Limnol. Oceanogr., 49(4, part 2), 2004, 1355–1364
© 2004, by the American Society of Limnology and Oceanography, Inc.

Haunted by the past: Evidence for dormant stage banks of microparasites and epibionts of *Daphnia*

Ellen Decaestecker, Christophe Lefever, and Luc De Meester
Laboratory of Aquatic Ecology, Catholic University of Leuven, 3000 Leuven, Belgium

Dieter Ebert
Ecologie et Evolution, Université de Fribourg, CH-1700 Fribourg, Switzerland

Abstract

Microparasites and epibionts have important implications for the ecology and evolution of their zooplankton host populations. Many parasites and epibionts produce resistant spores that infect new hosts upon intake. We explored the hypothesis that these spores build up dormant stage banks that remain infective for several years (decades). In laboratory experiments, we exposed *Daphnia magna* to sediments taken from different depths in sediment cores from four different shallow water bodies. All samples analyzed contained infective stages of epibionts, suggesting that dormant stage banks remain infective for decades. Microparasite infections from old sediments were only obtained in one of the four ponds studied. We found mainly the bacterium *Pasteuria ramosa* but also a yet undescribed microsporidium. We discuss the implications of long-lasting spore banks for the disease dynamics and coevolution in the *Daphnia*–microparasite system.

Many organisms, including representatives from all kingdoms, produce long-lived dormant stages, such as cysts, spores, seeds, or eggs, capable of surviving for many years or decades (Hairston et al. 1996; McQuoid et al. 2002). More specifically, aquatic invertebrates, including *Daphnia* (Crustacea, Cladocera), produce diapausing stages (called ''eggs'' in the remainder of the text, but note that dormancy is arrested at the blastula stage; Zaffagnini 1987) in response to environmental changes associated with unfavorable conditions (Hobaek and Larsson 1990). In temperate regions, most *Daphnia* populations produce diapausing eggs as they go through a phase of sexual reproduction (De Meester 1996*a*). The production of diapausing eggs enables zooplankton to occur in temporarily unfavorable habitats, as these diapausing eggs survive harsh conditions. Temporarily unfavorable conditions can be caused by abiotic (e.g., ephemerality of the habitat; salinity; temperature) and/or biotic (antagonists, such as competitors, predators, and parasites) factors. As not all diapausing eggs hatch in the season following their production, they may accumulate in the sediments, forming a diapausing egg bank (DeStasio 1989; Brendonck et al. 1998; Caceres 1998). In addition to the evolutionary and ecological importance of the presence of a diapausing egg bank in the stratified sediments of lakes and ponds, it also represents a unique archive of the history of the population through time (Hairston et al. 1999; Brendonck and De Meester 2003).

Antagonists such as predators and competitors play an important role in the structuring of aquatic communities and are able to influence evolution within populations (Kerfoot and Sih 1987; DeMott 1989). Different studies have shown within and among population genetic differentiation for predator-induced defenses, with patterns strongly indicating local genetic adaptation to predation pressure (Parejko and Dodson 1991; De Meester et al. 1995; De Meester 1996*b*). Analysis of a *Daphnia* diapausing egg bank revealed that, in a time span of approximately less than a decade, a *Daphnia* population can show a significant adaptive evolutionary response to a change in predation pressure by fish (Cousyn et al. 2001). Studies on the adaptation and evolution of defenses in evolutionary ecology have mainly focused on predation and competition. However, it recently became clear that parasites possess features that influence evolutionary and ecological processes of host populations as well (Ebert 1994; Sheldon and Verhulst 1996; Hudson et al. 1998). These features include their ubiquity, their narrow host range, their negative effects on host reproduction and survival, host-density dependence of transmission, and their host genotype-dependent infectivity (Anderson and May 1979; Ebert et al. 1997; Little 2002). More in particular, data from several studies indicate that parasites of *Daphnia* populations have the potential to influence the ecology and evolution of their host population. Zooplankton populations are often heavily infected with parasites (Green 1974; Stirnadel and Ebert 1997). Both within and among population genetic variation for resistance in a *Daphnia magna* population have been shown, as well as local adaptation of parasites to their host population and genotype-dependent susceptibility of *Daphnia magna* toward both multiple strains of one parasite species and multiple parasite species (Ebert et al. 1998; Little and Ebert 1999; Carius et al. 2001; Decaestecker et al. 2003). Most of the zooplankton parasites have adverse ef-

Acknowledgments

We thank Joachim Mergeay, Ria Vanhoudt, Jeroen Verhack, and An Wollebrants for practical assistance; Peter Appleby and Dirk Verschuren for help in dating some of the cores; Luc Brendonck, Steven Declerck, Robby Stoks, Joost Vanoverbeke, Karl Wouters, Nelson Hairston, and two anonymous reviewers for providing valuable criticism on earlier versions of the manuscript. Financial support for this research was provided by the Flemish Institute of Scientific Research in Industry (IWT) to E.D., by the KULeuven Research Fund (OT/00/14) to L.D.M., and by the Swiss Nationalfonds to D.E.

fects on host fecundity and survival (Brambilla 1983; Mangin et al. 1995; Stirnadel and Ebert 1997; Ebert et al. 1998, 2000; Bittner et al. 2002; Decaestecker et al. 2003). Next to infections of endoparasites, the presence of ectoparasites or epibionts is a common phenomenon in zooplankton populations. As epibionts infect only external parts of the zooplankton and have only little or no effect on host reproduction, their influence on zooplankton individuals and populations is much smaller than that of endoparasites (Green 1974; Allen et al. 1993; Chiavelli et al. 1993; Threlkeld and Willey 1993; Stirnadel and Ebert 1997; Bareo-Arco et al. 2001). Therefore, they are also called ectosymbionts (Willey et al. 1990). However, they have been shown to increase host vulnerability to predation such that host fitness is indirectly reduced (Willey et al. 1990; Chiavelli et al. 1993; Bareo-Arco et al. 2001). Because of the difference in their characteristics and impact, we kept (endo-)parasites and epibionts separated in the remainder of the text. In laboratory experiments, transmission of *Daphnia* parasites and epibionts has been shown to be mainly waterborne, horizontal, and dependent on host density (Ebert 1994, 1995; Ebert et al. 2000).

Planktonic populations typically undergo fluctuations in density and disappear from the active community and survive as diapausing eggs. The resulting bottlenecks in host density pose a problem for horizontally transmitted parasites, and it has been suggested that plankton parasites should also have persisting transmission stages, which can endure phases of low host density (Green 1974; Ebert et al. 1997; Lawrence et al. 2002). It has indeed been shown that pond sediments can serve as parasite spore banks and that parasites can survive periods of low host density (Ebert 1995; Ebert et al. 1997; Decaestecker et al. 2002). So far, however, *Daphnia* have only been exposed to the upper layers of stratified sediments. It is not known whether old sediment layers still contain infective parasite transmission stages. Nor is it known how long these stages can persist and be infective under natural conditions. Parasite spores are known to be resistant, as they can be stored for a few years at low temperature (4°C; Dieter Ebert pers. obs.). Even after storage at −80°C for 6 months, some parasites (e.g., *P. ramosa*) are still infective (Ellen Decaestecker unpubl. results). Moreover, it can be expected that, in aquatic systems, parasite spores can remain infective for a longer time than in terrestrial habitats because the aquatic environment protects them against desiccation and ultraviolet radiation (Ebert et al. 1997).

In this study, sediments from three Belgian ponds and one Danish shallow lake are investigated on the presence and infectivity of dormant stages of parasites and epibionts along a depth (age) profile. Under standardized laboratory conditions, we exposed *D. magna* clones to sediment isolated from different depths. The aim of the present study is to determine whether old parasite and epibiont spores, as present in natural sediments, are still infective. If old parasite and epibiont spores are still capable of infecting *Daphnia*, this may have important consequences for the disease dynamics. In addition, by screening sediments covering several decades, our aim was to obtain an estimate of the upper limit of survival of infective spores.

Methods

Study sites and sampling—Four shallow eutrophic ponds and lakes inhabited by *Daphnia magna* were sampled: OM2, Oud Heverlee (OH), Driehoeksvijver (DV), and Lake Ring (LR). OM2, OH, and DV are located in Belgium, LR in Denmark. OM2 and OH are located at a distance of about 10 km from each other, in the province of Vlaams-Brabant. DV is located at approximately 90 km from OM2 and OH, in the province of Oost-Vlaanderen. These are all man-made, shallow, eutrophic ponds. OM2 has a surface area of 2.5 hectare, OH 8.7 hectare, and DV 0.75 hectare. Lake Ring has a surface area of 22.5 hectare and is a shallow eutrophic lake in Central Jutland (Berg et al. 1994). From all habitats, the vertical profile of the *Daphnia magna* diapausing egg bank has been described before (OM2, OH, and DV: Cousyn and De Meester 1998; Cousyn et al. 2001; LR: H. Michels unpubl. data).

Sediment cores were recovered from all ponds with a standard sediment corer consisting of a Plexiglas tube (5.2 cm inner cross-section), of which the lower end was reinforced with a metal cutting edge. Cores from OH and DV were taken between February and April of 1997 (Cousyn and De Meester 1998), cores from LR in October of 2000, and cores from OM2 in November of 2001 (Table 1). The cores were sliced in depth increments of 1 cm (approximately 20 cm^3). The outer millimeters of the increments were removed to exclude contamination of parasite and epibiont spores between different depths.

One of the sediment cores from each of the habitats has been subjected to ^{210}Pb and ^{137}Cs radiodating (P. Appleby, University of Liverpool). Radiodating results were straightforward for LR, but were difficult to interpret for OH, OM2, and DV. Dating of the core from OH has been performed based on the assumption of constant sedimentation rates in terms of mass of sediments and using the time the pond was created as a reference point (see Cousyn et al. 2001). To interpret the vertical profile of OM2, we have taken the same approach and assumed a similar sedimentation rate to OH (OM2 is older, so that the sediment core taken does not reach the transition zone to the mineral soil, resulting in the loss of this reference point). OM2 was characterized by a very similar history during the past decades as OH, as it was also used as a fish culture pond and is equally productive. DV partially dries out in summer and is thus strongly different from the two other ponds. No statements will be made regarding the age of the parasite and epibiont spores for this pond. The relations between depth and age for the sediment of LR, OH, and OM2 are indicated in the figures in the result section. Given the above, the relations are straightforward for LR and OH but should be interpreted with more caution for OM2. In OM2, the time axis should be considered indicative, and only rough statements will be made. The age of the deepest sediment layer studied in the different habitats are LR: 25 cm = approximately 96 yr (SE of age estimate for upper 40 yr: 2–4 yr); OH: 22 cm = approximately 30 yr (age of this pond); OM2: 32 cm = approximately 37 yr.

D. magna clones of a given habitat were exposed to sediments (parasite spores; different depths) of their own hab-

Table 1. Summary of the data on the cores of the four ponds.

	OM2	OH	DV	LR
Period cores were taken	Nov 2001	Feb–Apr 1997	Feb–Apr 1997	Oct 2000
Core dated?	Yes (a)*	Yes (a)*	No	Yes (b)*
Age of deepest sediment layer	37 years	30 years	—	96 years
No. of *Daphnia* clones	6 (2+4)†	6 (6+0)†	6 (6+0)†	6 (6+0)†
No. of depth treatments	12 depths (cm)	3 depths (cm)	3 depths (cm)	3 depths (cm)
	0–2	1–4	1–2	0–5
	2–4			
	4–6			
	6–8			
	8–10			
	10–12	9–12	9–10	10–15
	12–14			
	14–16			
	16–18	18–22	20–24	20–25
	20–22			
	24–26			
	30–32			
No. of core slices used in final volume suspension	6–8‡ 300 ml	8 300 ml	8 300 ml	5 1 liter

* (a) Dating based on the assumption of constant sedimentation rate, (b) ^{210}Pb radiodating.
† First number indicates clones hatched from sediments, second clones isolated from active population.
‡ In-depth treatments 0–2 cm, 10–12 cm, 30–32 cm; eight core slices, in all other depth treatments; six core slices used to make the sediment suspension.

itat. This increases the likelihood of infection success, as *Daphnia* microparasites have been shown to adapt locally to their host population. Clones from OH, DV, and LR were obtained by hatching diapausing eggs isolated from different depth layers from one core from each of the habitats studied. From each habitat, six clones were isolated. For OH, hatchlings were obtained from 1, 5, 7, 9, 16, and 18 cm depth; for DV from 1, 5, 9, 13, 17, and 21 cm, and for LR from 3, 5, 10, 15, 20, and 26 cm depth. From OM2, two clones were obtained by hatching diapausing eggs from the upper sediment layers in 1996; the other clones were directly isolated from the active field population: clone 3 and 4 in 1999, clone 5 and 6 in 2000. After hatching or isolating the *Daphnia* clones, all clones were kept in the laboratory as clonal lineages. This is possible as *D. magna* is a cyclical parthenogen and reproduces by amictic parthenogenesis as long as environmental conditions are favorable. We used clones of different times to take temporal dynamics into account. If parasites track genetic changes in their host population through time, using clones from different sediment layers will increase the likelihood of infection success.

In DV, LR, and OH, we exposed the *Daphnia* to sediments from 3 depths, in OM2 to sediments from 12 depths (Table 1). For each depth treatment, a sediment suspension was made. In the sediment suspensions, core slices of different cores were pooled to ensure enough mud sediment. The sediment suspension of each depth treatment in OH and DV contained eight core slices of 1 cm (in total, approximately 160 cm^3 on a total volume of 300 ml). In LR, sediment suspensions of different depth treatments contained five core slices of 1 cm (in total approximately 100 cm^3 on a total volume of 1 liter). In OM2, we used eight core slices of 1 cm for sediment suspensions (final volume of 300 ml) of the depth treatments 0–2 cm, 10–12 cm, and 30–32 cm; in other depth treatments, six core slices of 1 cm were used. In OH, DV, and LR, sediment volumes of the different treatments were equal, but in OM2, they were not. In OM2, we therefore corrected infection rates and spore loads for the volume of mud to which the experimental animals were exposed. In all cases, we also determined the amount of dry weight of mud present in the sediment suspensions; dry weight was determined after drying at 100°C for 12 h.

In each experimental vial, we placed 10 ml (OM2, DV, and OH) or 40 ml (LR) of homogenized sediment suspension and diluted it with dechlorinated tap water to 250 ml. We then allowed the sediments to settle for 3 d and removed hatched *Daphnia* and *Chaoborus* sp. larvae before the experimental *Daphnia* were added. *Daphnia* that hatched later were much smaller than the experimental *Daphnia* and could easily be distinguished and removed from the experimental jars (no *Chaoborus* larvae were noticed after the first 3 d of collection).

Experiment—We controlled for maternal effects by keeping isofemale lines for two generations under standardized conditions prior to the experiment. From each of the DV, OH, and LR clones, 4 isofemale lines were isolated; from each OM2 clone, 12 isofemale lines were initiated. In each isofemale line, one female was kept individually in a 250-ml jar that we filled with dechlorinated tap water. The *Daphnia* were fed daily: the first generation maternal lines with 40×10^3 cells ml^{-1} of the alga *Scenedesmus acutus*, the second generation with 160×10^3 cells ml^{-1} of the same algal species. Both generations were kept under standardized conditions, i.e., a temperature of 19 ± 1°C and a 16 : 8 light : dark cycle. The water was replaced every 3 d.

The neonates of the second clutch of the second generation maternal lines were isolated and used in the experiment.

In OH, DV, and LR, each of the isofemale lines yielded 18 experimental animals that were used to establish three experimental units of six individuals, each group of six animals being allocated to a different depth treatment. The four isofemale lines thus resulted in four independent replicates for each depth treatment. In OM2, each of the 12 isofemale lines yielded 24 individuals that were used to establish four experimental units of six individuals. These were allocated to treatments such that a given isofemale line provided animals for four different depth treatments to ensure that all replicate observations for a given depth treatment were independent. For OH, DV, and LR, there were in total 72 vials with six *Daphnia* each; for OM2, there were in total 288 vials with six *Daphnia* each.

We exposed the experimental *Daphnia* to the sediment suspensions for 6 d. Each day, the vials were gently turned a few times along their longitudinal axis to stir up parts of sediment and spores. This was done to increase the encounter rates of the *Daphnia* with parasite spores as well as to reduce chance effects associated with these encounter rates. The *Daphnia* were fed daily with 160×10^3 algal cell ml^{-1}.

On day 7, the *Daphnia* were placed in 250-ml jars with fresh dechlorinated water without sediment. They were fed daily with 160×10^3 algal cells ml^{-1}. Every fourth day, the medium was refreshed. On these occasions, the number of offspring produced was determined for three replicates of all treatments. On day 38, females were examined for infection by parasites and epibionts. Females that died earlier were investigated for infection at death. In each vial, we calculated the proportion of infected *Daphnia* (infection rate). On day 38, all living *Daphnia* infected with *P. ramosa* were placed in tubes with a volume of 0.2 ml medium to quantify the number of parasite spores and stored at −80°C until quantification. To quantify spore loads (number of *P. ramosa* spores per infected *Daphnia*), the animals were homogenized and spores were counted with a counting chamber (0.1 mm depth, Bürker) using phase-contrast microscopy at ×400 magnification.

Data analysis—We used Kruskal–Wallis analysis of variance to test whether sediment depth had a significant effect on infection rate. Kruskal–Wallis analysis of variance was used because infection rate did not meet the assumptions of normality. We used average values for each clone per depth treatment as input data ($n = 6$, as there are six clones). On the *P. ramosa* spore loads (angular transformed) of all replica of all clone–depth treatment combinations, a two-way analysis of variance (ANOVA; clone and depth as main effects) was performed. The two deepest sediment layers were not used in the ANOVA because none of the clones showed infections in these sediment layers.

We calculated correlation coefficients between average infection rate, average *P. ramosa* spore load and sediment depth. We also correlated average *P. ramosa* infection rate and average spore load. We used average values per clone–depth treatment combination as input data. We further correlated the average amount of dry weight of mud with sediment depth and with infection rate of all parasites and epibionts using average values per depth treatment. For all tests, we used Spearman rank correlations.

To investigate the influence of the parasites and epibionts on the fitness of the host, we calculated the average number of juveniles per *Daphnia* in vials with infected *Daphnia* and in vials in which none of the *Daphnia* were infected. All vials with one or more infected *Daphnia* were considered infected. As a result, our analysis underestimates the real reduction in fitness. For parasites, we only investigated *Daphnia* from the OM2 treatments; for epibionts, *Daphnia* from all ponds were included. Vials with *Daphnia* infected with parasites were compared with vials in which all *Daphnia* were not infected by parasites, irrespective of whether they were infected with epibionts. In our analysis of the effect of epibionts, the controls were free of epibionts as well as parasites. The effect of the epibionts on the number of juveniles was investigated by Wilcoxon matched pairs tests because data sets were too small or did not meet the assumptions of normality. In the analysis, the average number of juveniles per *Daphnia* of vials with infected individuals were compared with the average number of juveniles per *Daphnia* of vials in which none of the *Daphnia* were infected, matching data according to treatment (pond, clone, and depth combination). The effect of *P. ramosa* on the number of juveniles (log transformed) in OM2 was tested in a three-way ANOVA with infection (presence/absence of *P. ramosa*), sediment depth, and year of host clone isolation as main factors. To enable inclusion of more data while keeping a balanced design, we pooled the years of host clone isolation into two categories: one category with host clones isolated in 1996, and one with host clones isolated in 1999 and 2000. Depths 10–12, 12–14, 24–26, and 30–32 cm were left out of the analysis in order to generate a balanced design. Further, we correlated spore load (angular transformed) of *P. ramosa* in infected *Daphnia* with the number of juveniles (log transformed) per *Daphnia* by Pearson correlation per depth treatment and clone. The effect of Microsporidium 2 on *Daphnia* fecundity could not be tested because the number of replicate observations in which single infections of this parasite was present were too low (only 2 replica).

Results

Overall, we observed two parasite and three epibiont species in our experiment: the parasites were *P. ramosa* and an undescribed Microsporidium species, here called Microsporidium 2; the epibionts: *Amoebidium parasiticum*, *Vorticella* sp., and *Brachionus rubens* (Fig. 1). The investigated ponds differed in number of parasites or epibionts and in their average infection rates (Fig. 1). OM2 showed the highest parasite and epibiont richness as well as the highest infection rates. In DV, *P. ramosa* was the only microparasite, and infection rates were much lower than in OM2. In OH and LR, no parasites were present. Epibionts were found in all ponds. However, only in OM2 and DV were all three epibiont species present. In OH, only *A. parasiticum* and *B. rubens* were found; in LR, only *A. parasiticum* and *Vorticella* sp. were observed (Fig. 1).

Overall, epibiont infection rates tended to decline with increasing depth (Fig. 2). In OM2, OH, and LR, infection rates of *A. parasiticum* differed significantly across depths;

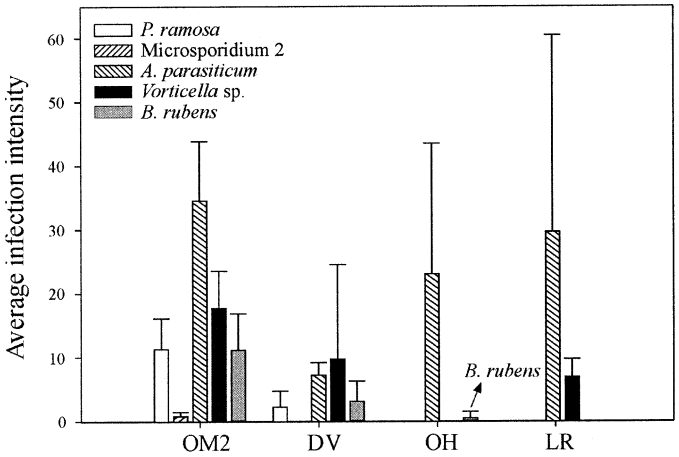

Fig. 1. Average (over clones and depths) infection rate (percentage of infected *Daphnia* in each vial) of different parasite (*P. ramosa* and Microsporidium 2) and epibiont (*A. parasiticum, Vorticella* sp. and *B. rubens*) species, induced by exposing *Daphnia* to different sediment layers of four different ponds (OM2, DV, OH, and LR).

and in the former two ponds, infection rates were negatively correlated with depth (Table 2, Fig. 2a,c,d). *Vorticella* sp. infection rates differed significantly across depths in DV and declined significantly with increasing depth in OM2 and DV (Table 2, Fig. 2a,b). For *B. rubens*, the pattern was less clear. There was only a significant depth effect for infection rates in OM2, and there was no negative correlation between infection rate and depth (Table 2, Fig. 2a).

In OM2, infection rates of both Microsporidium 2 and *P. ramosa* differed between depths, with a negative correlation between depth and infection rate (Table 2; Fig. 3a). In DV, we also observed *P. ramosa* infections, but infection rates were very low and limited to the sediment layer of 9–10 cm (data not shown). In contrast with the infection rates, the spore load of *P. ramosa* in infected *Daphnia* did not differ among sediment depth treatments in OM2 (Table 3, Spearman rank correlation between sediment depth and average spore load: $n = 39$, $r^2 = 0.09$, $P = 0.57$, Fig. 3b). There was no correlation between infection rate and spore load of infected *Daphnia* either (Spearman rank correlation: $n = 39$, $r^2 = 0.11$, $P = 0.48$).

The amount of dry weight of mud was in all ponds higher in deeper layers (figures not shown, Spearman rank correlation in OM2: $n = 12$, $r^2 = 0.88$, $P < 0.0001$; the number of data points for other ponds is too small to perform statistical tests). In OM2, correlations between infection rate and dry weight of mud, matched according to depth, were negative and significant for *Vorticella* sp. (Spearman rank correlation: $n = 12$, $r^2 = -0.72$; $P = 0.007$) and for Microsporidium 2 (Spearman rank correlation: $n = 12$, $r^2 = -0.68$; $P = 0.0153$), and marginally significant for *A. parasiticum* (Spearman rank correlation: $n = 12$, $r^2 = -0.55$; $P = 0.0625$).

From all parasite and epibiont species tested in our study, only *P. ramosa* infections reduced the number of juveniles significantly (three-way ANOVA on the number of juveniles (log transformed) per *Daphnia*: degrees of freedom = 1, MS = 0.18, $F = 5.66$, $P = 0.02$; results of other parasite and epibiont species not shown). *P. ramosa* spore load was not correlated with fecundity of the host (Pearson correlation: $n = 34$, $r^2 = 0.049$, $P = 0.7845$).

Discussion

Our results show that parasites and epibionts of *Daphnia* produce dormant stage banks, which have the potential to stay vigorous over considerable time periods. When *Daphnia* are exposed to these dormant stages, parasites and epibionts caused infections in the *Daphnia.* Epibiont infections were by far more common than infections by microparasites. *A. parasiticum* was abundant and was obtained with sediments from all ponds. *A. parasiticum* has been described as a common epibiont of Cladocera populations (Green 1974). *Vorticella* sp. and *B. rubens* were less abundant in our study. There were no infections present of *Vorticella* sp. in OH or of *B. rubens* in LR. Concerning parasites, exposure of *Daphnia* to sediments mainly lead to infections of *P. ramosa,* and most strongly in OM2. Low infection rates (<10%) of *P. ramosa* were also found on exposure to sediments from DV. The dominance of *P. ramosa* infections among the parasites was not surprising because, in several field studies, *P. ramosa* has been reported to occur in high prevalence (Stirnadel and Ebert 1997; Little and Ebert 1999; including in OM2: Decaestecker 2002). Moreover, an earlier study has already shown that exposure of *Daphnia* to recent mud sediments from OM2 results in high infection levels of *P. ramosa* (Decaestecker et al. 2002). A field survey has also documented high prevalences for Microsporidium 2 in the *Daphnia* population of OM2 (Decaestecker 2002), infection rates of this parasite upon exposure to mud was much lower compared with that of *P. ramosa* in our experiment. Three explanations for this difference are possible: spore density in the mud was much lower for Microsporidium 2 than for *P. ramosa,* infectivity of the spores of Microsporidium 2 was lower than that of *P. ramosa* spores, or the host clones used in the experiment were more resistant to Microsporidium 2 than to *P. ramosa.* Our experiment does not allow differentiation among these possibilities.

Our observations show that many parasite and epibiont dormant stages remain infective for decades. However, we did not find evidence for dormant propagules from all parasite and epibiont species that have been present in past standing field populations. A field survey of OM2 (Decaestecker 2002) indeed showed that the parasite and epibiont richness is higher than the number from which we observed dormant propagules. This may be due to the fact that these do not produce long-lasting dormant propagules or because we failed to detect them in our study. One difficulty of detection may be that the dormant propagules are not equally distributed but rather are accumulated in specific places due to disease dynamics, host aggregation, and wind-induced water currents. In addition, it is possible that the *Daphnia* clones used in our experiment are resistant the parasite species that we did not find.

Analysis of our data suggests that the infection rate of the observed parasites and epibionts decline with age of the sed-

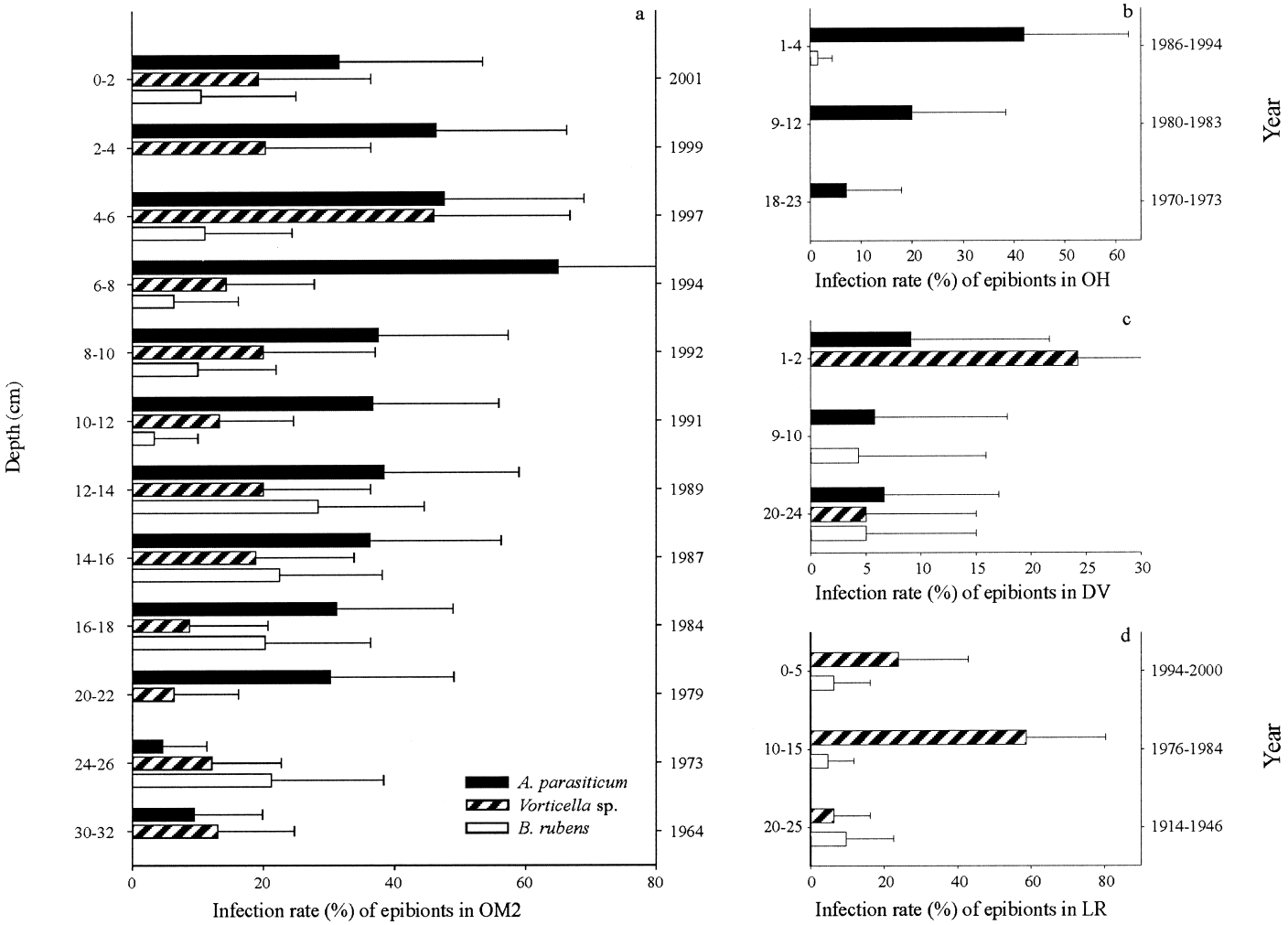

Fig. 2. Infection rate (%) of epibionts across different depths of the sediment layers in (a) OM2, (b) OH, (c) DV, and (d) LR. Dating of the LR core is based on ^{210}Pb radiodating, of OH and OM2 on the assumption of constant sedimentation rates in terms of mass of sediments (see text). In OM2, the average year corresponding with the sediment depth range of the different slices is given.

iments. This suggests that infectivity of the dormant stages declines with age. This latter statement assumes that the density of dormant stages originally deposited in the sediment does not differ with depth in the sediment layers in a similar way as is apparent from our data on infection rates, but such a gradual increase in the production of dormant stages with time is unlikely. Although our data are suggestive of a decline in infectivity with age, our data on parasite (*P. ramosa*) spore loads suggest that, once the host gets infected, there is no decline in parasite fitness, averaged over host clones with age.

We observed striking differences among ponds in infection rates upon exposure to sediments. This is likely related to differences in prevalence of the parasites and epibionts in the studied habitats. We did not quantify prevalence for the different parasites in any of the ponds studied except in OM2 (Decaestecker 2002), but anecdotal observations in the other populations indicate that infection levels are lower than in OM2, with DV having still relatively high infection rates (ED and LDM pers. obs.).

In our experiment, we standardized infection rates and spore loads within ponds to the volume of mud to which the *Daphnia* were exposed. Our procedure did not correct for the different amounts of sediments in terms of weight. Due to sediment compaction, the weight of solid material may differ with the sediment depth. However, infection rates did not show a clear association when plotted against the amount of dry weight of mud (data not shown). Within ponds and across depths, dry weight of mud was not positively correlated with infection rate. On the contrary, for some parasites and epibionts, correlations between infection rate and dry weight of mud, matched according to depth, were significant and negative. These negative correlations observed for some parasites and epibionts between infection rate and dry weight of mud can be explained by the positive association between dry weight of mud and the depth of the sediment layers combined with the negative association between infection rate and depth of the sediment layers. Deeper layers indeed showed higher amounts of dry weight of mud, possibly through compaction. We can thus be confident that the differences in infection rates between different depth treatments within ponds are due to other reasons (e.g., differences in

Table 2. Results of Kruskal–Wallis analysis of variance testing for the effect of depth on infection rate in *Daphnia* for all parasite and epibiont species in all investigated ponds. Results of Spearman rank correlations between sediment depth and average infection rate of all parasites and epibionts.

	Kruskal–Wallis				Spearman rank	
	n	df	*H*	*P*	r^2	*P*
OM2						
A. parasiticum	72	11	31.5	0.0009	−0.47	<0.0001
Vorticella sp.	72	11	13.8	0.2444	−0.25	0.0359
B. rubens	72	11	21.8	0.0262	0.03	0.8159
P. ramosa	72	11	30.1	0.0015	−0.37	0.0015
Microsporidium 2	72	11	18.5	0.0697	−0.36	0.0018
DV						
A. parasiticum	18	2	0.12	0.9433	−0.06	0.8049
Vorticella sp.	18	2	7.39	0.0249	−0.50	0.0353
B. rubens	18	2	1.06	0.5879	0.22	0.3881
P. ramosa	18	2	4.23	0.1203	−0.20	0.4165
OH						
A. parasiticum	18	2	6.51	0.0386	−0.61	0.0066
B. rubens	18	2	2.00	0.3679	−0.29	0.2313
LR						
A. parasiticum	18	2	11.82	0.0027	−0.27	0.2819
Vorticella sp.	18	2	0.27	0.8737	0.02	0.9434

infectivity of spores) rather than to a different amount of mud present in the experimental jars of the different depth treatments. It is also unlikely that the difference in amount of dry weight of mud between the different ponds (DV, OH, and OM2) strongly determined our results. Even though the amount of mud was much higher in DV and OH than in OM2 (averaged over all depths: in OH: 5.4 times that in OM2; and in DV: 5.9 times that in OM2), we did find higher infection rates in OM2. For LR, the amount of dry weight of mud was very low (0.12 times that in OM2). For this pond, it is thus possible that we did not find parasite infections because spore concentrations in the experimental vials were below the critical concentration to obtain infections.

In accordance with other studies, our results show that *P. ramosa* has a negative effect on the reproduction of the *Daphnia* host (Ebert et al. 1998; Carius et al. 2001). We did not find such a negative host fitness effect for Microsporidium 2 and the epibiont species. Note that, in the analysis of our experiment, we may have underestimated the real reduction in the number of juveniles (see Material and Methods). As a result, only strong fitness effects could be detected. This may be particularly the case for Microsporidium 2, as in an earlier study (Decaestecker et al. 2003), we have shown that this parasite indeed has a negative effect on *Daphnia* reproduction, although to a smaller extent than *P. ramosa*. The absence of a negative effect on the number of *Daphnia* juveniles from epibiont infections may be caused by an underestimation in the analysis, but may also reflect

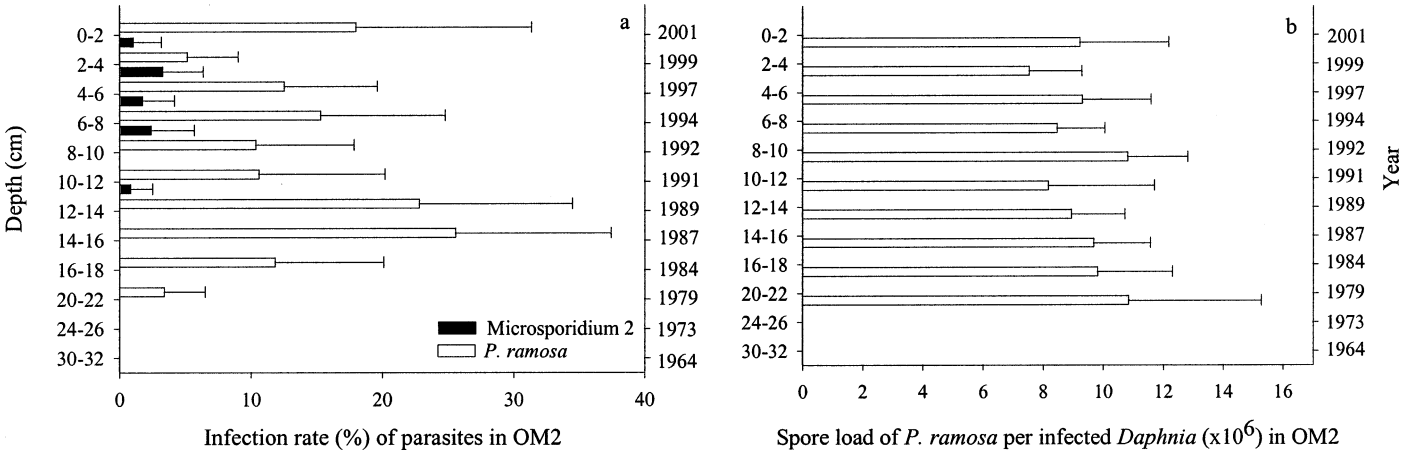

Fig. 3. (a) Infection rate (%) of parasites and (b) spore load of *P. ramosa* per infected *Daphnia* across different depths in OM2. Dating of the core is based on the assumption of constant sedimentation rates in terms of mass of sediments. The average year corresponding with the sediment depth range of the different slices is given.

Table 3. Results of two-way ANOVA to test whether spore loads (angular transformed) of *Daphnia* infected with *P. ramosa* differed across clone and depths in OM2.

Effect	df	MS	*F*	*P*
Clone	5	0.031	6.7	<0.0001
Depth	9	0.007	1.5	0.1326
Error	117	0.005		

that epibiont infections often have little or no negative effect on the *Daphnia* reproduction (Threlkeld and Willey 1993).

The presence of infective spore banks has an important influence on the epidemiology of *Daphnia* parasites. This epidemiology is characterized by low prevalence in winter and early spring. It is suggested that, after the *Daphnia* population peak in spring, food shortage induces the *Daphnia* to a browsing behavior on the bottom sediments by which they pick up parasite spores from spore banks (Ebert et al. 1997). Alternatively, predator-induced shifts to a deeper day-depth may also increase the contact between the *Daphnia* and the sediments (Decaestecker et al. 2002). Once the first hosts are infected by this density-independent sediment-borne transmission, the epidemic spreads by density-dependent host-to-host (waterborne) transmission and prevalence increases with fluctuations during summer (Green 1974; Ebert 1995). Ebert et al. (1997) modeled the aquatic host–parasite system and concluded that, by the uptake of spores from the sediment, the parasite will persist in the *Daphnia* population even if the parasite is not able to spread by waterborne transmission. The uptake of spores from pond sediments diminishes the influence of free-floating spore-derived density-dependent infection and dampens parasite dynamics.

The fact that parasite and epibiont spores remain infective for an extended period (>one growing season) has important ecological and evolutionary consequences. First, *Daphnia* parasites can survive long periods of low host density without losing the capacity to infect the host population when conditions return to favorable again. Second, increased spore longevity will increase the spread of an infection in the host population (Ebert et al. 1997). Third, the storage effect associated with a prolonged dormant phase may allow a more diverse community of epibionts and microparasites to coexist in a given habitat than in the absence of a dormant spore bank (Chesson 1983; Caceres 1997). To the extent that frequency-dependent selection is important in host–microparasite interactions in natural populations, the presence of a dormant propagule bank may increase the rate of adaptation of microparasites to changes in genotype frequencies of host populations by increasing genotype diversity in the parasites (Hamilton 1980). This may result in highly dynamic interactions. Moreover, the existence of infective spore banks in stratified sediments provides a way to reconstruct these dynamics.

Theoretical models of antagonistic coevolution predict that the short generation time and the large population sizes of parasites provide them with high evolutionary potential and that this enables parasites to track the resistance mechanisms of contemporary hosts in a time-lagged and frequency-dependent way (Thompson 1994; Dybdahl and Lively 1998; Little and Ebert 2001). Empirical evidence has indeed shown that new crop varieties have to be renewed frequently because old ones are susceptible to their evolving pest populations (Thompson and Burdon 1992). New diseases emerge and mutate (e.g., HIV), and the genetic compositions of traditional diseases (influenza, malaria) change continuously (Nesse and Williams 1996; Stearns 1999). However, only a few studies (Lenski and Levin 1985; Dybdahl and Lively 1998; Lively and Dybdahl 2000; Buckling and Rainey 2002) could clearly demonstrate time-lagged frequency-dependent host–parasite coevolution. In general, demonstrations of host–parasite coevolution in the field are rare and are at best consistent with theories of coevolution (i.e., spatial local adaptation; Little 2002). Until now, it has been difficult to come to straightforward conclusions with respect to temporal adaptation in host–parasite interactions, mostly because long-term time-series studies are lacking and difficult to perform (Lythgoe and Read 1998; Woolhouse et al. 2002). Spatial local adaptation of parasites to *Daphnia* has been shown (Ebert 1994), but clear-cut patterns of reciprocal temporal adaptation between *Daphnia* and its parasites have not been recorded and are difficult to show (Little and Ebert 1999, 2001). Stratified parasite spore banks with vigorous and infective spores open interesting avenues to investigate important questions on host–parasite coevolution. The reconstruction of the *Daphnia* diapausing egg and parasite spore bank may allow one to investigate whether parasites are able to track host defenses and whether they can cause evolutionary responses in the *Daphnia* host population. Although we have used clones from different sediment layers in our experiment, our present data do not allow us to conclude whether host genotype tracking by parasites was present in the studied ponds, as data sets were too small. Further studies that reconstruct changes through time (years, decades) in the genetic structure of natural *Daphnia* populations using diapausing egg banks, combined with studies on the virulence of parasites from different growing seasons (sediment depths) to host clones that did or did not coexist in time with the parasite, may reveal patterns of temporal adaptation between *Daphnia* and their parasites.

References

Allen, Y. C., B. T. De Stasio, and C. W. Ramcharan. 1993. Individual and population level consequences of an algal epibiont on *Daphnia.* Limnol. Ocanogr. **38:** 592–601.

Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases: Part I. Nature **280:** 361–367.

Barea-Arco, J., C. Pérez-Martinez, and R. Morales-Baquero. 2001. Evidence of a mutualistic relationship between an algal epibiont and its host, *Daphnia pulicaria.* Limnol. Oceanogr. **46:** 871–881.

Berg, L., E. Jeppesen, M. Sondergaard, and E. Mortensen. 1994. Environmental effects of introducing whitefish, *Coregonus lavaretus* (L.) in Lake Ring. Hydrobiologia **275/276:** 71–79.

Bittner, K., K.-O. Rothhaupt, and D. Ebert. 2002. Ecological interactions of the microparasite *Caullerya mesnili* and its host *Daphnia galeata.* Limnol. Oceanogr. **47:** 300–305.

BRAMBILLA, D. J. 1983. Microsporiodosis in a *Daphnia pulex* population. Hydrobiologia **99:** 175–188.

BRENDONCK, L., AND L. DE MEESTER. 2003. Egg banks in freshwater zooplankton: Evolutionary and ecological archives in the sediment. Hydrobiologia **491:** 65–84.

———, ———, AND N. G. JR. HAIRSTON. 1998. Evolutionary and ecological aspects of crustacean diapause. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. **52.**

BUCKLING, A., AND P. B. RAINEY. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. Proc. R. Soc. Lond. B Biol. Sci. **269:** 931–936.

CACERES, C. E. 1997. Temporal variation, dormancy, and coexistence: A field test of the storage effect. Proc. Natl. Acad. Sci. USA **94:** 9171–9175.

———. 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology **79:** 1699–1710.

CARIUS, H. J., T. LITTLE, AND D. EBERT. 2001. Genetic variation in a host–parasite association: Potential for coevolution and frequency-dependent selection. Evolution **55:** 1146–1152.

CHESSON, P. L. 1983. Coexistence of competitors in a stochastic environment: The storage effect, p. 188–198. *In* H. I. Freeman and C. Strobeck [eds.], Population biology. Springer.

CHIAVELLI, D. A., E. L. MILLS, AND S. T. THRELKELD. 1993. Host preference, seasonality, and community interactions of zooplankton epibionts. Limnol. Oceanogr. **38:** 574–583.

COUSYN, C., AND L. DE MEESTER. 1998. The vertical profile of resting egg banks in natural populations of the pond-dwelling cladoceran *Daphnia magna* Straus (Crustacea: Cladocera). Arch. Hydrobiol. Spec. Issues Advanc. Limnol. **52:** 141–161.

———, L. DE MEESTER, J. K. COLBOURNE, L. BRENDONCK, D. VERSCHUREN, AND F. VOLCKAERT. 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proc. Natl. Acad. Sci. USA **98:** 6256–6260.

DECAESTECKER, E. 2002. Evolutionary ecology of host–parasite interactions: *Daphnia* and its parasites as a model. Ph.D. thesis, Univ. of Leuven.

———, L. DE MEESTER, AND D. EBERT. 2002. In deep trouble: Habitat selection constrained by multiple enemies. Proc. Natl. Acad. Sci. USA **99:** 5481–5485.

———, A. VERGOTE, D. EBERT, AND L. DE MEESTER. 2003. Evidence for strong host clone–parasite species interactions in the *Daphnia*–microparasite system. Evolution **57:** 784–792.

DE MEESTER, L. 1996*a*. Local genetic differentiation and adaptation in freshwater zooplankton populations: Patterns and processes. Ecoscience **3:** 385–399.

———. 1996*b*. Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, *Daphnia magna.* Evolution **50:** 1293–1298.

———, L. J. WEIDER, AND R. TOLLRIAN. 1995. Alternative antipredator defenses and genetic polymorphism in a pelagic predator–prey system. Nature **378:** 483–485.

DEMOTT, W. R. 1989. The role of competition in zooplankton succession, p. 195–252. *In* U. Sommer [ed.], Plankton Ecology: Succession in plankton communities. Springer-Verlag.

DESTASIO, B. T. 1989. The seed bank of a freshwater crustacean—copepodology for the plant ecologist. Ecology **70:** 1377–1389.

DYBDAHL, M. F., AND C. M. LIVELY. 1998. Host–parasite coevolution: Evidence for rare advantage and time-lagged selection in a natural population. Evolution **52:** 1057–1066.

EBERT, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. Science **265:** 1084–1086.

———. 1995. The ecological interactions between a microsporidian parasite and its host *Daphnia magna.* J. Animal Ecol. **64:** 361–369.

———, M. LIPSITCH, AND K. L. MANGIN. 2000. The effect of parasites on host population density and extinction: Experimental epidemiology with *Daphnia* and six microparasites. Am. Nat. **156:** 459–477.

———, R. J. H. PAYNE, AND W. W. WEISSER. 1997. The epidemiology of parasitic diseases in *Daphnia*, p. 91–111. *In* K. Dettner, G. Bauer, and W. Völkl [eds.], Vertical food web interactions: Evolutionary patterns and driving forces. Springer-Verlag.

———, C. D. ZSHOKKE-ROHRINGER, AND H. J. CARIUS. 1998. Within-and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa.* Proc. R. Soc. Lond. B Biol. Sci. **265:** 2127–2134.

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

HAIRSTON, N. G., JR., C. M. KEARNS, S. P. ELLNER. 1996. Phenotypic variation in a zooplankton egg bank. Ecology **77:** 2382–2392.

———, AND OTHERS. 1999. Rapid evolution revealed by dormant eggs. Nature **401:** 446.

HAMILTON, W. D. 1980. Sex versus non-sex parasite. Oikos **35:** 282–290.

HOBAEK, A., AND P. LARSSON. 1990. Sex determination in *Daphnia magna.* Ecology **71:** 2255–2268.

HUDSON, P. J., A. P. DOBSON, AND D. NEWBORN. 1998. Prevention of population cycles by parasite removal. Science **282:** 2256–2258.

KERFOOT, W. C., AND A. SIH. 1987. Predation, direct and indirect impacts on aquatic communities. University Press of New England.

LAWRENCE, J. E., A. M. CHAN, AND C. A. SUTTLE. 2002. Viruses causing lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae) are widespread in coastal sediments of British Columbia, Canada. Limnol. Oceanogr. **47:** 545–550.

LENSKI, R. E., AND B. R. LEVIN. 1985. Constraints on the coevolution of bacteria and virulent phage—a model, some experiments, and predictions for natural communities. Am. Nat. **125:** 585–602.

LITTLE, T. J. 2002. The evolutionary significance of parasitism: Do parasite-driven genetic dynamics occur ex-silico? J. Evol. Biol. **15:** 1–9.

———, AND D. EBERT. 1999. Associations between parasitism and host genotype in natural populations of *Daphnia* (Crustacea: Cladocera). J. Anim. Ecol. **67:** 134–149.

———, AND ———. 2001. Temporal patterns of genetic variation for resistance and infectivity in a *Daphnia*–microparasite system. Evolution **55:** 11146–11152.

LIVELY, C. M., AND M. F. DYBDAHL. 2000. Parasite adaptation to locally common host genotypes. Nature **405:** 679–681.

LYTHGOE, K. A., AND A. F. READ. 1998. Catching the Red Queen? The advice of the rose. Trends Ecol. Evol. **13:** 473–474.

MANGIN, K. L., M. LIPSITCH, AND D. EBERT. 1995. Virulence and transmission modes of two microsporidia in *Daphnia magna.* Parasitology **111:** 133–142.

MCQUOID, M. R., A. GODHE, AND K. NORDBERG. 2002. Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. Eur. J. Phycol. **37**: 191–201.

NESSE, R., AND G. C. WILLIAMS. 1996. Evolution and healing. Phoenix.

PAREJKO, K., AND S. O. DODSON. 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus.* Evolution **45:** 1665–1674.

SHELDON, B. C., AND S. VERHULST. 1996. Ecological immunology: Costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol. Evol. **11:** 317–321.

STEARNS, S. C. 1999. Evolution in health and disease. Oxford University Press.

STIRNADEL, H. A., AND D. EBERT. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. J. Anim. Ecol. **66:** 212–222.

THOMPSON, J. N. 1994. The coevolutionary process. The University of Chicago Press.

———, AND J. J. BURDON. 1992. Gene-for-gene coevolution between plants and parasites. Nature **360:** 121–125.

THRELKELD, S. T., AND R. L. WILLEY. 1993. Colonization, interaction, and organization of cladoceran epibiont communities. Limnol. Oceanogr. **38:** 584–591.

WILLEY, R. L, P. A. CANTRELL, AND S. T. THRELKELD. 1990. Epibiotic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. Limnol. Oceanogr. **35:** 952–959.

WOOLHOUSE, M. E. J., J. P. WEBSTER, E. DOMINGO, B. CHARLESWORTH, B. R. LEVIN. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature Genetics **32:** 569–577.

ZAFFAGNINI, F. 1987. Reproduction in *Daphnia.* Mem. Ist. Ital. Idrobiol. **45:** 245–284.

Received: 17 January 2003
Accepted: 18 July 2003
Amended: 1 August 2003