Limnol. Oceanogr., 49(4, part 2), 2004, 1401–1407

Do parasites lower *Daphnia* hybrid fitness?

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Abstract

In Greifensee (Switzerland), *Daphnia galeata* × *hyalina* hybrids cooccur with both parental taxa. Hybrids are the most abundant taxon, suggesting that hybrids have greater fitness. In addition to many known factors that promote hybrids, specific environmental conditions favoring parentals must also exist to explain their cooccurrence. We investigated the influence of the protozoan gut parasite *Caullerya mesnili* on the *D. galeata* × *hyalina* species complex. Up to 22% of the *Daphnia* population was infected with *C. mesnili* in October 2002. *C. mesnili* dramatically reduced the fecundity of its hosts. Only 2% of infected individuals carried eggs compared with 70% in the uninfected group, which suggests that *C. mesnili* exerts a strong selection pressure. Our results indicate that hybrids were frequently infected, whereas parental *D. galeata* were almost never infected. We also found genetic variation for infection within hybrids, evidenced by significant differences in clonal composition between the infected and uninfected parts of the taxon. Resistance of *D. galeata* might counterbalance the greater fitness of hybrids and therefore contribute to the maintenance of coexistence of hybrids and parentals in this lake.

Dominance of *Daphnia* hybrids within the *D. galeata*/*hyalina*/*cucullata* complex has been reported for several lakes (Wolf and Mort 1986; Spaak et al. 2001). Persistence of hybrid zones is explained by hybrid inferiority theories (e.g., ''tension zone models'' of Barton and Hewitt 1985) that assume a dynamic equilibrium between hybrid dispersal and selection against hybrids due to genetic incompatibility of parental genomes. According to these theories, fitness values are independent of the environment. The alternative ''hybrid superiority model'' (Moore 1977) supposes that in intermediate environments, hybrids have higher fitness than their parental taxa. This model could be applied to aquatic environments characterized by temporally changing conditions, especially to organisms with ameiotic parthenogenetic reproduction (such as *Daphnia,* Hebert and Ward 1972), which circumvents the deleterious effects of reduced sexual fertility. Indeed, field and laboratory studies have revealed that under specific environmental conditions, *Daphnia* hybrids might have higher fitness than their parentals (reviewed in Schwenk and Spaak 1997). For example, *D. cucullata* × *galeata* hybrids combine specific life history traits (high intrinsic rate of population increase and small body size) of both parentals. This combination of traits may give them a selective advantage in the presence of planktivorous fish that predate mainly on larger daphnids (Spaak and Hoekstra 1997). Moreover, under high food conditions, the intrinsic rate of population increase of these hybrids is greater than that of *D. galeata* (reviewed in Schwenk and Spaak 1997). Spaak and Hoekstra (1995) called this phenomenon ''temporal hybrid superiority.'' Essentially, due to fluctuating environmental conditions, during certain periods of the year hybrids are more fit than parentals.

Daphnia offspring represent genetic replicates of their parents (Hebert and Ward 1972), and, once established, hybrid clones can be maintained for several generations (*see* Weider and Stich 1992; Spaak 1997). Parental taxa then do not have to produce new hybrids each season. Moreover, it has been shown that in unstable conditions hybrids can reproduce sexually (Spaak 1997) and are able to backcross (Schwenk and Spaak 1997). Since parentals are often present in populations dominated by hybrids (Schwenk and Spaak 1997), and are present in conditions that promote hybrids (e.g., temperature, food levels, predator regimes; reviewed in Schwenk and Spaak 1997), environmental conditions that also favor parentals must exist.

One potential condition that is known to have a strong impact on *Daphnia* populations but has never been studied in a *Daphnia* hybridization context is parasitism (reviewed in Little and Ebert in press). In other animal hybrid systems it has been shown that interactions between parasites and their hosts can be modified in hybrid zones (reviewed in Moulia 1999). Two studied systems showed that hybrids were more resistant than parentals, whereas six other systems supported either the ''hybrid additive hypothesis'' (hybrids are intermediately infected) or the ''hybrid susceptibility hypothesis'' (hybrids are the most infected group) (reviewed in Moulia 1999; Jackson and Tinsley 2003). One explanation for a higher infection rate of hybrids is that their different spatial and trophic position makes them more exposed to parasites (e.g., cyprinid fishes; Dupont and Crivelli 1988). Alternative explanations for higher hybrid infection rates in cyniprids are that hybrids contain attractive substances from each of their parentals in their mucus or that immune defenses expressed in parental mucus might be impaired in

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Acknowledgments

We thank Esther Keller for counting the quantitative samples and Richard Illi for support in the field. We thank an anonymous reviewer for very constructive comments on the manuscript and Lisa Shama for linguistic help. J.W. thanks Dieter Ebert for introducing her to the topic of *Daphnia* parasites and Tadeusz Kawecki for helpful discussions about statistical analyses. P.S. thanks Charles Kerfoot for the invitation to the Biocomplexity symposium in Ann Arbor. This work was supported by grant 31-65003.01 from the Swiss National Science Foundation to P.S.

hybrids (Dupont and Crivelli 1988). An experiment conducted on mice hybrids confirmed that greater hybrid susceptibility was caused by genetic dysfunction in their defense mechanisms (Moulia et al. 1993).

Daphnia is a known host of a wide range of different parasites, among which bacteria, fungi, and protozoan microparasites are common (Green 1974). Laboratory experiments and field studies (reviewed in Little and Ebert in press) have shown that parasites often have strong impacts on *Daphnia* fitness by decreasing fecundity and increasing host mortality. Some studies have shown that variation for infection exists within species, both among allopatric (e.g., Ebert 1994) and sympatric clones (e.g., Little and Ebert 1999). However, few studies have compared infection of sympatric species (Stirnadel and Ebert 1997; Bittner 2001). Infection loads of coexisting *D. magna*, *D. pulex*, and *D. longispina* species have been shown to differ considerably for a wide range of parasites (Stirnadel and Ebert 1997). Bittner (2001) investigated the *Daphnia galeata* × *hyalina* species complex in Lake Constance (Germany), focusing on infection of parental taxa. Parentals exhibited differences in susceptibility to many parasites; however, in general, *D. hyalina* were infected to a larger extent than *D. galeata.* The protozoan gut parasite *Caullerya mesnili* Chatton was found to strongly reduce fecundity and survival of its *Daphnia* host (Bittner 2001). *C. mesnili* is a horizontally transmitted parasite that infects *Daphnia* by free-floating spore stages (Bittner et al. 2002). *C. mesnili* is regularly found in several natural *Daphnia* populations (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999) with prevalences as high as 43% for *D. pulex* (Little and Ebert 1999).

Given that differences exist in *C. mesnili* loads between parental taxa within the *D. galeata* × *hyalina* species complex (Bittner 2001), we expect there to be variation for infection between those parentals and hybrids. If hybrids are more infected than parentals, the extent of introgression might be limited. To test this, we investigated parasitism of the *D. galeata* × *hyalina* species complex in Greifensee. Prevalence of *C. mesnili* and one pathogenic bacterium were compared between parentals and hybrids. Additionally, we tested for clonal variation for infection and for effects of *C. mesnili* on *Daphnia* host fecundity and population density.

Material and methods

Study site—We studied the *Daphnia* population in Greifensee (Switzerland), a eutrophic, prealpine lake of medium size (8.5 km^2). *D. galeata* × *hyalina* hybrids dominate the *Daphnia* population of Greifensee (Spaak et al. 2001), with an average proportion of 75% during 1998–2002 (Keller and Spaak 2004). Moreover, it cannot be excluded that *D. hyalina* clones are backcrossed hybrids, whereas pure *D. galeata* occur in the lake (Spaak et al. 2001). Several planktivorous fish (e.g., *Coregonus* sp.) that inhabit Greifensee predate mainly on *Daphnia* (B.K., pers. obs.). *C. mesnili* is a common *Daphnia* parasite in the lake.

Sampling—To assess the prevalence of *C. mesnili*, we sampled the *Daphnia* population during June–August 2001 and June 2002–January 2003. Samples were collected with a 95-μm net in 2001 and a 250-μm net in the second sampling period. Samples were taken at three different positions within the whole water column and were taken at the deepest point of the lake (maximum depth 32 m). The lake was sampled weekly (every other week in winter); however, on a few occasions the interval between sampling was longer. Samples were cooled with ice during transport to the laboratory. Analyses were carried out on the same day to avoid selective mortality of infected animals. Zooplankton was concentrated by filtration through a 250-μm mesh sieve. The length of randomly chosen *Daphnia* was measured to the nearest 0.05 mm, from the top of the eye to the base of the spine. Since size at maturity of *D. galeata* × *hyalina* daphnids is higher than 1.1 mm (regardless of food conditions; Weider 1993), only females larger than 1 mm were analyzed to ensure that the sample is representative for the adult population of all taxa. Daphnids were dissected and the gut contents investigated using a phase-contrast microscope at 100–400 × magnification. Presence or absence of *C. mesnili* spores was documented. We analyzed 60 to 120 individuals per sample. Quantitative samples were taken from the same locations with a 95-μm net and preserved in 95% ethanol.

To determine whether the prevalence of *C. mesnili* varied among different *Daphnia* taxa we took additional samples between September–October 2001 (*Caullerya* 2001, four sampling dates) and October–December 2002 (*Caullerya* 2002, five sampling dates). During 2001, we took two subsamples from the population of females larger than 1 mm: randomly picked individuals (uninfected group, *n*: 112–118) and those with external signs of infection (observed in transmitted light with ×25 magnification) (infected group, *n*: 17–65). Additionally, in June 2001, a high abundance of pathogenic bacteria (hereafter referred to as unidentified bacterium) was found in the haemocoel of infected *Daphnia.* This bacterium was easily detected since infected daphnids were whitish. To see whether infection varied among taxa, we used the same method as with *C. mesnili* (*unidentified bacterium* 2001, two sampling dates). In 2002, we dissected the *Daphnia* first and classified them to the *C. mesnili*–infected or uninfected group after microscopic inspection. More infected animals were picked ($n \sim 50$) to increase statistical power. All individuals were frozen (−80°C) for subsequent electrophoretic analysis.

To estimate the impact of *C. mesnili* on host fecundity, presence or absence of eggs in the brood pouch for each infected and uninfected female was documented throughout the June 2002–January 2003 sampling period. Additionally, fecundity was documented for females from the infected and uninfected groups from the *Caullerya* 2002 population (October–December 2002). *Daphnia* with filled ovaries were considered to contain eggs.

Allozyme electrophoresis—*Daphnia* individuals were assayed for four enzyme loci: aldehyde oxidase (*AO*, enzyme commission number [EC] 1.2.3.1), aspartate amino transferase (*AAT*, EC 2.6.1.1), phosphoglucose isomerase (*PGI*, EC 5.3.1.9), and phosphoglucomutase (*PGM*, EC 5.4.2.1). *AAT* and *AO* loci are known diagnostic markers that distinguish between *D. galeata* and *D. hyalina* (Wolf and Mort 1986; Gießler 1997). *D. galeata* is fixed for the *f* (fast) alleles at

the *AAT* locus and *f* or f^+ (very fast) alleles at the *AO* locus. *D. hyalina* is fixed for the *s* (slow) alleles at both loci. Specimens were assigned (*see* criteria in Nason and Ellstrand 1993) to six possible genealogical classes (taxa): both parentals (*D. galeata* and *D. hyalina*), both hybrid generations (F_1 and F_2), and first generation backcrosses (BP_{gal}, BP_{hyl}). This classification system is conservative, however, since each given taxon contains a fraction of misclassified individuals (Nason and Ellstrand 1993). Hereafter we use the term ''taxon'' for each of the six *Daphnia* forms without addressing the systematic or phylogenetic significance. Variation at *PGI* (*f, m*—medium), *PGM* (*s, m, f*), and *AO* loci (although it is a diagnostic marker, within each taxon different combinations of alleles are expected; e.g., for *D. galeata*: *ff*, f^+f^+, or ff^+) made it possible to identify a number of three-locus genotypes within each taxon. Individuals sharing the same three-locus genotype are referred to as clones, but it should be considered that a clone defined as such could represent a clonal group whose members share the same allozyme pattern but differ in loci not assayed.

Data analysis—Parasite prevalence: Parasite prevalence, defined as the percent of infected hosts in a population, is a commonly used parameter in *Daphnia* parasitological studies (e.g., Stirnadel and Ebert 1997; Little and Ebert 1999). Correlation between *C. mesnili* prevalence (June 2002–January 2003) and *Daphnia* population density was examined.

Fecundity reduction: We determined the size of adult females per sampling date as the length of the smallest size class in which at least 50% of uninfected females carried eggs (as in Stirnadel and Ebert 1997). The proportions of gravid infected and gravid uninfected females in adult size classes was calculated for each sampling date, as well as the overall mean proportions for the entire June 2002–January 2003 period.

Taxa variation for infection: Differences in taxa composition between the infected and uninfected group were determined with an $R \times C$ test of independence (Sokal and Rohlf 1995). Analyses were done within populations (*Caullerya* 2001, *Caullerya* 2002, and *unidentified bacterium* 2001) and separately for each date. Since F_2 hybrids and *D. hyalina* taxa had low frequencies or were absent in the *Caullerya* 2001 and *Caullerya* 2002 populations, they were excluded from the analyses. Sequential Bonferroni correction was used to calculate significance levels for simultaneous statistical tests. When significant differences in taxa composition were detected between the infected and uninfected groups, further $R \times C$ tests were undertaken (Sokal and Rohlf 1995) to determine which taxa were overinfected or underinfected (as in Little and Ebert 1999).

Clonal variation for infection: Differences in clonal composition between the infected and uninfected groups of taxon were determined with a pairwise test of differentiation (Fstat program, version 2.9.3.2; Goudet 2002). Analyses were done for each sampling date separately. Owing to the limited number of individuals, it was only possible for the most frequent taxon, F_1 hybrids.

Results

Host density and parasite prevalence—*Daphnia* densities were similar during both summers (Fig. 1A,B). Density was lowest during the first 2 weeks in August (~600 individuals per m^3; Fig. 1A,B) and increased thereafter. *C. mesnili* was only found on two sampling dates in summer 2001 (Fig. 1A), whereas during the second sampling period (June 2002–January 2003) *C. mesnili* was present in all samples (Fig. 1B). Prevalence varied between 1% and 21% ($\bar{x}$ = 8.3%). When *Daphnia* density was highest (~8600 individuals per m^3), only 1% of the population was infected (3 September 2002, Fig. 1B). Prevalence of *C. mesnili* reached 20% when *Daphnia* density decreased sixfold (7 November 2002, Fig. 1B). The observed negative relationship between prevalence and host density was not significant ($r^2 = 0.20$, $n = 17$, $P = 0.07$). An unidentified bacterium was found on five sampling dates in summer 2001 (prevalence 1.7–6.8%, Fig. 1A) and only once during the June 2002–January 2003 sampling period (data not shown).

Fecundity reduction—Only 2.4% of *C. mesnili*–infected adult females carried eggs, compared to 70.4% of uninfected adult females (June 2002–January 2003). Moreover, we noticed that eggs in the brood pouch of two out of nine infected gravid females were degenerated.

Taxa variation for infection—Significant differences in taxa composition between the infected and uninfected groups were found for both populations: *Caullerya* 2001 (*p* values significant after sequential Bonferroni correction for three out of four sampling dates; Fig. 2A) and *Caullerya* 2002 (three out of five sampling dates; Fig. 2B). In the *Caullerya* 2001 population, significant differences between both groups ($G = 22.2$, $P < 0.01$; $G = 25.8$, $P < 0.01$; $G = 10.2$, $P < 0.05$; Fig. 2A) resulted from the fact that almost no *D. galeata* were infected. The average proportion of *D. galeata* in the uninfected group was 17.5%, whereas in the infected group only one *D. galeata* individual was found. All other taxa were homogeneously distributed between infected and uninfected groups ($G = 5.7$, not significant [NS]; $G = 7.8$, NS; $G = 2.4$, NS; respectively). In the *Caullerya* 2002 population, differences in taxa composition were also observed ($G = 14.0$, $P < 0.01$; $G = 9.2$, $P < 0.05$; $G = 13.2$, $P < 0.01$; Fig. 2B). On 24 October, *D. galeata* was the underinfected taxon, whereas the other taxa were homogeneously distributed ($G = 5.1$, NS). During the last two sampling dates *D. galeata* were rare in the lake (only two individuals were found), and significant differences in taxa composition were mainly due to the lack of BP_{hyl} in the infected group (other taxa were homogeneously distributed; $G = 1.1$, NS; $G = 4.7$, NS). In the *unidentified bacterium* 2001 population, significant differences were found for one out of two sampling dates ($G = 28.8$, $P < 0.01$; Fig. 2C). BP_{gal} were less abundant in the infected (8.6%) than in the uninfected (34.7%) group, whereas *D. hyalina* showed a tendency to be overinfected (15.5% and 2.0%, respectively). Other taxa were homogeneously distributed ($G = 7.0$, NS).

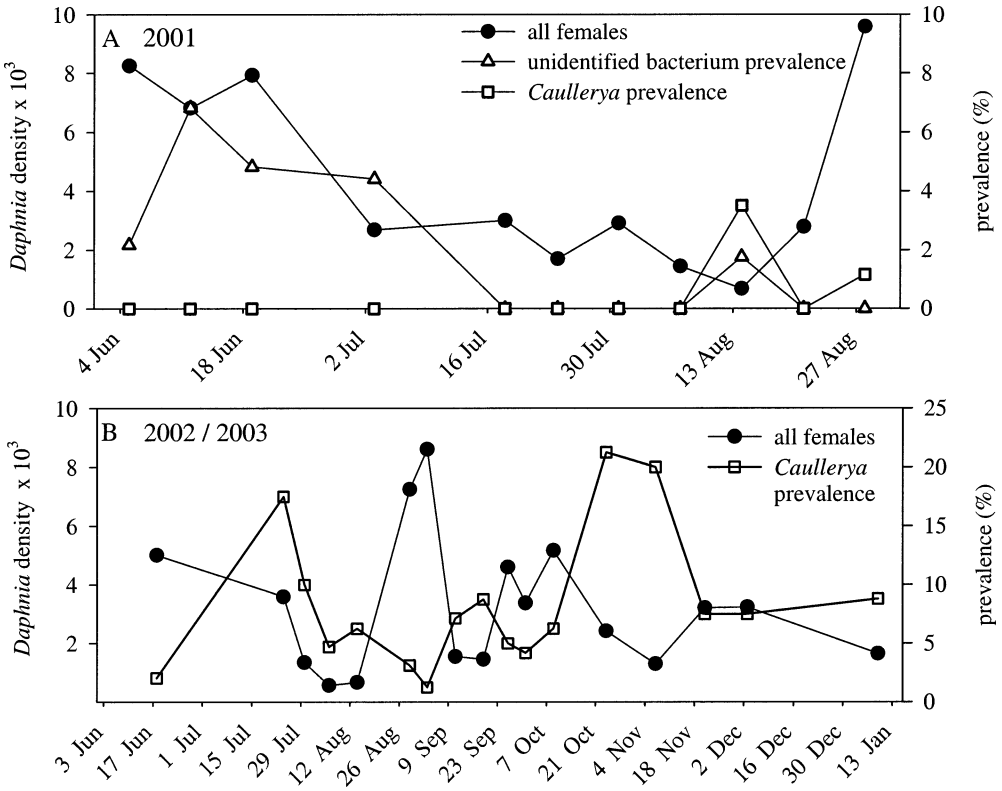

Fig. 1. Densities (per m^3) of *Daphnia* females in Greifensee and prevalence (%) of *Caullerya mesnili* and an unidentified bacterium in the *Daphnia* population. (A) during a 3-month period in 2001, population infected by *C. mesnili* and unidentified bacterium; (B) during an 8-month period in 2002, population infected by *C. mesnili.*

Clonal variation for infection—Significant differences in clonal composition between the infected and uninfected part of the F_1 taxon were found (for two sampling dates in *Caullerya* 2001, one in *Caullerya* 2002, one in the *unidentified bacterium* 2001 population; Table 1). As stated in the methods section, it was impossible to analyze the other taxa due to low numbers of individuals per sampling date. To identify the reason why the BP_{hyl} taxon was significantly overinfected in the last two sampling dates (whereas it was not in the three previous ones, *Caullerya* 2002; Fig. 2B), clonal composition of the BP_{hyl} taxon was analyzed for the pooled infected and uninfected group. Significant differences were found between those groups ($n_{inf} = 18$, $n_{un} = 37$, $P < 0.001$). Throughout the entire sampling period 12 different BP_{hyl} clones were found, but in the last two sampling dates, three clones that had never been infected took over the BP_{hyl} population and the other clones went extinct.

Discussion

Our study shows that host–parasite interactions can differ among *Daphnia* hybrids and parentals. Specifically, throughout the entire first sampling period (*Caullerya* 2001, Fig. 2A), *D. galeata* were less infected than hybrids. In 2002 (Fig. 2B), after the peak density of *D. galeata* disappeared, we again observed that hybrids were susceptible to *C. mesnili* infection.

We suspect that *C. mesnili* represents a strong selection pressure against infected daphnids within the *D. galeata* × *hyalina* complex. In Greifensee, only 2.4% of infected *Daphnia* females carried eggs, and in Lake Constance (Bittner 2001), *C. mesnili* was found to be highly virulent and almost castrated its hosts. In contrast, however, a study of *D. pulex* found only slightly reduced fecundity of *C. mesnili*–infected females and no genetic differences between the infected and uninfected groups (Little and Ebert 1999).

Parasite–*Daphnia* systems are often characterized by variation for parasite infectivity and for host resistance. For example, Carius et al. (2001) found that no tested *D. magna* clone was resistant to all bacterial endoparasite *Pasteuria ramosa* strains and no parasite isolate could infect every host clone. Therefore, clonal variation for infection can also be expected within the *D. galeata* × *hyalina* complex and was found on some sampling dates for F_1 hybrids (Table 1). On other sampling dates those associations were not detected. In a study of infected *Daphnia* in small ponds (Little and Ebert 1999), the significant parasitism–genotype association also varied with time; the same clones that were significantly overinfected or underinfected in one sample did not show differences a few days later. They suggested that populations at a single time point, due to dynamic clonal oscillations, may be at a stage of the cycle where detection of clonal variation for infection is simply not possible (Little and Ebert 1999). This might explain why significant clonal dif-

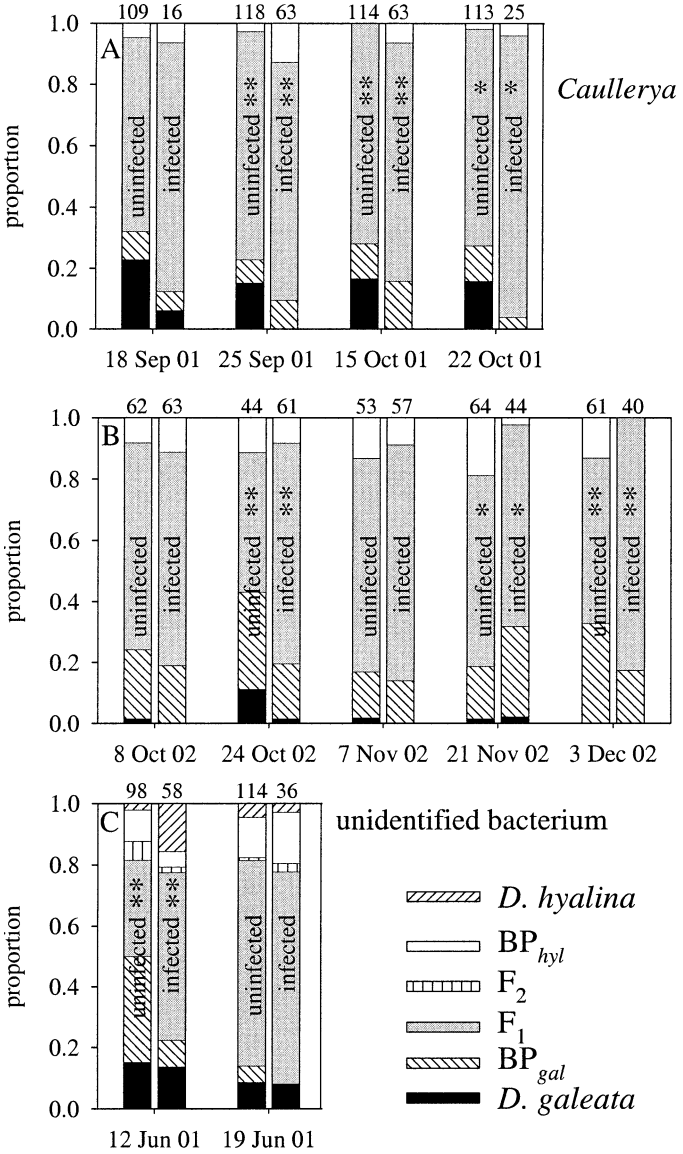

Fig. 2. Differences in taxa composition between the infected and uninfected group of the *Daphnia* female population of Greifensee; (A) infected by *Caullerya mesnili* in 2001; (B) infected by *C. mesnili* in 2002; (C) infected by unidentified bacterium in 2001 (for *C. mesnili*–infected populations, *D. hyalina* and F_2 taxa were excluded from analyses, due to their low frequency). For each sampling date an $R \times C$ test was done; significant differences after sequential Bonferroni correction denoted as * $P < 0.05$, and ** $P < 0.01$. Sample sizes are given at the top of each bar.

ferences within F_1 hybrids were found only at specific dates in Greifensee.

Prevalence of *Daphnia* parasites is known to be highly variable (e.g., Little and Ebert 1999). In Lake Constance, prevalence of *C. mesnili* seems to have an epidemic character. During a 3-yr study period, *C. mesnili* was only found in two winters (Bittner 2001). In Greifensee, *C. mesnili* was detected in late summer 2001 (Fig. 1A) and in each sampling date during the 8-month study period the year after (Fig. 1B). Therefore, *C. mesnili* seems to be able to infect *Daphnia* hosts throughout the year. This is in contrast to the seasonality of other parasites, e.g., microsporidia, whose growth and transmission were found to be reduced at low temperatures, and for which a minimum temperature of disease development exists (Ebert 1995). Moreover, our results indicate that *C. mesnili* transmission in Greifensee is not impaired at low host densities (Fig. 1B), which is in contrast to laboratory experimental results with *C. mesnili* (Bittner et al. 2002) and field studies with other parasites (reviewed in Ebert et al. 1997). After *Daphnia* density peaked in 2002 (3 September 2002, Fig. 1B), the host population crashed (from 8600 to 1500 individuals per m^3 within 1 week), whereas the prevalence of *C. mesnili* increased seven times during that period. Since high *Daphnia* density usually leads to declining food conditions (Lampert and Sommer 1999), it may be that food resources play an important role in impairing *C. mesnili* epidemics. It was shown in a laboratory experiment that *C. mesnili* harms well-fed daphnids more strongly than poorly fed ones, in that more spore clusters were found in well-fed than poorly fed individuals (Bittner et al. 2002). This could result from differences in feeding rate, since well-fed animals are larger, have a higher filtration rate, and therefore are more likely to ingest waterborne, horizontally transmitted infection stages (Ebert 1995).

In some natural systems parasites play a key role in causing population density fluctuation cycles (e.g., nematodes in red grouse populations; Hudson et al. 1998). Regulation of *Daphnia* populations by epibionts or parasites has also been suggested (e.g., Green 1974; Allen et al. 1993). Brambilla (1983) argued that although parasites reduce *Daphnia* fecundity, food level might be the single most important condition that influences population dynamics. It is unlikely that *C. mesnili* regulates the growth of *Daphnia* population in Greifensee (nonsignificant relation between prevalence and host density, Fig. 1B). In Lake Constance (Bittner 2001), *Daphnia* density fluctuations were similar during the winters of 1997 and 1998 (although during 1997 *C. mesnili* infected up to 30% of the population, whereas in the 1998 no *C. mesnili* was found). Variation in predation rates, food level,

Table 1. Comparison of clone distribution (pairwise test; Goudet 2002) within the infected and uninfected group of F_1 *Daphnia* hybrids in Greifensee. F_1 hybrids were infected by *Caullerya mesnili* in 2001 and 2002 and an unidentified bacterium in 2001. n_{inf}, number of infected individuals; n_{un}, number of uninfected individuals; * $P<0.05$, *** $P<0.001$, NS, not significant.

Population	Sampling date	n_{un}	n_{inf}	P
Caullerya 2001	18 Sep	69	13	NS
	25 Sep	88	49	***
	15 Oct	82	49	***
	22 Oct	80	23	NS
Caullerya 2002	8 Oct	42	44	***
	24 Oct	20	44	NS
	7 Nov	37	44	NS
	21 Nov	40	29	NS
	3 Dec	33	33	NS
Unidentified bacterium 2001	12 Jun	31	32	NS
	19 Jun	71	25	*

or other conditions that have not been considered may influence population density more strongly than parasitism in *C. mesnili–Daphnia* systems.

When *C. mesnili* was not present in Greifensee (June–July 2001, Fig. 1A), an epidemic of an unidentified bacterium occurred. As with the protozoan parasite *C. mesnili*, bacterial diseases often castrate their hosts (e.g., Green 1974). In contrast, however, the unidentified bacterium occurred sporadically and with low prevalence (Fig. 1A). Seasonality of bacterial diseases is most likely due to temperature-dependent transmission (reviewed in Ebert et al. 1997). Additionally, the unidentified bacterium makes *Daphnia* less transparent and therefore more conspicuous for visually hunting predators. Specifically, we expect a large impact of juvenile planktivorous fish. Selective fish predation may explain the low prevalence of unidentified bacterium and the disappearance of the epidemic in midsummer 2001 (Fig. 1A). In contrast to our results with *C. mesnili*, we did not notice any *D. galeata* resistance to unidentified bacterium (Fig. 2C). Resistance to one parasite species, however, does not necessarily confer resistance to another (Capaul and Ebert 2003). On the first sampling date, *D. galeata* backcrosses (BP_{gal}) were significantly underinfected, whereas *D. hyalina* were overinfected (Fig. 2C). In Lake Constance, *D. hyalina* were also more infected by an unknown bacterium (description similar to the one founded in Greifensee) than *D. galeata* (Bittner 2001).

Variation for infection loads might be caused by behavioral differences. In another hybrid complex (cyprinid barbel fishes) one parental species lives in habitats that are more exposed to platyhelminth gill parasites than the other, whereas hybrids with intermediate levels of genomic introgression live in intermediate habitats and consequently have an intermediate level of infection (Le Brun et al. 1992). Differences in behavioral traits are also known for the *D. galeata* × *hyalina* hybrid complex. In Lake Constance, *D. hyalina* and hybrids perform diel vertical migrations, whereas *D. galeata* does not (Weider and Stich 1992). Decaestecker et al. (2002) demonstrated that migrating daphnids could be infected by bacterial *P. ramosa* spores from the sediment. *C. mesnili* probably does not form spore banks in the sediment (exposure to sediment does not cause infection; K.B., pers. obs.), but a nonrandom distribution of spores in the water column may exist, due to a density gradient associated with the thermocline (as suggested by Decaestecker et al. 2002). In Greifensee, it was found that *D. galeata* × *hyalina* hybrids have genotype-specific migration strategies (Spaak et al. 2001), and, therefore, clones may differ in their exposure to free-floating *C. mesnili* spores.

Assuming that selection by a rapidly evolving parasite would give an advantage to rare clones (Little and Ebert 1999), parasites might adapt to F_1 hybrids because they are the most common taxon in the lake. If F_1 hybrids are susceptible, it could prevent parasites from adapting to the parental host, which would then be eliminated from the host spectrum of the parasites (''hybrid sink hypothesis'' of Whitham 1989). Alternatively, parasites may adapt to individual host clones rather than to an entire taxa, as evidenced by clonal variation for infection in F_1 hybrids from Greifensee (Table 1). *D. galeata* has been infected under laboratory conditions (Bittner 2001; Bittner et al. 2002); however, those few particular clones were selected from a large group of clones (K.B. pers. obs.). It may be that in Greifensee only the most resistant *D. galeata* clones remain. A strong example of parasite-mediated selection is the changes in the clonal composition of the BP_{hyl} population. The heavily infected clones dropped dramatically in frequency, whereas uninfected clones took over the population.

In Lake Constance, *D. galeata* is reported to survive the winter as resting eggs (Straile and Geller 1998), and one could suggest that *D. galeata* is less infected than *D. hyalina* (Bittner 2001) because parasites have to adapt to new *D. galeata* clones each spring. In Greifensee, only a small part of the population switches to sexual reproduction; however, taxa-specific differences have been observed. Mostly *D. galeata* clones hatch from ephippia, whereas F_1 hybrid clones seem to be maintained by asexual reproduction (Keller and Spaak 2004).

Detailed infection experiments under controlled laboratory conditions are necessary to test whether *Daphnia* disease patterns are determined environmentally or by genetic variation for resistance. For example, daphnids that were infected in field samples but were subsequently cured with antibiotics (and then raised in the laboratory) were more susceptible compared to those that were uninfected (Little and Ebert 2000). From this it was concluded that resistance has a genetic basis.

From our data, we cannot distinguish between the ''hybrid susceptibility'' and ''hybrid additive hypothesis,'' since it was impossible to compare F_1 hybrid infection loads with the second parental *D. hyalina* (which were rarely found). Since *D. galeata* is the main hybrid competitor in Greifensee, it is expected that the overinfected F_1 hybrids, having a cost of infection, would decline in frequency, and consequently *D. galeata* should take over the *Daphnia* population (as clonal fluctuation within BP_{hyl} taxon). Given that F_1 hybrids have dominated the population for the entire monitored period (since 1998, Keller and Spaak 2004), it is clear that either *D. galeata* resistance is both genetically determined and costly or other factors must strongly favor F_1 hybrids (*see* Spaak and Hoekstra 1997). Even if *C. mesnili* parasites are not able to regulate *Daphnia* density, they are strong candidates to alter the outcome of species (and clonal) competition and promote coexistence. Observed lower infection rates of *D. galeata* might explain why *D. galeata* cooccurs with hybrids in this lake.

References

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

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. **16:** 113–148.

Bittner, K. 2001. Parasitismus bei *Daphnia* im Bodensee, p. 120. Fakultät für Biologie, Universität Konstanz.

———, 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. Microsporidiosis in a *Daphnia pulex* population. Hydrobiologia **99:** 175–188.

CAPAUL, M., AND D. EBERT. 2003. Parasite-mediated selection in experimental *Daphnia magna* populations. Evolution **57:** 249–260.

CARIUS, H. J., T. J. LITTLE, AND D. EBERT. 2001. Genetic variation in a host-parasite association: Potential for coevolution and frequency-dependent selection. Evolution **55:** 1136–1145.

DECAESTECKER, E., L. DE MEESTER, AND D. EBERT. 2002. In deep trouble: Habitat selection constrained by multiple enemies in zooplankton. Proc. Natl. Acad. Sci. U.S.A. **99:** 5481–5485.

DUPONT, F., AND A. J. CRIVELLI. 1988. Do parasites confer a disadvantage to hybrids? A case study of *Alburnus alburnus* × *Rutilus rubilio,* a natural hybrid of Lake Mikri Prespa, northern Greece. Oecologia **75:** 587–592.

EBERT, D. 1994. Genetic differences in the interactions of a microsporidian parasite and four clones of its cyclically parthenogenetic host. Parasitology **108:** 11–16.

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

———, R. PAYBE, AND W. WEISSER. 1997. The epidemiology of parasitic diseases in *Daphnia,* pp. 91–111. *In* K. Dettner, G. Bauer, and W. Völkl [eds.], Vertical food web interactions, evolutionary patterns, and driving forces. Ecological studies. Springer.

GIEßLER, S. 1997. Analysis of reticulate relationships within the *Daphnia longispina* species complex. Allozyme phenotype and morphology. J. Evol. Biol. **10:** 87–105.

GOUDET, J. 2002. Fstat(version 2.9.3.2), a program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics. Institut de Zoologie et Ecologie Animale, Université Lausanne.

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

HEBERT, P. D. N., AND R. D. WARD. 1972. Inheritance during parthenogenesis in *Daphnia magna.* Genetics **71:** 639–642.

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

JACKSON, J. A., AND R. C. TINSLEY. 2003. Parasite infectivity to hybridising host species: A link between hybrid resistance and allopolyploid speciation? Int. J. Parasitol. **33:** 137–144.

KELLER, B., AND P. SPAAK. 2004. Nonrandom sexual reproduction and diapausing egg production in a *Daphnia* hybrid species complex. Limnol. Oceanogr. **49:** 1393–1400.

LAMPERT, W., AND U. SOMMER. 1999. Limnoökologie, 2nd ed. Georg Thieme Verlag.

LE BRUN, N., F. RENAUD, P. BERREBI, AND A. LAMBERT. 1992. Hybrid zones and host-parasite relationships—effect on the evolution of parasitic specificity. Evolution **46:** 56–61.

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

———, AND ———. 2000. The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): The role of host genetics. Proc. R. Soc. Lond. B **267:** 2037–2042.

———, AND ———. In press. Evolutionary dynamics in *Daphnia* and their microparasites. *In* K. Dronamraju [ed.], Infectious disease and host-pathogen evolution. Cambridge Univ. Press.

MOORE, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. Q. Rev. Biol. **52:** 263–277.

MOULIA, C. 1999. Parasitism of plant and animal hybrids: Are facts and fates the same? Ecology **80:** 392–406.

———, N. LEBRUN, J. DALLAS, A. ORTH, AND F. RENAUD. 1993. Experimental-evidence of genetic determinism in high susceptibility to intestinal pinworm infection in mice—a hybrid zone model. Parasitology **106:** 387–393.

NASON, J. D., AND N. C. ELLSTRAND. 1993. Estimating the frequencies of genetically distinct classes of individuals in hybridized populations. J. Hered. **84:** 1–12.

SCHWENK, K., AND P. SPAAK. 1997. Ecology and genetics of interspecific hybridization in *Daphnia,* pp. 199–229. *In* B. Streit, T. Städler, and C. M. Lively [eds.], Evolutionary ecology of freshwater animals. Birkhäuser Verlag.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry, 3rd ed. W.H. Freeman.

SPAAK, P. 1997. Hybridization in the *Daphnia galeata* complex: Are hybrids locally produced? Hydrobiologia **360:** 127–133.

———, L. EGGENSCHWILER, AND H. BÜRGI. 2001. Genetic variation and clonal differentiation in the *Daphnia* population of the Greifensee, a pre-alpine Swiss lake. Verh. Int. Ver. Theor. Angew. Limnol. **27:** 1919–1923.

———, AND J. R. HOEKSTRA. 1995. Life history variation and the coexistence of a *Daphnia* hybrid with its parental species. Ecology **76:** 553–564.

———, AND ———. 1997. Fish predation on a *Daphnia* hybrid species complex: A factor explaining species coexistence? Limnol. Oceanogr. **42:** 753–762.

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.

STRAILE, D., AND W. GELLER. 1998. Crustacean zooplankton in Lake Constance from 1920 to 1995: Response to eutrophication and re-oligotrophication. Arch. Hydrobiol. Beih. Ergeb. Limnol. **53:** 255–274.

WEIDER, L. J. 1993. Niche breadth and life history variation in a hybrid *Daphnia* complex. Ecology **74:** 935–943.

———, AND H. B. STICH. 1992. Spatial and temporal heterogeneity of *Daphnia* in Lake Constance: Intra- and interspecific comparisons. Limnol. Oceanogr. **37:** 1327–1334.

WHITHAM, T. G. 1989. Plant hybrid zones as sinks for pests. Science **244:** 1490–1493.

WOLF, H. G., AND M. A. MORT. 1986. Interspecific hybridization underlies phenotypic variability in *Daphnia* populations. Oecologia **68:** 507–511.

Received: 5 February 2003
Accepted: 31 August 2003
Amended: 12 November 2003