

Supplementary material

Can pathogens optimize both transmission and dispersal by exploiting sexual dimorphism in their hosts?

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Production of male and female *Daphnia*

Naturally, female *Daphnia* can shift to producing male and female offspring given certain environmental conditions (Ebert 2005). However, in the laboratory, we can induce the production of males and females by exposing mothers to a small amount of juvenoid hormone, methyl farnesoate (Echelon Biosciences, product number S-0153). Specifically, experimental mothers were prepared by collecting juvenile female *Daphnia magna* from stock cultures and cultured individually in 60 mL jars filled with 50 mL artificial *Daphnia* medium (ADaM, Klüttgen et al. 1994; modified by Ebert et al. 1998) for three generations, to minimize maternal effects. *Daphnia* were transferred into fresh ADaM twice a week, maintained under standard conditions (20°C, 16L: 8D) and fed up to 5 million *Scenedesmus* algal cells daily; steadily increased to accommodate growing needs of the animal. Trays were rearranged every three days to minimise any positional effects.

To produce experimental male and females, the experimental mother generation were exposed to 300 µg L⁻¹ methyl farnesoate hormone, after producing their first clutch, and then transferred into fresh hormone-treated media every 2 days. Subsequent clutches were collected, and the sex of all offspring determined and used as experimental animals. *Daphnia* sex were determined by the presence of a “modified first leg” as per Ebert 2005, since sexual dimorphism in body size is minimal in juveniles (Ebert 2005). Finally, this method can be used to reliably produce male and female *Daphnia* while having no detectable impact on lifespan, fecundity, infection rates or spore loads (Thompson et al. 2017).

Manipulation of host density signals

In a variety of zooplankton, including *Daphnia magna*, info-chemicals and metabolic waste released from conspecifics, influence life-history investment in a variety of traits. Furthermore, presence of info-chemicals as a cue of stress, has been used in a variety of studies, exposing individuals to “stress conditioned water” (Folt & Goldman 1981; Goser & Ratte 1994; Burns 2000; Lüring *et al.* 2003; Michel *et al.* 2016). In this experiment, we exploit release of info-chemicals to simulate cues related to high density in a two-patch experimental microcosm system. Conditioned water was produced by incubating healthy *Daphnia* in 500 mL glass jars (~250 adult *Daphnia* L⁻¹). After 5 days of incubation, we collected the conditioned water by using a coarse-meshed plankton net (mesh size 0.1 mm) to remove *Daphnia*, and then pumping the conditioned water through a 0.45 µm filter to remove debris and algae cells, following (Michel *et al.* 2016).

Table S1 Probability of dispersal from a crowded habitat to an empty neighbouring habitat. Results show the analysis of variance from a generalized linear model using infection status (yes or no), host sex (male or female), and their interaction as fixed effects.

Source	χ^2	d.f.	P-value	Sign. code
Sex	68.621	1	<0.001	***
Infection status	28.802	1	<0.001	***
Sex x Infection status	16.219	1	<0.001	***

Table S2 ANCOVA results from a fitted linear mixed effect model using accumulated number of patches as a continuous response variable and time (in days) as a covariate, and host sex, infection treatment (uninfected control, pathogen C19 and C1) and their interaction, as fixed effects, and individual id as random effect.

Source	χ^2	d.f.	P-value	Sign. code
Sex	0.285	1	0.594	
Infection treatment (Trt)	4.437	2	0.109	
Day number	13251.183	1	<0.001	***
Day number x Sex	2507.064	1	<0.001	***
Day number x Trt	1464.637	2	<0.001	***
Sex x Trt	9.724	2	0.008	**
Day number x Sex x Trt	666.494	2	<0.001	***

Table S3 ANOVA results for model predicting square root transformed total number of patches travelled, using host sex and infection treatment (uninfected control, pathogen C19, and C1) as fixed effects.

Source	d.f.	F	P-value	Sign. code
Sex	1,93	37.820	<0.001	***
Infection treatment (Trt)	2,93	70.349	<0.001	***
Sex x Trt	2,93	13.489	<0.001	***

Table S4 ANOVA results of two factor analysis of variance with square transformed pathogen spore load as response and using host sex and pathogen genotype (Gp, pathogen C1 or C19) as fixed effects.

Source	d.f.	F	P-value	Sign. code
Sex	1, 64	106.717	<0.001	***
Pathogen genotype (Gp)	1, 64	13.923	<0.001	***
Gp x Sex	1, 64	1.425	0.237	

Size corrected spore load in male and female hosts

When accounting for standard body sizes obtained from the literature (male = 2 mm and female = 5 mm, Benzie, J. A. H., 2005), we find evidence that pathogens were able to produce more transmission spores in female hosts, compared to male hosts. This suggest that the pathogens are better at utilising female resources, or, that females provide a higher quality resource patch, for pathogen proliferation.

Table S5 ANOVA results of two factor analysis of variance using relative pathogen spore production in male and female hosts, corrected for standard body sizes. Relative spore load is predicted using host sex, pathogen genotype (C1 and C19) and their interaction as fixed effects.

Source	d.f.	F-value	P-value	Sign. code
Sex	1,64	23.0245	<0.001	***
Pathogen genotype	1,64	22.182	<0.001	***
Sex x Pathogen genotype	1,64	1.417	0.238	

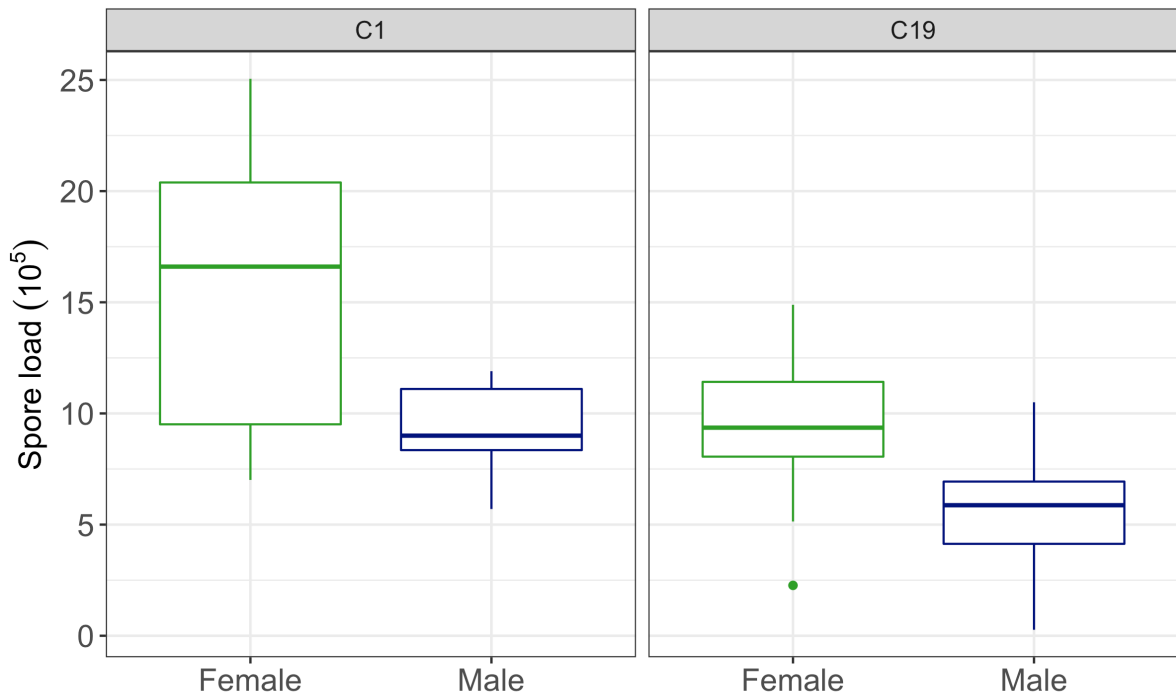


Figure S1 Relative pathogen spore production corrected by standard body size (left: pathogen C1, right: pathogen C19) of males (blue) and females (green).

Cited literature in supplementary material

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