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Published in:
Journal of Evolutionary Biology

DOI:
10.1046/j.1420-9101.2001.00249.x

Parthenogenetic flatworms have more symbionts than their coexisting, sexual conspecifics, but does this support the Red Queen?

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Keywords:

cost of sex;
Dugesia;
mutation hypothesis;
parasite hypothesis;
parthenogenesis;
Platyhelminthes;
protozoa;
recombination.

Abstract

The Red Queen hypothesis predicts that sexuality is favoured when virulent parasites adapt quickly to host genotypes. We studied a population of the flatworm *Schmidtea polychroa* in which obligate sexual and parthenogenetic individuals coexist. Infection rates by an amoeboid protozoan were consistently higher in parthenogens than in sexuals. Allozyme analysis showed that infection was genotype specific, with the second most common clone most infected. A laboratory measurement of fitness components failed to reveal high infection costs as required for the Red Queen. Although fertility was lower in more infected parthenogens, this effect can also be explained by the accumulation of mutations. We discuss these and other characteristics of our model system that may explain how a parasite with low virulence can show this pattern.

Introduction

Rapid adaptation of parasites to common clones is a major disadvantage for asexual populations and is seen as one of the major reasons as to why sexuality and not asexuality is the most prevalent mode of reproduction (Hamilton *et al*., 1990). More generally, the Red Queen hypothesis states that organisms are under selection to recombine their genotypes in the face of co-adapting species, of which parasites are an important subset (Van Valen, 1973; Hamilton, 1980; Bell, 1982). The other main hypothesis that can explain the success of sex emphasizes that, irrespective of the environment, clonal genomes lose fitness over time because of the inevitable accumulation of deleterious mutations (Muller, 1964; Kondrashov, 1982; Gabriel *et al*., 1993). Whether mutations can explain sex or not critically depends on the mutation rate in deterministic models (Kondrashov, 1988) or population size in stochastic models (Muller, 1964). More recently, it has been argued that a combination of relative and absolute genome deterioration through Red Queen and mutations respectively, is powerful enough to explain the success of sex under a wider range of conditions (Howard & Lively, 1994, 1998; West *et al*., 1999).

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Although the costs and benefits of sexuality have been modelled mathematically from many different angles, there is a remarkable lack of empirical studies (Wuethrich, 1998). This is particularly true for the parasite hypothesis, as the detection of host–parasite dynamics in a field population requires detailed, long-term studies. One such investigation is by Lively and co-workers of the freshwater snail *Potamopyrgus antipodarum*. They showed that asexual snails are more parasitized than their coexisting, sexual conspecifics and that infection is genotype specific (Lively, 1987, 1992; Dybdahl & Lively, 1995a) and a consequence of local adaptation by parasites to common host genotypes (Lively & Dybdahl, 2000). This was shown to result in negative frequency dependent selection by parasites (Dybdahl & Lively, 1998), resulting in time-lagged fluctuations in the frequencies of clones and their adapted parasites as predicted by Hamilton (1980). Such a possibility has instigated the view that highly polymorphic clonal populations may actually be able to coexist with

parasites, also on a long-term (Dybdahl & Lively, 1995b). Other examples from snails are increased outcrossing in the face of increased parasitism (Schrag *et al*., 1994) and parasite-linked geographical parthenogenesis (Johnson, 1994). Higher parasite loads in asexuals relative to sexuals have also been found in fish (Lively *et al*., 1990) and geckos (Moritz *et al*., 1991). Other studies have found rapid host-genotype dependent adaptation in parasites, but did so in species that were either sexual (Jaenike, 1993) or in which bouts of parthenogenetic reproduction alternate with sexuality (Ebert, 1994; Ebert *et al*., 1998; Little & Ebert, 1999). Good field systems in which sexual and asexual animals can be compared directly are exceedingly rare. Both types need to coexist ecologically and should not differ in anything but their mode of reproduction. Moreover, asexuals should ideally not have arisen through interspecific hybridization (as in *Poecilia* and *Gecko*) as this may lead to unpredictable positive (heterosis) or negative (hybrid dysgenesis) effects (Cullum, 1997).

Schmidtea (Dugesia) polychroa is a freshwater planarian with obligate sexual and parthenogenetic forms. Both types coexist in lakes in northern Italy (Beukeboom *et al*., 1996; Weinzierl *et al*., 1999b). Sexuals are obligatory outcrossing, diploid hermaphrodites. Parthenogens are polyploid, usually triploid, and produce polyploid eggs and haploid sperm. Parthenogens are sperm dependent, which means they require allosperm to trigger embryogenesis in their oocytes, but without making a genetic contribution (Beukeboom & Vrijenhoek, 1998). Allocation to sperm is lower and fecundity higher in parthenogens relative to sexuals (Weinzierl *et al*., 1998, 1999a). Parthenogens and sexuals also mate readily with each other (Storhas *et al*., 2000). Fertilization of haploid, sexual eggs, with haploid 'parthenogenetic' sperm results in diploid, sexual F1 offspring, which occasionally gives rise to new parthenogenetic lineages in the F2 generation (Benazzi Lentati, 1970; Weinzierl *et al*., 1999a). A comparison of allele frequencies within and between populations confirmed occasional local origin of parthenogens (Pongratz *et al*., 1998). Storhas *et al*. (2000) showed that parthenogens suffer from low fertility as a result of high embryo mortality. Inheritance studies suggest that this developmental instability is caused by deleterious mutations accumulated in parthenogens (Storhas, M., Carter, K. & Michiels, N., in preparation). Because parthenogens are sperm-dependent, asexuality cannot be related to low density as suggested by the reproductive assurance hypothesis (Lively, 1992). Yet, as a result of the production of cocoons in which offspring compete for a common yolk mass, parthenogens can limit the cost of unviable offspring (Lively & Johnson, 1994; Greeff *et al*., 1999).

In this study, we test some of the predictions made by the parasite hypothesis in *Schmidtea polychroa*. Because nothing was known about parasites in this species and very little about planarians in general, it was preceded by a survey of nine different Italian and German *S. polychroa* populations (Zeitlinger, J., unpublished data). An undescribed amoeba (see Methods) was particularly abundant in northern Italian populations and easily quantifiable. Here, we investigate whether parthenogens are more often and stronger infected than sexuals. We also determined allozyme genotypes to check for genotype-specific infection. In order to reveal short-term fitness costs that would confirm whether the symbionts are indeed parasitic, several fitness related measures were taken related to patterns of infection.

Materials and methods

Collection

Spatially separated samples were taken from nine different localities in northern Italy, east of Trento. Seven of these were different sites within Lago di Caldonazzo, our focal study population (Beukeboom *et al*., 1996; Pongratz *et al*., 1998; Weinzierl *et al*., 1999b; Table 1), whereas another two were from the nearby Lago di Toblino and Lago di Levico, both of which contain a purely sexual population. Samples were taken on 1 May 1996, which coincides with peak reproduction. Three additional samples were collected from locality HD ('Happy Days')

Table 1 Frequency of infection by amoebae in sexual and parthenogenetic *S. polychroa* in three different neighbouring lakes. In Lake Caldonazzo, seven different sites were sampled. Codes refer to the labels used in Pongratz *et al*. (1998). Total number of individuals, number infected (in parentheses), and mean *N* amoebae ± SD for infected individuals are shown separately for sexuals and parthenogens.

			Sexual		Parthenogenetic	
Locality	Code	Shore	*N* individuals (infected)	$\bar{X}$ amoebae	*N* individuals (infected)	$\bar{X}$ amoebae
Caldonazzo						
PE	C12	E	27 (7)	4.00 ± 4.16	–	–
MR	C13	E	43 (8)	1.63 ± 0.74	6 (2)	1.00 ± 0.00
LS	C14	SE	24 (8)	3.13 ± 1.73	57 (34)	7.71 ± 8.04
PV	C15	SW	–	–	21 (12)	23.3 ± 39.3
MD	C16	W	–	–	19 (6)	8.50 ± 10.60
HD	C17	W	51 (25)	5.40 ± 8.64	46 (43)	62.8 ± 46.2
HP	C18	NW	–	–	17 (2)	1.00 ± 0.00
Levico	C2		9 (0)	0 ± 0	–	–
Toblino	C3		22 (10)	25.3 ± 61.6	–	–

within Caldonazzo (C17 in Pongratz *et al*., 1998). This site is known to host a mixed population of sexual and parthenogenetic *S. polychroa* in more or less equal proportions. These samples were taken on 13 March, 15 May and 3 September 1996, yielding a total of four temporally separated samples for this locality. Collection was carried out by rinsing worms off stones picked in shallow water. Individuals were either colchicine-treated and fixed in the field (see below), or transported to the laboratory alive. Within each site, collection was always carried out in an area of no more than a few square metres. Within sites, sexuals and parthenogens occur spatially mixed (Weinzierl *et al*., 1999b). Hence, sexuals and parthenogens from a single location must have experienced very similar environmental conditions prior to collection.

Whole-mount preparation

Animals were fixed in Carnoy fixative (1 part acetic acid, 3 parts absolute ethanol), DNA-stained with Schiff's reagent and bleached with acetic acid according to the protocol described by Weinzierl *et al*. (1998) and Michiels & Bakovski (2000). This procedure renders worms transparent with cell nuclei stained dark purple and is particularly suitable to visualize protozoans. As the first samples clearly showed that amoeboids clustered in the head, with none or few in the caudal body half, we decided to use the caudal half for genetic analyses in the final sample on 3 September. Although it may have lowered the symbiont count per individual, it is very unlikely to have resulted in infected animals scored as uninfected.

Description of the symbiont

The organism under investigation is a 40–50 μm sized protozoan, characterized by strong vacuolization and a large nucleus with distinct nucleolus; and an amorph, roughly ellipsoid body shape and rarely with parapodia-like elongations (Fig. 1a–c). After Feulgen staining of host specimens, they can be counted easily (Fig. 1d). The characteristics suggest a member of the Gymnamoebea (Amoebozoa), most probably *Acanthamoeba* sp. (Page & Siemensma, 1991; Personal communication W. Foissner, Salzburg; J. Lom, Budweis). This is one of the commonest genera and is known to facultatively parasitize freshwater invertebrates (Martinez, 1985). Amoeba preferentially infect the nervous system of their hosts, which coincides with our observation that they were most abundant in the head region, clustered around the ventral commissure that links the two ventral nerve strands at their anterior end. There was never an obvious link with the reproductive system (ovaries, testes), suggesting that vertical transmission to offspring is unlikely. Exact identification requires culturing the

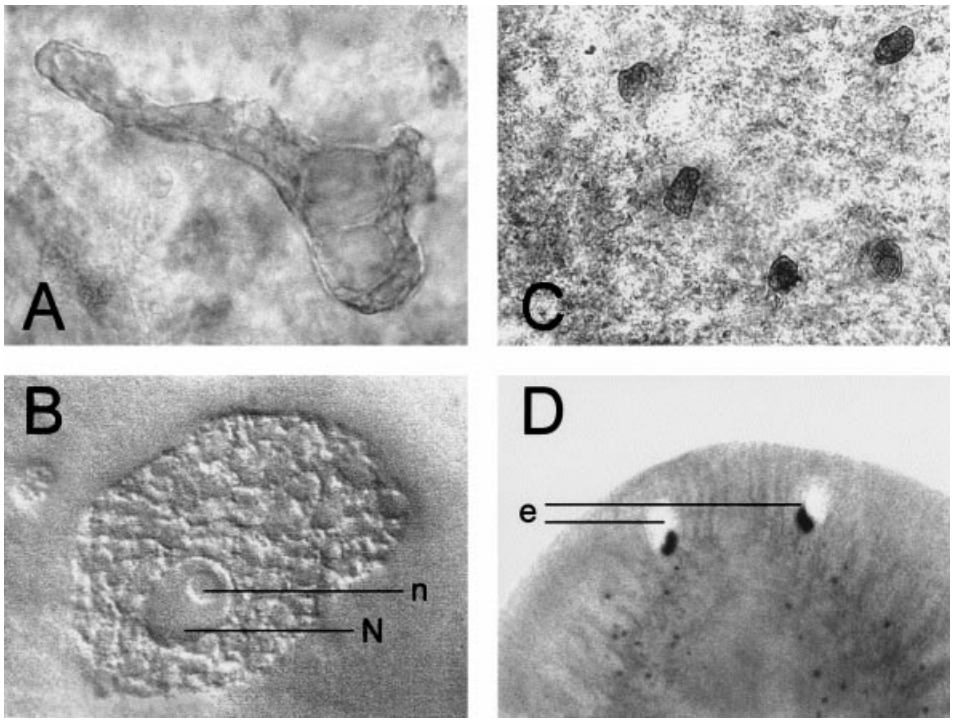

Fig. 1 General appearance and within-host distribution of amoeboid symbionts. (A) Feulgen-stained parasite in elongated shape. (B) Differential phase contrast picture of an amoeboid in its typical shape, showing large nucleus (N) with nucleolus (n). (C) Several amoebae in Feulgen-stained tissue. (D) Feulgen-stained head of *S. polychroa*, showing numerous amoeboids as dark spots behind the eyes (e).

amoebae, which will be attempted in the future, so that infection experiments in the laboratory can be performed.

Karyology

To distinguish between diploid sexuals and polyploid parthenogens, chromosome counts were carried out for each individual. We applied an established protocol (Beukeboom *et al*., 1996) for preparing chromosomes from regenerated blastemas from freshly cut tail-tips (*ca.* 3 mm) with extended colchicine treatment (7 h). Karyotypes were determined from metaphase nuclei. Individuals that could not be karyotyped reliably, could be assigned to either reproductive mode unambiguously when Feulgen stained, as sexual individuals always have significantly more testes and larger sperm ducts than parthenogens (Weinzierl *et al*., 1998).

Allozyme analysis

Genotypes were determined electrophoretically using eight polymorphic enzyme loci described by Pongratz *et al*. (1998), of which only four were polymorphic in this sample: two isocitrate dehydrogenase loci (*Idh1* and *Idh2*), L-iditol dehydrogenase (*Iddh*) and glucose-6-phosphate isomerase (*Gpi*) (see Pongratz *et al*., 1998 for details). It was not possible to always unambiguously distinguish *aab* from *abb* di-allelic heterozygote triploids. We therefore used a di-allelic representation (*ab*) for triploids throughout. We did not expect to see genotype-specific infection because of recombination in sexuals, as this would imply linkage between the allozyme loci and relevant resistance traits, which is unlikely. We merely present results for sexuals as well as parthenogens to indicate the relatedness between clones and sexuals.

Fitness measurements

Planarians produce cocoons in which they enclose several small oocytes with a large mass of extra-cellular yolk. Cocoon volume is therefore a good indicator of female fecundity, irrespective of fertility. The effect of amoebae on reproduction was assessed in an additional sample collected from the same locality on 15 May. Animals were immediately isolated in separate vials. In the laboratory, they were kept in isolation in small containers with two holes at opposite sites covered with mesh and placed in larger containers that were interconnected by a continuous water-flow system (Storhas *et al*., 2000). Twice a week, vials were taken out of circulation and animals were fed mixed, raw beef liver. Food remnants were removed and water was exchanged before turning on the water-flow system again.

During 21 days, cocoons were collected each morning and kept individually at 14 °C. They were opened after 19–21 days, shortly before hatching, using the method described in Storhas *et al*. (2000). A total of 507 cocoons and 1740 embryos were scored. Problems with embryogenesis are common. We therefore counted the total number of embryos, and subdivided them into undeveloped and viable. Undeveloped embryos are small, spherical and show no sign of head formation or eyes. They resemble a 4-day-old normal embryo. Viable hatchlings are motile and have eyes, but can be slightly deformed or small. Anecdotal observations have shown that deformed hatchlings may grow into normal juveniles later. Because the number of undeveloped embryos is probably an underestimate, we used the number of viable embryos per cocoon volume as a measure of (maternal) fertility. Total cocoon volume produced over the experimental period was used as a measure for female fecundity. Cocoons, hatchlings and undeveloped embryos were measured by drawing them under a camera lucida and determining their area using an image analysis system. Cocoon diameter was used to calculate cocoon volume (cocoons are spherical). The adults were measured at the end of the experiment by taking 3–5 digital pictures of the live individual gliding in a Petri dish, from which the average area was determined. A controlled infection experiment would have been more appropriate, but would require sterile culture conditions for the parasite and the host, neither of which have as yet been established.

Statistical analysis

We used SPSS for Windows v. 9.0.0 (SPSS Inc. 1999) including the *Exact* probability module which was applied wherever possible for nonparametric statistics. If memory space was insufficient, we estimated Exact *P* using a Monte-Carlo approximation. In such cases, we show the 99% confidence interval (CI) for *P*. Probabilities are always two-tailed. Contingency tables were tested using the likelihood ratio test (LRT). When variances were unequal, means were compared using an alternative Student's *t*-test that does not assume homogeneity of variances, albeit with a reduction in the degrees of freedom. Box-plots show median (line), interquartile range (box) and overall range excluding outliers. Means are shown ±SD. All analyses were performed separately for infection rate (proportion of infected animals) and symbiont count per infected animal. Although both are linked, the first may be more linked to the likelihood of infection, whereas the second may be more a measure of reproduction within the host.

Results

Spatially separated samples

The incidence of amoeboids varied strongly between sites (Table 1). Although infection was widespread in sexuals as well as in parthenogens, parthenogens were more

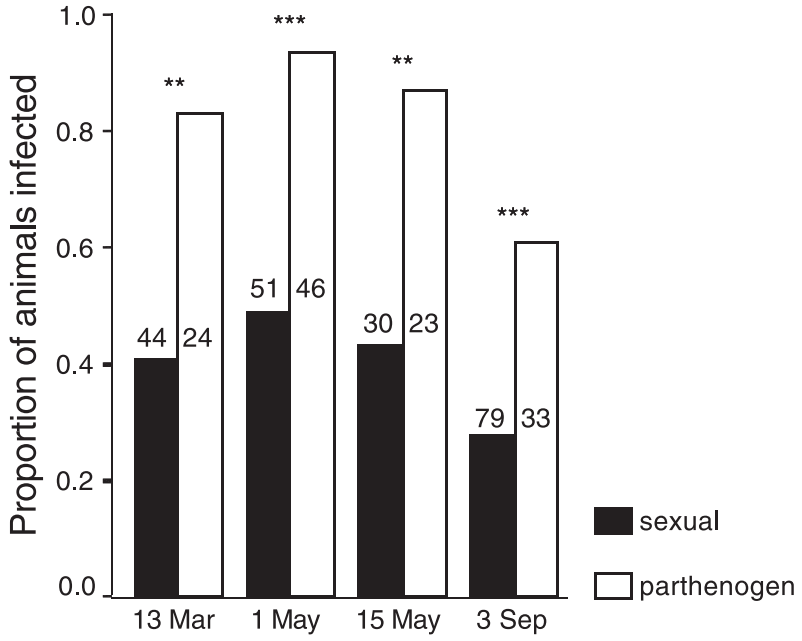

Fig. 2 Proportion of infected individuals in four mixed samples of sexuals (black) and parthenogens (white) from locality HD in Lago di Caldonazzo. Numbers on bars indicate total sample size for each reproductive mode. The results of LRTs are indicated (**$P < 0.01$; ***$P < 0.001$).

liable to be infected than sexuals at the two sites where both are present in reasonable numbers (Site LS: LRT $G = 4.74$; d.f. $= 1$; Exact $P = 0.050$; Site HD: see 1 May in Fig. 2). The same applied to the number of amoebae per infected individual (Site LS: Mann–Whitney $U = 75.5$; $n = 8$ and 34; Exact $P = 0.051$; Site HD: see 1 May in Fig. 3). The frequency of infected vs. noninfected individuals among mixed localities varied less strongly in sexuals (LRT $G = 9.24$; d.f. $= 3$; Exact $P = 0.030$) than in parthenogens (LRT $G = 28.0$; d.f. $= 3$; Exact $P < 0.001$). When comparing the number of amoebae per infected individual, the difference is even more pronounced (sexuals: Kruskal–Wallis $\chi^2 = 5.10$; d.f. $= 2$; Exact $P = 0.076$; parthenogens: Kruskal–Wallis $\chi^2 = 41.3$; d.f. $= 2$; Exact $P < 0.001$). These results indicate that infections have more variable effects on parthenogens than sexuals.

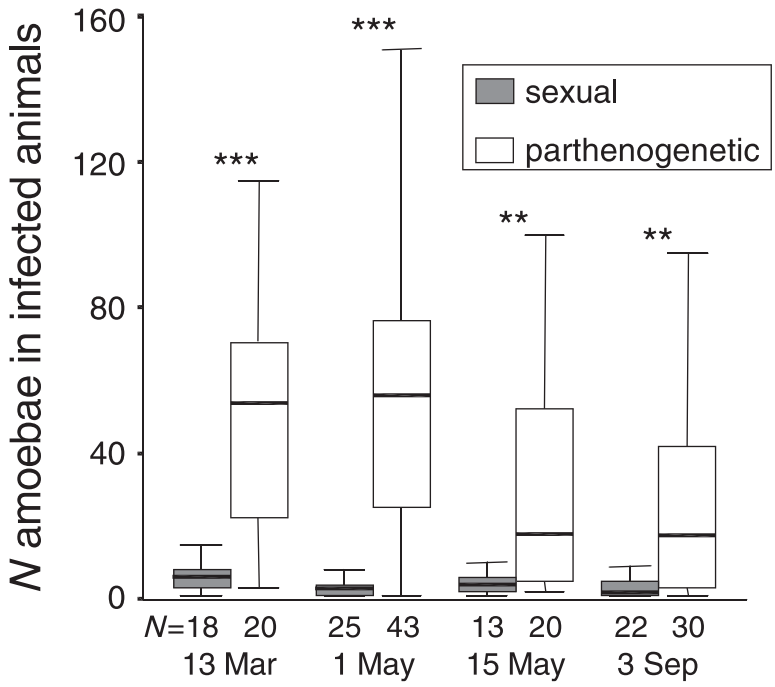

Fig. 3 Number of amoebae per infected individual in four mixed samples of sexuals (shaded) and parthenogens (white) from one locality in Lago di Caldonazzo. Samples sizes are shown below *X*-axis. The results of Mann–Whitney *U*-tests are indicated (**$P < 0.01$; ***$P < 0.001$).

Table 2 Clonal genotypes identified from four polymorphic allozyme-loci, genotype rank according to abundance across genotypes, number of individuals for each genotype (infected individuals in parentheses) and mean number of amoebae in individuals with at least one symbiont. Data from 3 September sample. Data for sexuals confirm that the allozyme loci are not linked to resistance traits (see text).

		Sexuals		Parthenogens	
4-locus genotype *Idh1-Idh2-Iddh-Gpi*	Genotype rank	*N* individuals (infected)	$\bar{X}$ amoebae	*N* individuals (infected)	$\bar{X}$ amoebae
bb–bb–bb–bb	1	46 (11)	2.55 ± 1.92	1 (0)	–
ab–bb–bb–bb	3	11 (3)	4.00 ± 4.36	2 (2)	13.50 ± 17.68
bb–bb–bb–ab	5	5 (2)	2.00 ± 1.41	–	–
bb–bc–bb–bb	6	4 (1)	12	–	–
ab–bc–bb–bb	8	4 (1)	1	–	–
aa–bb–bb–bb	9	2 (1)	5	–	–
bb–bc–bb–ab	10	1 (0)	–	–	–
ab–bb–bc–bb	11	–	–	1 (0)	–
ab–bb–bb–ab	4	1 (1)	57	11 (11)	49.18 ± 30.45
ab–bb–bc–ab	2	1 (1)	23	21 (13)	13.62 ± 17.06
ab–bc–bc–bb	7	–	–	4 (1)	2
ab–bc–bc–ab	12	–	–	1 (1)	6

The data do not suggest a simple relationship between relative abundance of sexuals and parthenogens and infection. Interestingly, infection of sexuals was lowest and highest in the two separate, purely sexual populations Levico and Toblino.

Temporally separated samples from one site

The proportion of each reproductive mode fluctuated between samples, with the earliest and latest samples being the ones with most sexuals and least parthenogens (Fisher Exact $P = 0.046$). In all four samples, parthenogens were much more likely to be infected than sexuals (Fig. 2). Fluctuations between samples were significant in parthenogens (LRT $G = 9.29$; d.f. $= 3$; Exact $P = 0.038$), less so in sexuals (LRT $G = 6.67$; d.f. $= 3$; Exact $P = 0.088$). In infected individuals, the number of amoebae again differed dramatically between sexuals and parthenogens (Fig. 3). Fluctuations between samples were significant in parthenogens (Kruskal–Wallis $\chi^2 = 18.4$; d.f. $= 3$; Exact $P < 0.001$), but not in sexuals (Kruskal–Wallis $\chi^2 = 5.27$; d.f. $= 3$; Exact $P = 0.15$).

Genotype specific infections

Allozyme genotypes could be determined in 75 sexual and 41 parthenogenetic individuals (Table 2). The frequency of parasitized vs. nonparasitized individuals did not differ between genotypes in sexuals (Likelihood Ratio Test $G = 6.94$; d.f. $= 8$; Exact $P = 0.77$), as expected for a recombining genome. Genotype-specific infection was, however, explicit in parthenogens (LRT $G = 18.8$; d.f. $= 6$; Exact $P = 0.003$). When comparing the amoeba count per infected individual between genotypes, sexuals showed no difference (Kruskal–Wallis $\chi^2 = 0.16$; d.f. $= 2$; Exact P 99% CI $= 0.917$–0.923), whereas the difference was significant in parthenogens (Kruskal–Wallis $\chi^2 = 8.29$; d.f. $= 2$; Exact P 99% CI $= 0.005$–0.007).

Fitness experiment

After 3 weeks in the laboratory, individuals had more amoebae than expected from those individuals that were caught at the same time and location, but fixed immediately (Table 3). The infection rate increased significantly in sexuals. Such effect was absent in parthenogens, because they had already a high infection rate at the start. Interestingly, the number of amoebae per infected individual increased only in parthenogens, but not in sexuals. On the one hand, the results indicate cross-infection in the course of the experiment and on the other, amoebae appear to reproduce faster in parthenogens than in sexuals, but this may also be the result of a higher initial number of amoebae in parthenogens.

Although 37 of 140 animals died in the course of the experiment, the proportion of sexuals and parthenogens at the end did not differ from that in the field sample from the same date (Fisher Exact $P = 0.72$), suggesting that mortality did not differ between the two groups.

For an analysis of fitness components, we ignored individuals that did not produce at least one fertile

Table 3 Comparison of infection rates and amoeba counts in two groups of animals collected on the same day: one part was fixed immediately (*field*), the other was kept in the laboratory (*experiment*) for another 3 weeks for fitness measurements. Mean amoeba count is based on individuals with at least one amoeboid.

Group	*N* not infected	*N* infected	Fisher Exact *P*	$\bar{X}$ amoebae	Student's *t*-test *P*
Sexuals					
field	17	13		5.46 ± 4.96	
experiment	1	37	<0.001	8.62 ± 10.74	0.31
Parthenogens					
field	3	20		33.05 ± 37.64	
experiment	0	35	0.057	109.9 ± 102.6	<0.001

Table 4 General summary of maternal reproduction of sexuals and parthenogens after 3 weeks in the laboratory. Fertility is expressed as number of viable offspring produced per mm^3 cocoon. Means were compared using a Student's *t*-test. Note that degrees of freedom vary not because of varying sample sizes, but because some tests involved an algorithm that allows for unequal variances, which reduces d.f. (see Methods).

	Sexual	Parthenogenetic	*t* (d.f.)	*P*
Body size (mm^2)	17.80 ± 3.69	19.59 ± 5.19	1.67 (66)	0.10
N cocoons	5.82 ± 2.39	7.80 ± 3.46	2.68 (49)	0.010
Avg. cocoon volume (mm^3)	0.81 ± 0.23	1.15 ± 0.31	5.24 (66)	<0.001
Fertility	4.67 ± 1.52	2.63 ± 1.06	6.24 (66)	<0.001
N undeveloped embryos	0.60 ± 0.10	1.35 ± 0.25	4.05 (38)	<0.001
N viable embryos	22.2 ± 12.2	25.1 ± 17.0	1.06 (50)	0.29
Mean viable offspring size (mm^2)	0.764 ± 0.139	0.997 ± 0.231	5.15 (66)	<0.001

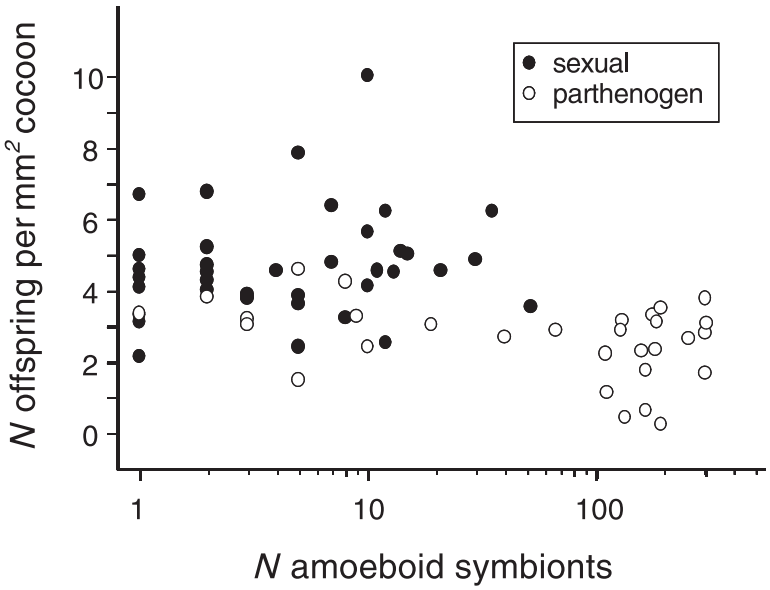

Fig. 4 Number of viable embryos produced per mm^3 cocoon volume (= yolk) as a function of the log amoeba count at the end of a 3-week period in the laboratory. Filled circles: sexuals, open circles: parthenogens. Individuals that produced only infertile cocoons are excluded.

cocoon, as complete infertility may have miscellaneous causes (immaturity, allosperm depletion, parasites, true infertility, etc.). Before looking at parasites, we first discuss overall differences in reproduction between sexuals and parthenogens (Table 4). They did not differ in mean body size. As expected from previous studies, sexuals produced fewer and smaller cocoons than parthenogens. Yet, sexuals had a higher overall fertility than parthenogens, defined as the number of viable embryos per cocoon volume. This can be attributed to the higher number of undeveloped embryos in parthenogens. The total number of viable embryos produced by sexuals and parthenogens did not differ. However, because parthenogens produced more yolk, their young were approximately 1.30 times larger.

There was no relationship between log amoeba count and body size at the end of the experiment (sexuals: Pearson $r_p = -0.12$; $n = 38$; $P = 0.49$; parthenogens: $r_p = -0.03$; $n = 30$; $P = 0.87$), indicating that individuals did not lose weight or that larger or smaller individuals were more prone to infection. Large individuals produced a larger total cocoon volume in parthenogens ($r_p = 0.56$; $n = 30$; $P = 0.001$), but not in sexuals ($r_p = 0.22$; $n = 38$; $P = 0.19$). Total cocoon volume did not decrease with log amoeba parasite count (sexuals: $r_p = -0.160$; $n = 38$; $P = 0.34$; parthenogens $r_p = 0.134$; $n = 30$; $P = 0.48$). This suggests that parasites did not influence resource allocation to (female) reproduction. Fertility, however, decreased with log amoeba count in parthenogens ($r_p = -0.385$; $n = 30$, $P = 0.036$, Fig. 4), but not in sexuals ($r_p = 0.163$; $n = 38$; $P = 0.33$). This coincides with an increase in SD of offspring size with log amoeba count in parthenogens ($r_p = 0.445$; $n = 27$; $P = 0.020$; Fig. 5), but not in sexuals ($r_p = -295$; $n = 38$; $P = 0.073$). Figures 4 and 5 suggest that this effect may be the result of a cluster consisting of a large number of parthenogens with many amoebae and lower overall fertility.

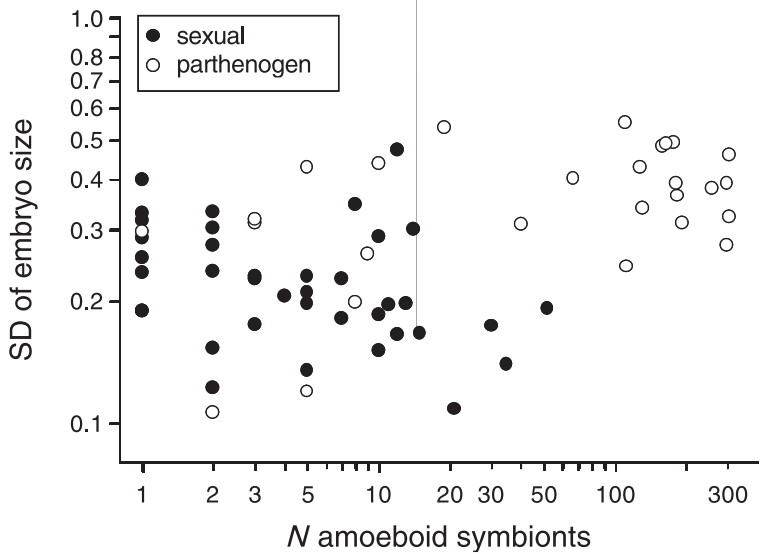

Fig. 5 Log SD of viable offspring size as a function of log amoeba count. Filled circles: sexuals; open circles: parthenogens. One (parthenogenetic) outlier with an SD of 1.39 was excluded.

Discussion

Despite strong temporal and spatial fluctuations, parthenogenetic *S. polychroa* had a consistently higher likelihood of being infected than sexuals in localities where both coexist. When comparing infected individuals only, parthenogens had up to 12 times as many parasites than sexuals. Infection rates and parasite counts varied more in time and space in parthenogens than in sexuals. This may be explained by the fact that parasitism was found to be genotype specific in parthenogens, and that these genotypes have a highly heterogeneous spatial distribution within Lago di Caldonazzo (Pongratz *et al*., 1998). Cost of infection in terms of body shrinkage or fecundity loss could not be detected within a 3-week laboratory experiment, but heavily parasitized parthenogens had lower fertility. Whether the amoebae cause the latter effect is unknown but appears unlikely for the following reasons.

The absence of an effect on fecundity and growth implies that, in order to explain infertility by infection, amoebae would need to have a direct, specific effect on the ovaries or the survival of offspring in cocoons. Yet, amoebae were never seen to be associated with the reproductive system, neither with the two (small) ovaries nor with the numerous yolk glands. Infection of cocoons by amoebae as an explanation for infertility appears equally unlikely, as this should result in infection of all offspring within a cocoon. Unviable embryos typically appear in low numbers next to otherwise normal siblings in the same cocoon, a pattern already described by Storhas *et al*. (2000). They suggested that embryo mortality may be because of accumulation of deleterious mutations in parthenogens. This possibility is currently under investigation.

Despite the absence of clear fitness costs caused by amoeba infection, we nevertheless propose to consider the symbionts 'parasitic', which is also traditional for amoebae facultatively infecting multi-cellular animals.

Red Queen?

At first sight, our results appear to confirm basic predictions of the Red Queen hypothesis: parthenogenetic individuals were more parasitized and infection was genotype-specific. Unfortunately, we had no opportunity to establish whether time-lagged genotype-specific parasite-host dynamics exist (Dybdahl & Lively, 1995a, 1998). An indirect indication may be that the second most common clone had highest infection rates (Table 1). One might speculate that this clone may have been more frequent in the past, and was now being displaced by an uninfected clone that used to be rare.

Some important requirements for the Red Queen hypothesis, however, were not fulfilled. Most importantly, amoeba infection caused weak, if any, fitness costs. The time window may have been too short for more distinct effects. Yet, the Red Queen requires considerable virulence in order to yield an advantage to sexuals that is big enough to compete with asexuals (Howard & Lively, 1998). It has to be emphasized that this is the case when no other factor (e.g. mutations) can be invoked.

Another discrepancy with the Red Queen is the fact that the parasite considered here is free-living and only facultatively parasitic (Page & Siemensma, 1991). Although genetic recombination is known from the amoeba *Naegleria* (Cariou & Pernin, 1987) it is generally accepted that reproduction in the Gymnamoebea is predominantly asexual. Facultative parasitism and asexuality may slow down adaptation of parasites to specific host genotypes. More knowledge of the biology and reproduction of the amoebae is needed to shed light on this aspect.

The current data do not allow us to explain the discrepancy between the observed pattern and theory in more detail. Yet, the link with infertility suggests an intriguing possibility. If parthenogens suffer from higher embryo mortality, for instance because of accumulation of deleterious mutations or polyploidy, it may be that their immunocompetence is compromised as well. An important alternative explanation is therefore that parthenogens suffer from reduced immunocompetence, facilitating infection by unspecific and facultative parasites. In the current study, such a link is suggested by the observation that amoebae were more common in parthenogens with low fertility and high variance in hatchling size. The clustered appearance of the data points in Figs 4 and 5 also suggests genotype-specificity, which is likely, given the fact that parasitism on its own is genotype specific (Table 2) and that the same applies to embryo mortality (M. Storhas, K. Carter and N. K. Michiels, in preparation). The fact that amoeba number did not increase in sexuals, whereas it did in parthenogens in the course of the experiment, also hints at immunocompetence problems in the latter. If true, long-term data should reveal that particular parthenogenetic genotypes are always heavily infected, irrespective of their relative frequency in the population.

Acknowledgments

Thanks to Martin Storhas, Hinrich Schulenburg, Iris Vorndran and two anonymous referees for useful comments on earlier drafts. This study greatly benefited from interactions and assistance from all members of our former research group at the Max Planck Institute for Behavioural Physiology in Seewiesen, in particular Jaco Greeff, Letizia Gerace, Angel Martin Alganza, Tim Sharbel, Andrea Streng, Martin Storhas, Babsi Wehner and Rolf Weinzierl. Thanks also to all the participants to the Summer School on the Evolution of Sex in August 1997, for a very stimulating meeting.

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Received 10 August 2000; accepted 20 October 2000