Evolutionary Ecology Research, 2009, **11**: 965–973

Parasitism and environmental sex determination in *Daphnia*

Alison B. Duncan, Sarah A. Hall and Tom J. Little

Institute of Evolutionary Biology, School of Biology,
University of Edinburgh, Edinburgh, UK

ABSTRACT

Hypothesis: *Daphnia* exposed to cues associated with the onset of a parasite epidemic will have more males and resting eggs.

Organisms: Seven clones of *Daphnia magna* (chosen for high levels of sexual reproduction) and the bacterial parasite *Pasteuria ramosa*.

Methods: We explored how parasite infection (simulated by creating crowding conditions using infected hosts) might influence male and resting egg production compared with crowding conditions created using healthy hosts. We also explored the effects of putting bacterial spores in water.

Conclusions: Both crowding and crowding with infected hosts led to higher numbers of males and resting eggs. Direct exposure to parasite transmission spores had no effect. Male production in response to treatment was host-clone specific, with some clones responding strongly to the presence of infected hosts, but others not responding or only responding to water crowded with healthy *Daphnia*. Resting-egg production in response to treatment was also host-clone specific, but differences were not affected by crowding conditions.

Keywords: crowding, evolution, males, *Pasteuria ramose*, resistance, resting egg, sexual reproduction.

INTRODUCTION

Theory predicts that the most successful parasites are those adapted to infect the most common genotypes in their host populations. Sex, via recombination, may then combat parasites through the creation of novel host genotypes to which the parasite is not yet adapted (Haldane, 1949; Jaenike, 1978; Hamilton *et al.*, 1990; Peters and Lively, 1999). However, in some taxa sexual reproduction occurs only periodically, and it is tied to the production of resting eggs that enable populations to avoid unfavourable environments (Hairston and Kearns, 1996; Grishkan *et al.*, 2003). Parasitism is a ubiquitous source of environmental hostility, and if the presence of parasites can induce sexual reproduction, then in organisms where sex is linked to

---

Correspondence: A.B. Duncan, Institut des Sciences de l'Evolution (ISEM), UMR 5554 (CC065), Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France. e-mail: alison.duncan@univ-montp2.fr

diapause, sex may serve as a mechanism to hide from, rather than combat, parasites through recombination.

If diapause via sex serves as a mechanism to avoid parasites, selection may favour genotypes that detect cues associated with parasite epidemics, and subsequently enter diapause. This will have implications for parasite-mediated dynamics in natural populations. In particular, sexually produced resting eggs contain novel genotypes, but they will not contribute immediately to the active portion of the population when a sustained period of diapause occurs before hatching. Thus resting eggs are predicted to be important in maintaining genetic diversity by replacing genetic diversity that may have previously been lost to selection (Gomez and Carvalho, 2000; Berg, 2005).

The present study focuses on the cyclical parthenogen *Daphnia magna*, and in particular a long-term study population that experiences a strong annual epidemic of the bacterial pathogen *Pasteuria ramosa* (Mitchell *et al.*, 2004; Duncan *et al.*, 2006; Duncan and Little, 2007). Like most *Daphnia* species, *D. magna* produce daughters mitotically throughout most of the year. Occasionally, however, in response to certain environmental conditions, they switch to sexual reproduction (Carvalho and Hughes, 1983; Hobaek and Larsson, 1990; Kleiven *et al.*, 1992; Slarsarczyk *et al.*, 2005). Mitotically produced eggs then develop into males and both sexes produce gametes by meiosis. Mating results in the production of a resting egg encased in a structure called an ephippium that can remain in the sediment for considerable periods of time before hatching. There are clone-specific cues for sexual reproduction in *Daphnia*, with different clones having different levels of male and resting egg production (Yampolsky and Kalabushkin, 1992; Boersma *et al.*, 1999). Previously, Duncan *et al.* (2006) showed that both male and resting egg frequencies were highest in a natural population just before the annual *P. ramosa* epidemic. A laboratory infection experiment indicated that clonal lines that originated from females carrying resting eggs when collected from the field were more susceptible to this parasite, which suggests that some genotypes avoid the annual epidemic by diapausing.

Sexual reproduction was principally observed by Duncan *et al.* (2006) as the pathogen was emerging in the *Daphnia* population, but this was also at a time of high host population density. Thus it is not clear from this earlier work which cues in the environment stimulated these *Daphnia* to switch to sexual reproduction and the generation of their resting stage. It is established that high *Daphnia* population density (crowding conditions) can enhance resting egg production (Hobaek and Larsson, 1990; Kleiven *et al.*, 1992). In this study, therefore, we explored how crowding conditions by infected individuals might influence sexual reproduction compared with crowding conditions by healthy individuals. We also wished to determine whether direct exposure to parasite spores might increase the production of resting stages.

MATERIALS AND METHODS

Organisms and collections

Daphnia magna is a planktonic crustacean found in still freshwater bodies and is host to numerous bacterial, microsporidian, and fungal parasites (Green, 1974; Stirnadel, 1994; Little and Ebert, 1999). *Pasteuria ramosa* is a bacterial, spore-forming, obligate endoparasite of *D. magna* that greatly reduces host fecundity and causes gigantism (Ebert *et al.*, 2004). Transmission is horizontal, achieved by the release of spores from the decomposing cadavers of previously infected hosts (Ebert *et al.*, 1996).

Daphnia magna and *P. ramosa* were collected from a farm pond near Leitholm, Scottish Borders (2°20.43′W, 55°42.15′N). Seven *D. magna* individuals seeded the clonal lines used in this experiment. All seven isolates used were reproducing sexually at time of collection, as indicated by the presence of resting eggs. These isolates were part of a larger live sample studied previously (Mitchell *et al.*, 2004; Duncan *et al.*, 2006). They were maintained in the laboratory as clonal lines since their collection. The clones used are designated J1, J11, J8, M12, M5, M6, and M8.

Experimental procedures

Daphnia were exposed to one of four water treatment types, indicating: (1) crowding conditions by healthy hosts; (2) crowding conditions by infected hosts; (3) parasite spores; and (4) normal water (control). Treated water that indicated crowding conditions was achieved by keeping a mixture of clones of healthy *Daphnia* (isolated from the same pond as the focal *Daphnia*) in seventeen 1.5-litre jars at a density of 40 *Daphnia* per litre. Offspring were removed daily to maintain a constant number of individuals contributing to the crowding environment, and each female was fed 3.5×10^6 algae cells per day. Water from these donor jars was collected every 3 days, and the *Daphnia* placed in clean water. After collection, the water from each of the donor jars was combined and filtered through a 0.2-μm Whatman filter to remove algal cells and debris. Water was then stored in dark tanks for up to a week until needed for the experiment. The same protocol was used to make crowding conditions that indicated the presence of infected hosts. Infections were achieved using standard methods similar to those described by Duncan *et al.* (2006). The mean proportion of *Daphnia* that became infected in these jars was 0.62 ± 0.03 (standard error). Densities were identical in treatments (1) and (2), the only difference between the treatments being that infected *Daphnia* were used in (2). *Daphnia* exposed to parasite spores received 1×10^4 parasite spores per jar at each change of water. Such a dose is unlikely to lead to infection, and indeed did not in the present study.

Seven replicates of each of the seven clonal lines were used in this experiment. From each replicate, groups of five offspring (always from the same clutch) were randomly assigned to one of the four water treatment types [crowding by healthy hosts, crowding by infected hosts, parasite spores or normal water (control)]. On day 1, hosts were placed in a 200-ml jar containing the appropriate type of water. The water was then changed on day 5, and then on alternate days after observation of the first clutch. Every time the water was changed, each group of five females was moved to a clean jar containing the appropriate type of water, and any offspring were sexed and counted. The presence of resting eggs was also recorded. The number of adult females remaining (i.e. out of the original five) in the jar was also recorded at each change of water such that all analyses were based on the number of offspring produced per surviving adult.

All treatment groups were exposed to a variable food regime. From day 1 until the first clutch of offspring was observed, each *Daphnia* was fed 3.5×10^6 algal cells per day. After this time, the food was reduced to 1.5×10^6 algal cells per *Daphnia* per day. Pilot studies established that this food regime promotes sexual reproduction. All replicates were kept at 20°C, and subjected to a 16-h/8-h light/dark photoperiod. Jars were stirred daily, and the experiment lasted for 30 days.

Our two principal response variables were the proportion of offspring that were male and the proportion of offspring contained in resting eggs (each fertilized *Daphnia* ephippium contains two eggs) per adult female. We used generalized linear models (Link function = logit, distribution = binomial) to (separately) study the two proportions in response to water type (four levels) and host clone (seven levels). Replicate was included in each model nested within host clone. Host clone was included in the analyses as a fixed factor because each was purposely chosen from the field population for its higher propensity for sexual reproduction. We do not therefore necessarily expect variation in sexual reproduction between these clones to represent those that would be observed at the population level. We used general linear models to study the mean total number of offspring and total number of asexually produced female offspring per adult female. We used Spearman's rank correlation to investigate the relationship between male production and resting egg production for the different host clones in the different water treatment types. All analyses were done in JMP (version 8.0.1).

RESULTS

Table 1 provides summary statistics for the effect of 'water type' and 'host clone' on the total number of offspring produced, and for each offspring type separately. The proportion of offspring contained in resting eggs was affected by water type and host clone and there was a significant interaction between the two (Table 1; Fig. 1). We tested if the water type main effect and the water type × host clone interaction held in a comparison of the two crowded conditions only, and thus we analysed the data excluding control *Daphnia* and those directly exposed to parasite spores. This result reinforced that the quality of crowding conditions influences resting egg production ($\chi^2 = 14.48$, d.f. = 1, $P < 0.0001$). The interaction term, however, was no longer significant ($\chi^2 = 9.701$, d.f. = 6, $P = 0.1378$), indicating that differences in resting egg production between host clones were not due to crowding conditions.

The proportion of offspring that were male was largely determined by 'host clone', and an interaction between host clone and water type (Table 1; Figs. 1, 2). Again, we analysed the data excluding controls and *Daphnia* directly exposed to parasite spores. The clone × water treatment interaction remained significant in the reduced data set ($\chi^2 = 29.088$, d.f. = 6, $P = 0.0033$), thus reinforcing that the quality of crowding conditions mattered in a clone-specific way. Looking at the proportion of male offspring on a clone-by-clone basis, two clones (J11 and M6) increased the proportion of males in response to water crowded with infected hosts, while just one clone (J8) increased male production in response to water crowded with healthy hosts (Fig. 2).

There was no relationship between the proportion of offspring that were male and the proportion contained in resting eggs in any of the water treatment types (water crowded with healthy hosts: $r = -0.29$, $P = 0.053$; water crowded with infected hosts: $r = -0.37$, $P = 0.47$; parasite spores: $r = -0.29$, $P = 0.53$; control: $r = 0$, $P = 1$).

Mortality was low in our experiment, with 83% of jars still containing four or five of the original five hosts present by the end of the experiment at day 30. Nevertheless, we were concerned that differences in mortality, and thus differences in density, between jars could have affected sexual reproduction in relation to our treatments. We therefore repeated the analysis with the proportion of adults surviving in each jar entered into the model as a covariate. The proportion of adults surviving in each jar did not affect male ($\chi^2 = 8.93$, d.f. = 1, $P = 0.302$) or resting egg production ($\chi^2 = 0.674$, d.f. = 1, $P = 0.4116$) and in neither case did the proportion surviving interact with host clone or treatment. The three-way

Table 1. Results of ANOVA detailing the effects of water treatment, host clone, and their interaction

	N/D d.f.	F or χ^2	P
Total offspring			
Water type	3, 121	2.84	0.04
Host clone	6, 121	4.19	0.0007
Water type*host clone	18, 121	0.91	0.5678
Replicate (host clone)	42, 121	0.68	0.91
Total asexual daughters			
Water type	3, 121	1.14	0.33
Host clone	6, 121	3.13	0.0068
Water type*host clone	18, 121	1.24	0.24
Replicate (host clone)	42, 121	0.67	0.929
Proportion within resting eggs			
Water type	3, 121	3741	<0.0001
Host clone	6, 121	234.31	<0.0001
Water type*host clone	18, 121	35.70	0.0077
Replicate (host clone)	42, 121	82.47	0.0002
Proportion male			
Water type	3, 121	8.76	0.0327
Host clone	6, 121	49.81	<0.0001
Water type*host clone	18, 121	44.67	0.0005
Replicate (host clone)	42, 121	88.40	<0.0001

Note: Replicate was included in each model nested within host clone. N/D represents the numerator and denominator degrees of freedom, and the test statistic is either an F-ratio or a χ^2 (where appropriate). P is the level of significance.

interaction between parasite treatment, proportion surviving, and host clone was marginally significant for resting egg production ($\chi^2 = 29.129$, d.f. = 18, $P = 0.0468$). The analysis that excluded controls and *Daphnia* directly exposed to parasite spores revealed that the proportion of adults surviving did not influence variation in resting egg production between the crowding conditions (results not shown).

DISCUSSION

In this study, we investigated the production of sexual stages, measured as levels of male and resting egg production, in *Daphnia magna*. We assessed whether these were enhanced by (1) crowding conditions by healthy conspecifics, (2) crowding by infected conspecifics or (3) direct exposure to parasite transmission spores. Resting egg production was elevated in response to crowding conditions due to the presence of both healthy and infected individuals, although the effect was more pronounced in response to healthy individuals. Numbers of asexually produced female offspring did not differ across treatments, which

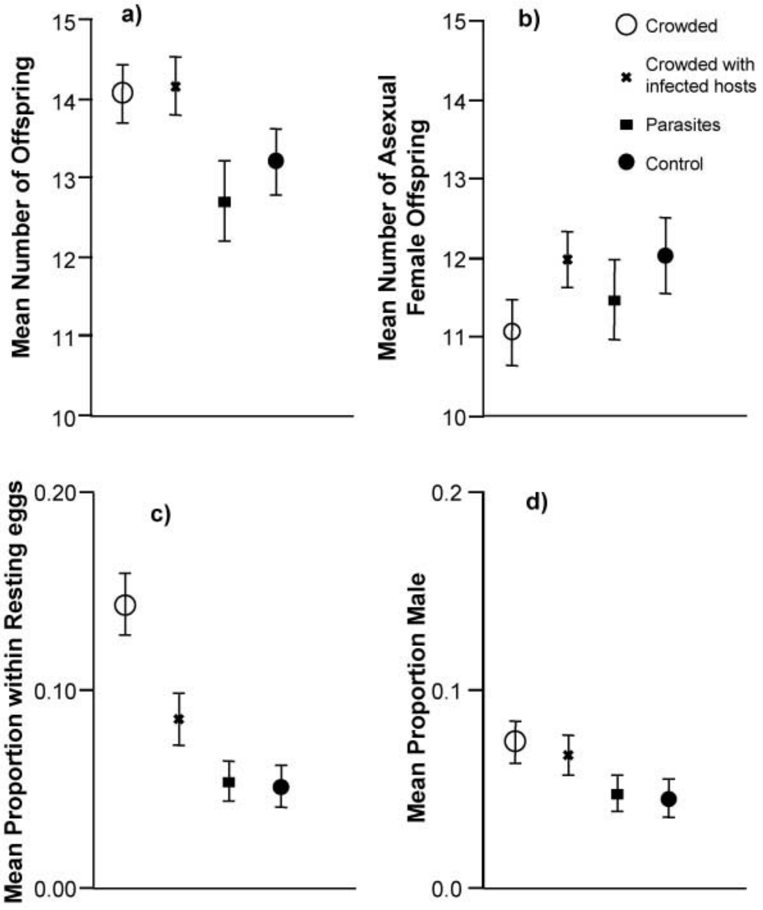

Fig. 1. Mean values for (a) total offspring, (b) asexual female offspring, (c) resting egg production, and (d) male production per female of *Daphnia* clones in different water treatments (± standard error).

suggests that changes in resting egg production reflect an overall increase in reproductive effort, rather than just a shift in allocation to sexually produced offspring.

Male production in response to 'water type' was host-clone specific, with some clones responding more to the presence of infected conspecifics, but others not responding or only responding to water crowded with healthy *Daphnia*. In contrast, resting egg production, which was also host-clone specific, showed a greater propensity for some host clones to produce resting eggs in the control treatments. Resting egg production and the production of males were independent of each other, in line with earlier studies (Boersma *et al.*, 1998).

It is intriguing that exposure to water crowded with healthy *Daphnia* would cause a different response to exposure to water crowded with infected *Daphnia* (e.g. the water type main effect on the proportion of offspring contained in resting eggs). The density of *Daphnia* in both types of donor jars was, in principle, identical. It could be that the body mass of individual infected hosts was different to that of healthy hosts, which influenced the degree of crowding. *Pasteuria*-infected hosts may show evidence of gigantism (Ebert *et al.*, 2004),

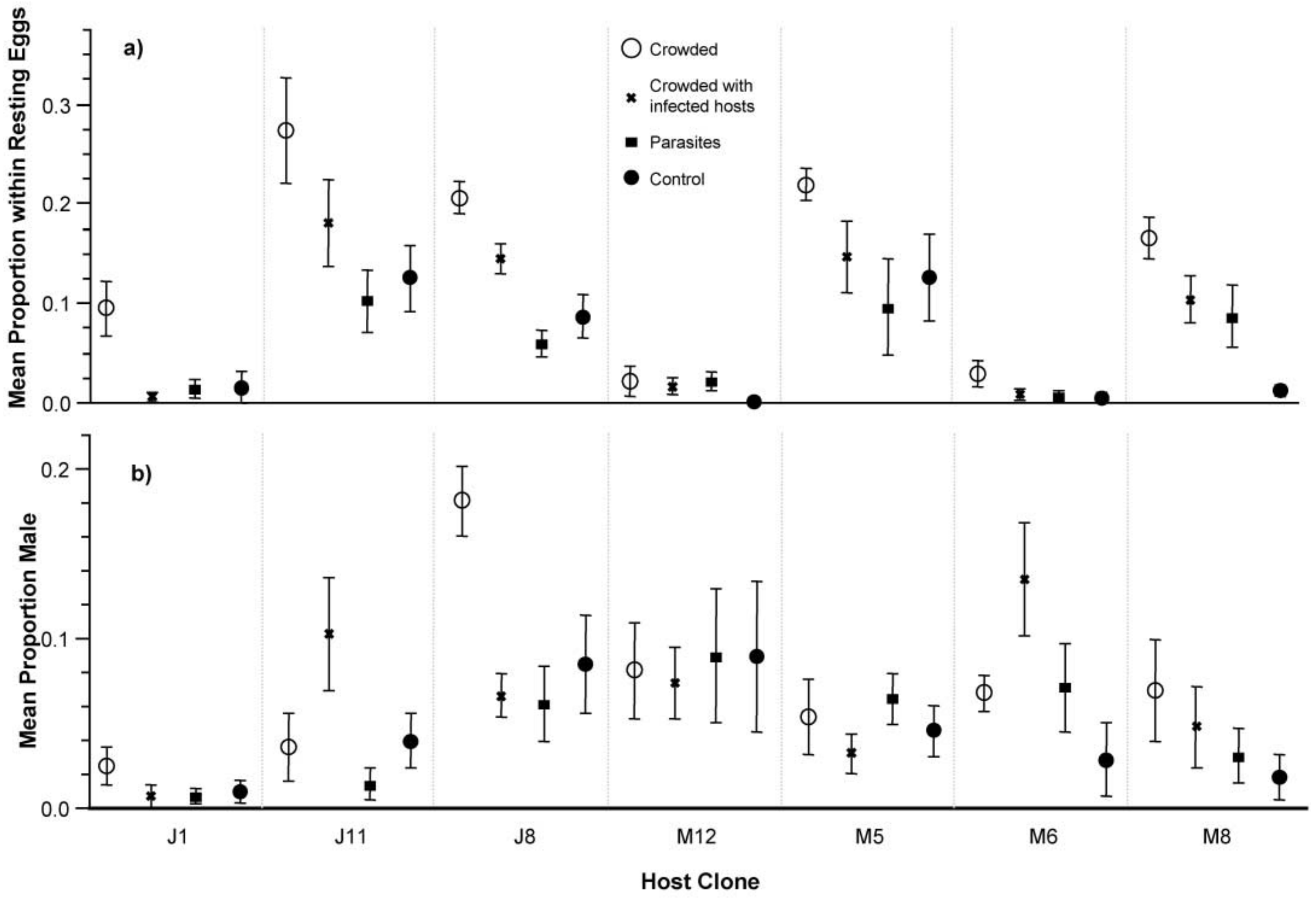

Fig. 2. Mean proportion of (a) resting eggs and (b) males produced by different *Daphnia* clones in different water treatments (± standard error).

and thus if anything we might expect body sizes to be consistently larger in the jars of infected hosts. The host clone × treatment interaction for male production, however, showed how some *Daphnia* increased investment in sex in response to water crowded with healthy conspecifics but not in response to water crowded with infected conspecifics. Thus, the difference between the two water types was not consistent, as one might expect if we had simply failed to make densities comparable. This result could, however, reflect a clone-specific response to the body size of the individuals contributing to the crowding environment. While this argument may not initially seem compelling, it is testable.

Regardless of the mechanism by which it is mediated, it does appear that some *Daphnia* have a specific life-history response to the presence of infected hosts, which presumably indicates a risk of infection in the environment. The solution to this threat, for some clones at least, is to invest in male production. If these males mate successfully, the bearers of these genes will be packaged into a resting egg that offers the opportunity to escape parasite epidemics. This interaction between male production and environment emphasizes the extent to which field dynamics might be influenced by genotype-specific effects. It appears that *Daphnia* populations may be comprised of reproductive specialists that shift their reproductive emphasis in response to environmental conditions, including the occurrence of infectious disease.

One obvious mechanism that would allow *Daphnia* to distinguish the presence of infected versus healthy individuals is through chemically mediated cues in the water. This has been observed in other species; for example, lobsters infected with a virus have been shown to induce behavioural changes in healthy conspecifics, and this is thought to be chemically mediated (Behringer *et al.*, 2006). *Daphnia* themselves are known to be sensitive to the chemical cues emitted by predators (Boersma *et al.*, 1998; Slarsarczyk *et al.*, 2005), but an analogous response to the threat of parasitism would be novel. Determining the benefit to a *Daphnia* of using infected conspecifics as a cue to engage in sex via male production will require study of the speed with which males can find mates and the speed with which resting eggs can be manufactured compared with how quickly epidemics spread.

In summary, this study is the first to explore how sexual reproduction in a *Daphnia* field population is influenced by environmental factors that might surround the onset of an annual parasite epidemic. Our results confirm that crowding conditions do enhance resting egg production. However, we also found that male production can be increased in the presence of infected individuals. It is interesting to speculate that when sexual reproduction is induced by the presence of infected conspecifics, in years when parasite presence is low, sexual reproduction may also be low. The population-wide consequences of sex would thus vary with the strength of epidemics.

ACKNOWLEDGEMENTS

We would like to thank Sharron Meaden and Rui Wang for help in the laboratory, Phil Agnew, Desiree Allen, and Yannis Michalakis for comments on the manuscript, and the Biotechnology and Biological Sciences Research Council, The Nuffield Foundation, Pfizer, and The School of Biology, University of Edinburgh for financial support.

REFERENCES

Behringer, D.C., Butler, M.J. and Shields, J.D. 2006. Avoidance of disease by social lobsters. *Nature*, **441**: 421.

Berg, L.M. 2005. Fluctuating selection, egg banks and population genetic structure in cyclically parthenogenetic species. *Hydrobiologia*, **549**: 287–295.

Boersma, M., Spaak, P. and De Meester, L. 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. *Am. Nat.*, **152**: 237–248.

Boersma, M., De Meester, L. and Spaak, P. 1999. Environmental stress and local adaptation in *Daphnia magna. Limnol. Oceanogr.*, **44**: 393–402.

Carvalho, G.R. and Hughes, R.N. 1983. The effect of food availability, female culture density and photoperiod on ephippia production in *Daphnia magna* (Crustacea Cladocera). *Freshw. Biol.*, **13**: 37–46.

Duncan, A.B. and Little, T.J. 2007. Parasite-driven genetic change in a natural population of *Daphnia. Evolution*, **61**: 796–803.

Duncan, A.B., Mitchell, S.E. and Little, T.J. 2006. Parasite-mediated selection and the role of sex and diapause in *Daphnia. J. Evol. Biol.*, **19**: 1183–1189.

Ebert, D., Rainey, P., Embley, T.M. and Scholz, D. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Phil. Trans. R. Soc. Lond. B*, **351**: 1689–1701.

Ebert, D., Carius, H.-J., Little, T.J. and Decaestecker, E. 2004. The evolution of virulence when parasites cause host castration and gigantism. *Am. Nat.*, **164**: S19–S32.

Gomez, A. and Carvalho, G.R. 2000. Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Mol. Ecol.*, **9**: 203–214.

Green, J. 1974. Parasites and epibionts of Cladocera. *Trans. Zool. Soc. Lond.*, **32**: 417–515.

Grishkan, I., Korol, A.B., Nevo, E. and Wasser, S.P. 2003. Ecological stress and sex evolution in soil microfungi. *Proc. R. Soc. Lond. B*, **270**: 13–18.

Hairston, N.G. and Kearns, C.M. 1996. Phenotypic variation in a zooplankton egg bank. *Ecology*, **77**: 2382–2392.

Haldane, J.B.S. 1949. Disease and evolution. *La Ricerca Scientifica*, **19** (suppl. A): 68–75.

Hamilton, W.D., Axelrod, R. and Tanese, R. 1990. Sexual reproduction as an adaptation to resist parasites. *Proc. Natl. Acad. Sci. USA*, **87**: 3566–3573.

Hobaek, A. and Larsson, P. 1990. Sex determination in *Daphnia magna. Ecology*, **71**: 2255–2268.

Jaenike, J. 1978. A hypothesis to account for the maintenance of sex within populations. *Evol. Theory*, **3**: 191–194.

Kleiven, O.T., Larsson, P. and Hobaek, A. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos*, **65**: 197–206.

Little, T.J. and Ebert, D. 1999. Associations between parasitism and host genotype in natural populations of *Daphnia* (Crustacea: Cladocera). *J. Anim. Ecol.*, **68**: 134–149.

Mitchell, S.E., Read, A.F. and Little, T.J. 2004. The effect of pathogen epidemic on the genetic structure and reproductive strategy of the crustacean *Daphnia magna*. *Ecol. Lett.*, **7**: 848–858.

Peters, A.D. and Lively, C.M. 1999. The red queen and fluctuating epistasis: a population genetic analysis of antagonistic coevolution. *Am. Nat.*, **154**: 393–405.

Slarsarczyk, M., Dawidowicz, P. and Rygielska, E. 2005. Hide, rest or die: a light-mediated diapause response in *Daphnia magna* to the threat of fish predation. *Freshw. Biol.*, **50**: 141–146.

Stirnadel, H.A. 1994. *The ecology of three* Daphnia *species – their microparasites and epibionts*. Masters diploma thesis, University of Basel, Basel.

Yampolsky, Y.L. and Kalabushkin, B.A. 1992. Genetical variability in an intermittent population of *Daphnia magna*. *Genetika*, **28**: 41–50.