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Coloured scanning electron microscopy image showing two waterfleas (Daphnia). Copyright: Martin Oeggerli 2008 / www.Micronaut.ch (with kind support of the University for Life Sciences, FHNW, Muttenz).

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LETTER

Genetic diversity of *Daphnia magna* populations enhances resistance to parasites

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Abstract

The diversity-disease hypothesis states that decreased genetic diversity in host populations increases the incidence of diseases caused by pathogens (= monoculture effect) and eventually influences ecosystem functioning. The monoculture effect is well-known from crop studies and may be partially specific to the artificial situation in agriculture. The effect received little attention in animal populations of different diversities. Compared with plants, animals are mobile and exhibiting social interactions. We followed the spread of a microsporidian parasite in semi-natural outdoor *Daphnia magna* populations of low and high genetic diversity. We used randomly selected, naturally occurring host genotypes. Host populations of low diversity were initially monoclonal, while the host populations of high diversity started with 10 genotypes per replicate. We found that the parasite spread significantly better in host populations of low diversity compared with host populations of high diversity, independent of parasite diversity. The difference was visible over a 3-year period. Host genotypic diversity did not affect host population density. Our experiment demonstrated a monoculture effect in independently replicated semi-natural zooplankton populations, indicating that the monoculture effect may be relevant beyond agriculture.

Keywords

Daphnia magna, density, epidemic, epidemiology, genotypic diversity, metapopulation, microsporidium, *Octosporea bayeri,* prevalence.

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INTRODUCTION

The diversity of species or genotypes influences the functioning and dynamics of natural ecosystems (Loreau *et al.* 2001). Several studies found a positive correlation between a higher diversity of species or functional groups and productivity and density within plant communities (Tilman *et al.* 1996; Hector *et al.* 1999; Engelhardt & Ritchie 2001). A higher genotypic diversity within species produces the same effect (Hughes & Stachowicz 2004; Reusch *et al.* 2005). Furthermore, a high diversity increases resilience, and more diverse ecosystems resist better to negative influences and recover faster after disturbances (Reusch *et al.* 2005). Parasites pose such a negative influence (Anderson & May 1979; Poulin 1998), and are therefore an important factor to consider when studying effects of host genetic diversity. Parasites are of general importance in all natural systems, and epidemics can lead to population extinctions (Anderson & May 1979; Weisser 2000; Ebert 2005; Pounds *et al.* 2006; Rauch & Weisser 2006). Parasites and parasitoids can reduce host density, host growth or productivity and may thus affect ecosystem functioning (Anderson & May 1979; Hudson *et al.* 1998; Ebert 2005; Pounds *et al.* 2006). However, the diversity of a system itself may influence parasites as well (Elton 1958; van der Plank 1963; Browning & Frey 1969; Leonard 1969; Garrett & Mundt 1999; Keesing *et al.* 2006). The question of how host genetic diversity influences parasite spread is the topic of this study.

The interest of genotypic or species diversity affecting the spread of parasites originated in agricultural research (Elton 1958; Leonard 1969). The rapid and devastating spread of diseases in agricultural monocultures is a well-known phenomenon (e.g. potato blight or rice blast Zhu *et al.* 2000; Pilet *et al.* 2006). The diversity-disease hypothesis states that decreased genetic diversity of hosts increases the incidence of diseases caused by specialist pathogens, a phenomenon called the monoculture effect (Elton 1958; van der Plank 1963; Browning & Frey 1969; Leonard 1969;

Garrett & Mundt 1999). Several agricultural or silvicultural studies empirically support the monoculture effect (Wolfe 1985; Hagle & Goheen 1988; Zhu *et al.* 2000; Cox *et al.* 2004; Pilet *et al.* 2006) and the effect is found for bacterial pathogens, fungal pathogens (Mitchell *et al.* 2002; Cox *et al.* 2004) and plant herbivores (Unsicker *et al.* 2006). However, crop monocultures or managed grasslands represent artificial situations and usually only one or a few cultivars are compared (Garrett & Mundt 1999; Zhu *et al.* 2000; Mitchell *et al.* 2002). The cultivars are selected lines for certain traits such as yield quantity or quality and not a random samples of genotypes (Zhu *et al.* 2000; Cox *et al.* 2004; Pilet *et al.* 2006). We wanted to know if the monoculture effect occurred also in natural and spatially less structured animal systems.

Little is known on the actual mechanism beyond a monoculture effect (reviewed in Keesing *et al.* 2006). Hosts in monocultures are not *per se* less resistant, but all individuals have a similar susceptibility to diseases. Pathogens transmit from one host individual to another without encountering large changes in the host genotype. Once a host genotype is infected, subsequent spread is facilitated because no genetic or physiological adaptations are required to infect another individual (Anderson & May 1986). In contrast, in genetically diverse host populations, a pathogen may encounter different host genotypes when it is transmitted from one host individual to another (Keesing *et al.* 2006). Genetic or physiological adaptations require some time and transmissions to resistant host genotypes are dead ends for the parasites (Anderson & May 1986). This slows down the spread of parasites. In agriculture, the strong spatial structure of plant genotypes grown in alternating rows may influence pathogen spread. In plots of higher diversity, each species or genotype has a lower relative density, and pathogen transmission within the susceptible individuals may be reduced by the less probable encounter of susceptible hosts (called dilution effect Schmidt & Ostfeld 2001; Mitchell *et al.* 2002; LoGiudice *et al.* 2003). In the long-term, a low genetic diversity is disadvantageous during coevolution, and parasites may adapt faster to less diverse host populations (Ebert 1998). Thus, the monoculture effect may promote the maintenance of genetic diversity and influence host–parasite coevolution.

Here, we experimentally tested the monoculture effect in a natural animal host–parasite system with realistic differences in genetic diversity. Up to now, the study of parasite spread within groups of animals differing in genetic diversity mostly focussed on social insects (Shykoff & Schmid-Hempel 1991; Schmid-Hempel & Crozier 1999; van Baalen & Beekman 2006; Hughes & Boomsma 2006). These animals live in close proximity within a colony, exhibit social interactions, and are closely genetically related, which all favours the spread of parasites. In bumblebees, colonies with a higher genetic diversity due to polyandry contain fewer parasites and show greater reproductive success, than low-diversity colonies (Baer & Schmid-Hempel 1999). Besides social insects, experimental data on the epidemiological effects of genetic diversity in animal populations are scarce.

We followed the epidemiology of the microsporidian parasite *Octosporea bayeri* Jírovec, 1936 in *Daphnia magna* Straus, 1820 host populations of low and high genetic diversity under outdoor conditions. Both hosts and parasites were collected in a natural metapopulation (Ebert *et al.* 2001; Pajunen & Pajunen 2003). In that metapopulation, host populations differ naturally in their genetic diversity (Haag *et al.* 2005). The *D. magna* populations were placed in mesocosms under outdoor conditions and parasites were allowed to spread in a natural way. We also used two diversity levels for the parasite to test if a monoculture effect depends on parasite diversity. We monitored the spread of the parasite over 3 years, which corresponds to *c.* 20–30 host generations and represents the average survival length of natural *Daphnia* populations in our metapopulation (Pajunen & Pajunen 2003). Parasites spread significantly better in host populations of low genetic diversity compared with host populations of high genetic diversity while the genetic diversity of the parasite population had surprisingly no significant effect on parasite spread.

MATERIALS AND METHODS

Daphnia magna and *Octosporea bayeri*

The freshwater crustacean *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) is widely distributed along the coast of the Baltic Sea, inhabiting water-filled rock pools (Pajunen & Pajunen 2003). These pools are spatially separated and genetic differentiation between the rock pool populations is strong (Haag *et al.* 2005) due to recurrent colonisations, immigrations and extinctions (Pajunen 1986; Pajunen & Pajunen 2003). Natural populations strongly differ in their age (Pajunen & Pajunen 2003). Populations founded by one or a few individuals are highly inbred and possess little genetic diversity (Ebert *et al.* 2002; Haag *et al.* 2002). Periods of asexual reproduction during summer are intermitted by sexual reproduction when resting eggs (ephippia) are produced. Only ephippia allow over-wintering (Ranta 1979).

The rock pool *Daphnia* populations harbour a wide spectrum of parasites, but differ naturally in their parasite community and prevalence (Green 1957; Bengtsson & Ebert 1998; Ebert *et al.* 2001; Ebert 2005). The microsporidium *Octosporea bayeri* Jírovec, 1936 is the most common parasite in our study area. *Octosporea bayeri* is specific to *D. magna* and is found in *c.* 45% of all populations (Ebert *et al.* 2001). *Octosporea bayeri* replicates inside the host and

infected animals contain up to 5×10^6 spores of the parasite (Ebert 2005; Vizoso *et al.* 2005). Parasite transmission is vertical or horizontal, and infected hosts have reduced fecundity, survival and competitive ability (Vizoso & Ebert 2004; Lass & Ebert 2006). Horizontal infection occurs by ingesting spores from dead infected hosts. Physical contact alone between infected and uninfected living animals does not allow transmission. Horizontal transmission is essential for the parasite to persist in a host population (Lass & Ebert 2006).

Setup

In spring 2003 and 2004, we took random samples of *D. magna* from 22 rock pool populations on 12 islands near Tvärminne Zoological Station, Finland (59°50′ N, 23°15′ E). We used single females to create clonal cultures in the lab. Each female represented a unique genotype that hatched from an over-wintering egg. Per population, either two (12 populations), three (nine populations) or four (one population) genotypes were isolated, giving in total 55 genotypes. We treated each genotype against possible infections of the microsporidium *O. bayeri* following the protocol of Zbinden *et al.* (2005). Infections of *O. bayeri* are easily seen when the host is dissected and investigated with phase-contrast microscopy (400-fold magnification). The success of the treatment was confirmed afterwards. No other parasites were found in the cultures. The natural infection status does not affect the competitive abilities of a genotype (Altermatt & Ebert 2007), and therefore the cured genotypes were not inferior to the naturally uninfected genotypes. We typed all *Daphnia*-lines for their allozyme genotypes at five loci. These loci were aspartate amino transferase (Aat, enzyme commission number EC 2.6.1.1), fumarate hydratase (Fum, EC 4.2.1.2), glucose-6-phosphate isomerase (Gpi, EC 5.3.1.9), phosphoglucomutase (Pgm, EC 5.4.2.2) and mannose-6-phosphate isomerase (Mpi, EC 5.3.1.8; Hebert & Beaton 1993). The genotypes were kept in 400 mL artificial medium (Klüttgen *et al.* 1994) at room temperature with a dark/light cycle of 10/14 h and were fed *ad libitum* with the green alga *Scenedesmus obliquus*.

To breed different parasite isolates, we collected from each of 11 rock pools one *D. magna* female that was naturally infected with *O. bayeri* in May 2005. To avoid a coevolutionary history, we collected the *D. magna* used as hosts in the experiment in different pools than the infected *D magna* used for parasite cultures. Because of the lack of genetic markers in *O. bayeri*, we could not test if single *D. magna* were infected by multiple parasite strains or not. To cultivate the parasite isolates, we placed the infected *Daphnia* singly in buckets containing 8 L of artificial medium. The buckets were put on an island next to natural pools and the *Daphnia* fed *ad libitum*. Vertical transmission of the parasite is 100% during the asexual reproduction of *D. magna* (Vizoso *et al.* 2005). Because of asexual reproduction, we could breed large numbers of *Daphnia* (> 1000) infected with the same parasite (herein called 'isolate') within a few weeks.

On 18 June 2005, we started the experiment. We released the uninfected *D. magna* into plastic buckets (volume 40 L) containing 20 L water from a rock pool free of *D. magna* and free of parasites filtered through a 20-μm filter. We added 1 L of sea-water, 700 mL artificial medium and 150 mL of a horse manure suspension (10 kg horse manure suspended in 60 L of sea-water) to increase salinity, increase the nutrient content and ameliorate water quality.

We established *D. magna* populations with a low genetic diversity (H_{low} = 1 genotype per host population) and with a high genetic diversity (H_{high} = 10 genotypes per host population). In both of these treatments we had five completely independent combinations in respect of their host genotype composition (Fig. 1). Every host genotype was only used once (five times one plus five times 10 = 55 *D. magna* genotypes). The genotypes in the high genetic diversity treatment were randomly chosen, but such that all genotypes per combination of 10 came from different pools of origin. Due to the random assignment of the host genotypes to the treatments, the genotypes did not differ systematically in any aspect between the two different diversity treatments. In the low genetic diversity treatment, 100 animals per genotype were released in buckets (replicated in triplicate), in the high-genetic diversity treatment 10 animals of each of the 10 genotypes per combination were released together (replicated in triplicate; this gave two treatments × five population × three replicates = 30 mesocosms). The mesocosm buckets were placed outdoor on an island next to natural rock pools. The chosen diversity levels are realistic and can be commonly found in the natural metapopulation: First, *c.* 20% of all populations are new colonisations (Pajunen & Pajunen 2003), and most of them are monoclonal (Haag *et al.* 2005). Over time, subsequent genotypes invade, facilitated by hybrid vigour, and genetic diversity of the Daphnia populations may reach similar levels as in our high genetic diversity treatment (Haag *et al.* 2005, 2006).

The host diversity levels were completely crossed with three parasite treatments: no parasite (control), low and high parasite diversity (Fig. 1). The parasite was added to the buckets by placing dead infected *D. magna* females (freshly killed with CO_2) from the parasite cultures to the appropriate replicates. We used only one parasite isolate in the low parasite diversity treatment and one cocktail of 10 parasite isolates in the high genetic diversity treatment (Fig. 1). We randomly chose one of the 11 cultured parasite isolates for the low genetic diversity treatment (P_{low}), while we added the other 10 together at equal proportions to

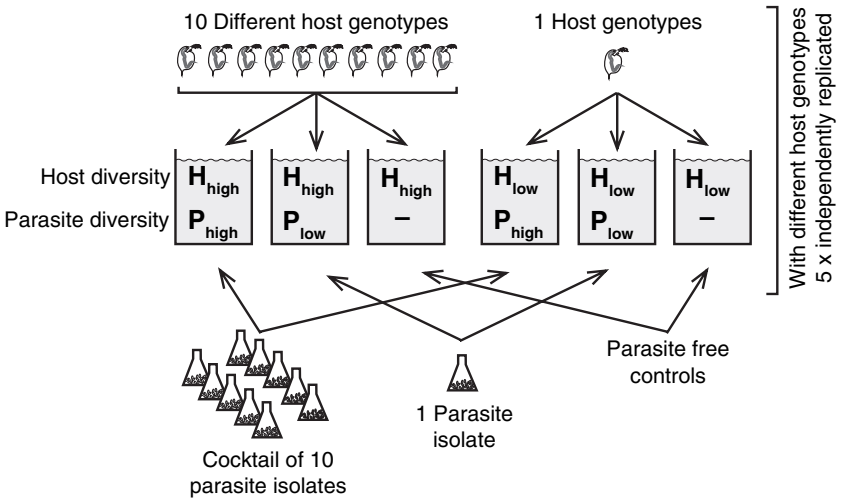

Figure 1 Setup of the experimental mesocosms in buckets. The experiment was started with uninfected *Daphnia magna* host populations of high and low genetic diversity (H_{high} and H_{low}). Each of the five different host populations of high genetic diversity started with 10 different genotypes at equal proportions. Each of the five different host populations of low genetic diversity started with one different genotype. All populations were setup in triplicate ([5 + 5]*3 = 30). Thereafter, we introduced the microsporidium *Octosporea bayeri*. Parasite isolates originated from 11 different natural populations. Within each triplicated host population, one population received a cocktail of 10 parasite isolates (P_{high}), one population received only one parasite isolate (P_{low}) and one population remained a parasite free control (–). The buckets were placed outdoors next to natural *D. magna* populations.

produce a cocktail of high genetic diversity (P_{high}). We added dead infected females to each replicate on 23 June 2005 (60 dead females per replicate) and again on 6 August 2005 (30 dead females per replicate). Parasite spores were passively released from the decaying cadavers of these hosts, allowing the infection to spread in the mesocosm populations (Vizoso *et al.* 2005). This represents the natural mode of horizontal infection. To the controls, we added the same number of dead *Daphnia* from parasite free laboratory cultures.

Over the summers, water volume in the buckets varied from 15 to 35 L due to evaporation and precipitation. We added deionised water during phases of long droughts to compensate for excessive evaporation-loss. In each autumn, we lowered the water level to *c.* 5 L. This prevented breaking of the buckets during freezing in winter. We covered the buckets with a lid and left them outdoors. Only ephippia and parasite spores survived the freezing in winter. In the subsequent year at the beginning of May, we filled the buckets with deionised water up to the previous level.

We estimated prevalence in random samples of the host populations on 1 August 2005 (summer 2005), 13 September 2005 (autumn 2005), 26 May 2006 (spring 2006), 11 July 2006 (summer 2006), 19 August 2006 (autumn 2006), 26 May 2007 (spring 2007) and 16 August 2007 (autumn 2007). In the year 2005, we estimated prevalence by dissecting 20 *D. magna* females per replicate and sampling date, while in the years 2006 and 2007 always *c.* 30 *D. magna* females per replicate and sampling date were dissected. On 11 July 2006 we took a random sample of all replicates to quantify the frequency of alleles and multi-locus genotypes at the five allozyme loci mentioned above. Per replicate, 66 *Daphnia* females were genotyped. We estimated *Daphnia* density on 30 July and 18 August 2006 by taking a volumetric sample. For the analysis, we used the mean of these two estimates per replicate to balance short temporal fluctuations. Furthermore, we roughly estimated the number of hatchlings at 26 May 2006 using the categories < 100 hatchlings, 100–500 hatchlings, 500–1000 hatchlings and > 1000 hatchlings.

As mentioned above, *D. magna* reproduces asexually during May to September but only survives the winter (beginning of October to end of April) in the sexually produced ephippia. Therefore, in 2005 only the initial sets of genotypes were present in the mesocosms, while in 2006 and 2007 all animals were recombinants. The monoclonal populations were all selfed from 2006 onwards, which is a common phenomenon in this metapopulation (Haag *et al.* 2005).

Analysis

We performed statistical analyses with R (R Development Core Team 2007) using the libraries Matrix (version 0.98-7), lme4 (version 0.98-1), asuR, Hmisc and base. We analysed prevalence in autumn 2005 with generalized linear mixed effect models and tested for an effect of host genetic diversity (quasibinomial error distribution used in the

model). At that time, no recombinant hosts were present. Host populations of low genetic diversity were still monoclonal, while host populations of high genetic diversity consisted of up to 10 genotypes. Clonal selection may have reduced the genotype richness in the high genetic diversity treatments (Capaul & Ebert 2003). We analysed prevalence changes over the whole 3 years time period using repeated measure ANOVAs with arcsin transformed prevalence data. We used generalized linear mixed effect models to analyse the total number of alleles, the density data and the heterozygosity data (quasipoisson error distribution used in the two first models, quasi-binomial error distribution in the latter). In all models, contrasts were chosen *a priori* to compare host populations of low and high genetic diversity and the parasite treatment of low and high genetic diversity. We tested the assumptions of all models (mostly with functions from package asuR) and chose the simplest model.

RESULTS

The regular samples revealed that the parasite established, spread and persisted over 3 years in all mesocosm populations where it had been added. All but one replicates of the parasite free control treatments stayed free of parasites. In one of the low genetic diversity controls we found an *O. bayeri* infection in summer 2006, indicating an immigration of either an infected *Daphnia* or of the parasite. This replicate was excluded from the analyses.

We found a significant monoculture effect. Parasites were more successful and reached a higher prevalence in the host populations of low genetic diversity compared with the host populations of high genetic diversity in autumn 2005 (indicated by the contrasts that we chose in the linear mixed effects model, $t_{16} = -2.6$, $P = 0.02$; Fig. 2). At that time, host populations had not yet recombined and still reflected the initial composition of genotypes. At the same time, the parasite diversity treatments had no effect on parasite prevalence (contrast as above, $t_{16} = -1.1$, $P = 0.30$). The interaction between the host and parasite diversity treatment is marginal non-significant (contrast as above, $t_{16} = 1.9$, $P = 0.06$). Variance estimates of the random term are 3.7×10^{-8} (intercept) and 73.6 (residual).

We then analysed prevalence over the whole 3 years. From the second year onwards, host populations were recombined and inbreeding effects may have occurred as well. Again, genetic diversity of the host population significantly influenced the spread of the parasite. The parasite *O. bayeri* spread significantly better in *D. magna* host populations of low genetic diversity compared with host populations of high genetic diversity over the 3 years' period ($F_{1,16} = 12.7$, $P = 0.0026$; Fig. 2). Parasite prevalence was higher in the host populations of low genetic diversity in all but one sample. In accordance with an earlier study, parasite prevalence showed a cyclic pattern with an increase during the asexual phase from spring to autumn, and a decrease during winter diapause (Lass & Ebert 2006), resulting in a significant time effect in our analysis ($F_{6,96} = 116$,

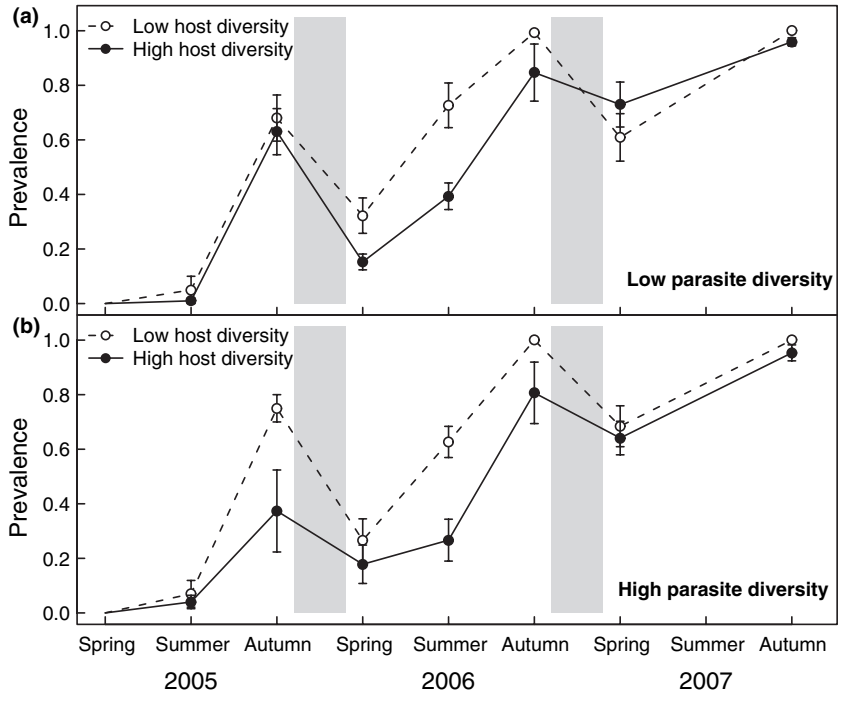

Figure 2 Prevalence of the parasite *O. bayeri* in *D. magna* host populations over time. *Daphnia magna* populations differed in their genetic diversity (low and high) and were all uninfected at the beginning of the experiment. The parasite spread significantly better in host populations of low genetic diversity (open dots) compared with host populations of high genetic diversity (filled dots). This difference was found both in the parasite treatment of low genetic diversity (a) and in the parasite treatment of high genetic diversity (b). Prevalence significantly changed over time. During winter diapause, indicated by the grey area, the hosts only survived in sexually produced resting stages.

$P < 0.0001$). The interaction between the effect of host genetic diversity and time is significant ($F_{6,96} = 4.2$, $P = 0.0009$). Prevalence between the parasite treatment of low and high genetic diversity was not significantly different ($F_{1,16} = 0.3$, $P = 0.59$; Fig. 2).

In all replicates, > 300 hatchlings were present in spring 2006 (data not shown). Most populations had *c.* 500–1000 hatchlings and a few populations had even > 1000 hatchlings. Each hatchling is a unique recombinant. The number of hatchlings between host populations of low genetic diversity and host populations of high genetic diversity was not significantly different (Wilcoxon signed rank test, $W = 87$, $P = 0.24$). Population size increased by subsequent asexual reproduction. We then measured population density at mid-summer. We did not find a significant difference between the density of host populations of low genetic diversity and host populations of high genetic diversity (contrast as above, $t_{24} = 1.16$, $P = 0.26$, Fig. 3). Also, the density of the uninfected host populations does not significantly differ from the density of infected host populations (contrast as above, $t_{24} = -1.5$, $P = 0.14$, Fig. 3). Variance estimates of the random term are 0.068 (intercept) and 7.5 (residual). All populations contained at least 1000 individuals (and up to 30 000 individuals) and only towards the end of the summer population sizes decreased. In some replicates as few as 50 individuals occurred in September/October (personal observation). However, sexual reproduction had already taken place at that time and the majority of ephippia are produced before end of July (F. Altermatt, unpublished data). Thus, the number of hatchlings and the subsequent population sizes were always large enough to prevent a substantial genetic drift effect over our experimental period of 20–30 generations (Hartl & Clark 1997). Prevalence and density, both depending variables, significantly correlate with each other ($R_S = 2030$, rho = −0.53, $P = 0.017$, the parasite free controls were excluded in this analysis; Fig. 4).

In the first year of the experiment (2005), the host populations of low genetic diversity were monoclonal and had a mean number of 5.6 alleles at the five loci assessed (SE ± 0.6). The host populations of high genetic diversity contained 10 different genotypes at start (Fig. 1). At the same five loci, they had a mean of 10 alleles (SE ± 0.51). As expected, the heterozygosity between the host populations of low and high genetic diversity did not differ at start (Fisher exact test, $P = 0.74$). In the subsequent years, all populations consisted of recombinants, and we could only distinguish a fraction of the genotypes with allozymes. In summer 2006, we confirmed that the diversities in the two different host population treatments still differed according to the initial setup. As expected, the difference in the number of alleles remains highly significant between the two diversity treatments (contrast as above, $t_{24} = 6.2$, $P < 0.0001$; Fig. 5a). New, host populations of low genetic diversity have a significantly lower heterozygosities (contrast as above, $t_{24} = 3.3$, $P = 0.003$; Fig. 5b) than host populations of high genetic diversity. Variance estimates of the random term are 0.027 (intercept) and 0.0067 (residual). We did not find new alleles occurring in any of the replicates and both allele number and heterozygosity were in accordance with the expected values under a model without

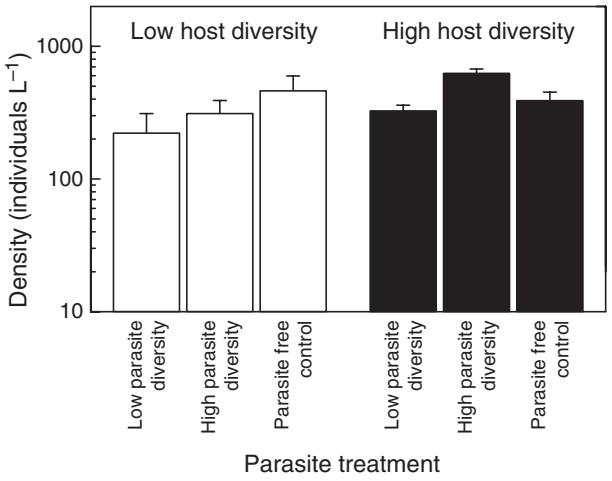

Figure 3 Mean density (± SE) of *D. magna* in summer 2006. Density varied considerably between the different replicates, though there was no significant effect of host or parasite diversity. The mesocosms contained *c.* 25 L of water at the time of density estimation.

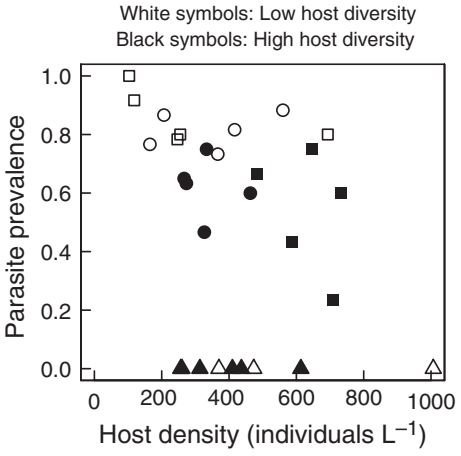

Figure 4 Correlation between the two dependent variables host density and parasite prevalence. The significant negative correlation does not imply causality. Squares represent replicates infected with a parasite isolate of low genetic diversity and circles represent replicates infected with a parasite isolate of high genetic diversity. Triangles represent parasite free controls and are only shown only for comparison and were excluded from the analysis.

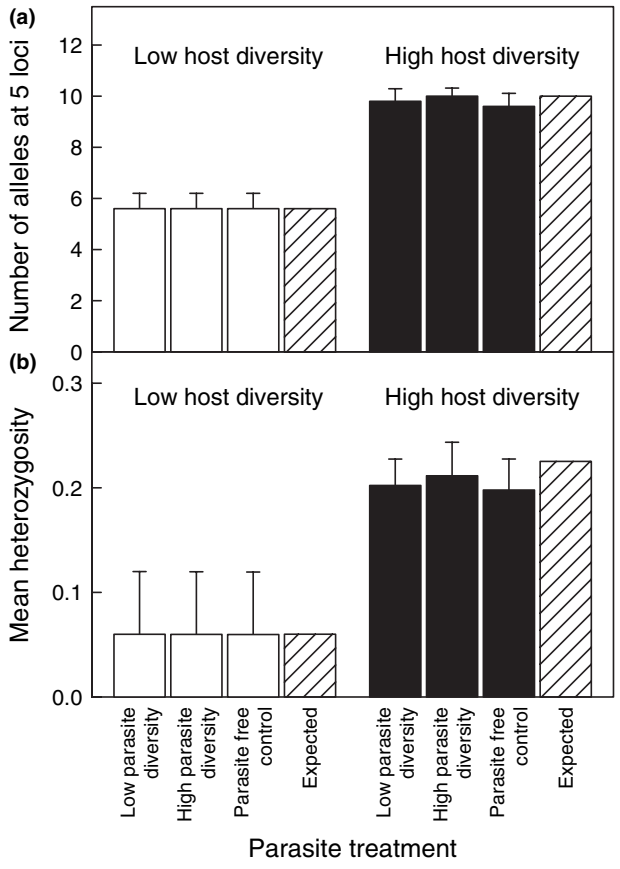

Figure 5 (a) Mean number of alleles (± SE) at five loci in the experimental *D. magna* populations in the second year (summer 2006). Accordingly to the initial setup, host populations of low genetic diversity (white bars) still contained significantly fewer alleles than host populations of high genetic diversity (black bars). There was no effect of the parasite treatment. We did not find new alleles that would indicate immigration of hosts into the mesocoms. The shaded bars show the initial and thus expected mean number of alleles. (b) Mean heterozygosity (± SE) of *D. magna* populations in the second year (summer 2006). Accordingly to the initial setup, host populations of low genetic diversity (white bars) still had a significantly lower heterozygosity than host populations of high genetic diversity (black bars). There was no effect of the parasite treatment. The shaded bars show the expected heterozygosity assuming no genetic drift or selection.

drift and selection. The occurrence of new alleles would have indicated immigration of hosts from other populations or, though much less likely, *de novo* mutations. The number of alleles in the populations was not influenced by the parasite treatment (contrast as above, $t_{24} = 0.82$, $P = 0.42$; Fig. 5a). Also, heterozygosity of the host populations was not influenced by the parasite treatment (contrast as above, $t_{24} = 0.35$, $P = 0.73$; Fig. 5b). Variance estimates of the random term are 6.1 (intercept) and 20.8 (residual).

DISCUSSION

We set up a multigeneration experiment (Fig. 1) to test if the monoculture effect typically seen in spatially structured agricultural systems also occurs in spatially intermixing animal populations. Parasites spread significantly better in host populations of low genetic diversity compared with host populations of high genetic diversity. We first discuss the monoculture effect found at the end of the first summer (summer 2005, Fig. 2). At that time, we could exclude inbreeding effects and attribute the monoculture effect to different levels of genetic diversity alone. All host genotypes used were randomly selected, natural isolates and were independently replicated within the treatments. Thereby, we excluded that the effect was due to a specific feature of the chosen genotypes itself. By using different genotypes of only one host species, we showed that the monoculture effect occurred at the genotypic level (see also Reusch *et al.* 2005).

The different genetic diversities of the host populations give a mechanistic explanation for our findings. Spatial structure alone can be safely excluded as mechanistic explanation (Mitchell *et al.* 2002; Keesing *et al.* 2006), because our plankton populations are spatially intermixing. Keesing *et al.* (2006) suggested five mechanisms by which genetic diversity reduces the disease risk in a specialist host–pathogen system and results in a monoculture effect. In their models, diversity is increased by the presence of an additional non-host species. We defined diversity more stringently by using different host genotypes within one host species. Our setup is comparable to models using multiple host species (Keesing *et al.* 2006). From previous experiments with the *Octosporea-Daphnia* system, we know that *Daphnia* genotypes from this metapopulation differ in susceptibility to the parasite, but full resistance has never been reported (Vizoso *et al.* 2005; Lass & Ebert 2006; Altermatt & Ebert 2007). Furthermore, *Octosporea bayeri* is not transmitted by contact between living hosts alone. Thereby, encounter reduction, transmission reduction and susceptible host regulation are excluded as mechanisms by which diversity could reduce disease risk (Keesing *et al.* 2006), because all three require the presence of totally resistant host genotypes. We could also exclude recovery augmentation (Keesing *et al.* 2006) in our system, because hosts do not recover from infections during the asexual phase (Vizoso *et al.* 2005; Lass & Ebert 2006; Ebert *et al.* 2007). From the five suggested mechanisms (Keesing *et al.* 2006), only differential mortality and reproduction of infected hosts in genetically diverse compared with less diverse populations may be used as mechanistic explanation of how parasite incidence is reduced. In that scenario, infected individuals are less able to tolerate competitive interactions (Keesing *et al.* 2006) in diverse populations compared with single-host populations. Infected hosts die

faster or do not reproduce. Another mechanism, which may lead to a reduced spread in diverse host populations would be when horizontal host-to-host transmission is more likely among similar hosts than dissimilar hosts. Then, a parasite may adapt phenotypically, but not necessarily genetically, to the current host genotype. This adaptation results in a higher transmission success to hosts of the same or a similar genotype. The mechanism would be independent of parasite genetic diversity, and would be consistent with our findings. Further, not yet specified mechanisms may arise in systems involving multiple host species or genotypes (Keesing *et al.* 2006). In the natural metapopulation two other *Daphnia* species are occurring, both of which are not host species of *O. bayeri*. It would be interesting to see if the presence of these species may further reduce parasite spread (Keesing *et al.* 2006).

We now discuss the effect seen over all 3 years (2005–2007, Fig. 2). We can eliminate population level effects caused by strong drift or selection as an alternative explanation for the difference in prevalence because genetic diversity of the host populations stayed in accordance with the setup (Fig. 5). From the second year onwards, all host populations in the low diversity treatment were inbred as a result of selfing, which is an inherent and naturally occurring situation in *D. magna* populations (Haag *et al.* 2006). Vertical transmission of *O. bayeri* through resting stages is more successful in inbred populations compared with outcrossing populations (Ebert *et al.* 2007). Previous work suggests that inbreeding depression alone does result in increased parasitism (Altermatt & Ebert 2007). Rather, inbreeding in a population increases the similarity between host individuals, and parasites may adapt faster to the host populations (Ebert 1998; Ebert *et al.* 2007). From the second year onwards, inbreeding and monoculture effect together may have consistently influenced parasite prevalence and may have allowed for accelerated parasite adaptation. In the natural system, the two mechanisms are intrinsically tied to each other.

The spread of parasites has an evolutionary component when hosts encounter pre-adapted parasites. Parasites infect and spread better in their sympatric host population compared with allopatric populations (Lively 1989; Ebert 1994; Gandon *et al.* 1996; Refardt & Ebert 2007), also seen in herein studied metapopulation (Altermatt *et al.* 2007). We started the experiment with uninfected host populations and used allopatric parasites to avoid the confounding effects of local adaptation and coevolutionary history. The experiment thus represented the situation after a new colonisation of a rock pool. Compared with sympatric combinations, parasite fitness strongly varies on allopatric hosts and depends on the specific host–parasite combination (Ebert 1994). By chance, the rapid spread of the parasite in host populations of low genetic diversity may be due to an *a priori* presence of parasite genotypes that could easily infect the host genotype in the monoculture (Carius *et al.* 2001). As we used five independent replicated host populations, it seems unlikely that pre-adapted parasites were present in several replicates of either low or high host genetic diversity. However, the general increase of parasite prevalence over time in both treatments (Fig. 2) may reflect some local adaptation of the parasite over time. Prevalence reached very high values in all replicates after 3 years and the effect of host genetic diversity vanished. This also suggests that the observed effect is not permanent and may depend on frequent disturbances that reset the system to initial conditions with low prevalence. Such disturbances are common for metapopulation systems. Indeed, the turn-over rates in the herein studied metapopulation are very high and less than one third of all populations get older than 3 years at all (Pajunen & Pajunen 2003). Newly founded populations are usually uninfected (Ebert *et al.* 2001). Thus, the natural situation is comparable to our experiment.

In our study, the monoculture effect was considerably smaller (*c.* 20% reduction in prevalence, Fig. 2) than in crop studies. For example, Zhu *et al.* (2000) found a 94% blast reduction in mixtures compared with monocultures (but see Cox *et al.* 2004). Part of the monoculture effect size in the plant studies is mechanistically explained by the spatial structure in plant cultivations (Zhu *et al.* 2000; Mitchell *et al.* 2002; Cox *et al.* 2004). In diverse cultures, susceptible plants may grow at distances that are too large for the pathogen to spread successfully. The reduction in relative density of the susceptible host is mechanistically equivalent to encounter reduction (Keesing *et al.* 2006). In mobile hosts the transmission and spread of parasites is favoured (Anderson 1995; Altizer *et al.* 2003). Consequently, the monoculture effect can be expected to be smaller.

Contrary to other studies (Engelhardt & Ritchie 2001; Hughes & Stachowicz 2004; Reusch *et al.* 2005 1431), we did not find a positive effect of the genetic diversity on host density (Fig. 3). However, a high genetic diversity in the host populations suppressed parasite prevalence but interacted with other parameter such as productivity (Fig. 4). In our experiment, we could not control for these two dependent variables.

We expected a higher prevalence in infections with diverse parasite mixtures. A high genetic diversity in the parasites may both have increased the probability of matching parasites to be present (Carius *et al.* 2001) as well as facilitated subsequent parasite adaptation (Gandon & Michalakis 2002). However, the effect was not found (Fig. 2). There are several, non-excluding explanations for the lack of a significant difference in our experiment. First, even though each of the 11 parasite strains was bottlenecked by starting with only one infected *D. magna* (as successfully applied in the same system by Vizoso & Ebert 2005), each

isolate could still have been genetically diverse. In case of a high within isolate diversity, mixing different isolates in the cocktail would have little effect and the parasite treatment of either low or high genetic diversity would differ less than expected. Second, contrary to the host populations, we did not replicate the parasite treatment of low and high genetic diversity. For the parasite, we had only one cocktail of high genetic diversity and one isolate of low genetic diversity. If the single isolate used in the low genetic diversity treatment was by chance exceptionally virulent or infectious, the difference may vanish. However, this is unlikely and would require the parasite to be generally virulent on all independently replicated host genotypes. A generally superior parasite is contrary to all current evidence in *Daphnia*–parasite systems (Ebert 1994; Carius *et al.* 2001; Altermatt & Ebert 2007; Refardt & Ebert 2007).

Our study contributed realistic data on both the generality and effect size of the monoculture effect in animal populations. Compared with agricultural studies we also included randomly selected host genotypes and a multigenerational setting under outdoor conditions. Host populations of high genetic diversity had an advantage over a period of 3 years. Thus, our study empirically shows that the monoculture effect may be important beyond the known agricultural situation. The epidemiological advantage of a high genetic diversity in host populations will in the long run also hamper parasite adaptation (Ebert *et al.* 1998; Hughes & Boomsma 2006).

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