Fine-scale association between parasites and sex in *Potamopyrgus antipodarum* within a New Zealand lake

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Abstract: Why sexual reproduction is so common despite major costs remains a widely debated evolutionary question. One of the most plausible potential explanations is the Red Queen hypothesis, which proposes that coevolving parasites can generate a selective advantage for sex. This hypothesis predicts that sexual reproduction should be most common where individuals experience a relatively high risk of parasitic infection. Here, we test this prediction at a very fine spatial scale by evaluating whether variation in the frequency of infection by sterilising trematode parasites within a single New Zealand lake population is positively associated with similar variation in the relative frequency of sexually vs. asexually reproducing *Potamopyrgus antipodarum*, a native New Zealand snail. We found that sexual snails were significantly more common in sites within the lake that were subject to a higher frequency of parasite infection, consistent with the prediction of the Red Queen hypothesis. This result extends previous research on the connection between sexual reproduction and infection in *P. antipodarum* to a new lake and a finer spatial scale than previously documented.

Keywords: asexual; coevolution; Lake Grasmere; parasites; Red Queen; sexual reproduction; snail; trematodes

Introduction

Potamopyrgus antipodarum is a freshwater snail native to New Zealand that is characterized by reproductive mode polymorphism, with obligately sexual and obligately asexual individuals coexisting at variable relative frequencies in many lake populations (Lively 1987). Why the sexual individuals persist at all and what accounts for the across-lake variation is puzzling in light of the major costs of sexual reproduction (e.g. male production) relative to asexual reproduction (Maynard Smith 1978). There is clear evidence that some of the across-lake variation in the relative frequency of sexual *P. antipodarum* in New Zealand is associated with the frequency of *P. antipodarum* infected by sterilising trematodes. In particular, multiple studies have shown that asexual *P. antipodarum* tend to be more common in New Zealand lakes where the snails experience lower frequencies of infection by the sterilising trematode *Microphallus* sp. (e.g. Lively 1987, 1992; Lively & Jokela 2002). A similar pattern has been documented for New Zealand stream populations of this snail-trematode system (King et al. 2011a). These results are broadly consistent with the expectations of the Red Queen hypothesis for sex (Jaenike 1978; Hamilton 1980; reviewed in Neiman & Koskella 2009), which postulates that virulent parasites can impose negative frequency-dependent selection by favouring rare hosts, thereby giving an advantage to sexually vs. asexually produced individuals.

While many of the studies linking trematode infection and sexual reproduction in *P. antipodarum* are focused on comparing the frequency of parasitism and the relative frequency of sex at an across-lake scale (e.g. Lively 1987,

1992; Lively & Jokela 2002; Neiman et al. 2005), several studies at the within-lake level (Jokela & Lively 1995a, b; King et al. 2009; Vergara et al. 2013; Paczesniak et al. 2014) have revealed that fine-scale variation in the relative frequency of sexual *P. antipodarum* is also associated with parasitism. Most notably, multiple empirical studies (King et al. 2009, 2011b; Vergara et al. 2013) find a higher frequency of sexual *P. antipodarum* in shallow vs. deep habitats in some lakes. This pattern is likely driven by a higher intensity of coevolutionary interactions between *Microphallus* and *P. antipodarum* in the shallow regions of these lakes (Vergara et al. 2013), which itself may arise from the shallow-water foraging of the dabbling ducks that are *Microphallus*'s final host (King et al. 2009). Nearly all of these within-lake studies have focused on Lake Alexandrina (MacKenzie District, Canterbury, South Island; e.g. Jokela & Lively 1995a, b; Paczesniak et al. 2014; Vergara et al. 2014); whether the patterns uncovered in Lake Alexandrina are broadly generalizable to other New Zealand lakes remains unclear.

Here, we provide an important extension of this body of work by quantifying the relative frequency of sexual reproduction and trematode infection in *P. antipodarum* in another South Island lake, Lake Grasmere (-43.062 S, 171.77 E). Because asexual *P. antipodarum* are nearly always female (>95%; Neiman et al. 2012) while sexual *P. antipodarum* are typically ~50% male and ~50% female (Wallace 1992), the relative frequency of males serves as a useful proxy for the relative frequency of sexual reproduction in this system (Jokela et al. 2003). Vergara et al. (2013) sampled *P. antipodarum* populations from a variety of locations and depths within Lake Grasmere from 1985–2008. This study revealed that,

similar to Lake Alexandrina, males were uncommon overall (3.5%, 59 of 1696 adults sampled), and their frequency varied with depth. In shallow habitats, males made up 5.4% of the population (49 of 900 snails sampled), with markedly lower male frequencies at intermediate (1.0%; 3 of 300 snails) and deep (1.4%; 7 of 496 snails) habitats.

Based on these results, we focused on shallow habitats and investigated how the relative frequency of sexual individuals and the frequency of trematode parasitism varied in *P. antipodarum* sampled from the entire periphery of Lake Grasmere. We tested the hypothesis that the relative frequency of males (as a proxy for sexual reproduction) would be positively correlated with the frequency of trematode infection even within a single relatively small lake (0.63 km^2 vs. Lake Alexandrina, which is 6.4 km^2).

Methods

Lake Grasmere is a well-studied glacial lake (Stout 1975) located at 583 m elevation near the upper Waimakariri River in the central South Island. The adjacent terrestrial habitat ranges from steep forested slopes on the northeast side to swampy open areas on the southwest (Figure 1). A more complete description of the terrestrial vegetation surrounding the lake can be found in McClymont (1997).
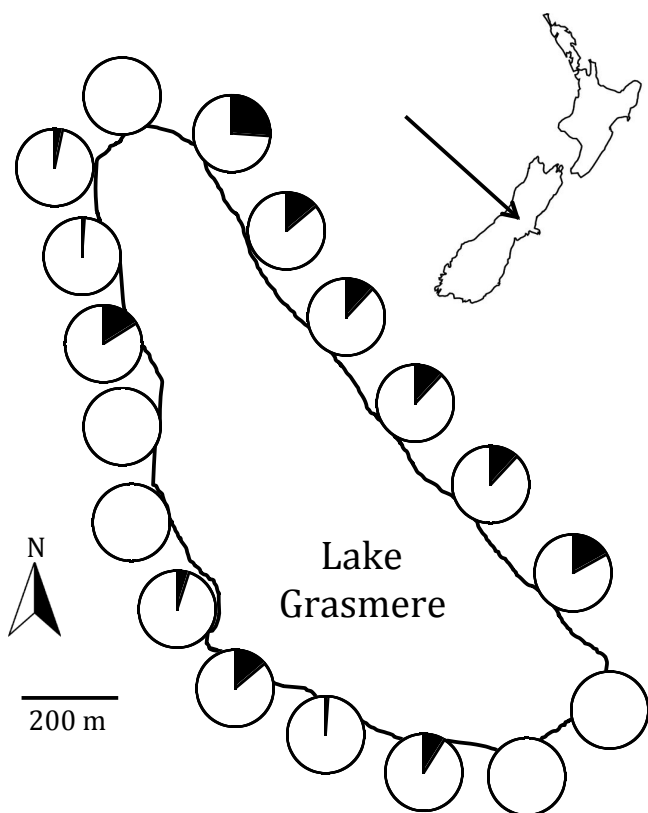
Figure 1. The relative frequency of males (black; proxy for the relative frequency of sexual *P. antipodarum*) and females (white) in samples of about 100 snails from 18 locations spaced approximately 150–200 metres apart around the periphery of Lake Grasmere. Map locations are based on field observations and are only approximate. The northeast side of the lake (with six sample points) is bordered by steep forested slopes; the rest of the lake periphery is more open, bordered by grasses, sedges, and wetlands. Location of Lake Grasmere indicated in the inset.

In January 2014, we collected snails from 18 sites that were approximately evenly spaced around the perimeter of Lake Grasmere (Figure 1), about 150–200 m apart. Sites on the steep northeast side of the lake were accessed by boat, and sites around the rest of the lake were accessed by foot.

Approximately 100 adult *P. antipodarum* were collected from each site from rocks near the shore and < ~0.5 m below the surface. This depth corresponds to the 'shallow' habitat as defined in previous investigations of *P. antipodarum* in New Zealand lakes (e.g. Vergara et al. 2013; Paczesniak et al. 2014). Snails were collected from a minimum of five different rocks per sampling site.

We then brought the *P. antipodarum* samples to the laboratory to quantify the relative frequency of male vs. female snails in each sample and the frequency and type of trematode infection. We began by using a dissecting microscope to first sex each snail (males have a penis behind their right tentacle) and then opened the body cavity of the snails to determine whether they were infected with sterilising trematode parasites. *P. antipodarum* serves as the first intermediate host for at least 20 sterilising trematode species (Winterbourn 1973; Hechinger 2012). All types of trematode observed were included in counts of parasite infection. The two most common types found in our samples were identifiable to genus: *Microphallus* sp. and *Notocotylus* sp., both of which use waterfowl as their definitive host (Osnas & Lively 2011).

We used chi-square analyses to address whether there were significant across-site differences in the relative frequency of males and trematode-infected snails, respectively. We then used Spearman rank correlations to test for an association of the relative frequency of males with both the total prevalence of sterilising trematodes and the prevalence of *Microphallus* infection among our 18 samples. We performed this second Spearman analysis because *Microphallus* in particular has been implicated as a likely player in the maintenance of sexual reproduction in many previous *P. antipodarum* studies (e.g. Lively 1987; Jokela et al. 2009; Vergara et al. 2014).

Results

The frequency of male snails differed significantly among the 18 sample sites (chi-square = 142.9, 17 d.f., *p* < 0.0001), ranging from 0% males (five sites) up to 26% males (one site). Males were particularly common in six samples from the forested northeast side of the lake (Figure 1) but also occurred in a variety of other locations.

Of the 1800 snails that we sampled, 91 (~5.1%) were infected with sterilising trematodes. Of the 44 parasites that could be identified with confidence, 30 were *Microphallus* and 14 were *Notocotylus*. Parasitised snails were non-randomly distributed among sites (chi-square = 40.0, 17 d.f., *p* = 0.0013), with a range from 0% to 14% parasitised individuals.

There was a positive association between the frequency of infection by trematode parasites and the relative frequency of males among the 18 sites (Spearman rank correlation, ρ = 0.593, *p* = 0.009) (Figure 2). The association remained when the infection rates of females only were used (ρ = 0.643, *p* = 0.004), demonstrating that this parasite-sex connection was not driven by potential differences in susceptibility between males and females (also see Vergara et al. 2014). We also detected a significant positive correlation between the frequency of *Microphallus* infection alone and the relative frequency of males (ρ = 0.535, *p* = 0.022).
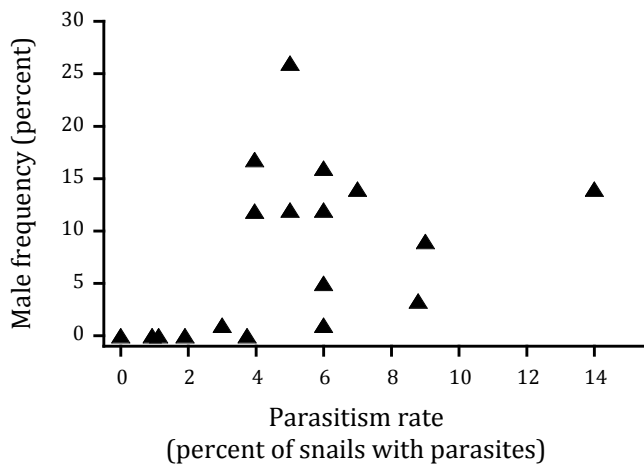
Figure 2. Relationship between the percent of *P. antipodarum* infected with sterilising trematode parasites and the relative frequency of male *P. antipodarum* (a proxy for the relative frequency of sexual *P. antipodarum*) at 18 sites around Lake Grasmere. Two overlapping points in the lower left of the figure were offset slightly for visibility. There was a significant and positive relationship between the prevalence of trematode infection and the relative frequency of male *P. antipodarum* (Spearman rank correlation, ($\rho = 0.593$, $p = 0.009$).

Discussion

We found a significant positive correlation between the frequency of sterilising trematode parasitism and the relative frequency of sexual reproduction in *P. antipodarum* within Lake Grasmere, similar to patterns previously observed in the larger Lake Alexandrina (Jokela et al. 2009; Vergara et al. 2013). In both lakes, pressure from virulent coevolving parasites apparently contributes to the maintenance of sexual *P. antipodarum* on a within-lake spatial scale (e.g. Jokela et al. 2009; Vergara et al. 2013; Paczesniak et al. 2014). Our data extend evidence for the well-documented among-lake association between trematode infection and sexual *P. antipodarum* (e.g. Lively 1987, 1992; Lively & Jokela 2002; Vergara et al. 2013) to a smaller geographic scale within multiple lake populations (Grasmere: this study; Alexandrina: Jokela & Lively 1995a, b; Paczesniak et al. 2014; Vergara et al. 2014).

The correlation between sexual reproduction and parasitism observed within Lake Grasmere is particularly striking in light of the small size of this lake. Lake Alexandrina is 6.4 km² in area, with sampled locations often separated by multiple kilometres (see Figure 1 of Jokela & Lively 1995b). By contrast, Lake Grasmere is only 0.63 km² and our adjacent samples were <200 m apart. Significant variation in both the relative frequency of sexual *P. antipodarum* and the frequency of parasitism were nonetheless evident on this scale (Figure 1).

Why do the rates of trematode infection vary so widely among sites around Lake Grasmere? Variation in prevalence of trematode infection in shallow-water populations of *P. antipodarum* in other lakes has been tied to the distribution of the dabbling ducks that act as the final host for *Microphallus* sp. and other sterilising trematodes (Lively & Jokela 1996; Jokela et al. 2009; King et al. 2009). It is possible that the pattern of parasite abundance among sites is similarly related to the abundance of ducks in different areas of Lake Grasmere.

Some insight into this question is provided by McClymont (1997), who carried out extensive surveys of the birds present

at Lake Grasmere from March 1995 through January 1996. Duck species used as final hosts by trematode parasites of *P. antipodarum* (Osnas & Lively 2011) were commonly observed (McClymont 1997), especially New Zealand scaup (*Aythya novaeseelandiae*) and *Anas* species; including grey ducks (*A. superciliosa*), mallards (*A.* platyrhynchos) and their hybrids. McClymont (1997) divided the lake into five separate zones and found that host duck abundance varied significantly among these zones. Such within-lake variation in duck host abundance could have influenced the site-to-site variation in trematode abundance that we observed. Quantification of bird distribution on the scale of our sites (150–200 m apart) instead of the larger scale used by McClymont (each of her five zones encompassed at least three of our study sites) would be necessary to determine if bird host distribution is likely to contribute directly to the varying abundance of trematode infection in Lake Grasmere.

Conclusions

Population genetic (Dybdahl & Lively 1995; Paczesniak et al. 2013) and phylogenetic (Neiman & Lively 2004; Neiman et al. 2011; Paczesniak et al. 2013) data suggest that asexual *P. antipodarum* are generated repeatedly by sexual *P. antipodarum*. The implications are that any sexual *P. antipodarum* population is potentially subject to invasion by asexual descendants. The within- and across-population links between trematode infection frequency and the relative frequency of sexual *P. antipodarum* documented here and in previous work suggests that local parasite pressure imposes selection that can effectively prevent asexual lineages from being successful. The fine-scale spatial association between sex and parasitism that we detected in Lake Grasmere suggests that parasite-imposed selection pressures vary markedly at a very local level. Further investigation at such fine spatial scales will provide critical tests of the explanatory power of the Red Queen hypothesis for the maintenance of sex in this system.

Acknowledgments

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