Evolutionary Ecology Research, 2000, **2**: 1031–1043

Parasite heterogeneity affects infection success and the occurrence of within-host competition: An experimental study with a cestode

Claus Wedekind* and Andreas Rüetschi

Abteilung Verhaltensökologie, Zoologisches Institut, Universität Bern, CH-3032 Hinterkappelen, Switzerland

ABSTRACT

It has been suggested that a main benefit of sex is genetic heterogeneity among offspring, so as to be better prepared for unpredictable interactions faced in the next generation. This may be especially important in the co-evolutionary arms race between parasites and their hosts. The aim of this study was to determine whether parasite heterogeneity influences transmission and growth of the cestode *Schistocephalus solidus* in its copepod host. *S. solidus* can either reproduce by selfing or by outcrossing, producing different degrees of genetic heterogeneity in the offspring. It is, however, not sufficient to compare selfed with outcrossed parasites, because these two types may also vary, for example in their load of genetic damage caused by inbreeding depression. We therefore used 10 parasite sibships to infect copepods, each with six larvae, that came from either a single clutch ('pure' exposure; i.e. low heterogeneity) or a mixture of two clutches ('mixed' exposure; i.e. high heterogeneity). We found that infection was more likely in mixed exposures (increase in prevalence >50%). We also found that multiple infections were more common in pure exposures. Parasite transmission rates were therefore only slightly increased in mixed exposures (22%). Since parasite growth was reduced in multiple infections, parasites from mixed exposures were on average more than 50% larger than those from pure exposures at a time when they were infectious to the next intermediate host. One day after exposure, we distributed the copepods between two different housing conditions, and let the parasites grow within the hosts for 2 weeks. These two conditions resulted in different parasite growth rates and different host mortalities. However, the conditions did not have a significant influence on the prevalence and the parasite transmission rates either in the pure or the mixed exposures. Sexual reproduction in this parasite appears to be advantageous because heterogeneity increases the range of hosts that can be infected, while it decreases the occurrence of within-host competition. The parasite–host interaction that determines how many worms reach their infectious stage appears to take place during the very early stages of infection.

Keywords: antagonistic co-evolution, copepod, frequency-dependent selection, lottery model, *Schistocephalus solidus*.

* Address all correspondence to Claus Wedekind, Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. e-mail: c.wedekind@ed.ac.uk

INTRODUCTION

One group of theories predicts that diversity-generating mechanisms like sex enable the spread or even the creation of advantageous traits (see reviews in Michod and Levin, 1987; Stearns, 1987; Kondrashov, 1993; Howard and Lively, 1994; Hurst and Peck, 1996). These hypotheses typically require that the direction of selection is continuously changing; that is, the main source of mortality is short-term environmental changes. This condition is fulfilled in particular in co-evolving systems, such as parasite–host communities (e.g. Jaenike, 1978; Hamilton, 1980; Hamilton *et al.*, 1990; Little and Ebert, 1999).

The effect of genetic diversity on parasite–host co-evolution has been studied almost exclusively from the host's point of view (see, for example, reviews in Ladle, 1992; Sorci *et al.*, 1997). However, the argument can be turned around to predict that sexual reproduction in parasite populations is maintained as a diversity-generating mechanism to counteract the rapidly changing selection imposed by the hosts' immune system (Wedekind, 1999; for examples in vertebrate hosts, see Read and Viney, 1996; Gemmill *et al.*, 1997; Taylor *et al.*, 1997, 1998).

We tested experimentally whether parasite heterogeneity within a multiple exposure has an influence on transmission and growth in a host. Our model species were the pseudophyllidean cestode *Schistocephalus solidus* and its first intermediate host, the cyclopoid copepod *Macrocyclops albidus*. *S. solidus* has a complex life cycle. It matures and reproduces within a few days in the gut of piscivorous birds. The eggs pass out with the faeces into water. After several weeks of development, a free-living larva, the coracidium, hatches and is taken up by a copepod. Development to the procercoid stage and growth occur in the body cavity of the crustacean and, if the latter is swallowed by a stickleback (*Gasterosteus aculeatus*), the parasite develops into the plerocercoid stage and grows in the body cavity of the fish. The life cycle is completed if the infected stickleback is swallowed by a bird and the plerocercoid develops to the adult worm (Hopkins and Smyth, 1951; Clarke, 1954). *S. solidus* causes a reduction in fitness in its copepod host (Clarke, 1954; Wedekind and Milinski, 1996; Wedekind, 1997; Jakobsen and Wedekind, 1998) and in its fish host (literature summarized by LoBue and Bell, 1993), while it appears to cause little harm to its bird host (Clarke, 1954; Tierney and Crompton, 1992).

Adult *S. solidus* live for only a few days in the gut of a bird. Therefore, birds will spread eggs for only a few days (Tierney and Crompton, 1992; Schärer and Wedekind, 1999). This should happen where the two intermediate hosts of *S. solidus* are common, typically in small ponds or shallow regions of larger ponds where the dispersion of eggs is often not very widespread. We observed that, under laboratory conditions, coracidia from a clutch typically hatch quite synchronously (i.e. within about a week) and normally live for only a few hours but up to about one day. It is therefore likely that multiple infections in copepods are often by sib-mates.

Adult *S. solidus* are simultaneous hermaphrodites that can reproduce either by selfing or by outcrossing (Smyth, 1946, 1954; Wedekind *et al.*, 1998; Schärer and Wedekind, 1999; Schärer *et al.*, submitted). This produces different degrees of genetic heterogeneity in the offspring, which could influence the transmission dynamics in exposure. However, for testing the effect of parasite heterogeneity on transmission, it is not sufficient to compare selfed with outcrossed individuals, because these different types are also likely to vary in their load of genetic damage caused by inbreeding depression (Charlesworth and Charlesworth, 1987). Outcrossing is also likely to result in a generally increased degree of

heterozygosity. This could also covary with a general viability (Brown, 1997). Wedekind *et al.* (1998) found that eggs of *S. solidus* that were forced to reproduce by selfing reached lower hatching rates than outcrossed eggs from two-worm cultures 10 weeks after egg production. They also found that outcrossers and selfers differed in their maternal investment per egg. Eggs of selfers were on average smaller and contained even smaller embryos per given egg size than eggs of outcrossers (see also Schärer and Wedekind, 1999). Here, we controlled for all these potential confounding variables by using the larvae of 10 different clutches (sibships of *S. solidus*) to experimentally infest copepod hosts either by six larvae of a single clutch or by three larvae from each of two clutches.

It takes procercoids a week or more to develop a cercomer and thereby reach the infective stage in the copepod host; that is, the stage at which the larva has the potential to successfully establish itself in the next host (Clarke, 1954; Wedekind, 1997). The procercoids in this study were allowed to grow for 14 days, and their hosts were distributed between two housing conditions, which began 1 day after exposure. This also allowed us to test whether the degree of parasite heterogeneity in a multiple exposure influences early establishment or later parasite growth under two different housing conditions.

METHODS

We caught naturally infected sticklebacks from a pond in Bochum, Germany, and kept them for several months in the laboratory until we began the experiments. We used 17 fish that contained 1–8 *S. solidus* plerocercoids weighing 52–693 mg. The copepods stem from cultures that have been maintained in our laboratory for several years following techniques described by Orr and Hopkins (1969).

We obtained cestode eggs of known sibship by letting the worms reproduce in an *in vitro* apparature that replaces the bird host (modified from Smyth, 1954). The plerocercoids were removed aseptically from sticklebacks (on a balance to measure their weight simultaneously) and immediately inserted into seamless semi-permeable tubes. One or two tubes were suspended in a 500-ml bottle filled with sterilized culture medium at 40°C and shaken continuously for 4 days during incubation (for more details, see Wedekind, 1997). The eggs of each culture (later designated as 'clutches') were collected and kept at 20°C in tap water until they hatched.

To limit the genetic heterogeneity within a sibship as much as possible in our experiments, we mainly used eggs of one-worm cultures that were expected to be selfed (Smyth, 1954). However, we know from our own experience that selfed eggs of *S. solidus* normally do not hatch very well under laboratory conditions (see also Wedekind *et al.*, 1998), whereas clutches of multi-worm cultures that are likely to be largely outbred normally achieve good hatching rates. To ensure enough hatchlings, we also initiated some multi-worm cultures. We began 22 cultures with one worm, one culture with three worms, and four cultures with four worms each. In the multi-worm cultures, we combined worms that were of similar weights and, if possible, from different donor fish (the exception was the four-worm culture 'D', in which two worms came from two fish each). Any potential differences between single-worm and multi-worm cultures would not influence the conclusions of our study. These potentially confounding differences were controlled for by the way the experiment was set up and by the data analysis we chose (see below and Results section).

Before infestation, copepods without egg sacks were filtered from the culture tanks and transferred singly into wells (volume of water ~ 2 ml) of ELISA plates where they were kept throughout the experiment. Six coracidia of *S. solidus* (retrieved with a micropipette) were then added to the individual copepods. These six coracidia were either from the same clutch ('pure' exposure) or from two different clutches ('mixed' exposure, three coracidia of each). It should be noted that there is still some genetic heterogeneity within the offspring of a clutch. This is especially so in the clutches of multi-worm cultures, but also in the clutches of one-worm cultures, since selfing is still a form of sexual reproduction. However, to give the two treatments a name, we use the terms 'pure' and 'mixed' exposure here.

Pure and mixed exposures were balanced with respect to the well in the ELISA plates, and randomized with respect to the copepods' capture order (which might be correlated with a number of traits; see Wedekind and Milinski, 1996). Infections took place over several days, but all copepods within an ELISA plate were exposed to coracidia on the same day. The rate of pure and mixed exposures per day was similar; on average, 36.2% (standard error = 2.1%) of the copepods per ELISA plate were exposed to a mixed set of coracidia. Which coracidia were used for pure and mixed exposure was dependent on the hatching pattern of the different cultures and hence on the availability of coracidia. That this resulted in a non-random mixing was taken into consideration in the analyses (see Results). In several parasite sibships, only a few coracidia hatched during the time the copepods were exposed. Sibships in which less than five copepods could be exposed per pure and mixed exposure were not included in the analyses (the 'worst' culture included in the analyses was sibship 'C' with 20 exposed and surviving copepods). We could therefore only use six one-worm cultures (plerocercoid weight, $\mu = 335.5 \pm 58.2$ mg; mean ± standard error) and all four four-worm cultures ($\mu = 300.5 \pm 15.2$ mg). They were not significantly different in terms of their mean weight (*t*-test for separated variances, $t = 0.54$, $P = 0.61$, two-tailed). The six one-worm cultures came from six different donor fish and the four-worm cultures came from 11 different donor fish (12 donor fish in total).

The copepods were not fed in the ELISA plates before exposure and for 24 h after exposure. At that time, about half of the ELISA plates (containing 53.3% of all copepods) were closed with a net and sunk in a tank containing plants and stones in aged pond water with *Paramecium* sp. added as a constant food supply (22.5°C, constant light and current; 'first housing condition'). The other ELISA plates with copepods were placed in a climate chamber (20°C, 12 h light/12 h dark) and fed every 4 days with two freshly hatched *Artemia*, starting from day 1 after exposure ('second housing condition').

To determine infection success and the sizes of the hosts and the parasites, all copepods were filmed under a microscope 14 days after exposure. The number of procercoids was recorded and, after removing them from the copepod with two fine needles, they were filmed for subsequent determination of their size (by A.R., who did not know which copepods had been exposed to pure or mixed sets of coracidia). The size of the copepods was determined by measuring the distance from the base of the first antenna to the end of the 4th thoracic segment to the nearest 1 μm and converting to the copepod volume using the formula:

$$\text{copepod volume} = e^{-4.485} \cdot \text{length}^{3.383} \quad (1)$$

(Wedekind *et al.*, in press). The size of the cercomer correlates well with the size of the rest of the procercoid (Wedekind *et al.*, in press). Therefore, if the procercoid possessed a cercomer, procercoid volume (which includes the cercomer) was estimated by measuring the

maximal area of the longitudinal section of its body, excluding the cercomer, and converting it to procercoid volume using the formula:

$$\text{procercoid volume} = e^{0.533} \cdot \text{area}^{1.363} \quad (2)$$

For the few procercoids that had not yet developed their cercomer, we used the formula:

$$\text{procercoid volume} = e^{0.279} \cdot \text{area}^{1.385} \quad (3)$$

(Wedekind *et al.*, in press). To measure the severity of an infection, we calculated the total volume of all parasites in a copepod as the percentage of the body volume of its host (= 'parasite index').

RESULTS

Of the 462 copepods, 49 (10.6%) died for unknown reasons. Whether or not the copepods were infected by a pure or mixed set of coracidia did not correlate significantly with mortality (Fisher's exact test, $P = 0.43$, two-tailed). However, the two housing conditions resulted in different mortalities, 14.0% mortality when submerged in a tank and 6.8% when kept in a climate chamber (Fisher's exact test, $P = 0.015$, two-tailed).

Of the 413 surviving copepods, 74 were infected with 1–4 parasite larvae. The prevalence and the number of larvae per infected copepod were not significantly different between the two housing conditions (in both comparisons, $P > 0.30$). One-worm and four-worm cultures did not differ significantly with respect to their prevalence in pure exposures (Mann-Whitney $U = 11.5$, $P = 0.92$) or with respect to the number of larvae per infected host ($U = 2.5$, $P = 0.08$, two-tailed) (Tables 1 and 2). However, the analysis of differences between these two mating groups lacked statistical power, as they consisted of very few sibships; with the sample sizes here ($n_1 = 4$, $n_2 = 6$) and a two-tailed α-value of 0.05, the probability of finding a medium effect of $d = 0.5$ is below 10% and of finding a strong effect of $d = 0.8$ it is still below 25% (Cohen 1988, p. 36).

When examining the effect of mixed versus pure exposure, we had to control for possible differences of the 10 sibships used. This was necessary because, when exposing the copepods, the different sibships could not be mixed randomly but had to be mixed according to the availability of coracidia. The outcome of mixed exposures was therefore compared to a null-expectancy calculated from the outcome of the various pure exposures (see more detailed explanation in the respective table headings). This resulted in 10 paired replicates (i.e. observations and calculated null-expectancies for 10 sibships). However, these replicates were not fully independent as required by the *t*-statistics. To test the influence of this violation of the model assumption of the *t*-test, we performed a randomization test. In this procedure, the partners for mixed exposures were newly assigned for each sibship using a random number generator, and a new null-expectancy for this assignment was calculated. This was repeated 1000 times for each sibship. In this way, we obtained a frequency distribution of possible null-expectancies to which the observed and expected values could be compared. Tables 1–3 and Table 5 show the means (± standard deviation, *s*) of these frequency distributions and the percentage of respective simulation runs that resulted in differences of the same sign (i.e. the difference given in the tables and used for the *t*-tests). In nearly all cases, this was over 50% and was often 100% (i.e. there is no possible way of calculating a null-expectancy that would lead to a qualitatively different result as that shown).

Table 1. Frequencies of infected copepods (prevalence, in %) in pure and in mixed exposures. The numbers of dissected copepods per sibship are given in parentheses (each copepod that had two types of coracidia appears twice here, i.e. once for each sibship). The expected prevalences for mixed exposures were calculated according to how often sibships had been used for mixed exposures, including the observed prevalances from the pure exposures (e.g. for 'C': $(6 \cdot (6.3 + 15.4)/2 + 1 \cdot (8.6 + 15.4)/2)/7 = 11.0$). Since this method results in replicates that are not fully independent as required for the *t*-test, a randomization test was performed in which mean ($\pm s$) expectancy for mixed exposure was calculated by randomly assigning new sibships to the given ones (1000 runs each). To indicate the stability of the *t*-test, the percentage of runs that resulted in relative differences of the same directions as the ones given in the last column are given in curly brackets

Cestode sibship	Observed prevalence		Mixed with:	Expected prevalence for mixed exposure	Randomization test: mean expectancy $\pm s$ {% runs with same sign of difference}	Relative difference*
	Pure exposure	Mixed exposure				
A	7.7 (13)	9.1 (11)	B(4), D(1), G(2), J(4)	11.6	10.7 ± 2.4 {69.9%}	–21.6
B	6.3 (16)	18.5 (27)	A(4), C(6), G(15), J(2)	15.3	10.3 ± 3.0 {100%}	+20.9
C	15.4 (13)	14.3 (7)	B(6), F(1)	11.0	14.0 ± 4.5 {70.2%}	+30.1
D**	7.7 (39)	18.5 (54)	A(1), E(14), F(7), G(4), I(1), J(27)	10.4	10.9 ± 2.7 {100%}	+77.5
E**	0.0 (9)	17.6 (17)	D(14), G(1), H(1), J(1)	4.8	7.4 ± 3.9 {100%}	+264.3
F	8.6 (35)	22.2 (27)	C(1), D(7), I(4), J(15)	12.7	11.2 ± 3.0 {100%}	+75.1
G	33.3 (18)	29.2 (24)	A(2), B(15), D(4), E(1), J(2)	20.3	22.1 ± 2.4 {100%}	+43.5
H	5.9 (17)	21.4 (14)	E(1), J(13)	11.2	10.2 ± 4.9 {100%}	+91.2
I**	29.4 (34)	38.5 (26)	D(1), F(4), J(21)	22.7	20.5 ± 3.7 {100%}	+69.4
J**	17.8 (73)	25.9 (85)	A(4), B(2), D(27), E(1), F(15), G(2), H(13), I(21)	15.6	15.3 ± 2.0 {100%}	+65.8
Average	13.2	21.5		13.6	13.2	+58.6

* (Observed prevalance in mixed exposure – expected prevalence)· 100/(expected prevalence).
** Offspring from four-worm cultures.

Table 2. Average number of parasite larvae per infected copepod in pure and in mixed exposures. The numbers of infected copepods per sibship are given in parentheses. The expected number of parasite larvae per mixed infection and the randomization tests were calculated analogously to the procedure described in Table 1

Cestode sibship	Observed		Expected for mixed exposure	Outcome of randomization test: mean expectancy ± *s* {% runs with same sign of difference}	Relative difference*
	Pure exposure	Mixed exposure			
A	1.0 (1)	1.0 (1)	1.50	1.00 ± 0.08 {56.7%}	–33.3%
B	2.0 (1)	1.0 (5)	1.74	1.62 ± 0.12 {100%}	–42.5%
C	1.5 (2)	1.0 (1)	1.68	1.51 ± 0.20 {99.9%}	–40.5%
D**	2.0 (3)	1.2 (10)	1.70	1.72 ± 0.11 {100%}	–29.4%
E**	— (0)	1.0 (3)	0.96	0.76 ± 0.18 {87.7%}	+4.2%
F	1.0 (3)	1.5 (6)	1.52	1.28 ± 0.12 {3.3%}	–1.3%
G	1.5 (6)	1.7 (7)	1.68	1.44 ± 0.15 {99.0%}	+1.8%
H	1.0 (1)	1.0 (3)	1.54	1.29 ± 0.20 {99.5%}	–35.1%
I**	1.5 (10)	1.4 (10)	1.77	1.52 ± 0.19 {64.3%}	–20.9%
J**	2.2 (13)	1.4 (22)	1.85	1.79 ± 0.07 {100%}	–22.7%
Average	1.52	1.22	1.59	1.39	–22.0%

* (Observation in mixed exposure – expected) · 100/(expected).
** Offspring from four-worm cultures.

The prevalence of infected copepods was on average 58.6% higher in mixed exposures than in pure exposures (Table 1, Fig. 1a). However, since the expected prevalences for mixed exposures were calculated from the pure exposures, and the numbers of infected copepods resulting from pure exposures were often very low (see Table 1), the relative differences should be regarded as rough estimates for some of the sibships.

If infected, copepods from the mixed exposures harboured on average 22.0% less parasites than would have been expected from pure exposures (Table 2, Fig. 1b). The transmission rate – that is, the percentage of parasite larvae that survived and could establish in the host – was therefore only slightly higher in mixed exposures (compared to calculated null-expectancies: $t_9 = 1.93$, $P = 0.086$; compared to pure exposure: $t_9 = 2.58$, $P = 0.03$, two-tailed) (Table 3, Fig. 1c).

As a second control for the robustness of the *t*-statistics, we assessed whether parasite sibships that achieved higher prevalence were used more often for mixed exposures (Table 1, Fig. 1a). This was not the case (Spearman's $r_s = 0.19$, $P = 0.601$, two-tailed). When assessing whether parasite sibships that achieved higher numbers of parasites per infected copepod were used less often for mixed exposures, we found that they tended in fact to be used *more* often ($r_s = 0.66$, $P = 0.055$), which would obscure the effect described in Table 2 and Fig. 1b rather than artificially produce it. The transmission probabilities of the 10 sibships did not correlate significantly with how often they had been used for mixed exposures ($r_s = 0.32$, $P = 0.374$).

The parasite index used to estimate the severity of an infection – that is, the total parasite volume as a percentage of copepod volume – ranged from 0.06% to 8.67%. This index was

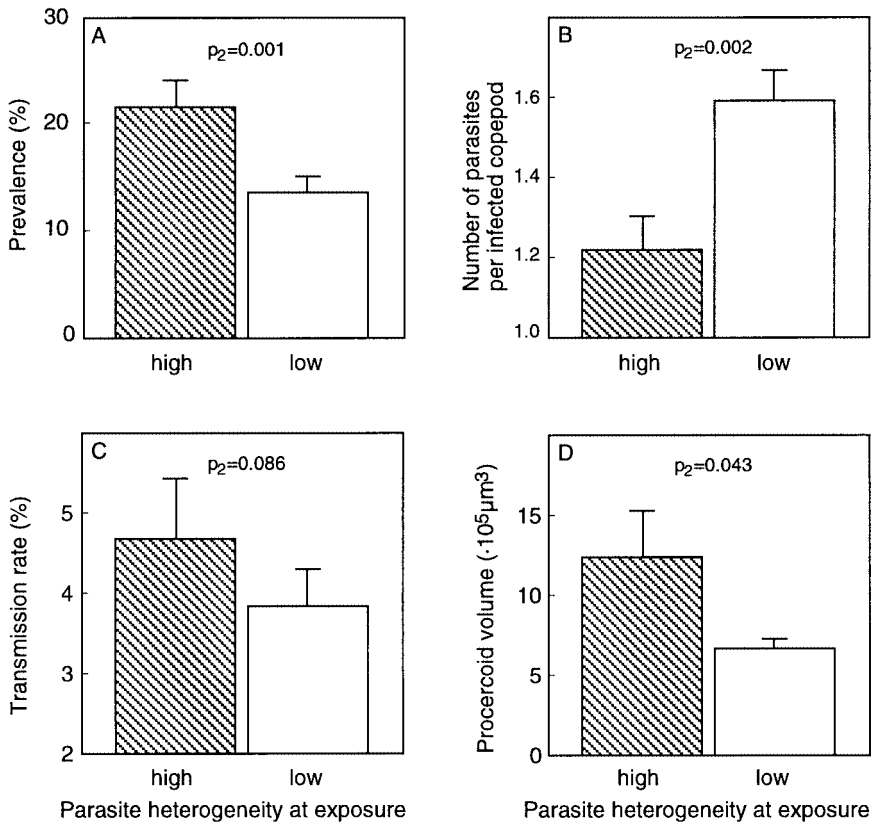

Fig. 1. Performance of the cestodes in their copepod host when the parasite heterogeneity at exposure was high (mixed exposure) or low (expected values calculated from pure exposure; see text for explanation). (A) Prevalence (paired t-test, $t = 4.75$, $n = 10$); (B) average number of procercoids per infected copepod ($t = 4.25$, $n = 10$); (C) transmission rates, i.e. the survival per individual coracidia ($t = 1.93$, $n = 10$); (D) the average procercoid volume per infected copepod ($t = 2.35$, $n = 10$). All values are reported as the mean ± standard error. P-values are two-tailed.

significantly different between the two housing conditions and whether or not a copepod was infected by one or several parasite larvae (Table 4). However, the parasite index did not appear to depend on whether the exposure was a mixed or a pure one (Table 4). When submerged in a tank the parasite index was on average 1.13% (standard error = 0.15), while when kept in the climate chamber it was on average 2.71% (standard error = 0.33). Singly infected copepods had an average parasite index of 1.39% (standard error = 0.17), while the parasite index of multiple infected copepods was on average 3.34% (standard error = 0.47).

Although the parasite index was higher in multiple infected copepods, the mean size of the individual parasites was smaller with higher numbers of competitors within a host (ANOVA, $F = 6.787$, d.f. = 3, $P < 0.001$). This result, combined with that in Table 2, leads to the prediction that the mean size of the parasites per copepod should be larger in mixed exposures than pure exposures. This was the case; the increase in procercoid size in mixed exposures was on average 98.1% (median = 56.5%) (Table 5, Fig. 1d). In singly infected copepods, the procercoids of mixed and of pure exposures were of similar size ($t = 0.71$, $P = 0.48$).

Table 3. Transmission rate (i.e. the percentage of parasite larvae that survived and could establish themselves in the host) in pure and in mixed exposures. The expected number of parasite larvae per mixed infection and the randomization tests were calculated analogously to the procedure described in Table 1

Cestode sibship	Observed: Pure exposure	Observed: Mixed exposure	Expected for mixed exposure	Randomization test: mean expectancy ± *s* {% runs with same sign of difference}	Relative difference*
A	1.28	2.38	3.10	2.41 ± 0.68 {52.9%}	–23.1
B	2.08	3.09	4.02	2.81 ± 0.81 {36.4%}	–23.3
C	2.94	2.08	2.47	3.18 ± 1.26 {80.3%}	–15.5
D**	2.56	3.70	3.42	2.98 ± 0.76 {78.8%}	+8.3
E**	0.00	2.94	1.52	1.88 ± 1.10 {70.3%}	+92.9
F	1.43	5.56	3.48	2.52 ± 0.85 {100%}	+59.4
G	8.33	8.00	5.36	5.57 ± 0.70 {100%}	+49.2
H	0.98	3.57	3.56	2.29 ± 1.30 {69.2%}	+0.2
I**	7.35	8.97	6.51	5.15 ± 1.05 {100%}	+37.9
J**	6.62	6.25	4.98	4.81 ± 0.47 {100%}	+25.5
Average	3.36	4.65	3.84	3.36	+21.2

* (Observation in mixed exposure – expected) · 100/(expected).
** Offspring from four-worm cultures.

Table 4. Results of a three-way analysis of variance on the parasite index of infected copepods (i.e. the total parasite volume as a percentage of copepod volume)

Source of variation	SS	d.f.	*F*	*P*
Housing condition	21.65	1	10.3	0.002
Pure *vs* mixed exposure	0.05	1	0.03	0.88
Single *vs* multiple infection	27.74	1	13.1	0.001
All possible interactions	3.05	4	0.02–0.67	0.42–0.89
Error	135.08	64		

DISCUSSION

We tested experimentally whether parasite heterogeneity affects infection success. When the six coracidia used to expose the copepod originated from two different parasite sibships ('mixed exposure'), we observed a more than 50% increase in prevalence compared with exposures with six coracidia of one sibship only ('pure exposure'). However, this cannot be translated directly to a higher parasite transmission. Copepods that were successfully infected by a pure exposure harboured on average more parasites than those infected by a mixed exposure. Parasite transmission rates were therefore only slightly and not significantly increased in mixed exposures.

This slightly increased transmission rate is not the only potential advantage for parasites in mixed exposures. Procercoid growth is limited by the size of its copepod host and by

Table 5. Procercoid volume ($\times 10^5$ μm^3) per infected copepod (averaged over all parasites within one copepod and over all infected copepods per cell). The expected parasite size for mixed exposures and the randomization tests were calculated analogously to the procedure described in Table 1. The numbers of infected copepods per type of mixing are given in parentheses (data are given per sibship, i.e. each copepod that had two types of coracidia appear twice here)

Cestode sibship	Observed		Infected from mixing with:	Expected for mixed exposure	Randomization test: mean expectancy ± s {% runs with same sign of difference}	Relative difference*
	Pure exposure	Mixed exposure				
A	7.43	1.06	J(1)	6.04	7.51 ± 1.70 {100%}	–82.52%
B	6.60	22.42	C(1), G(4)	9.89	7.26 ± 1.34 {100%}	+126.61%
C	12.04	30.94	B(1)	9.32	9.74 ± 1.55 {100%}	+231.90%
D**	2.73	12.06	E(3), F(1), G(2), I(1), J(3)	4.67	5.57 ± 0.52 {100%}	+158.26%
E**	—	14.35	D(3)	2.73	7.60 ± 3.27 {100%}	+425.75%
F	8.52	9.70	D(1), J(5)	6.42	8.15 ± 1.40 {78.0%}	+51.07%
G	13.47	15.15	B(4), D(2), J(1)	9.34	10.23 ± 0.78 {100%}	+62.12%
H	5.60	3.39	J(3)	5.12	6.79 ± 1.64 {100%}	–33.85%
I**	8.50	8.21	D(1), J(9)	6.48	8.06 ± 1.50 {62.7%}	+26.71%
J**	4.65	6.96	A(1), D(3), F(5), G(1), H(3), I(9)	6.07	6.15 ± 0.66 {88.3%}	+14.58%
Average	7.73	12.42		6.61	7.70	+98.1% (median = +56.5%)

* (Observation in mixed exposure – expected) × 100/(expected).
** Offspring from four-worm cultures.

the number of competitors within a host (Wedekind, 1997). Although the overall parasite index – that is, the total volume of the parasites as a percentage of copepod volume – increased with increasing number of parasites, the individual parasites of multiple infections did not grow as large as those that were alone in a host. Since procercoids from mixed exposures were more often alone in hosts, they would be expected to grow larger; this was the case. The difference in growth was clearly more than 50% in favour of parasites that came from mixed exposures. However, it is unclear what influence this size difference has on transmission and growth in the next intermediate hosts – that is, how it translates into fitness. There are no data available on this aspect.

The two housing conditions used resulted in different mortalities and in different parasite growth. The copepods appeared to be better fed and less stressed when kept in closed 2-ml wells and fed with *Artemia* than when kept in net enclosures submerged in an 80-litre tank with *Paramecium* sp. added to the water. Nevertheless, the prevalence and the number of parasites per infected copepod were not significantly different between the two conditions. Moreover, the outcome of the exposure by pure or by mixed groups of parasites did not interact significantly with the two housing conditions (see interactions in Table 4). This suggests that the housing conditions influenced the growth rate of the parasites but not their survival, although sometimes procercoids die during the first 14 days in their host (L. Schärer and C. Wedekind, unpublished observations). The main interaction between the parasites and their copepod host (i.e. the interaction that determines how many worms are successfully transmitted) appeared to take place during the very early stages of infection. Differences between the two housing conditions started 24 h after exposure and did not significantly influence parasite transmission as measured 14 days after exposure.

That pure exposures resulted in multiple infection more often than mixed exposures indicates that there may be a kind of a strain-for-strain interaction between *S. solidus* and its copepod host. It would appear that, if a parasite is able to establish itself in the host, siblings of this successful individual have a higher probability of establishing themselves than non-siblings. An alternative explanation for our findings is that coracidia or early procercoids possess mechanisms to directly compete (or even kill) each other in the copepod host. If such competition exists, it could potentially be stronger between non-siblings than between siblings.

Our results show that heterogeneity in parasites plays a crucial role in their infection success and in the occurrence of within-host competition. The amount of heterogeneity that we produced in our experiments cannot yet be compared to that which pairing would produce, especially because helminths often perform some selfing even in the presence of a partner (Nollen, 1983). The amount of heterogeneity that we produced could, therefore, be more than sex between worms in the same bird would generate. Nevertheless, offspring of an outbred clutch can be assumed to be genetically more heterogeneous than those of a selfed clutch. The important benefits from outbreeding in this parasite appear to be (1) that the offspring can infect a broader range of hosts and achieve even slightly higher transmission rates (supporting the 'lottery model', Williams, 1975) and (2) that infections result in larger procercoids because multiple infections, and hence within-host competition, are less likely. These benefits of outcrossing would add to the likely benefits of reduced inbreeding depression (Charlesworth and Charlesworth, 1987) and a potential general heterozygosity advantage (Brown, 1997).

ACKNOWLEDGEMENTS

We thank Tatjana Hadermann for laboratory assistance and Mark Dybdahl, Curtis Lively, Manfred Milinski, Lukas Schärer and Dora Strahm for discussion or comments on an earlier draft of the manuscript. The project was supported by the Swiss National Science Foundation.

REFERENCES

Brown, J.L. 1997. A theory of mate choice based on heterozygosity. *Behav. Ecol.*, **8**: 60–65.

Charlesworth, D. and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.*, **18**: 237–268.

Clarke, A.S. 1954. Studies on the life cycle of the pseudophyllidean cestode *Schistocephalus solidus*. *Proc. Zool. Soc. Lond.*, **124**: 257–304.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Gemmill, A.W., Viney, M.E. and Read, A.F. 1997. Host immune status determines sexuality in a parasitic nematode. *Evolution*, **51**: 393–401.

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

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

Hopkins, C.A. and Smyth, J.D. 1951. Notes on the morphology and life history of *Schistocephalus solidus* (Cestoda: Diphyllobothriidae). *Parasitology*, **41**: 283–291.

Howard, R.S. and Lively, C.M. 1994. Parasitism, mutation accumulation and the maintenance of sex. *Nature*, **367**: 554–557.

Hurst, L.D. and Peck, J.R. 1996. Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.*, **11**: 46–52.

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

Jakobsen, P.J. and Wedekind, C. 1998. Chemical reaction to odor stimuli influenced by cestode infection. *Behav. Ecol.*, **4**: 414–418.

Kondrashov, A.S. 1993. Classification of hypotheses on the advantage of amphimixis. *J. Heredity*, **84**: 372–387.

Ladle, R.J. 1992. Parasites and sex: Catching the Red Queen. *Trends Ecol. Evol.*, **7**: 405–408.

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

LoBue, C.P. and Bell, M.A. 1993. Phenotypic manipulation by the cestode parasite *Schistocephalus solidus* of its intermediate host, *Gasterosteus aculeatus*, the threespine stickleback. *Am. Nat.*, **142**: 725–735.

Michod, R.E. and Levin, B.R., eds. 1987. *The Evolution of Sex: An Examination of Current Ideas*. Sunderland, MA: Sinauer Associates.

Nollen, P.M. 1983. Pattern of sexual reproduction among parasitic platyhelminths. *Parasitology*, **86**: 99–120.

Orr, T.S.C. and Hopkins, C.A. 1969. Maintenance of *Schistocephalus solidus* in the laboratory with observations on rate of growth of, and proglottid formation in, the plerocercoid. *J. Fish. Res. Board Can.*, **26**: 741–752.

Read, A.F. and Viney, M.E. 1996. Helminth immunogenetics: Why bother? *Parasitol. Today*, **12**: 337–343.

Schärer, L. and Wedekind, C. 1999. Life time reproductive output in a hermaphrodite cestode when reproducing alone or in pairs: A time cost of mating. *Evol. Ecol.*, **13**: 381–394.

Smyth, J.D. 1946. Studies on tapeworm physiology. I. The cultivation of *Schistocephalus solidus in vitro*. *J. Exp. Biol.*, **23**: 47–73.

Smyth, J.D. 1954. Studies on tapeworm physiology. VII. Fertilization of *Schistocephalus solidus in vitro*. *Exp. Parasitol.*, **3**: 64–71.

Sorci, G., Møller, A.P. and Boulinier, T. 1997. Genetics of host–parasite interactions. *Trends Ecol. Evol.*, **12**: 196–200.

Stearns, S.C., ed. 1987. *The Evolution of Sex and its Consequences*. Basel: Birkhäuser.

Taylor, L.H., Walliker, D. and Read, A.F. 1997. Mixed-genotype infection of the rodent malaria *Plasmodium chabaudi* are more infectious to mosquitoes than single-genotype infections. *Parasitology*, **115**: 121–132.

Taylor, L.H., Mackinnon, M.J. and Read, A.F. 1998. Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution*, **52**: 583–591.

Tierney, J.F. and Crompton, D.W.T. 1992. Infectivity of the plerocercoids of *Schistocephalus solidus* (Cestoda: Ligulidae) and fecundity of the adults in an experimental definitive host, *Gallus gallus*. *J. Parasitol.*, **78**: 1049–1054.

Wedekind, C. 1997. The infectivity, growth, and virulence of the cestode *Schistocephalus solidus* in its first intermediate host, the copepod *Macrocyclops albidus*. *Parasitology*, **115**: 317–324.

Wedekind, C. 1999. Parasite-driven sexual selection and the evolution of virulence. In *Evolution in Health and Disease* (S.C. Stearns, ed.), pp. 102–107. Oxford: Oxford University Press.

Wedekind, C., Christen, M., Schärer, L. and Treichel, N. in press. Relative helminth size in crustacean hosts: *In vivo* determination and effects of host gender and within-host competition in a copepod infected by a cestode. *Acquatic Ecol*.

Wedekind, C. and Milinski, M. 1996. Do three-spined sticklebacks avoid consuming copepods, the first intermediate host of *Schistocephalus solidus*? An experimental analysis of behavioural resistance. *Parasitology*, **112**: 371–383.

Wedekind, C., Strahm, D. and Schärer, L. 1998. Evidence for strategic egg production in a hermaphroditic cestode. *Parasitology*, **117**: 373–382.

Williams, G.C. 1975. *Sex and Evolution*. Princeton, NJ: Princeton University Press.