
Time 1 (12 days)	21,628	3.52	0.110
Time 2 (19 days)	18,225	2.97	0.136
Time 3 (26 days)	59,133	9.63	0.021

SS, sum of squares.

Fig. 7. Prevalences (±standard error) of *Octosporea bayeri* in populations of *Daphnia magna* that differed in initial densities. Populations were initially set up with 50% infected and 50% uninfected individuals, and prevalence among their offspring was measured after 28 days.

2001; McKean and Nunney, 2001). Reduced immune defence in males may be explained by differences in selection acting on the two sexes, leading to gender-specific resource allocation (Grossmann, 1985; Zuk, 1990; König and Schmid-Hempel, 1995; Rolff, 2002). Currently, it is not known if male *Daphnia* are generally more susceptible than females. Further experiments are necessary to determine whether differences in the parasite's resource allocation or in the immune defence between males and females can explain the increased spore-load in *D. magna* males.

to invest all resources into spores, which allow horizontal transmission.

The second explanation is based on sex-specific immunity. It is possible that the higher spore-load in males compared to females results from reduced immune defence in males. This has been reported for other invertebrates (Kurtz et al., 2000; Sheridian et al., 2000; Kurtz and Sauer,

As a consequence of the higher spore-load in males, one would expect the parasite to be more virulent in males than in females. Our results do not support this corollary. Parasite-induced reduction in host survival was not greater in males than in females. However, the parasite may be affecting fitness components other than host longevity, e.g. reproductive success. Our experimental approach that investigates fitness components in hosts kept individually does not allow the measurement of reproductive success in males. The proxy for reproductive success would be sperm production in males and egg production in females, being two different units and thus not comparable between males and females.

Males infected with *O. bayeri* initially had the same number of sperm as uninfected males, but after about 20 days, the number of sperm did not increase in infected males (Fig. 6 and Table 4). A reduction in sperm numbers has, to our knowledge, never been shown before in a host–parasite system with a microsporidian parasite, but as microsporidian infections are poorly investigated, it is likely that effects on sperm number may also exist in other systems. In our host–parasite system the effect on sperm production was not unexpected, as the parasite typically builds up large spore numbers with a considerable biomass in the host (Vizoso and Ebert, 2004). The lower number of sperm, together with the reduced lifespan of infected males (Fig. 3), is clearly expected to decrease lifetime reproductive success. The fact that infected males are able to reproduce (e.g. Ebert et al., 2007) still allows for the possibility that infected mothers have uninfected grand-progeny through their sons.

A male-biased sex-ratio distortion may indicate an adaptive response of infected *Daphnia* to parasitism with *O. bayeri*. By shifting the sex ratio towards sons, the infected mothers increase their chances to have uninfected grand-progeny. During sexual reproduction, microsporidian parasites are known to be passed on through the female line (Becnel and Andreadis, 1999; Baldauf, 2003; Terry et al., 2004). Thus, mating of infected sons with uninfected females results in uninfected offspring. Further benefits may arise because genetic recombination is believed to be beneficial in developing defences to rapidly co-evolving parasites (the Red Queen Hypothesis) (Hamilton, 1980). A previous study demonstrating lower prevalence in sexual versus asexual offspring and in outbred versus inbred progeny, provides evidence for such benefits in the *D. magna–O. bayeri* system (Ebert et al., 2007).

To estimate more comprehensively the benefit of male-biased offspring production, further aspects have to be taken into account. Firstly, male-biased sex-ratio distortion can only be beneficial if the males have the opportunity to mate in competition with other males. Secondly, male induction only makes sense if the host population is in a phase of sexual reproduction. Males without the presence of receptive females (i.e. females in the state of resting egg production) have no value. Thirdly, we have recently shown that the benefits of being uninfected as a spring hatchling (hatching of the resting stages produced in the previous season) can be very high, but depend strongly on the background prevalence in the population (Ebert et al., 2007). Being uninfected among many infected hatchlings is highly advantageous. Thus, we would expect that producing males can only be advantageous for the host during times of sexual reproduction and at times of high parasite prevalence.

Vertical parasite transmission is a safe way for *O. bayeri* to infect the next host generation (Vizoso and Ebert, 2005). Male-biased sex-ratio distortion, therefore, seems disadvantageous for the parasite. However, this disadvantage may be overcome by benefits resulting from horizontal transmission. Spore-production in male *Daphnia* is higher than in females and males have a shorter life-span than females (Fig. 3). Thus, horizontal transmission through water-borne spores released from male cadavers may result in a higher rate of horizontal transmission. This is advantageous during phases of horizontal spread, because early transmission is favoured (Turner et al., 1998). If the parasite, for instance, invades a *Daphnia* population, horizontal transmission is responsible for parasite spread (Lass and Ebert, 2006). Likewise, after the hatching of *Daphnia* from resting eggs, the parasite starts to spread mainly by means of horizontal transmission (Lass and Ebert, 2006). Thus, we believe that a male-biased sex-ratio is advantageous for the parasite when horizontal transmission is important.

The conditions under which a male-biased sex-ratio is advantageous differ for the host and the parasite. In spring, when *Daphnia* hatch from resting eggs and the populations grow exponentially, a male-biased sex-ratio is beneficial for the parasite, as it may allow for rapid spread by means of horizontal transmission, but it is disadvantageous for the host, as it slows down its asexual exponential growth phase. During the sexual phase of *Daphnia*, which takes place when conditions deteriorate because of high *Daphnia* density and low food availability, production of sons is beneficial for the host, but it is disadvantageous for the parasites, as the parasite will only be passed on to sexually produced resting stages through the female line. Thus, the two antagonists are likely to have a conflict of interest that is dependent on the population growth phase. During host parasite co-evolution the realised offspring sex-ratio may never be optimal for any of the antagonists.

Our laboratory experiments were performed with single individuals in small vials. We think it is not possible to answer the question whether this is more similar to spring or summer conditions in the native habitat of this host–parasite system. Our field experiment was conducted under conditions of variable population growth by starting with different initial densities. However, we did not find a treatment effect, suggesting that there is not a plastic response for sex-ratio with regard to the population growth phase. Under all initial density conditions, we found a male-biased sex-ratio in infected populations (Fig. 7). Possibly, the initial densities did not vary enough for a treatment effect to show up, although a density effect was clearly visible in the

form of delayed reproduction at higher initial densities. Alternatively, the host–parasite conflict results in a scenario with increased male production in infected populations under all conditions.

Another line of evidence suggests that the offspring sex-ratio may be co-evolving. Previous experiments in the same host–parasite system, using artificially created host–parasite combinations, showed strong variation in the sex-ratio of the host, some combinations increasing the proportion of males, others decreasing it (Vizoso and Ebert, 2005; D. Vizoso, unpublished data). In the current study, we only used natural combinations and generally found a more male-biased sex-ratio. A possible explanation for the discrepancies among these two sets of experiments may relate to the co-evolutionary history of the present host–parasite combinations. If the offspring sex-ratio is a trait with a different optimum for the host and the parasite, one may find different results in novel than in co-evolved combinations. However, to reach better conclusions these differences must be tested in direct comparisons between novel and co-evolved combinations.

Acknowledgements

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