Published in final edited form as:

Am Nat. 2018 November ; 192(5): 537–551. doi:10.1086/699829.

Periodic, parasite-mediated selection for and against sex

Amanda K Gibson^{1,2,*}, Lynda F Delph¹, Daniela Vergara^{1,3}, Curtis M Lively¹

¹Department of Biology, Indiana University, Bloomington, IN, USA

²present address: Department of Biology, Emory University, Atlanta, GA, USA

³present address: Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA

Abstract

Asexual lineages should rapidly replace sexual populations. Why sex then? The Red Queen hypothesis proposes that parasite-mediated selection against common host genotypes could counteract the per-capita birth rate advantage of asexuals. Under the Red Queen, fluctuations in parasite-mediated selection can drive fluctuations in the asexual population, leading to the coexistence of sexual and asexual reproduction. Does shifting selection by parasites drive fluctuations in the fitness and frequency of asexuals in nature? Combining long-term field data with mesocosm experiments, we detected a shift in the direction of parasite selection in the snail *Potamopyrgus antipodarum* and its coevolving parasite *Microphallus* sp. In the early 2000's, asexuals were more infected than sexuals. A decade later, the asexuals had declined in frequency and were less infected than sexuals. Over time, the mean infection prevalence of asexuals equaled that of sexuals, but varied far more. This variation in asexual infection prevalence suggests the potential for parasite-mediated fluctuations in asexual fitness. Accordingly, we detected fitness consequences of the shift in parasite selection: when they were less infected than sexuals, asexuals increased in frequency in the field and in paired mesocosms that isolated the effect of parasites. The match between field and experiment argues that coevolving parasites drive temporal change in the relative fitness and frequency of asexuals, potentially promoting the coexistence of reproductive modes in *P. antipodarum*.

Keywords

Asexual reproduction; coevolution; evolutionary maintenance of sex; Red Queen hypothesis; sexual reproduction

*corresponding author: amanda.gibson@emory.edu.

Author Contributions

AKG conducted field collections from 2013–2016, ran the experiment from 2013–2016, collected and analyzed the data, and wrote the manuscript; LD conducted field collections, contributed to running the experiment, and collected data; DV collected field and experimental data in 2012; CML conducted field collections, contributed to running the experiment, and conceived and directed the project. All authors contributed to revising the manuscript.

Conflicts of Interest

DV is the founder and president of the non-profit organization Agricultural Genomics Foundation, and the sole owner of CGRI, LLC.

“...it seems to me that we need environmental fluctuations around a trend line of change.” “For the source of these we may look to fluctuations and periodicities... generated by life itself.”

~W.D. Hamilton (1975)

Introduction

Sexual reproduction is not an efficient strategy. Sexual females produce male offspring, while asexual females do not. This investment in sons can reduce the per-capita birth rate of a sexual lineage by one-half relative to that of an asexual lineage (the two-fold cost of males). Rare asexual mutants should thus rapidly increase in frequency, driving sexual lineages extinct in populations where sexual and asexual lineages initially coexisted (Maynard Smith 1971; Maynard Smith 1978). This prediction contrasts with the reality of nature: sexual reproduction is the dominant strategy amongst animal taxa, and obligately asexual lineages are rare and short-lived in evolutionary time (Bell 1982; Suomalainen et al. 1987; Vrijenhoek 1998).

In species where a cost of sex is upheld, coexistence of sexual and asexual forms requires the presence of a strong selective force favoring sex. The Red Queen hypothesis proposes that coevolving parasites impose this force (Haldane 1949; Levin 1975; Jaenike 1978; Hamilton 1980; Bell 1982; Hamilton et al. 1990). The hypothesis assumes that parasites rapidly adapt to infect common clones in a host population (Chaboudez and Burdon 1995; Lively and Dybdahl 2000; Koskella and Lively 2009; Wolinska and Spaak 2009). With a sufficient fitness cost of infection (May and Anderson 1983; Howard and Lively 1994), parasite selection will then drive these common clones down in frequency (Dybdahl and Lively 1998; Jokela et al. 2009; Koskella and Lively 2009). On the other hand, sex enables the continual production of rare genotypes, which are more likely to evade infection by coevolving parasites (Haldane 1949; Jaenike 1978; Hamilton 1980; Bell 1982). The Red Queen hypothesis therefore predicts that asexual lineages will be more infected than sympatric sexual lineages (i.e. over-infected), at least periodically. In a mixed population of sexual females and multiple asexual clones, the hypothesis predicts that the asexual subpopulation as a whole should be periodically more infected than the sexual subpopulation.

The Red Queen hypothesis is founded upon a temporally dynamic coevolutionary process. When we consider these temporal dynamics, the Red Queen hypothesis makes an unusual and often overlooked prediction: asexual lineages can periodically be less infected than their sympatric sexual lineages (as in Lively 2009). The predicted coevolutionary sequence is as follows: if parasites can drive common clones down in frequency, the asexual subpopulation may experience periods of rarity. A core assumption of the Red Queen hypothesis is that parasites are under selection to preferentially infect common host genotypes. Lineages within the rare asexual subpopulation would then, on average, evade coevolving parasites. This pattern would manifest as asexual under-infection, meaning that the prevalence of infection for asexual individuals is less than the population mean and, by extension, less than that for coexisting sexuals. Over time, we expect to see fluctuations in asexual infection prevalence, from over-infection (greater than the population mean) to under-infection (less

than the population mean), resulting in higher variance in infection prevalence for asexuals relative to sexuals (see Fig. 4 in Lively 2009). When the asexual population is under-infected, asexuals (as a group) should increase in frequency. If they fail to do so, we could reject the Red Queen hypothesis, as some force other than parasites must be preventing asexuals from spreading and outcompeting sexuals.

Our long-term monitoring of a natural host-parasite system provided us with the opportunity to evaluate these predicted shifts in the prevalence and parasitism of asexual lineages. In the freshwater snail *Potamopyrgus antipodarum*, sexual females pay a two-fold cost of sex (Gibson et al. 2017). Yet sexual and asexual females commonly coexist in natural populations (Lively 1987; Lively 1992). Field and experimental studies consistently support the underlying assumptions of the Red Queen hypothesis: *Microphallus* rapidly evolves to infect common clones in both laboratory experiments and in natural populations, driving them down in frequency (Dybdahl and Lively 1998; Jokela et al. 2009; Koskella and Lively 2009). A long-term field study at our focal lake, Lake Alexandrina, supported the Red Queen's prediction of periodic over-infection of asexual lineages: asexual females were significantly more infected than sexual females from 2001 to 2004 (Vergara et al. 2014). In 2005, however, the pattern reversed: asexual females were significantly less infected than sexual females (Vergara et al. 2014). The latter result is interesting because it shows that asexuals are not inherently more susceptible to infection (see also Lively et al. 2004). It also suggests that there may be periodicity in selection for and against sexual reproduction that plays out over long periods of time.

In the present study, we repeated the survey, starting about one decade after the beginning of the first survey. We combined the survey with a mesocosm experiment, where we could compare populations with and without exposure to infection. First, we compared the first (2001–2005) and second (2012–2016) field collections to test the prediction that the asexual subpopulation becomes under-infected as it declines in frequency. In juxtaposing these two distinct time periods, we aimed to capture the general trends that characterize this highly dynamic natural system. Second, we tested the critical prediction that asexual females increase in frequency when the asexual subpopulation is under-infected. We paired field observations with a concurrent mesocosm experiment that manipulated hosts and parasites from the natural population to measure the direction and magnitude of parasite selection on asexual fitness from 2012–2015. Throughout, we compared the sexual subpopulation against the asexual subpopulation as a whole, rather than against individual clonal lineages. This approach is the most direct way to measure the overall performance of sexual vs. asexual reproduction. Our results suggest that there are long-term periodicities in parasite-mediated selection, both for and against sex.

Methods

The raw data for this research and the R codes for statistical analysis are available at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.77v1s92> (Gibson et al. 2018b) and the GitHub repository [periodic_parasites](#) (Gibson et al. 2018a).

Natural history

The prosobranch snail *Potamopyrgus antipodarum* is native to New Zealand, where it dominates the invertebrate communities of sub-alpine streams and lakes (Forsyth and McCallum 1981; Biggs and Malthus 1982; Talbot and Ward 1987). In many populations, obligately sexual males and females coexist with obligately asexual females, while other populations are strictly asexual (Winterbourn 1970; Lively 1992). In mixed populations, the asexual subpopulation of a lake comprises a diversity of clonal lineages that have arisen from the local sexual lineages (Dybdahl and Lively 1995; Fox et al. 1996; Paczesniak et al. 2014). The transition to asexual reproduction is associated with an increase in ploidy: sexual males and females are diploid, while asexual females are typically triploid (higher ploidy levels are occasionally found - Neiman et al. 2011). A recent four-year study of experimental mesocosms showed that sexual subpopulations pay a full two-fold cost of males (Gibson et al. 2017). Hence, there must be some countervailing selective force that prevents the fixation of asexual reproduction.

At least 20 different species of digenean trematodes naturally infect *P. antipodarum* (Hechinger 2012). Among these, *Microphallus* sp. is particularly well-studied, and it is by far the most prevalent parasite at Lake Alexandrina (Jokela et al. 2009; Gibson et al. 2016a). Snails ingest eggs of *Microphallus* while foraging. The parasite then replicates within the snail, replacing host tissue with larval trematodes (metacercariae). The snail is ultimately sterilized as its gonads are consumed by the parasite. This severe fitness cost is the primary impact of the parasite on the host. *Microphallus* requires a second host, waterfowl, to complete its life cycle. When a duck (*Anas platyrhynchos*, *superciliosa*, and hybrids) or scaup (*Aythya novaeseelandiae*) ingests a snail infected with metacercariae (Osnas and Lively 2011), the hermaphroditic parasites rapidly mature to adulthood and cross-fertilize in the bird's intestine. The bird then sheds *Microphallus* eggs in its feces.

The coevolutionary interaction of *P. antipodarum* and *Microphallus* has been studied for over 20 years at Lake Alexandrina, a waterfowl preserve. Lake Alexandrina is a small (6.4 km²) glacial lake located at an altitude of 730 m in the Mackenzie Basin of the central South Island of New Zealand. Gibson et al. (2016a; 2016b) and Vergara et al. (2013) describe substantial variation in the strength of host-parasite coevolution within the shallow zone (<0.5 m) of this relatively small lake. The feeding behavior of ducks, the definitive host of *Microphallus*, may drive this site-level divergence by promoting coevolution at sites where they preferentially feed (King et al. 2009; King et al. 2011). This variation in coevolution persists in the face of gene flow at neutral loci: there is no evidence of genetic structure at neutral loci between shallow sites for either clonal or sexual lineages (Fox et al. 1996; Paczesniak et al. 2014).

Field surveys for infection prevalence and reproductive mode

We aimed to replicate the field sampling of Vergara et al. (2014) and to complement the survey with replicated experiments. Every year from 2012 through 2016, we collected snails from the shallow water zones of six sites: the four sites sampled in Vergara et al. (2014) (Camp, Swamp, Halfway and West Point) plus two additional sites (Southwest End and JMS) (Fig. B1). Collections were performed in January and February of each year, with the

exception of the 2013 collections of Southwest End, Camp, JMS, and Halfway – these were performed one month early, in December of 2012. Over the course of the five years of this study, we sampled additional sites at Lake Alexandrina (as reported in Gibson et al. 2016a; Gibson et al. 2016b), but here we have included only those six sites for which we have data for every year.

We obtained large samples of snails by sweeping a net through the vegetation along the bank of the lake. To obtain adult snails, we separated field collections using a 1.7 mm sieve, which retains snails greater than ~3 mm in length (reproductively mature females are > 3.0 mm). We transported live snails to the University of Canterbury's Edward Percival Field Station in Kaikoura, New Zealand and subsequently to Indiana University. We dissected a random subset of adults from each site and determined length, sex, and infection status using a stereomicroscope. We individually froze the head tissue of females at -20°C. Dissections were performed at the Edward Percival Field Station or at Indiana University. Tissue was ultimately stored at -80°C for flow cytometry. Males were assumed to be sexual diploids and not stored for flow cytometry (Neiman et al. 2011).

We used flow cytometry to distinguish sexual from asexual females (Osnas and Lively 2006). When DNA is stained with a fluorescent dye (propidium iodide), the nuclei of asexual (triploid) females exhibit ~50% more fluorescence than the nuclei of sexual females (diploid). Flow cytometry was performed as outlined in Gibson et al. (2016b) at the Indiana University-Bloomington Flow Cytometry Core Facility. Data was collected with FACSDiva software v6.1.3 (BD Biosciences) using an LSRII flow cytometer (BD Biosciences; Franklin Lakes, NJ, USA) with the High Throughput Sampler option. For each site in each year, we excluded a small proportion of individuals with ambiguous DNA content ($2.50 \pm .95\%$ SEM).

Statistical analyses of field data

All statistical analyses were performed in R v3.3.1 (R Core Team 2013). Models were fit with a binomial response variable using a logit link function (infection status or reproductive mode). Here, binomial or proportional response variables (e.g. infection prevalence, specified as numbers of infected and uninfected individuals) are used to predict the probability of observing an event (e.g. sampling an infected female) (Crawley 2013a; Crawley 2013b). We fit each model using all six sites sampled from 2012 to 2016. To ensure our findings were comparable to those from the 2001–2005 field study, we also replicated each analysis using only data from the four sites shared between the two study periods.

In analyzing both field and experimental data, we largely focus on lake-wide patterns, rather than site-specific ones. The dynamics of individual sites are more sensitive to random biological variation than the lake as a whole. We therefore adopted the conservative approach of averaging site-specific trends to obtain a more robust, lake-wide estimate of temporal change.

Temporal dynamics of relative infection prevalence—We first compared relative infection prevalence of asexual and sexual females in the 2012–2016 field collections. Using the function `glm` in R, we fit a logistic model with site, year, and ploidy (diploid or triploid

as proxies for sexual and asexual reproduction, respectively) as predictors of the probability of *Microphallus* infection of a female snail. We also included as predictors the interactions of site with ploidy and year with ploidy (sole possible interactions). Shell length was included as a covariate; shell length increases with snail age, so this term controls for the increase in infection risk with snail age (Jokela and Lively 1995). Models with and without the interaction effects were compared using likelihood-ratio tests. Interaction effects were excluded if they did not improve the fit of the model. We used McFadden's pseudo- R^2 to quantify the explanatory power of the final model. For this estimate, values between 0.2 and 0.4 reflect strong explanatory power (McFadden 1974; McFadden 1979).

We then directly tested for a change in the relative infection prevalence of asexual vs. sexual females from the first (2001–2005) to second (2012–2016) sampling periods. We fit a generalized estimating equation (GEE) using the function `geeglm` in the R package `geepack` (Halekoh et al. 2006). Site, sampling period, ploidy, the interaction of site and ploidy, and the interaction of sampling period and ploidy were predictors of the probability of *Microphallus* infection. Length was included as a covariate, as described above. Using the GEE framework, we included year as a grouping (subject) variable with an exchangeable correlation structure; hence, individuals sampled in the same year were treated as correlated and the within-year correlation was identical for all years (Zuur et al. 2009). We excluded insignificant interaction effects based upon the results of Wald tests (Zuur et al. 2009). To evaluate reproductive modes independently of one another, we repeated this analysis with females of each reproductive mode alone and thus removed ploidy as a factor. To compare variation in the rate of *Microphallus* infection in asexual and sexual females, we calculated the coefficients of variation for sexual and asexual infection rates across all sampled sites in all 10 years. We calculated confidence intervals on the coefficients of variation by bootstrapping the data set 10,000 times with replacement.

Relative frequency of asexual females during sampling periods—The Red Queen hypothesis is based upon the idea that the relative infection prevalence of host lineages is a function of their frequency in the host population, with relative infection prevalence declining as a lineage becomes rare. It is not clear what frequencies constitute “rare” and “common” – rather, these are relative concepts best studied by comparison. We therefore approached the question of rarity of asexuals by comparing asexual frequency in the first and second sampling periods. In the absence of clonal genotype data, this global comparison serves as a relative indicator of the presence of common clones.

Specifically, we tested for a change from the first to the second sampling period in the overall proportion of females that were asexual. We fit a logistic model with site and period as predictors of the probability of sampling a triploid (asexual) female. Our response variable was the numbers of diploid and triploid females sampled per site per year, with each site*year combination treated as an independent replicate. Similar to the GEE, this model structure effectively nests year within period and accounts for the non-independence of individuals sampled from the same site in the same year. Here, the more complex GEE was not necessary: reproductive mode is not a function of the length of individual females, so the model did not require data at the level of the individual. We tested if the binomial distribution was appropriate by measuring overdispersion: the ratio of the squared Pearson

residuals to the residual degrees of freedom (variance inflation factor, $\hat{c}$) should be approximately 1 (Venables and Ripley 2002). We found evidence for significant overdispersion ($\hat{c} = 8.01$), indicating that the variance in our data exceeded that assumed by the binomial distribution. Therefore, we scaled the standard errors by the variance inflation factor using a quasi-GLM model (Zuur et al. 2009; Crawley 2013a).

Change in asexual frequency during period of under-infection—We then focused upon the second sampling period to test if the proportion of asexual females increased from the start of sampling in 2012 to the end in 2016. We fit a logistic model with site, year and their interaction as predictors of the probability that an individual female was triploid. To facilitate interpretation, we excluded the interaction effect in calculating odds ratios and performing linear contrasts between years.

Experimental test of parasite-mediated selection

We complemented the field collections above with a concurrent mesocosm experiment replicated in four years, from 2012–2015. This experiment was conducted with hosts and parasites collected from Lake Alexandrina at the time of the above field collections. Therefore, the experiment contained the same natural variation present in the field at the time of field collections. The aims of the experiment were to 1) test if local parasites differed in their ability to infect sexual and asexual hosts and 2) to measure the relative fitness of asexuals in a controlled environment that isolated the effect of parasite selection on the total cost of sex.

Establishing experimental replicates—In January 2012–2015, we visited Lake Alexandrina and collected snails by sweeping a net through *Isoetes kirkii* plants at ~1-meter depth. We collected from Swamp and Camp in 2012, and from 1st Fence, Swamp, 2nd Fence, and West Point in 2013 and 2014. In 2015, we collected from Halfway instead of West Point (Fig. B1). To obtain juvenile snails, we sieved the collections at 1.7 mm on the lake shore and retained the snails below this size cut-off (i.e. < 3mm in length). Juveniles are ideal for measuring infectivity and fitness because they have had limited opportunity for parasite exposure and reproduction in the field. To obtain parasite eggs, we collected duck feces from the lake shore. In 2013 and 2015, we combined duck feces from multiple sites around the lake. In 2012 and 2014, duck feces came from the southern end of the lake only. We transported our field collections to Kaikoura, where we rinsed and sieved the duck feces to create a homogenous slurry.

Each year from 2013–2015, we combined 200 juvenile snails from each of the four sampled sites to create 12 experimental replicates, each with 800 snails. We maintained replicates in 20 L trays. Six of the 12 replicates were exposed to 10 doses of parasites through daily additions of homogenized duck feces (12–20 mL) to the water of the tray for 10 days. Each snail was exposed to ~650 *Microphallus* eggs in 2013, ~1900 in 2014, and ~3985 in 2015. Snails in the 6 control replicates were fed dried spirulina algae *ad libitum*. We took a different approach in 2012, the pilot year. We established two large populations of snails, ~1/3 from Swamp and ~2/3 from Camp. One population was given 8 large, daily doses of homogenized duck feces (50 mL). We did not estimate parasite egg concentrations, but our

intent was to use enough inoculum to achieve a saturating dose (King et al. 2011). The other (control) population was fed spirulina algae. After completing the exposure, we divided each of the two large populations into 6 replicates of 300 snails, for a total of 6 exposed and 6 control replicates.

At the end of January in each year, we transferred the six control and six experimental replicates to 12 different 1000 L Dolav box pallets outside the field station. These mesocosms were filled with ~800 L of water and covered with shade cloth. The mesocosms were successfully seeded with *Daphnia* and ostracods collected from Lake Alexandrina. In addition, 8–10 large boulders were placed on the bottoms of the tanks. The snails received daily feedings of spirulina for ~2 weeks, after which they were left undisturbed until January of the following year. Because they were based in New Zealand, mesocosm populations experienced seasonal variation in temperature and photoperiod similar to that of the field population. Importantly, we thoroughly washed the exposed snails to remove parasite eggs prior to adding them to the mesocosms. Moreover, infected snails could not transmit the infection to other snails, because completion of the trematode life cycles require passage through a duck. Therefore, no new infections arose in the experimental mesocosms, and members of the parental generation could not infect members of the offspring generation.

By January of the following year, two generations of snails were present in the mesocosms: the original snails, now reproductively mature, and their offspring. At this point, we removed the entire population of each mesocosm and sieved it using a 1.4 mm sieve to separate parents from their much smaller offspring.

Data collection—We randomly sampled 150 adults from each replicate and processed them for infection status and flow cytometry as described above. We also randomly sampled a large number of offspring from each mesocosm and transported these alive to Indiana University, where they were frozen for flow cytometry. The offspring were too young to sex, so we ran flow cytometry on a random subsample, irrespective of sex. For each experimental replicate, we excluded $5.83 \pm 0.94\%$ of parents and $6.49 \pm 0.74\%$ of offspring with ambiguous DNA content.

Statistical analyses of experimental data

Unless otherwise noted, all models were fit with a binomial response variable using a logit link function. We analyzed data from 12 mesocosms across 4 years, for a total of 48 replicates.

Relative infectivity of local parasites—To compare the ability of local parasites to infect sexual vs. asexual females, we compared the rate of *Microphallus* infection in sexual and asexual females of the parental generation. We focused on parental snails here because they were experimentally exposed to parasites, and we measured infectivity as the proportion of hosts that become infected following controlled exposure to the local parasite population. It is possible that snails had acquired infection in the field prior to collection for the experiment. We controlled for this baseline level of infection by including control replicates, where hosts were not experimentally exposed to parasites. The baseline level of infection in the control tanks was found to be low (see Results).

We fit a generalized estimating equation with ploidy, experimental treatment (exposed, control), experimental year (2012–2015), and their interactions as predictors of the probability of *Microphallus* infection. Our response variable was the number of infected and uninfected females per experimental replicate, with the sexual and asexual females within an experimental replicate grouped independently of one another. We then included experimental replicate as a grouping variable with an exchangeable correlation structure to account for the non-independence of sexual and asexual females from the same replicate. Because our parasite inocula contained eggs of other trematode species in addition to *Microphallus* (Osnas and Lively 2011), we replicated this analysis using the numbers of females infected with any castrating trematode.

Change in asexual frequency in a single generation—We then tested for the effect of parasite selection on the fitness of asexual females. First, we compared the proportion of asexual offspring in replicate mesocosms that were or were not subject to parasite exposure. We fit a logistic model with experimental treatment, year, and their interaction as predictors of the probability of sampling a triploid (asexual) offspring. Our response variable was the number of diploid and triploid offspring sampled from an experimental replicate. We fit a quasi-GLM because of overdispersion ($\hat{c} = 3.43$). We repeated this analysis excluding the pilot year of our experiment, 2012, in which we used a different experimental approach.

Experimental estimate of the cost of sex—In Gibson et al. (2017), we used experimental estimates of asexual frequency in the parent and offspring generations of our control mesocosms to estimate the cost of sex. In that study, we developed the following equation for the frequency of asexual individuals in the offspring generation:

$$q_{t+1} = \frac{cq_t}{1 + q_t(c - 1)}$$

(1)

where q_t is the starting frequency of asexuals in the parental generation and c is the net cost of sex. Using this approach, we found that the asexual subpopulation present at Lake Alexandrina from 2012–2015 had a per-capita birth rate advantage over the sexual subpopulation that was consistent with at least a two-fold fitness cost of sex. This prior test was conducted in the absence of parasite selection. Here, we extend our approach to exposed mesocosms to ask if parasite-mediated selection changed the total cost of sex. We formulated four candidate models based upon equation (1): (1) no cost of sex ($c=1$), (2) a two-fold cost ($c=2$), (3) the maximum likelihood estimated (MLE) of the cost, and (4) the MLE of a cost that varies with parasite selection. Model 4 was specified by allowing the net cost of sex to vary between control and exposed mesocosms.

We fit these models using our experimental estimates of the proportion of asexuals in the parent (q_t) and offspring (q_{t+1}) generations of the 48 mesocosms. The number of asexual and sexual offspring came directly from flow cytometry data. For the parental generation, we

performed flow cytometry on female snails only. To incorporate parental males into this data set, we randomly sampled a fraction of males equivalent to the fraction of females subsampled for flow cytometry for each experimental replicate (Gibson et al. 2017). We then estimated the proportion of asexual parents from these numbers. In order to account for parasite selection, we did not exclude infected individuals when estimating asexual frequency in the parents. As a result, our estimate of the cost of sex in control mesocosms is expected to differ slightly from that in Gibson et al. (2017), where we excluded the few infected individuals that were present in control mesocosms. For our likelihood function, we assumed a beta-binomial distribution (R package emdbook, Bolker 2008) and found maximum likelihood estimates of parameters using the mle2 function (R package bbmle). To estimate 95% confidence intervals for parameter estimates, we boot-strapped the data set 10,000 times with replacement.

We compared our four models using Akaike's information criteria corrected for small sizes (AIC_c), the difference in AIC_c of the focal and best models (ΔAIC_c), and Akaike weights, w_i . Traditionally, models with ΔAIC_c below 2.0 have strong support, while models with ΔAIC_c from 4.0 to 7.0 have substantially less support. To complement this rule of thumb, we calculated the ΔAIC_c value below which fell the 95% confidence set of models for our data by bootstrapping our data set 10,000 times with replacement (pg. 128–129 in Burnham and Anderson 1998). Akaike weights give the probability that the focal model is the best of the candidate models (Burnham and Anderson 1998).

Results

Field patterns

Temporal shifts in relative infection prevalence—From 2001 to 2005, asexual females at Lake Alexandrina had a higher mean prevalence of *Microphallus* than sexual females (as reported by Vergara et al. 2014). From 2001 to 2004, *Microphallus* prevalence was $55.89 \pm 7.05\%$ SEM for asexuals and $37.61 \pm 4.38\%$ for sexuals. Hence, on average, sexual females were 1.41-fold more likely to be uninfected than asexual females. In some sites in some years, the parasite-mediated advantage for sexual females was greater than two-fold (Vergara et al. 2014). In 2005, relative infection rates flipped, with mean *Microphallus* prevalence falling to $30.44 \pm 13.53\%$ in asexuals and increasing to $55.24 \pm 12.22\%$ in sexuals (Fig. 1a).

We replicated this field study, surveying infection prevalence of sexual and asexual females at Lake Alexandrina from 2012 to 2016. Each year, we sampled the same four sites as Vergara et al. (2014) (Camp, Halfway, Swamp, West Point), plus an additional two sites (JMS, Southwest End) (Fig. B1). In contrast to the prior sampling period, we found that asexual females had a significantly lower mean prevalence of *Microphallus* infection ($14.35 \pm 4.03\%$) than sexual females ($24.83 \pm 3.45\%$) (Table 1a, Fig. 1b). Sites varied in the degree of difference between sexual and asexual infection prevalence (Table 1a: site*ploidy interaction): while asexuals were less infected than sexuals at all sites, at Halfway the mean infection prevalence of asexuals was only one-tenth that of sexuals. This large difference between sexual and asexual infection prevalence at Halfway significantly exceeded the differences in infection prevalence between sexuals and asexuals at Swamp ($\chi^2=6.3$, $df=1$,

$p=0.012$) and JMS ($\chi^2=4.8$, $df=1$, $p=0.029$) and marginally exceeded the differences at Camp ($\chi^2=3.1$, $df=1$, $p=0.080$), Southwest End ($\chi^2=3.1$, $df=1$, $p=0.076$), and West Point ($\chi^2=3.5$, $df=1$, $p=0.062$). Swamp, Camp, Southwest End, and West Point did not differ from one another in the relative infection prevalence of sexuals and asexuals ($p>0.2$) (Fig. B2).

Overall infection prevalence varied significantly with site (Table 1a): mean infection prevalence of all females was highest at Swamp ($46.37 \pm 8.23\%$) and lowest at Southwest End ($4.24 \pm 2.72\%$). It also varied significantly with year, with mean infection prevalence highest in 2013 ($33.46 \pm 9.55\%$) and lowest in 2012 (17.95 ± 3.61) and 2015 ($16.71 \pm 7.00\%$). The probability of infection increased with shell length (coefficient = 1.04 ± 0.13 , $z = 8.14$, $p<0.001$; Table 1), consistent with an increasing cumulative infection risk as snails age (as in Jokela and Lively 1995). These results were unchanged by restricting the analysis to the four sites that overlapped with the 2001–2005 study (Table B1a).

We then evaluated variation in *Microphallus* prevalence across both sampling periods, 2001–2005 and 2012–2016. This analysis confirmed a temporal shift in the relative infection prevalence of asexual vs. sexual females: the interaction of reproductive mode and sampling period contributed significantly to variation in infection prevalence (Table 1b) (Fig. 1a,b). Though the infection prevalence of sexual and asexual females differed markedly from one another within sampling periods, we detected no overall difference between sexual and asexual females across the two sampling periods (Table 1b). Over the 10 sampling years, mean *Microphallus* prevalence was $29.85 \pm 4.49\%$ for asexuals and $31.35 \pm 2.92\%$ for sexuals (Fig. 1c).

The Red Queen predicts oscillations between over-infection and under-infection of asexual lineages (Winterbourn 1970; Bell 1982; Lively 2009). This fluctuation should lead to higher variance in the infection prevalence of asexuals. We found support for this prediction: the coefficient of variation for infection prevalence across all sites in all years was 1.02, 95% CI [0.80, 1.30] for asexual females, which substantially exceeded that for sexual females (0.66, 95% CI [0.53, 0.79]). Moreover, the temporal shift in relative infection prevalence resulted from a decline in the infection prevalence of asexuals: asexual infection prevalence dropped significantly between sampling periods (GEE: $\chi^2=18.2$, $df=1$, $p<0.001$; Table B2a), while sexual infection prevalence did not change (GEE: $\chi^2=1.94$, $df=1$, $p=0.164$; Table B2b)(Fig. 1a,b).

Overall infection prevalence of females at Lake Alexandrina varied substantially between sampling periods: mean prevalence of *Microphallus* in females decreased from $44.73 \pm 4.51\%$ in 2001–2005 to $22.71 \pm 3.24\%$ in 2012–2016 (Fig. 1d). Though sampling period was a significant factor in explaining variation in infection prevalence (Table 1b), the coefficient for sampling period was insignificant (-0.39 ± 0.26 SEM, $z=2.15$, $p=0.143$). This weak effect may reflect substantial variation between sites and years within periods. These results were unchanged by restricting the 2012–2016 analysis to the four sites that overlapped with the 2001–2005 study (Table B1b).

Asexual frequency declined between sampling periods—At Lake Alexandrina, asexual females shifted from being more infected than sexual females in 2001–2005 to less

infected in 2012–2016 (Fig. 1 a,b). The Red Queen hypothesis predicts that rarity protects host genotypes from infection (Haldane 1949; Clarke 1976; Jaenike 1978). Accordingly, we tested if the shift to under-infection of asexual females was accompanied by a decline in the frequency of asexual females. We compared the frequency of asexual females in the first and second sampling periods. The frequency of asexual females varied significantly with sampling period (quasi-GLM: $F=5.46$, $df=1$, $p=0.024$) and site ($F=6.98$, $df=5$, $p<0.001$) (Table B3a). In support of the prediction, the mean frequency of asexual females from 2012–2016 ($22.52 \pm 3.45\%$) was substantially lower than that from 2001–2005 ($34.90 \pm 4.99\%$) (Fig. 1e). Strikingly, asexual females were absent from half (Southwest End, Halfway, and West Point) of the six sampled sites in 2012. We obtained identical results when restricting the 2012–2016 analysis to the four sites that overlap with the 2001–2005 study (Table B3b).

Experimental test

To complement the field patterns outlined above, we conducted a concurrent set of experiments in outdoor, semi-natural mesocosms using field-collected hosts and parasites.

Coevolving parasites have reduced infectivity to asexual hosts—The Red Queen hypothesis assumes that parasites are under strong selection to attack common host genotypes, and are thus relatively poorly adapted to infect rare host genotypes (Jaenike 1978). This is one possible explanation for the low infection prevalence of asexual females relative to sexual females from 2012–2016. To test this explanation, we compared the infection rates of asexual and sexual snails from Lake Alexandrina following exposure to a fixed dose of local *Microphallus* eggs.

In each year, from 2012 to 2015, we exposed six replicate populations of field-collected, juvenile hosts to field-collected *Microphallus* and compared these with six control replicates. Exposure to local *Microphallus* substantially increased infection rate (GEE: $\chi^2=104.9$, $df=1$, $p<0.001$; Table B4a): the infection rate of all females increased 5.4-fold from control ($4.31 \pm 0.01\%$, $N=24$) to exposed replicates ($23.06 \pm 2.17\%$, $N=24$). As observed in the natural population, *Microphallus* parasites were more infective to sexuals than to asexuals ($\chi^2=45.6$, $df=1$, $p<0.001$; Fig. 2A). In exposed replicates, the infection rate of asexuals ($11.23 \pm 1.68\%$) was one-third that of sexuals ($31.34 \pm 3.03\%$). This difference in infection rate between sexuals and asexuals did not vary with treatment ($\chi^2=2.10$, $df=1$, $p=0.147$; Table B4a): in control replicates, $2.44 \pm 0.64\%$ of asexuals and $5.76 \pm 0.90\%$ of sexuals were infected. Infection rate varied with experimental year and with the interaction between year and reproductive mode (Table B4a, Fig. B3). Yearly variation may reflect variation in the controlled exposure itself, as parasite dose varied between years. We obtained similar results when we considered infection with all castrating trematode parasites (Table B4b).

Parasites can increase the cost of sex—Experimental exposures demonstrated that, from 2012–2015, local parasites were more successful at infecting sexual females than asexual females at Lake Alexandrina. Under these conditions, we predict that asexual females have a fitness advantage over sexual females. To test this prediction, we populated semi-natural mesocosms with hosts from the control and exposed replicates introduced

above and tracked changes in the frequency of asexuals from this initial generation of hosts to the offspring generation.

First, we compared the proportion of asexual offspring produced by parents that were or were not subject to parasite selection. Averaging across all experimental replicates ($N=48$), the proportion of asexual females in the parental generation was $27.91 \pm 1.39\%$. In control replicates, the proportion of asexuals in the offspring generation was $45.87 \pm 2.16\%$. This is a 1.64-fold (± 0.11 SE) increase in the proportion of asexuals from parent to offspring generations. Parasite selection marginally increased the fitness of asexual parents (quasi-GLM: $F=3.57$, $df=1$, $p=0.066$; Table B5a): in exposed replicates, the proportion of asexuals increased 1.87-fold (± 0.14 SE), to $52.04 \pm 3.03\%$ (Figure 2b). The experiment's pilot year, 2012, differed substantially in experimental conditions. Excluding this year showed a significant increase in the proportion of asexual offspring in exposed relative to control tanks ($F=4.56$, $df=1$, $p=0.041$; Table B5b, Fig. B4).

Second, we estimated the cost of sex from our experimental estimates of the proportion of asexual snails in parent and offspring generations (q_t and q_{t+1} , respectively). We formulated four candidate models: (1) no cost of sex, (2) a two-fold cost of sex, (3) the maximum likelihood estimated (MLE) of the cost, and (4) the MLE of a cost that varies with experimental treatment (Table 2). A cost of sex that varied with parasite exposure (model 4) was the best approximation to our experimental measures of asexual frequency. For control mesocosms, where parasite selection was absent, the likelihood of model 4 was maximized at a cost of sex that slightly exceeded two ($c_0 = 2.18$, 95% CI [1.85, 2.57]), consistent with Gibson et al. (2017). For the exposed mesocosms, where parasite-mediated selection was present, the cost of sex increased to 3.15 (increase of $c_p = 0.97$, 95% CI [0.19, 1.97]).

There was also some support for model 3, in which the cost of sex was held constant across experimental treatments. The likelihood of model 3 was maximized at a relatively high cost of sex ($c = 2.61$, 95% CI [2.25, 3.06]), consistent with an elevation of the cost of sex with inclusion of replicates subject to parasite selection. Though the AIC for model 3 is reasonably low (<4), it exceeds the upper limit for our 95% confidence set of models (AIC = 2.28), lending further support to model 4. There was no support for a cost of sex less than or equal to two (models 1 and 2), though a two-fold cost of sex (model 2) was supported when parasite selection was absent (Gibson et al. 2017). These results provide direct experimental support for an increased cost of sex in the presence of parasite selection.

Return to field patterns

Asexual females increase in frequency—We have shown that the *Microphallus* population at Lake Alexandrina shifted from over-infection to under-infection of asexuals as the asexuals declined in frequency from 2001–2005 to 2012–2016. Our experimental exposures suggested that asexual under-infection from 2012–2016 resulted from the reduced ability of local *Microphallus* to infect asexual hosts relative to sexual hosts. We further demonstrated experimentally that, from 2012–2015, the asexual subpopulation had both a two-fold per-capita birth rate advantage over the sexual subpopulation (Gibson et al. 2017) and a smaller parasite-mediated fitness advantage. We therefore predicted that asexual females would have increased in frequency at Lake Alexandrina from 2012 to 2016.

To test this prediction, we evaluated the proportion of females that were asexual in field collections from 2012 to 2016. The proportion of asexual females varied significantly with year (Table 3). Specifically, the proportion of asexual females increased 2.6-fold over the sampling period, from $11.47 \pm 7.15\%$ in 2012 to $29.80 \pm 10.28\%$ in 2016 (Fig. 3A) (odds of sampling an asexual female in 2016 relative to 2012 = 2.66, 95% CI [1.81, 3.93]). This result holds if we limit the analysis to the four sites that overlap with Vergara et al. 2014 (Table B6). The proportion of asexual female increased significantly from 2012 to 2013 ($\chi^2=4.4$, $df=1$, $p=0.036$) and from 2013 to 2014 ($\chi^2=6.0$, $df=1$, $p=0.015$). There was no change in asexual frequency from 2014 to 2015 ($\chi^2=1.2$, $df=1$, $p=0.28$) and a marginal increase from 2015 to 2016 ($\chi^2=2.9$, $df=1$, $p=0.086$). If we treat yearly mean asexual frequencies as estimates of q_t and q_{t+1} , we can rearrange (equation 1) to estimate the cost of sex in the field (see eq. 4 in Gibson et al. 2017). Here the frequencies for asexuals would include snails from the previous generation, if the generations overlap to some degree. Based on the results of a previous model, an overlap of generations can decrease the cost of sex per reproductive time step (Lively 2010b). We estimated the lake-wide cost of sex to be 1.98 in 2012, 1.47 in 2013, 0.81 in 2014, and 1.39 in 2015.

The direction and rate of change of asexual frequency varied significantly across sites (Fig. 3B; Table 3). The proportion of asexual females increased dramatically at four sites, including at Southwest End, West Point, and Halfway, where asexual females were absent in 2012 (Fig. 3b). In contrast, the proportion of asexual females remained very low at JMS, and it declined at Swamp, from 28.75% of females in 2012 to 7.55% in 2016.

We did not find any evidence that the proportion of asexual females increased more at sites where asexuals were relatively less infected. Asexual frequency did not increase to a greater extent at Halfway, where the infection prevalence of asexual females was particularly low relative to sexual females (Fig. B2). Rather, the proportion of asexual females increased at all sites except those two where asexual females had periodically (JMS) or consistently (Swamp) high prevalence of *Microphallus* infection. Infection prevalence of asexual females reached 66.67% at JMS in 2016 ($N=3$ asexual females) and consistently exceeded 50% at Swamp (66.67% in 2013; 54.54% in 2015, 50.00% in 2016; $N=12$; 11; 4). At the four other sites, where the proportion of asexual females increased, the maximum infection prevalence of asexual females at any site was 25% and the mean infection prevalence of asexual females was $5.48 \pm 1.88\%$ (Fig. 3B, Fig. B2).

Inclusion of male snails

We excluded males from analyses of both field and experimental data, unless otherwise noted. The per-capita birth rate of the sexual subpopulation hinges upon female fecundity – it is not affected by infection in males, as long as the infection rate in males is not so high that females become sperm-limited. We did, however, replicate all analyses with males included. We found that inclusion of male snails did not alter the conclusions of the analyses presented above. Further details are presented in Appendix A.

Discussion

Long-term studies of natural populations facilitate tests of the Red Queen hypothesis for the maintenance of sex. In the present study, we conducted a five-year study of infection prevalence in sexual and asexual females in a natural population of freshwater snails (*Potamopyrgus antipodarum*). We combined these observational data with a concurrent controlled experiment using large outdoor mesocosms. By comparing these new data (2012–2016) to a previous long-term study of the same population (2001–2005), we were able to examine periodicity in selection for, or against, sex.

Between the first and second field collections, the average frequency of asexual *P. antipodarum* declined (Fig. 1e). In addition, the local population of *Microphallus* parasites shifted from over-infecting asexual females to under-infecting asexual females, relative to sexual females (Fig. 1a,b). Moreover, the controlled exposure of field-collected snails to parasites showed that the under-infection of asexuals in the field most likely resulted from a reduced ability of local parasites to infect asexuals (Fig. 2a). The reduced infection prevalence of asexual females relative to sexual females contributed to a substantial fitness advantage for asexuals: in the semi-natural mesocosms, sexuals paid both the two-fold cost of sex and the cost of higher infection prevalence (Fig. 2b, Table 2). This combination of costs for the sexual population suggested that the frequency of asexual females should have increased in the natural lake population between 2012 and 2016. Consistent with this expectation, we observed a 2.6-fold increase in the frequency of asexual females in the natural population over the five-year period of our study (Fig. 3a). This match between field data and the controlled experiment suggest that parasites are a significant selective force underlying the evolution of reproductive mode in this host-parasite system.

These long-term data seem to capture the inherently periodic process proposed by the Red Queen hypothesis. Common genotypes become over-infected and subsequently decline in frequency, while rare genotypes evade coevolving parasites and subsequently increase in frequency (Jaenike 1978; Hamilton 1980; Bell 1982). Simulation studies have shown that asexual lineages can fluctuate in frequency over time, and that they may be periodically less infected than sympatric sexual individuals (Lively 2009). Hence, parasites may periodically select against sex, even when parasite-mediated selection favors sex over the long term. Under this model, asexual infection prevalence should vary more than sexual infection prevalence. Averaged over this study's 10 years of field sampling, the prevalence of *Microphallus* infection in asexual females was similar to that in sexual females (Fig. 1c). Variance in infection prevalence was, however, far greater for asexuals than for sexuals (Fig. 1a-c). This result is consistent with the hypothesis that the coexistence of sexual and asexual *P. antipodarum* is at least partially maintained by parasite-mediated reductions in the geometric mean fitness of asexuals (as in Hamilton 1980; Vergara et al. 2014).

The experimental exposures showed that the local *Microphallus* population was less infective to asexual individuals than to sympatric sexual individuals from 2012–2015 (Fig. 2a). Hence, the relatively low infection prevalence observed in the natural subpopulation of asexuals from 2012–2016 (Fig. 1b) likely resulted from greater resistance in the asexual subpopulation as a whole. Based upon these experimental results, it is less likely that

reduced asexual infection prevalence resulted from differences in rates of exposure to parasites or in physiological conditions between sexuals and asexuals in the field. Taken together, the results suggest that the infectivity of the *Microphallus* population towards asexuals (relative to sexuals) declined after the asexuals decreased in frequency.

As asexuals declined in frequency and became under-infected, the infection prevalence of the entire host population fell from the first to second sampling periods (Fig. 1d). Lively (2010) proposes one potential explanation for this decline in overall infection prevalence. This simulation study shows that, as a parasite population adapts to infect a common clone, more parasites go on to reproduce, increasing the force of infection for all individuals. In contrast, as the clone declines in frequency and host genetic diversity (evenness) increases, the parasite population is less successful at tracking host genotypes. The force of infection and overall population prevalence accordingly declines (Lively 2010a; Lively 2016). Our field results provide some preliminary support for this model, suggesting that a direct experimental test would be worthwhile.

This study relies upon global comparisons of Lake Alexandrina's sexual and asexual subpopulations. Data on individual clonal lineages would provide a clearer view of the frequency-dependent mechanisms of the Red Queen. In particular, they would clarify the link between declines in asexual frequency and asexual under-infection. However, data on individual clonal lineages are rarely sufficient to demonstrate parasite-mediated selection on reproductive mode. If many clonal lineages are present in a population, as in *P. antipodarum* (Dybdahl and Lively 1995; Fox et al. 1996), infection prevalence of individual clonal lineages gives us limited information about the aggregate fitness of asexual reproduction. For example, clone-level data may provide support for frequency-dependent selection by showing that the most common clone is over-infected. This common clone may, however, represent a small fraction of the diverse asexual subpopulation (e.g. 10–20%). If the remaining rare clones are under-infected, the majority of the asexual subpopulation is expected to have higher fitness than the sexual subpopulation. Ultimately, demonstration of parasite-mediated selection on reproductive mode requires that we compare individuals reproducing asexually to those reproducing sexually. While it is possible to do so with clone-level data, it is impractical when the clonal subpopulation contains a diversity of rare clones. It is for this reason that we opted here for global comparisons of the sexual and asexual subpopulations.

We found that, as a group, asexuals were less infected than sexuals from 2012–2016, both in the field and in the mesocosm experiment. Thus, asexual reproduction should have had a significant fitness advantage over sexual reproduction and increased in frequency in the natural population. These circumstances offered a unique opportunity to falsify the Red Queen in a natural population: if a force stronger than parasites generally prevents the spread of asexuals in the field, asexuals would not have increased in frequency in response to their parasite-mediated fitness advantage. Asexuals did, however, increase in frequency during this period (Fig. 3), arguing that parasite selection contributes to the evolution of reproductive mode in the field. The concurrent experiment isolated the effect of parasite selection from that of other variables present in the field. The match between field and experiment therefore offers support to our interpretation of field patterns: if some

unmeasured environmental variable determined the relative fitness of sexuals and asexuals in the field, then we should not have seen similar dynamics unfold in the field and experimental mesocosms. Our yearly estimates of the mean costs of sex in the field are, however, lower than that estimated in the mesocosms. The presence of overlapping generations in the field may have reduced the cost of sex per reproductive time step (Doncaster et al. 2000; Lively 2010b).

In conjunction with Vergara et al. (2014), this study suggests that parasites favor asexual reproduction when asexuals are relatively rare and favor sexual reproduction when asexuals are more common. The Red Queen's concept of rarity, however, poses a challenge to interpreting field patterns. How rare must a genotype be to escape the sights of a coevolving parasite? We approached this problem by comparing lake-wide asexual frequency at different time periods. This is a conservative approach: significant temporal and spatial variation can hide or diminish real differences in asexual frequency. The geographic mosaic of coevolution suggests that what constitutes a rare genotype may vary across sites (Thompson 1994; Thompson 1999; Thompson 2005): at coevolutionary hotspots, clones may need to be rarer to evade coevolving parasites than at coevolutionary coldspots. Several studies of *Microphallus* and *P. antipodarum* demonstrated the existence of coevolutionary hotspots and coldspots around Lake Alexandrina (King et al. 2009; King et al. 2011; Vergara et al. 2013; Gibson et al. 2016b). This mosaic may explain the variation between sites in asexual infection and frequency change from 2012 to 2016 (Fig. B2a, 3b), though six sites is too few for a proper test of this idea.

On average, asexuals are increasing in frequency at Lake Alexandrina (Fig. 3). The Red Queen hypothesis predicts that the local *Microphallus* population should shift again to preferentially infect asexuals if common clones are emerging. This shift may already be in process. In 2012, asexuals were less infected than sexuals at all three sites at which asexuals were present. In 2013, asexual females were less infected than sexual females at half of sites. By 2016, asexual females were equally or more infected than sexual females at four of the five sites at which *Microphallus* was detected (Fig. B2). We do not know the precise timing of coevolutionary shifts in natural populations, so long-term field data remain fundamentally important. Only through combining two data sets, spanning a period of 16 years, could we see the extent of the dynamic interplay between parasite selection and asexual frequency in this natural system. Single snapshots in space and time miss the periodicity inherent to coevolution and the Red Queen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Christiane Hassel, the manager of the Indiana University-Bloomington Flow Cytometry Core Facility, for her assistance with flow cytometry. Amrita Bhattacharya, Eliza Oldach, and Andrew McKinney assisted with field collections. We also acknowledge Samantha Klosak, Peyton Joachim, and Julie Xu for contributions to flow cytometry. The staff of the University of Canterbury's Edward Percival Field Station in Kaikoura, New Zealand, including Ngaire Perrin, provided invaluable assistance with field collections. This work was funded by a US National Science Foundation grant to CML and Jukka Jokela (DEB-0640639), an award from Indiana University to

LD, a Doctoral Dissertation Improvement Grant (DDIG-1110437) and a National Geographic Society CRE Award (883510) to DV, and awards to AKG from the American Society of Naturalists (Student Research Award), the Society for the Study of Evolution (Rosemary Grant Student Research Award), Indiana University (Provost's Travel Award for Women in Science), the National Science Foundation (DDIG-1401281; GRFP) and the US National Institutes of Health (IU's T32 Common Themes in Reproductive Diversity Traineeship - 5T32HD049336-13; Emory University's IRACDA program Fellowships in Research and Science Teaching - K12GM000680).

References

- Bell G. 1982, *The Masterpiece of Nature: the Evolution and Genetics of Sexuality*. Berkeley, CA, University of California Press.
- Biggs B, and Malthus T. 1982 Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper Clutha Valley, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 16:81–88.
- Bolker B. 2008, *Ecological Models and Data in R*, Princeton University Press.
- Burnham KP, and Anderson DR 1998, *Model Selection and Inference: a Practical Information-Theoretic Approach*. New York, Springer.
- Chaboudez P, and Burdon J. 1995 Frequency-dependent selection in a wild plant-pathogen system. *Oecologia* 102:490–493. [PubMed: 28306892]
- Clarke B. 1976 *The ecological genetics of host-parasite relationships* in Taylor AER, and Muller R, eds. *Genetic Aspects of Host-Parasite Relationships*. London, U.K., Blackwell.
- Crawley MJ 2013a Chapter 16: Proportion Data *The R Book*. West Sussex, UK, John Wiley & Sons, Ltd. .
- Crawley MJ 2013b Chapter 17: Binary Response Variables *The R Book*. West Sussex, UK, John Wiley & Sons, Ltd.
- Doncaster C, Pound G, and Cox S. 2000 The ecological cost of sex. *Nature* 404:281–285. [PubMed: 10749210]
- Dybdahl MF, and Lively CM 1995 Diverse endemic and polyphyletic clones in mixed populations of the freshwater snail, *Potamopyrgus antipodarum*. *Journal of Evolutionary Biology* 8:385–398.
- Dybdahl MF, and Lively CM 1998 Host-parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. *Evolution* 52:1057–1066. [PubMed: 28565221]
- Forsyth D, and McCallum I. 1981 Benthic macroinvertebrates of Lake Taupo. *New Zealand Journal of Marine and Freshwater Research* 15:41–46.
- Fox J, Dybdahl MF, Jokela J, and Lively CM 1996 Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). *Evolution* 50:1541–1548. [PubMed: 28565708]
- Gibson AK, Delph LF, and Lively CM 2017 The two-fold cost of sex: experimental evidence from a natural system. *Evolution Letters* 1:6–15. [PubMed: 30233811]
- Gibson AK, Delph LF, Vergara D, and Lively CM 2018a Analysis files for: Periodic, parasite-mediated selection for and against sex, *American Naturalist*. GitHub repository *periodic_parasites*, https://github.com/amandakgibson/periodic_parasites.
- Gibson AK, Delph LF, Vergara D, and Lively CM 2018b Data and code from: Periodic, parasite-mediated selection for and against sex, *American Naturalist*. Dryad Digital Repository, 10.5061/dryad.77v1s92.
- Gibson AK, Jokela J, and Lively CM 2016a Fine-scale spatial covariation between infection prevalence and susceptibility in a natural population. *American Naturalist* 188:1–14.
- Gibson AK, Xu JY, and Lively CM 2016b Within-population covariation between sexual reproduction and susceptibility to local parasites. *Evolution* 70:2049–2060. [PubMed: 27402345]
- Haldane JBS 1949 Disease and evolution. *La Ricerca Scientifica* 19 68–76.
- Halekoh U, Højsgaard S, and Yan J. 2006 The R package *geepack* for generalized estimating equations. *Journal of Statistical Software* 15:1–11.
- Hamilton WD 1975 Gamblers since life began: barnacles, aphids, elms. *Quarterly Review of Biology* 50:175–180.
- Hamilton WD 1980 Sex versus non-sex versus parasite. *Oikos* 35:282–290.

- Hamilton WD, Axelrod R, and Tanese R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences* 87:3566–3573.
- Hechinger RF 2012 Faunal survey and identification key for the trematodes (Platyhelminthes: Digenea) infecting *Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae) as first intermediate host. *Zootaxa* 3418:1–27.
- Howard RS, and Lively CM 1994 Parasitism, mutation accumulation and the maintenance of sex. *Nature* 367:554–557. [PubMed: 8107824]
- Jaenike J. 1978 An hypothesis to account for the maintenance of sex within populations. *Evolutionary Theory* 3:191–194.
- Jokela J, Dybdahl MF, and Lively CM 2009 The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *American Naturalist* 174:S43–S53.
- Jokela J, and Lively CM 1995 Spatial variation in infection by digenetic trematodes in a population of freshwater snails (*Potamopyrgus antipodarum*). *Oecologia* 103:509–517. [PubMed: 28307000]
- King KC, Delph LF, Jokela J, and Lively CM 2009 The geographic mosaic of sex and the Red Queen. *Current Biology* 19:1438–1441. [PubMed: 19631541]
- King KC, Delph LF, Jokela J, and Lively CM 2011 Coevolutionary hotspots and coldspots for host sex and parasite local adaptation in a snail-trematode interaction. *Oikos* 120:1335–1340.
- Koskella B, and Lively CM 2009 Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution* 63:2213–2221. [PubMed: 19473396]
- Levin DA 1975 Pest pressure and recombination systems in plants. *American Naturalist* 109:437–451.
- Lively CM 1987 Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature* 328:519–521.
- Lively CM 1992 Parthenogenesis in a freshwater snail: reproductive assurance versus parasitic release. *Evolution* 46:907–913. [PubMed: 28564419]
- Lively CM 2009 The maintenance of sex: host-parasite coevolution with density-dependent virulence. *Journal of Evolutionary Biology* 22:2086–2093. [PubMed: 19732262]
- Lively CM 2010a An epidemiological model of host–parasite coevolution and sex. *Journal of Evolutionary Biology* 23:1490–1497. [PubMed: 20500364]
- Lively CM 2010b Parasite virulence, host life history, and the costs and benefits of sex. *Ecology* 91:3–6. [PubMed: 20380188]
- Lively CM 2016 Coevolutionary epidemiology: disease spread, local adaptation, and sex. *American Naturalist* 187:E77–E82.
- Lively CM, and Dybdahl MF 2000 Parasite adaptation to locally common host genotypes. *Nature* 405:679–681. [PubMed: 10864323]
- Lively CM, Dybdahl MF, Jokela J, Osnas EE, and Delph LF 2004 Host sex and local adaptation by parasites in a snail-trematode interaction. *American Naturalist* 164:S6–S18.
- May RM, and Anderson RM 1983 Epidemiology and genetics in the coevolution of parasites and hosts. *Proceedings of the Royal Society B: Biological Sciences* 219:281–313. [PubMed: 6139816]
- Maynard Smith J. 1971 The origin and maintenance of sex, Pages 163–175 in Williams GC, ed. *Group Selection* Chicago, Aldine Atherton.
- Maynard Smith J. 1978, *The Evolution of Sex*. Cambridge, UK, Cambridge University Press.
- McFadden D. 1974 Conditional logit analysis of qualitative choice behavior, Pages 105–142 in Zarembka P, ed. *Frontiers in Econometrics* New York, Academic Press.
- McFadden D. 1979 Quantitative methods for analyzing travel behavior of individuals: some recent developments, Pages 279–318 in Hensher D, and Stopher P, eds. *Behavioral Travel Modeling*. London, Croom Helm.
- Neiman M, Paczesniak D, Soper DM, Baldwin AT, and Hehman G. 2011 Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution* 65:3202–3216. [PubMed: 22023586]
- Osnas EE, and Lively CM 2006 Host ploidy, parasitism and immune defence in a coevolutionary snail-trematode system. *Journal of Evolutionary Biology* 19:42–48. [PubMed: 16405575]

- Osnas EE 2011 Using definitive host faeces to infect experimental intermediate host populations: waterfowl hosts for New Zealand trematodes. *New Zeal J Zool* 38:83–90.
- Paczynski D, Adolfsson S, Liljeroos K, Klappert K, Lively CM, and Jokela J. 2014 Faster clonal turnover in high-infection habitats provides evidence for parasite-mediated selection. *Journal of Evolutionary Biology* 27:417–428. [PubMed: 24417476]
- R Core Team. 2013 R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria: <http://www.R-project.org>.
- Suomalainen E, Saura A, and Lokki J. 1987, *Cytology and Evolution in Parthenogenesis*. Boca Raton, Florida, CRC Press, Inc.
- Talbot J, and Ward J. 1987 Macroinvertebrates associated with aquatic macrophytes in Lake Alexandrina, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 21:199–213.
- Thompson J. 1994, *The Coevolutionary Process*. Chicago, University of Chicago Press.
- Thompson JN 1999 Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist* 153:S1–S14.
- Thompson JN . 2005, *The Geographic Mosaic of Coevolution*. Chicago, IL, USA, University of Chicago Press.
- Venables WN, and Ripley BD 2002, *Modern Applied Statistics with S*. New York City, NY, Springer.
- Vergara D, Jokela J, and Lively CM 2014 Infection dynamics in coexisting sexual and asexual host populations: support for the Red Queen Hypothesis. *American Naturalist* 184:S22–S30.
- Vergara D, Lively CM, King KC, and Jokela J. 2013 The geographic mosaic of sex and infection in lake populations of a New Zealand snail at multiple spatial scales. *American Naturalist* 182:484–493.
- Vrijenhoek RC 1998 Animal clones and diversity. *BioScience* 48:617–628.
- Winterbourn M. 1970 Population studies on the New Zealand freshwater gastropod, *Potamopyrgus antipodarum*. *Proceedings of the Malacological Society of London* 39:139–149.
- Wolinska J, and Spaak P. 2009 The cost of being common: evidence from natural *Daphnia* populations. *Evolution* 63:1893–1901. [PubMed: 19228186]
- Zuur A, Ieno E, Walker N, Saveliev A, and Smith G. 2009 *Generalised Estimating Equations* in Zuur A, Ieno E, Walker N, Saveliev A, and Smith G, eds. *Mixed Effects Models and Extensions in Ecology with R*, Springer.

Figure 1: Temporal shifts in relative infection prevalence and reproductive mode.

The mean proportion of sexual and asexual females infected from (A) 2001–2005 and (B) 2012–2016. The proportions are means across (A) 4 or (B) 6 sampled sites, weighted by the number of sexual or asexual females sampled per site to account for the very low number of asexual females sampled at certain sites in certain years. Error bars are standard errors of the weighted mean. Inset boxplots are constructed from the proportion of sexual and asexual females infected at each site in each year from (A) 2001–2005 and (B) 2012–2016. Mean sample sizes per site per year: (A) 37.2 ± 2.5 sexual females ($n=20$), 22.9 ± 3.0 SEM asexual females ($n=19$ data points); (B) 44.6 ± 2.8 sexual females ($n=30$), 14.2 ± 2.0 SEM asexual females ($n=27$). The number of data points varied for asexual vs. sexual females because asexual females were absent from some sites in some years. (C) The proportion of sexual and asexual females infected with *Microphallus* at sites sampled in all 10 sampling years. Mean sample sizes per data point: 41.7 ± 2.0 sexual females per data point ($n=50$ data points); 17.8 ± 1.8 asexual females per data point ($n=46$). (D) The proportion of all females

infected with *Microphallus* and (E) the proportion of all females that were asexual at sites sampled in 2001–2005 ($n=20$ data points, 59.0 ± 2.5 females sampled per data point) and in 2012–2016 ($n = 30$, 57.4 ± 2.6). Box plots show median (black bar), upper and lower quartiles (limits of box), minimum and maximum (whiskers, excluding outliers), and outliers (dots).

Figure 2: Experimental test of asexual under-infection and fitness advantage.

(A) presents the proportion of sexual and asexual females infected with *Microphallus* in experimental replicates. Hosts in control replicates were not exposed to parasites, so these replicates reflect the background level of infection in field-collected juvenile hosts. The infection rate in exposed replicates reflects the infectivity of parasites to their local sexual and asexual hosts. The data come from six control and six exposed replicates in each of 4 years, for a total of 24 control and 24 exposed replicates. For each replicate, we determined the infection status of 37.77 ± 2.52 sexual females and 21.56 ± 1.04 asexual females. (B) presents the proportion of individuals identified as asexual in the parental (t) and offspring (t +1) generations of the semi-natural mesocosms. (A) summarizes the infection rates of female parents in these mesocosms; offspring were not exposed to parasites. For each replicate, we determined the reproductive mode of 81.83 ± 4.68 parents and 66.46 ± 1.13 offspring.

Figure 3: Asexual females increase in frequency from 2012 to 2016.

(A) presents the lake-wide trend, with each point showing the mean proportion of asexual females at six sites in the indicated sampling year. Error bars show the standard error of the mean. (B) presents the temporal change in asexual frequency at each of the six sites. Error bars shows the standard error of the proportion. The proportion of asexual females was estimated from a mean of 57.43 ± 2.62 females per site per year.

Table 1:Variation in the probability of *Microphallus* infection in the field

A.From 2012–2016			
	df	D	p-value
Site (n=6)	5	167.70	<0.001
Ploidy	1	29.09	<0.001
Year (2012–2016)	4	36.36	<0.001
Length	1	72.77	<0.001
Site*Ploidy	5	12.17	0.033
Residual	1706	1524.9	
$R^2_L = 0.173$			
Terms excluded based upon likelihood ratio test:			
Ploidy*Year	4	5.94	0.204
B.Across both sampling periods			
	df	χ^2	p-value
Site	5	58.3	<0.001
Period	1	10.9	<0.001
Ploidy	1	0.3	0.613
Length	1	151.2	<0.001
Period*Ploidy	1	11.9	<0.001
Terms excluded based upon Wald test:			
Site*Ploidy	5	4.23	0.520

We fit a logistic model with the infection status of an individual female as a binomial response variable. For each factor, we show the results of likelihood ratio (D) tests of models with and without the factor. R^2_L reflects the explanatory power of the model. Ploidy is a proxy for reproductive mode, with diploid females reproducing sexually and triploid females reproducing asexually.

We fit a generalized estimating equation with the infection status of an individual female as a binomial response variable. Sampling period denotes the 2001–2005 or 2012–2016 field collections, with 4 and 6 sites sampled, respectively. The Wald χ^2 statistics were obtained by sequentially adding each factor and comparing models with and without the factor of interest.

Table 2:

Estimating the cost of sex – candidate models and results

	Model		logL	Parameters	AIC _c	AIC _c	w
1	No cost $c = 1$	$q_{t+1} = q_t$	-211.4	1 (θ)	424.97	74.18	0.00
2	2-fold $c = 2$	$q_{t+1} = \frac{2q_t}{1+q_t}$	-179.6	1 (θ)	361.20	10.40	0.00
3	Estimate $c = \text{MLE}$	$q_{t+1} = \frac{cq_t}{1+q_t(c-1)}$	-174.8	2 (θ, c)	353.77	2.98	0.18
4	By treatment $c = \text{MLE}^*T$	$q_{t+1} = \frac{cq_t}{1+q_t(c-1)}$ Control: $c = c_0$ Exposed: $c = c_0 + c_p$	-172.1	3 (θ, c_0, c_p)	350.80	0.00	0.81

To estimate the cost of sex, we formulated four candidate models based upon equations developed in Gibson et al. (2017). These models are presented as variations of equation (1), where, q_t and q_{t+1} are the frequency of asexuals in the parent and offspring generations (estimated from experimental mesocosms) and c is the cost of sex (i.e. the per-capita birth rate advantage of asexual females, where the fitness of sexuals is multiplied by c to give the fitness of asexuals). When the cost of sex is allowed to vary with experimental treatment (model 4), c_0 is the baseline cost of sex in the absence of parasite selection, and c_p is the additional cost of sex conferred by parasite selection. If the maximum likelihood estimate of c_p deviates from 0, then the total cost of sex in exposed mesocosms differs from that in control mesocosms. For each model, the parameter θ is the overdispersion parameter used in fitting a beta-binomial distribution for the likelihood functions.

Table 3:

Variation in the probability of sampling an asexual female from 2012–2016.

	df	D	p-value
Site (n=6)	5	127.73	<0.001
Year (2012-2016)	4	32.48	<0.001
Site*Year	20	203.11	<0.001
Residual	1693	1464.8	
$R^2_L = 0.199$			

We fit a logistic model with the ploidy (reproductive mode) of an individual female as a binomial response variable. For each factor, we show the results of likelihood ratio (D) tests of models with and without the factor. R^2_L reflects the explanatory power of the model.