

Temporal and spatial distributions of parasites and sex in a freshwater snail

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ABSTRACT

The Red Queen hypothesis predicts that sexual reproduction should be favoured in geographic locations where the risk of infection is high. We surveyed 20 lakes on the South Island of New Zealand to determine whether an association exists between the presence of individuals sterilized by trematode larvae and the presence of males in a common freshwater snail (*Potamopyrgus antipodarum*). The snail is unique in that populations are often mixtures of triploid asexual females that reproduce by apomictic parthenogenesis and diploid sexual individuals that reproduce by cross-fertilization. We compared the results to a similar study conducted over 10 years ago. The results showed a highly significant correlation between the frequency of males in the past and present data. In addition, the results showed a strong significant correlation between the prevalence of trematodes in past and present data sets, indicating that any selection imposed by the parasites has remained reasonably constant. Finally, the frequency of males averaged over both data sets was significantly and positively correlated with the mean frequency of infected individuals. These results suggest that infection levels and male frequency may be relatively stable and that parasites may be a factor in selecting for sexual reproduction.

Keywords: *Microphallus*, New Zealand, *Potamopyrgus antipodarum*, Red Queen hypothesis, sexual reproduction.

INTRODUCTION

The presence of sexual reproduction remains an unsolved problem in evolutionary biology (Williams, 1975; Maynard Smith, 1978; Bell, 1982). The problem is especially clear in populations where sexual and asexual females co-exist and sexual females invest equally in male and female offspring. All else being equal, the asexuals should eliminate the sexuals, given the two-fold reproductive advantage of asexual reproduction (Maynard Smith, 1978). One possible solution, known as the Red Queen hypothesis, is that parasites evolve to infect the most common host genotypes; as such, they select against any asexual form of reproduction once the clone has become common (Jaenike, 1978, Bremermann, 1980; Hamilton, 1980, 1982; Lloyd, 1980; Hamilton *et al.*, 1990). The hypothesis has proven difficult to test

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directly, but it makes several falsifiable predictions. One key prediction is that, if wholly parthenogenetic (or self-fertilizing) populations exist, they should be found in locations that are relatively free of co-evolving parasites (Bell, 1982).

This prediction has support from a variety of studies (Glesener and Tilman, 1978; Bell, 1982; Lively, 1987, 1992; Schrag *et al.*, 1994). In one of these studies, sexual reproduction in a freshwater snail (*Potamopyrgus antipodarum*) was found to be positively and significantly correlated with prevalence of infection by larval trematodes (Lively, 1992). One weakness of this study, however, was that the data were collected in a short window of time, so it is unknown whether the pattern is temporally robust. Here we repeated the original survey by sampling more intensively a subset of the original populations ($n = 20$). Our main aims were: (1) to determine whether the frequency of sexual hosts or the prevalence of infection had changed over the 10–15 years between surveys; and (2) to determine whether the previously observed correlation between sex and infection was repeatable.

NATURAL HISTORY OF THE HOST AND PARASITE

We focus on the most common trematode that infects the snail. This species is presently undescribed, but is probably a new species in the genus *Microphallus* (S. Deblock, personal communication); we refer to this species here and elsewhere as *Microphallus* sp. Previous data show that *Microphallus* sp. infects *P. antipodarum* throughout New Zealand and that there is considerable gene flow among geographically separated lake populations of the worm (Dybdahl and Lively, 1996). Hence, the different populations of *Microphallus* do not appear to represent different species, although they show strong adaptation to local host populations (Lively, 1989).

The worm has a two-host life cycle. Snails are infected by ingesting eggs. A successful infection yields several hundred asexually produced larvae, which encyst in the host snail. Both sexes of this gonochoric, Prosobranch snail are sterilized during the course of the development of these larval cysts. The cysts (called metacercariae) ‘hatch’ when ingested by a final, vertebrate host (known to include dabbling ducks and black stilts) to yield mature hermaphroditic adults in about 24 h. The tiny worms (0.2 mm long) then produce eggs by cross-fertilization (as inferred from genetic data; Dybdahl and Lively, 1996), which are infective to *P. antipodarum*. The risk of infection is expected to vary among locations if the abundance of the final hosts also varies.

The host snail is a small (4–7 mm long) gastropod that is common in New Zealand’s freshwater habitats below tree line. Both sexuals and asexuals brood eggs in the mantle until they hatch and the young crawl out of the brood pouch. Asexual females are triploid and reproduction is by apomixis (Dybdahl and Lively, 1995). Sexual females are diploid and cross-fertilization is required for the production of young. Sexual and asexual females taken from the same lake and habitat mature at the same size and they produce the same number of offspring (Jokela *et al.*, 1997a). The frequency of developmental defects is also the same. Hence, there would appear to be a full two-fold cost of sexual reproduction.

METHODS

We selected lakes that were among the most intensively sampled between 1985 and 1988 (Table 1). These glacial lakes, which were all formed following the Pleistocene, are distributed on both sides of the Southern Alps in the central part of New Zealand’s South

Table 1. Sampling locations

Lake	Early mean (<i>n</i>)	Late mean (<i>n</i>)	Difference
Alexandrina	1986 (10)	2000 (20)	14
Catherine	1988 (1)	2001 (1)	13
Clearwater	1988 (2)	2000 (3)	12
Grasmere	1985 (2)	1998 (4)	13
Hauptiri	1987 (5)	1998 (4)	11
Hawdon	1987 (2)	1998 (11)	11
Ianthe	1987 (6)	2000 (2)	13
Ida	1987 (3)	1997 (2)	10
Kanieri	1986 (4)	1998 (9)	12
Katrine	1987 (1)	2001 (4)	14
Mapourika	1987 (11)	2000 (4)	13
Marymere	1987 (3)	2000 (3)	13
Moeraki	1988 (2)	2000 (3)	12
Paringa	1987 (2)	2000 (3)	13
Poerua	1987 (4)	2000 (1*)	13
Sarah	1985 (3)	1998 (6)	13
Selfe	1988 (1)	1997 (6)	9
Sumner	1987 (1)	2001 (2)	14
Taylor	1987 (1)	2001 (4)	14
Tennyson	1988 (5)	1996 (6)	8

Note: Entries give the lake names, the weighted mean year for the early (1985–88) and late (mostly 1997–2001) samples with sample sizes in parentheses. The difference between means is given in the last column. The average difference in years between the weighted means was 12.2.

* 234 snails from a 400 m transect along the shore.

Island. For these lakes, the correlation between (log) male frequency and (log) *Microphallus* prevalence was not statistically significant in the original survey ($r = 0.333$; $n = 20$; $P = 0.152$), although the correlation was significant in the larger sample (Lively, 1992).

We sampled the shallow-water (<0.5 m) margins of the lakes by washing snails from small rocks into kick nets, or by pushing the nets through the shallow-water vegetation. Most lakes were sampled between 1998 and 2001, with the exception that one of the lakes was sampled in 1995 and 1996 and two of the lakes in 1997. Since we knew from previous samples that there can be considerable spatial variation for infection in shallow water (Jokela and Lively, 1995a,b), we sampled from one to 20 (usually four) locations in each lake (depending on lake size and access). We then transported the snails to a field station in Kaikoura, New Zealand, where the snails were dissected ($n > 100$ for each sample). During dissection, we recorded the gender of each snail and whether it was infected by trematode larvae. We used male frequency as our measure of the frequency of diploid sexual females, as these variables are highly positively correlated (Fox *et al.*, 1996). The samples were then averaged to give a single datum for each lake; the data were log-transformed to meet the assumptions of parametric tests (Sokal and Rohlf, 1981). We report two-tailed probability values.

RESULTS

The results showed a strong positive correlation between male frequency in the early and late samples ($r = 0.875$; $n = 20$; $P < 0.001$). This finding indicates that lakes having high levels of sexual reproduction in the 1980s still have high levels of sexual reproduction (Fig. 1). Similarly, there was a strong positive correlation between the level of infection by *Microphallus* between the two sampling periods (Fig. 2), indicating that lakes previously having high levels of infection still have high levels of infection ($r = 0.864$; $n = 20$; $P < 0.001$). Thus, it would appear that, at least over this time period of 10–15 years, any selection that may have been imposed by parasites has remained reasonably constant. Finally, we found that the frequency of males and prevalence of *Microphallus* were positively and significantly correlated in the new survey ($r = 0.452$; $n = 20$; $P = 0.046$), which is consistent with the results from the earlier survey.

Based on these results, we reasoned that our best measures of parasite prevalence and male frequency would be the means of both sampling periods. Interestingly, these means for male frequency give a bimodal distribution, which is apparent from inspection of Fig. 1. One mode exists at very low male frequency. The mean (\pm standard error) of the populations associated with this mode is only 2.11 ± 0.43 . This low-male group is likely to represent all clonal populations, since some clones are known to produce up to 6% males. The populations associated with the second mode exists at a much higher male frequency (23.49 ± 2.41). This high-male group probably represents populations composed of both sexual and asexual individuals ('mixed' populations; see Dybdahl and Lively, 1995).

We then compared the prevalences of infection between these two groups of populations. The high-male group had a higher mean prevalence of infection by *Microphallus* (7.49 ± 2.05) than the low-male group (2.49 ± 0.60), and the difference between means

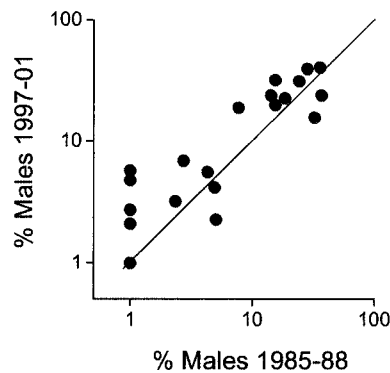


Fig. 1. Bivariate plot of *P. antipodarum* male frequency from samples collected in the mid-1980s against male frequency for samples collected primarily between 1997 and 2001. The solid line shows the line of no change; deviations from the line give the differences between the early and late samples. Note that the points fall into two groups: one group for which male frequency is less than 6% for both samples and another group for which male frequency is greater than 15%. The former (low-male) group is likely to represent all clonal populations, since some clones are known to produce up to 6% males. The latter (high-male) group represents populations composed of sexual and asexual individuals (mixed populations).

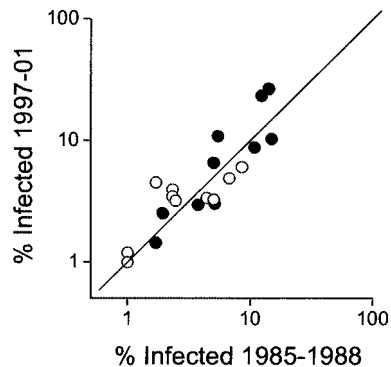


Fig. 2. Bivariate plot of prevalence of infection by *Microphallus* from samples collected in the mid-1980s against prevalence of infection for samples collected primarily between 1997 and 2001. The solid line shows the line of no change; deviations from the line give the differences between the early and late samples. Open circles show asexual populations; filled circles show mixed populations.

(log-transformed data) was statistically significant ($F_{1,18} = 4.74$; $P = 0.043$). The result can be seen in Fig. 2, which shows the prevalence of infection in 'mixed' and asexual populations for both sampling periods. The same result was obtained for total prevalence of infection for all trematode species. The congruence of results, however, is not surprising given that total prevalence and *Microphallus* prevalence are highly correlated.

DISCUSSION

In previous studies, we have used the snail *P. antipodarum* to test the hypothesis for the evolutionary maintenance of sexual reproduction. At present, our results provide no evidence in support of the lottery model (Lively, 1987), which is consistent with Bell's (1982) broad taxonomic survey of the literature and with Schrag and co-workers' (1994) study of the distribution of outcrossing potential in a hermaphroditic snail. Our previous results are also consistent with Bell's finding that sex is more common in stable habitats, which is consistent with both the tangled bank model and the Red Queen model, assuming parasites are also more common in stable habitats (Lively, 1987). However, our results also show that parasites account for more of the variation in male frequency than habitat *per se*, which favours the Red Queen model, but does not eliminate the tangled bank hypothesis (Lively, 1987). Finally, we have found no support for the deterministic mutation hypothesis (Kondrashov, 1988; Charlesworth, 1990; Howard, 1994), at least in terms of how it could be used to explain the distribution of parthenogenesis in these snails (Lively *et al.*, 1998).

The results of the study are also consistent with expectation under the Red Queen hypothesis. We found that the prevalence of infection by a digenetic trematode was correlated between samples taken over a 10–15 year period (Fig. 2), which suggests that any selection imposed by this worm is relatively constant over time. If this were not the case, it would be difficult to see how the parasites could be responsible for the maintenance of sex in the highly sexual populations. Similarly, we found that male frequency was highly correlated

between the two sampling periods and that there was no overall tendency for males to have decreased over time (Fig. 1).

In addition, we found that male frequency and parasite prevalence were positively correlated in both the new data set and over both data sets combined. We find the results from the combined data set to be particularly interesting, as it should give the best estimates of the variables, and because male frequency broke cleanly into two groups of populations: low-male populations, which are probably composed of asexual individuals, and high-male populations, which are at least partially sexual. Our finding that the high-male populations also have a significantly higher mean level of infection is consistent with the Red Queen hypothesis.

However, the result is also consistent with alternative hypotheses. For example, sex and parasites would be expected to covary if sexual individuals, which are diploid, were more susceptible to parasites than asexual individuals, which are triploid. We previously examined this idea in one lake where sexuals and asexuals co-exist, but found no indication that diploid individuals are inherently more infected by parasites (Jokela *et al.*, 1997b) or that males are inherently more infected than females (Lively, 1987, 2001). Hence, it is unlikely that the association between sexual reproduction and parasites in the wild is a simple consequence of sexuals being more susceptible to infection. In addition, we have found in laboratory experiments that parasites are adapted to infecting the most common triploid clones in a population composed entirely of asexual individuals (Dybdahl and Lively, 1998). Hence, even if ploidy has some effect on inherent infectivity, it would not appear to be sufficient to prevent parasite tracking of locally common triploid genotypes. Similarly, using reciprocal cross-infection experiments, we have found that parasites from a mostly diploid host population were more likely to infect snails from this same population than a remote triploid population of hosts and vice versa (Lively and Dybdahl, 2000). Thus, local co-evolution would appear more important than any inherent difference in susceptibility between diploids and triploids, even if such differences do exist.

Other alternatives to explain the observed association between sex and parasites will surely exist. These might be profitably sought in those lakes with high male frequencies and low parasite loads during both sampling periods (Fig. 2). However, in our minds, the existence of such alternatives would not undermine the need for biogeographic data. We feel that solid data on the distribution of asexual populations has been, and will continue to be, important to the evaluation of the different hypotheses for sex (e.g. Glesener and Tilman, 1978; Bell, 1982; Little and Ebert, 1999; Michiels *et al.*, 2001; see Kondrashov, 1993, for an alternative view). Our approach has been to combine field surveys with laboratory experiments. Based on our results to date, we cannot eliminate the Red Queen hypothesis.

In closing, we should point out an important subtlety. The Red Queen hypothesis predicts that sexual reproduction should be found where the *risk* of exposure to virulent parasites is high, but we have measured *prevalence* of infection instead of risk. It is reasonable to suspect that risk of exposure was underestimated. This may be especially true if, in fact, the Red Queen hypothesis is correct, because greater levels of sex reduce the levels of infection for any given value of risk (Lively, 2001). Thus, our measures may be disproportionately conservative for sexual populations. It would be interesting to discover whether the association between sex and parasites reported here would become stronger if risk of parasite exposure could be directly determined.

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