Title

Parasite resistance predicts fitness better than fecundity in a natural population of the freshwater snail *Potamopyrgus antipodarum*

2. Running title:

Fitness components of asexual lineages

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Abstract

The cost of males should give asexual females an advantage when in competition with sexual females. In addition, high-fecundity asexual genotypes should have an advantage over low-fecundity clones, leading to reduction in clonal diversity over time. To evaluate fitness components in a natural population, we measured the annual reproductive rate of individual sexual and asexual female *Potamopyrgus antipodarum*, a New Zealand freshwater snail, in field enclosures that excluded competitors and predators. We used allozyme genotyping to assign the asexual females to particular clonal genotypes. We found that the most fecund asexual clones had similar or higher fecundity as the top 10 % of sexual families, suggesting that fecundity selection, even without the cost of males, would lead to replacement of the sexual population by clones. Consequently, we expected that the clones with the highest fecundity would dominate the natural population. Counter to this prediction, we found that high annual reproductive rates did not correlate with the frequency of clones in the natural population. When we exposed the same clones to parasites in the laboratory, we found that resistance to infection was positively correlated with the frequency of clones in the population. The correlation between fecundity and parasite resistance was negative, suggesting a trade-off between these two traits. Our results thus suggest that parasite resistance is an important short-term predictor of the success of asexual *P. antipodarum* in this population.

Introduction

Theory on the maintenance of sex in natural populations emphasizes that any process favouring sexual reproduction has to overcome the short-term cost of producing males (Maynard Smith 1978; Lively and Lloyd 1990). Male-producing (sexual) lineages are expected to have lower population growth rates than all-female (asexual) lineages, assuming that life-history traits are otherwise equal. Testing the cost of sex hypothesis in natural conditions remains rare, because direct and rigorous tests require lifetime reproductive data on sexual and asexual lineages. Such data can elucidate the conditions required for sexual/asexual coexistence, as well as the conditions for coexistence of multiple asexual lineages.

Both evolutionary and ecological processes are expected to impact clonal diversity. The coexistence of multiple phenotypically similar asexual lineages calls for an explanation, firstly because fecundity selection is expected to reduce variation in fitness by favouring the best genotypes (Fisher 1930). Secondly, intraspecific competition is expected to lead to competitive exclusion, unless coexisting

clones rely on different resources (Gause 1934; Tilman 1977; Wilson et al. 2007). Nonetheless, many empirical studies report clonally diverse asexual populations (Table 1).

Table 1. Examples of empirical studies reporting clonally diverse asexual populations.

Taxonomic group	References
earthworms	(Jaenike et al. 1982; Christensen et al. 1989)
flatworms	(Pongratz et al. 1998)
rotifers	(Gomez and Carvalho 2000)
arthropods	(Menken and Weibosch-Steeman 1988; Honeycutt and Wilkinson 1989; Weeks and Hoffmann 1998; Stenberg et al. 2000; Vorburger 2006; Wilson and Sunnucks 2006; Ivens et al. 2012; Forbes et al. 2013; Fontcuberta Garcia-Cuenca et al. 2016)
nematodes	(Castagnone-Sereno 2006)
crustaceans	(Browne and Hoopes 1990; Theisen et al. 1995; Little and Hebert 1997; Butlin et al. 1998; Haileselasie et al. 2016)
molluscs	(Fox et al. 1996; King et al. 2011; Dagan et al. 2013)
bryozoans	(Hatton-Ellis et al. 1998)
fish	(Vrijenhoek 1984; Janko et al. 2012)
reptiles	(Bolger and Case 1994)
flowering plants	(Ellstrand and Roose 1987; van Dijk 2003; Paun et al. 2006; Majeský et al. 2012; Lovell et al. 2014)
unicellular algae	(Rynearson and Armbrust 2005; Richlen et al. 2012)
fungi	(Sanders et al. 1996; Milgroom et al. 2014)

While clonal diversity thus seems common, how clonal diversity is maintained and whether and how coexisting clones differ in their resource use and niche space remains unclear. Taken together, we should expect the elimination of sexual individuals, and a reduction in clonal diversity over time, unless there are countervailing selective forces that overcome fecundity variation and/or lead to the partitioning of resources by different genotypes.

Darwinian fitness is the genetic contribution to the next generation. Conceptually, fitness can be dissected into elements related to 1) basic life-history traits and 2) ecological interactions. The first component reflects the inherent genetic variation in the capacity of individuals to reproduce and survive (the “propensity of fitness” concept; Sober 1984; Brandon 1990). The second component - ecological interactions - tests competitive ability, defence against predators and parasites, and many

other suites of fitness-related traits that are relevant for success in intra- and interspecific ecological challenges (Wade and Kalisz 1990). For example, an inherent propensity for high fecundity may not be realized if an individual is a poor competitor or susceptible to virulent parasites. With some danger of oversimplifying a complex process, understanding the evolutionary success of coexisting lineages can be achieved by measuring relative contributions of these fitness components: basic life history traits and performance in ecological interactions.

In this study we examined the annual reproductive rates of asexual females and sexual females of freshwater snail *Potamopyrgus antipodarum* in experimental enclosures. We also performed laboratory infection experiments to determine genotype-specific resistance to infection. We used a well-studied population of the *P. antipodarum* snail from Lake Alexandrina (South Island, New Zealand). This population consists of a diverse set of independently derived asexual clones coexisting with a sexual population (Fox et al. 1996; Jokela et al. 2009; Paczesniak et al. 2014). The sexual population has been shown to pay the cost of males (Jokela et al. 1997; Gibson et al. 2017). In addition, asexual individuals are more common where parasites are rare or absent (Jokela and Lively 1995a; Vergara et al. 2013). Our results agree with earlier estimates of the cost of males in this population: the clones express high reproductive rates that should allow them to outcompete the sexuals. However, counter to our predictions, the reproductive rate of clonal genotypes was not correlated with their frequency in the population. Instead, our results suggest that parasite resistance is a more important predictor of clonal frequency in this population.

Methods

Study system

Potamopyrgus antipodarum is a Prosobranch snail that is native to the freshwater lakes and streams of New Zealand. Females brood their offspring and give birth to crawl-away juveniles. In laboratory conditions, the snails reach maturity in 4-12 months (Winterbourn 1970; Larkin et al. 2016). In natural populations, juveniles born in the spring may reach maturity in late summer, and before the next summer season, if born later in the season. Detailed studies of the lifespan in the natural populations are not available but some individuals can survive two summers (last author, personal observation). When field enclosures are started with adult snails in mid-summer (January), three cohorts are present in the enclosures after one year. The oldest cohort consists of the survivors of the original adults placed in the enclosure, the second cohort is the first-generation adult offspring (F1), and the third cohort are the recently born F2 juveniles that are distinguishable by their smaller size in a frequency distribution (Supporting information, Figure S1).

Many New Zealand *P. antipodarum* populations are characterized by coexistence between obligate sexual, dioecious, diploid individuals and obligate asexual, polyploid (usually triploid) individuals, which are predominantly female (Winterbourn 1970; Lively 1987; Neiman et al. 2011). Natural populations vary widely in the proportion of asexual females, and the proportion of asexuals tends to be higher in populations where virulent trematode parasites are rare (Lively 1987; Lively and Jokela 2002; Vergara et al. 2013). In mixed (sexual and clonal) populations, the clones comprise a genetically diverse assemblage that appears to be derived from the sympatric sexual population (Dybdahl and Lively 1995a; Fox et al. 1996; Jokela et al. 1999; Paczesniak et al. 2013).

We studied the snail population of Lake Alexandrina (South Island, New Zealand). Lake Alexandrina is a mesotrophic sub-alpine lake (Ward and Talbot 1984), and its key littoral habitats are arranged by depth and defined by dominant vegetation. We chose to study the mid-water habitat (1-3 m depth) because sexual and asexual snails coexist there in relatively stable proportions. Male frequency in this habitat was on average 13% over 14 years with standard deviation of 3.6% (last author, unpublished data). The exact mechanism of sex determination in *P. antipodarum* is not known, but based on the relationship between the frequencies of males and diploid females, a 13% male frequency corresponds to approximately 50% frequency of diploid females (Jokela et al. 2003). Clonal diversity among asexual lineages in Lake Alexandrina is high, with few common and many rare clones (Fox et al. 1996; Jokela et al. 2009; Paczesniak et al. 2013; Paczesniak et al. 2014). Lake Alexandrina is not special in this respect, as high clonal diversity has been reported for other *Potamopyrgus* populations in New Zealand (Dybdahl and Lively 1995a, 1998; Jokela et al. 1999; King et al. 2011).

Potamopyrgus antipodarum serves as an intermediate host for several species of common trematode flukes (Platyhelminthes: Digenea) (Winterbourn 1974; Hechinger 2012). The most common trematode in the Lake Alexandrina population of snails is *Microphallus* sp. (Winterbourn 1974; Hechinger 2012), which infects both sexual and asexual *P.antipodarum*. *Microphallus* reproduces asexually within the gonad of snail hosts and thus castrates (sterilizes) infected snails after 8-12 weeks post-infection. The parasite then develops into metacercarial cysts that hatch in the gut of the definitive waterfowl host. The adult worms produce eggs in the intestine of the waterfowl host. Eggs are then shed with the bird faeces into the environment.

Field experiment to measure fecundity of individual females. (For an overview of timeline and samples used in all field and laboratory experiments see Supporting Information, Figure S2.)

In January 2010, we collected a large random sample of *P. antipodarum* from the mid-water habitat of Lake Alexandrina by pushing a kick net through the bed of dominant macrophyte (*Isoetes kirkii*) while snorkelling (~200 m distance) between sites called “Camp” and “SW-End” (Jokela and Lively 1995b). The original sample consisted of several thousand snail individuals. Population density in this area was between 4000-7000 snails per square meter (the authors, unpublished data). We sieved the sample through a 2-mm sieve to retrieve snails larger than this threshold that were sexually mature or just approaching maturity (Negovetic and Jokela 2001). From this random sample, 200 individuals were separated for later genotyping in order to assess the genetic structure at the beginning of the experiment. We further refer to this sample as the “background sample” of the field experiment. The experiment was started the same day by randomly distributing 521 individuals into separate individual experimental enclosures.

The experimental enclosures (cages) consisted of a 250 ml plastic bottle with four cut-out holes (approx. 9x4 cm) on the sides to ensure water exchange. Similarly constructed cages have been used in an earlier study by Negovetic and Jokela (2001). To enclose the snail and its future offspring we attached a 250 µm mesh sleeve around the bottle frame using cable binders (Figure 1). The experimental (N=521) and control (N=5) cages (see below) were tied together on ropes (24 to 27

cages per rope), and the 21 ropes were anchored to the lake bottom at “Site 1” on the west shore of Lake Alexandrina (longitude 170.4415, latitude -43.9483). Cages were placed in the mid-water habitat (depth of 1.5 to 2m). We chose Site 1 for the location of this experiment because based on the long-term dataset on infection dynamics in Lake Alexandrina we knew that infection rates at this site are generally low. *Microphallus* sp. infection prevalences from 12 years between 2000-2017 were on average 7.0 % (standard deviation 4.0) in mid-water, and 10.1% (standard deviation 5.9) in shallow habitats (last author, unpublished data). We put a shoot (approx. 7 cm long) of *Elodea canadensis* macrophyte thoroughly washed using lake water in each cage to provide a food source for the snail before the periphyton could colonize the mesh surface of the cage. We collected the *Elodea* plants from the deep habitat zone of the lake (4-5 m) at Site 1. In order to account for the potential of accidental inclusion of juvenile *P. antipodarum* on *Elodea* shoots in the cages, we included five cages with only *Elodea canadensis*. These control cages were checked for presence of snails when we terminated the experiment (see below).

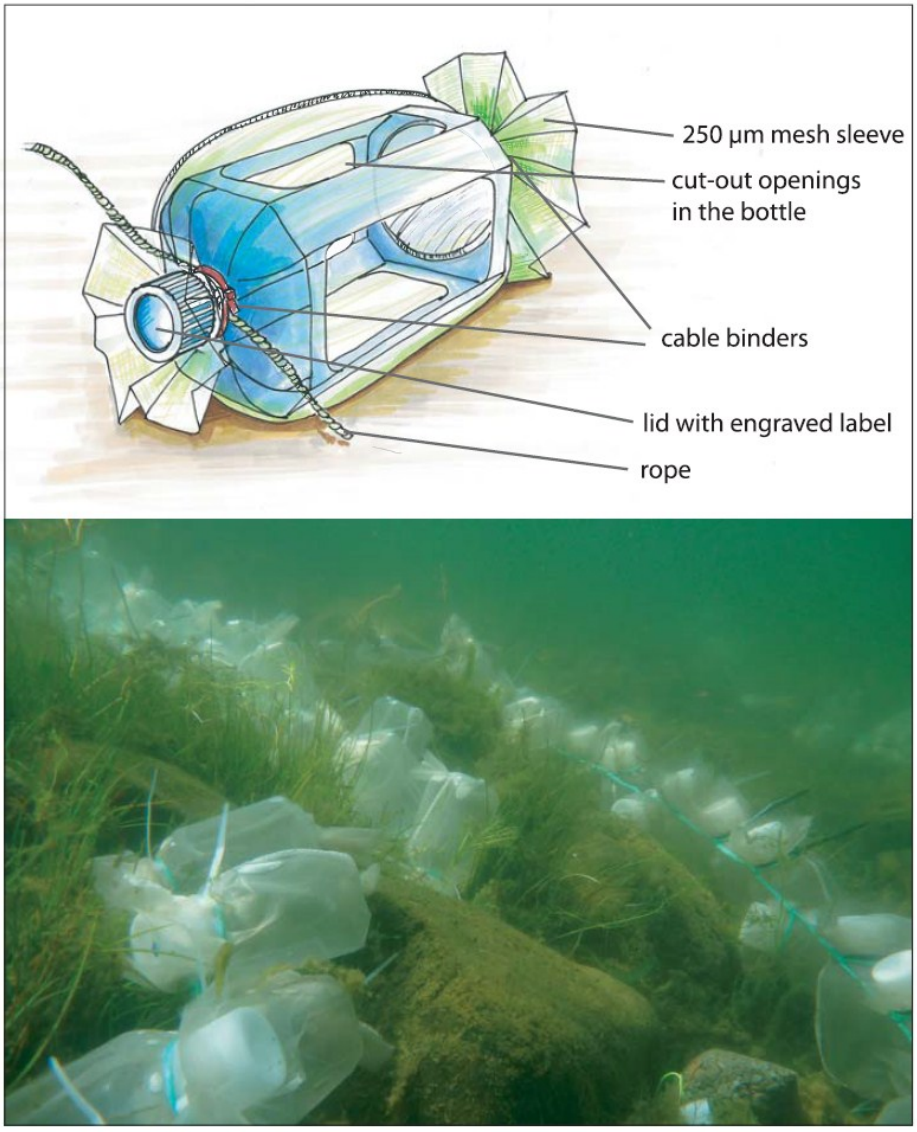

Figure 1. Top: a schematic drawing of an experimental enclosure. Bottom: an underwater photograph of the cage enclosures tied to ropes and anchored to the lake floor of Lake Alexandrina.

The field experiment lasted for one year. The experimental enclosures were emptied over 11 consecutive days in January 2011. Upon retrieval, each cage was visually inspected for holes or tears in the mesh. If the mesh was damaged, the cage was discarded. Intact cages were first washed from the outside in order to remove any snails that may have been crawling on them. We then cut the cages open in a tray filled with clean lake water. All snails retrieved from a single cage (later referred to as "family" or "isofemale lineage") were wrapped in a wet paper towel and transported in a cooler to Mt. John University Observatory near Lake Tekapo and Lake Alexandrina. Each family was then photographed with a digital camera against a white background with a millimetre-scale ruler for subsequent analyses. One to three individuals (if present) from each family were then frozen in liquid nitrogen for genotyping.

We analysed the digital photos of the families with ImageJ software (Rasband 1997-2012), using the function "Analyse Particles" with images converted to binary (black snails on white background). For each image, we recorded the number of snails (measure of reproductive output) and the length of each individual. As a measure of snail length we used Feret's diameter, which is the longest distance between any two points along the selection boundary.

Laboratory infection experiments to measure genotype-specific parasite resistance of field-collected snails

We conducted two laboratory infection experiments where we exposed field-collected snails from the mid-water habitat of Lake Alexandrina to eggs of the parasite *Microphallus* sp. Our purpose was to compare the susceptibility of common asexual snail genotypes in reference to average susceptibility of the sexual snails. We used a similar experimental design for the first (2009) and second (2011) experiments. The cage experiment in the field (see: "*Field experiment to measure fecundity of individual females*" for details) was started in 2010. As we collected snails in the same habitat for 3 consecutive years for these three experiments, we found the same common clones in these samples: all of the eleven common clones of the field cage experiment were also present in the 2011 infection experiment, and ten of these clones were represented in the 2009 infection experiment (Supporting Information, Table S1). These infection experiments were designed to answer separate questions about local adaptation of the snails and parasites; the results of the full experiment (including additional parasite sources) will be published elsewhere (last author, in preparation).

We collected faecal droppings of wild ducks at several sites around the shore of Lake Alexandrina to serve as a source for parasite eggs. The very small eggs (~10 µm) of *Microphallus* can be found in the millions in the faeces of ducks. We collected between 50 and 100 individual duck droppings in order to ensure that we sampled a diverse pool of faeces. In order to obtain parasite eggs we washed the faeces with water in flat-bottom trays and let the eggs sediment for several hours before pouring-off the water carefully and repeating the washing with fresh water. As a control for the parasite exposure, we boiled duck faeces to kill parasite eggs. Based on the infection success in previous controlled infection experiments using the same protocol (Lively et al. 2004), we were confident that we had a sufficient number of parasite eggs in the parasite treatment for all snails to be exposed. To directly confirm this expectation, in 2011 we counted parasite eggs with a Neubauer

hemocytometer, counting 16 grids per each of six independent samples. Based on these counts, we estimated that the sample of 3 L of diluted duck faeces used to expose the 900 snails in that experiment contained $39\times10^6 \pm 17\times10^6$ (SE) parasite eggs. This level of exposure should have been sufficient to expose all snails to parasites and infect most of the susceptible snails (Osnas and Lively 2004).

Target snails for the infection experiment were collected in the mid-water habitat of Lake Alexandrina from several sites around the lake. The same sites were sampled both in 2009 and 2011. We sieved the snails to retain adults (2 mm sieve, similarly to the field experiment described above). In 2009, we exposed 500 adult snails in each of two 10-L plastic trays over 12 days (total N=1000 snails), each day adding duck faeces suspension (or control suspension). Snails were then left to feed on the faeces (or control suspension) for two more days after last exposure (total exposure time 14 days). In 2011, we exposed 300 adult snails in each of three trays by adding faeces on two occasions, 3 days apart (total exposure time was 9 days, total N=900 snails). Similarly, we exposed control snails to boiled faecal suspension: 500 snails in each of 2 trays in 2009 and 300 snails in each of 2 trays in 2011 (N=1000 in 2009, N=600 in 2011). Faeces (or control boiled faecal suspension) were distributed by repeatedly pipetting several millilitres of homogenised suspension among the target trays in rotation to ensure that each tray received an approximately equal dose of parasite eggs. The total volume of faeces that was pipetted during exposure in these repeated pipetting rounds was 3.5 L in 2009 and 3 L in 2011. In 2009, the snails were exposed at the Edward Percival Field station (Kaikoura, New Zealand); and in 2011, the snails were exposed at EAWAG, The Swiss Federal Institute of Aquatic Science and Technology (Duebendorf, Switzerland), after transportation from New Zealand. The differences in the experimental protocols arose for practical reasons with handling the two experiments. We subsequently applied analytical solutions to the resulting data in order to compare the results between years (see “Statistical analysis” for details).

After exposure was completed, the snails were kept in standard conditions in the laboratory: temperature 18 ± 1 ˚C, with water changes and feeding (dry *Spirulina*) three times a week. Experiments were terminated by dissecting the snails at 12 weeks post exposure, which allowed us to definitively assess infection status and record snail sex. We then froze the heads of the snails in liquid nitrogen for genotyping.

Genotyping of snails from the field and infection experiments

We genotyped two individuals, if possible, from each family that returned from the field experiment. We also genotyped the 200 individuals of the random sample collected in 2010 (“background sample”) and all dissected snails from the two infection experiments with field-collected snails (2009 and 2011). The frequencies of clones in these samples are in Supporting Information, Table S1. All asexual (polyploid) genotypes were numbered (“genotype ID”) and genotype assignment was matched between the experiments. Snails were genotyped with nine polymorphic allozyme loci (6PGD, PEP-D, MPI, IDH1, IDH2, AAT1, AAT2, PGM1, PGM2) using cellulose acetate electrophoresis. We genotyped the individuals using methods described in Jokela et al. (2009). In short, we identified diploid individuals (i.e. sexual) by the presence of two (vs. three) alleles at least one or more loci, while polyploid heterozygotes showed three alleles or asymmetric banding patterns (indicative of AAB or ABB genotypes). Ploidy could not be assigned based on heterozygosity in the AAT1 and AAT2 loci alone. Therefore, the individuals that were completely homozygous for all loci, or heterozygous

only for the AAT (7.5% of 200 individuals of the random sample collected in 2010, and 3.3 % of 756 individuals from families in the field experiment) were treated as diploid.

We had to exclude 20% (104) of the original 521 experimental cages from any further analyses due to abrasion-related holes in the mesh. These exclusions were not specific to any of the 21 ropes to which the cages were attached. We found snails in 412 (98%) of the intact cages. We further excluded cages for which we failed to obtain a genotype (N = 9, 2.2 %), cages where we could not resolve the ploidy of the snails (N =67, 16.3 %), and cages where the genotypes of the two genotyped polyploid snails were not the same (N =23, 5.6 %). Altogether, after genotyping, we had to exclude 99 families of the 412 (24%) as unresolved. Together, we could confidently assign the reproductive mode and, in case of clones, the genotype of the mother (and, thus, the genotype of the daughters for asexual lines) for 313 families (162 sexual, 151 asexual) in the field experiment.

Assignment of common and rare clones

We classified the asexual families (151 polyploid families) to "common clone" (104 families) and "rare clone" groups (47 families). Assignment to common and rare clone groups was based on the relative frequencies of clones in the cages. We also used the random ("background") sample of 200 genotyped individuals from the same collection that was initially used to populate the cages to check if mortality during the experiment changed the frequencies of common clones. We assigned a clone as common if the clone was replicated by at least three independent families in the field experiment which resulted in 11 common clones. Each of these 11 clones represented at least 1.8 % of all asexual (polyploid) snails in the background sample. The frequencies of common clones found in the field experiment were positively correlated with the frequencies of these clonal genotypes in the background sample (Pearson $r = 0.94$, $n = 11$, $P < 0.001$), as expected if assignment of snails to cages was random and if the frequencies of clones in the cages at the end of the field experiment did represent the frequency of clones in the population as a whole. The two most common clones were each replicated in over 20 cages.

Assignment of best sexual lineages

Fecundity estimates of sexual snails are likely to be underestimated relative to asexuals because we had no means of identifying sexual vs. asexual females or male vs. female individuals in the field. Therefore, some of the cages have been initially populated with males, and we were not able to add mating partners for sexual females. Thus we cannot formally exclude the possibility that sexual females in the cages were affected by sperm limitation, although *Potamopyrgus* snails, like many other gastropods, can store sperm for many months (Wallace 1992). Because we cannot determine whether most sexual females would have carried sufficient sperm storage when entering the experiment, we examined the fecundity of the most productive 10% (N=16) of the sexual families separately. As these 10% of sexual families produced 60% of all diploid offspring in the experiment, we considered that these families provide the best comparison to the best-performing clonal lineages (in other words, they represent the group that are likely to pose the most competition to the asexuals in the mixed sexual/asexual population).

Statistical analysis

Fecundity estimates for each isofemale lineage (family) in the field cage experiment were based on the count of snails found in the cages after one year in the lake. We made no effort to identify the original female because the offspring were already adult when the cages were retrieved. Small juvenile snails were also observed in some of the retrieved cages. Because we could not determine whether these small juvenile snails represented a later brood produced by the original female or the second-generation offspring produced by the first generation adult offspring, we excluded juveniles that were smaller than 1.8 mm for all analyses of fecundity, delineating the second cohort with the information provided by a size-frequency distribution (see Supporting information, Figure S1).

We analysed variation in fecundity (number of offspring found in each cage) using generalized linear mixed models (GLMMs). We used Poisson-distributed errors and a log-link function as appropriate for count data. We used GLMMs to compare the fecundity of i) common clones, and ii) four types of lineages. Firstly, we contrasted the fecundity of 11 common clones. Secondly, we contrasted lineages that represented the 10% most productive sexual lineages (see above: "Assignment of best sexual lineages"), 90% less productive sexuals, common clones and rare clones (we call this fixed factor "lineage type" below). We alternatively used either i) "genotype identity" or ii) "lineage type" as fixed factors in these models. In both cases the GLMM model was corrected for the effect of rope by including rope as a random effect.

In the analysis of the infection experiments with field-collected snails, we first calculated the prevalence of infection for each of the clones that were common in the field cage experiment (PI_c) for both infection experiments of 2009 and 2011. Because we were not controlling for the parasite dose between the years, we could not compare the prevalence estimates for each clone directly between years. Therefore, we calculated relative susceptibility estimates (*RS*) for each clone using the mean prevalence of infection of the sexual snails (PI_s) in those experiments as a reference. Relative susceptibility (RS) was calculated as $RS = (PI_c - PI_s)/PI_s$. In other words, susceptibility of each clone was calculated relative to susceptibility of sexual snails in that particular experiment.

We calculated Pearson correlation coefficients to compare relative susceptibility (*RS*) of common clones between the 2009 and 2011 experiments. We also calculated Pearson correlation coefficients to test for correlations among the frequency of the clone in the population, resistance to parasites, and average fecundity.

All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp) or R version 3.5.0 (R Core Team 2018).

Results

In 2011, we retrieved 80 % (417) of the original 521 experimental cages and all five control cages intact. The losses were due to abrasion-related holes in the mesh, which were not specific to any of the 21 ropes to which the cages were attached. We found snails in 412 (98%) of the intact cages. Altogether, we counted and measured 10624 snails, of which 7133 were longer than 1.8 mm and therefore considered the first cohort of F1 offspring (see Methods for details). Of the five control

cages, which contained the food plant but were otherwise empty, three had no snails, one had three snails, and one had one snail (mean ± SE: 0.80 ± 0.58 snails). The presence of snails in some control cages indicates that some snails were able to invade either via attachment to initial food plant, or as newborn individuals through the 250-micron mesh, or via imperfections in cage assembly (e.g. loose cable tie attachment). As the mean number of invaders per cage was low and the invaders had to be very small at the point of invasion, and thus non-reproductive, we are confident that the estimates of reproductive rates of the enclosed adult females (mean ± SE: 17.11±1.06 offspring) were not significantly affected by the possible invaders. Of the experimental cages, five returned completely empty (1.2%) and 47 with one snail only (11.3%), which indicates that survival of snails in the cages was very high during the experiment. Two-thirds of the cages had more than three snails after the 12 months in the enclosure, indicating that most snails reproduced in the cages. Among the 313 families for which we knew the genotype of the mother, we found 162 diploid sexual (51.8%) and 151 polyploid asexual families. In the background sample (200 individuals of the random sample collected in 2010), we found 44.5 % diploid individuals (Supporting information, Table S1).

Fitness differences among the common clones

We retrieved 104 families belonging to the 11 most common clones, yielding 3236 offspring for the analysis of reproductive rate and variation in individual size. We found that the fecundity of the most productive clone (CL153) was more than four times that of the least productive common clone (CL92, mean ± SE: 62.4 ± 7.1 vs. 15.4 ± 1.7 offspring per female, Figure 2). We also detected significant among-clone variation in fecundity (Table 2). The number of offspring produced by these eleven common clones during one year was not significantly correlated with the frequency of the clone among experimental cages (Pearson r=-0.32, N=11, p =0.341; Figure 2), suggesting that lineage fecundity was not the main driver for the clonal frequencies in the population.

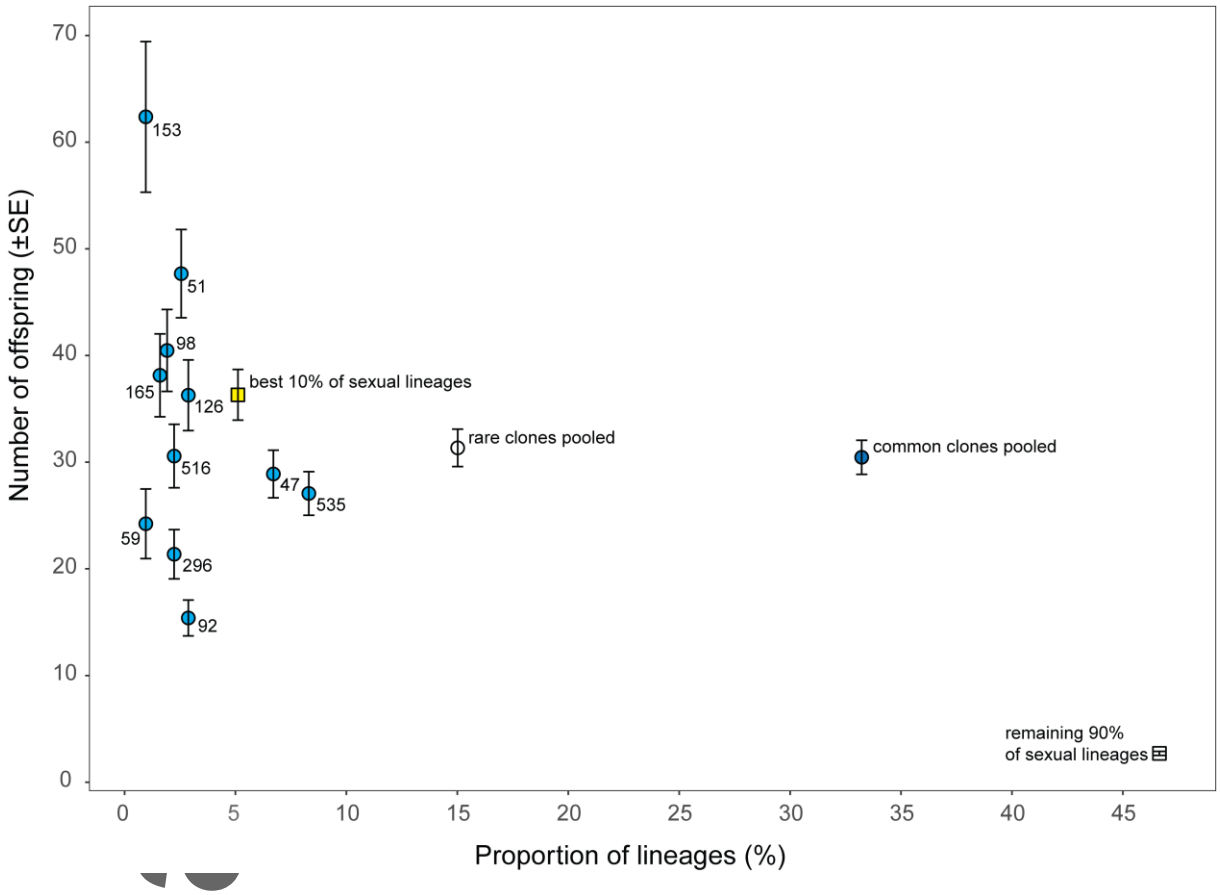

Figure 2. Mean number of offspring per female plotted against the frequency of each clone among the 313 experimental cages. Light blue circles depict the 11 most common clones, labelled by their respective genotype ID. Also shown are means for rare clones (open circle), all common clones pooled (dark blue circle), the most productive 10% of sexual lineages (yellow square), and the remaining 90% of sexual lineages (open square). Error bars represent one standard error of the mean.

Table 2. Analysis of variance of number of offspring within and among the common clones (N = 11).

Model: number of offspring ~ clone + rope Prob dist: Poisson, Link function: Log			AIC = 1025, BIC = 1027	
Fixed effects	*F*	Df1	Df2	*p*
Clone	20.90	10	93	<0.001
Random effects	Variance estimate	SE	Z	*p*
Rope	0.082	0.031	2.62	0.009

Life-history differences among common clones, rare clones, and sexual lineages

Overall, we detected significant variation in fecundity between lineage types: common clones, rare clones and sexual lineages (Table 3). The mean number of offspring did not differ among common and rare clones (adjusted mean ± SE: 31.34± 1.75 vs 30.460 ± 1.60, pairwise contrast: p = 0.379, Figure 2). Because we expected the estimate of offspring production by sexual females to be affected by sperm limitation and inclusion of males (see Methods: "Assignment of best sexual lineages" for details), we analysed the fecundity separately for the best 10%, and the remaining 90% of sexual lineages. The fecundity of the best 10% of sexual females (adjusted mean ± SE: 36.32 ± 2.38) was 17.5 % higher than the average fecundity of the common clones, but this difference was not significant. The fecundity of the remaining 90% of sexual lineages was lowest of all groups (adjusted mean ± SE: 2.700 ± 0.189, Figure 2). Nevertheless, the result suggests that when the male production is discounted (adult sex-ratio among sexuals in Lake Alexandrina population is approximately 1 male to 2 females (Jokela et al. 2003; Gibson et al. 2017)), the best sexual lineages still produce fewer females than the best asexual competitors (Figure 2). In other words, it appears that the sexual lineages pay the cost of males, as also found by Gibson et al. (2017).

Table 3. Analysis of variance of number of offspring among the common clones, rare clones, and sexual lineages.

Model: number of offspring ~ lineage type + type × rope Prob dist: Poisson, Link function: Log			AIC =2576, BIC = 2580	
Fixed effects	*F*	Df1	Df2	*P*
Type of lineage	741.3	3	309	<0.001
Random effects	Variance estimate	SE	Z	*p*
Rope	0.051	0.018	2.868	0.004

Correlation between susceptibility to parasites, fecundity, and clonal frequency – Eight of the 11 common clones in the field cage experiment were represented by at least 4 individuals in the infection experiments (Figure 3). The relative susceptibility of these eight clones was significantly and positively correlated across the 2009 and 2011 experiments (Pearson r=0.850, N=8, *P* =0.007). Relative susceptibility in the 2011 infection experiment was significantly and positively correlated with the productivity in the field cage experiment 2011 (Pearson r=0.76, N=8, *P* =0.029, Figure 3B), suggesting that more resistant clones were less productive. We did not detect a significant correlation between susceptibility in 2009 and productivity in 2011 (Pearson r=0.495, N=8, *P* =0.213, Figure 3A). Relative susceptibility in both the 2009 and 2011 experiments was negatively correlated with the frequency of the common clone in the random sample of 2011 (Pearson r=-0.783, N=8, *P* =0.022 for 2009; Figure 3C; Pearson r=-0.784, N=8, *P*=0.021 for 2011, Figure 3D). Together, these results suggest that, even though resistant clones had a lower baseline productivity than the susceptible clones, the resistant clones were nevertheless more successful in the population because their frequency was higher than that of susceptible clones.

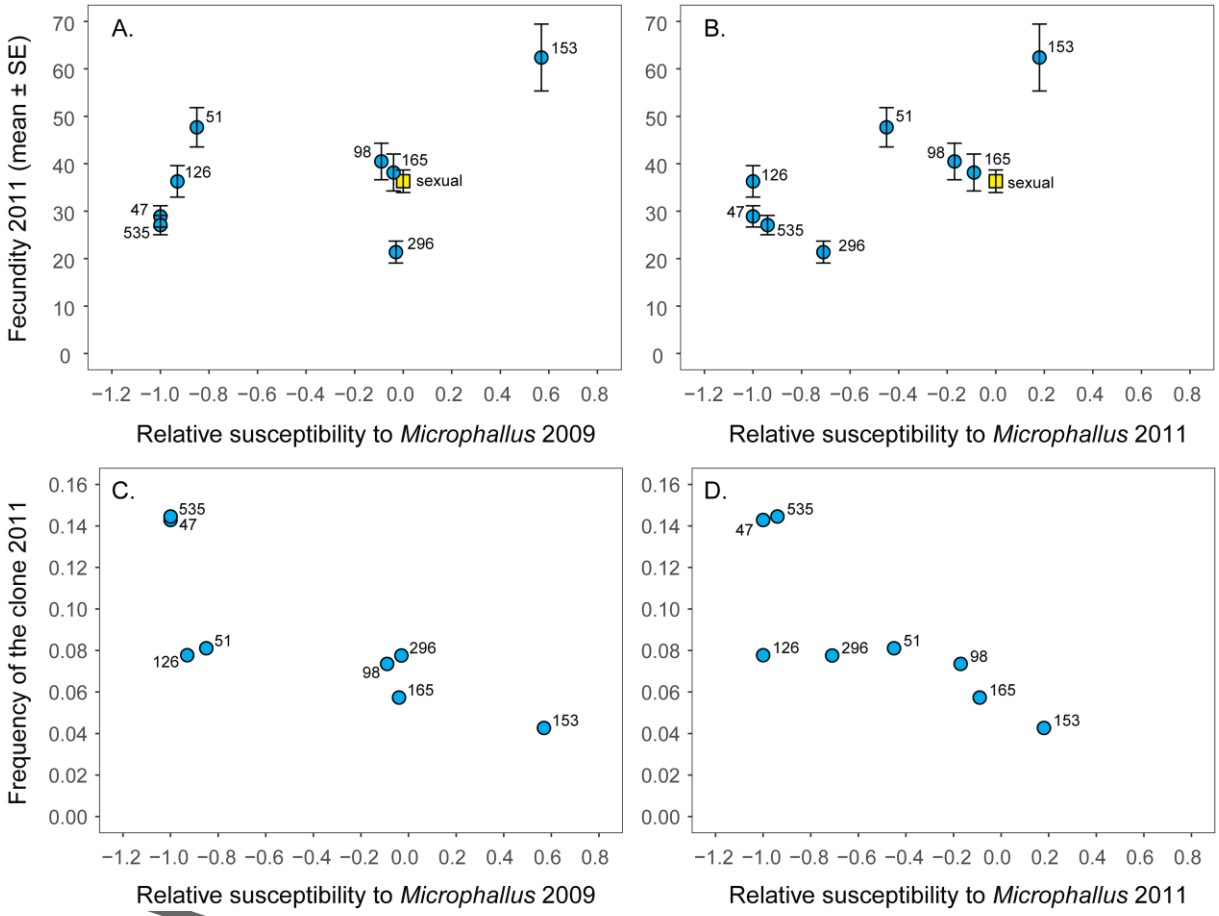

Figure 3. A., B. Relative susceptibility (*R*) to infection by *Microphallus* sp. in the infection experiments of 2009 and 2011 is positively correlated with the fecundity of common clones (adjusted means from the 2011 field cage experiment). Fecundity estimate for sexuals (A, B) is the value for the best 10% of sexual lineages in the field experiment. Relative susceptibility of the clone is calculated against the susceptibility of the sexuals in the experiment (yellow square). Using relative susceptibility as a measure allows comparisons across two infection experiments with potentially different parasite doses (see Methods). C. D. Frequency of the clone in the population in 2011 is negatively correlated with the clone's susceptibility to *Microphallus* sp. in the infection experiments of 2009 and 2011. Frequency of the clone is calculated as a proportion in the sample of asexual genotypes (control + experimentals, N = 256) used in the field cage experiment in 2011. The numbers in all panels represent clonal genotype IDs.

Discussion

Evolutionary success is easy to judge after the fact, but it is more difficult to identify the phenotypic traits that best predict success (Wade and Kalisz 1990). Intuitively, a high propensity to reproduce and survive is important, but do not necessarily fully determine variation in fitness. In natural populations, one has to account for the fact that the realized individual fitness includes the outcome of phenotype- and genotype-specific ecological interactions (Endler 1986; Wade and Kalisz 1990).

The key result of our study was that, in a mixed population of sexual and asexual snails, genotype-specific parasite resistance seems to be a better predictor of success than high performance in basic life-history traits.

Our initial goal was to contrast the fecundity of asexual (clonal) females to coexisting sexual females under near-natural conditions when excluding intra- and interspecific ecological interactions. In the short term, ecological and demographic costs of male production should favour clones when in competition with sexual lineages that produce males (Maynard Smith 1971, 1978). The cost of males is a problem-defining assumption for studies on the maintenance of sexual reproduction. This assumption is of central importance to the study of sex, because males reduce the per-capita birth rate in sexual populations. Thus, sexual reproduction needs to have significant short-term advantages that prevent asexual takeover of the population (Maynard Smith 1971, 1978). Earlier laboratory experiments have shown substantial variation in fecundity of asexual *P. antipodarum* lineages (Jokela et al. 2003) and that asexual lineages have significantly higher fecundity than their sexual counterparts in the laboratory (Jokela et al. 1997). Recently, Gibson et al. (2017) estimated that in competition experiments in large mesocosms, asexual *Potamopyrgus* lineages increased in frequency by 1.6-fold when competing against local sexuals, which corresponds to a two-fold cost of sex when the starting frequency of the asexuals is accounted for. Here, with enclosures in the natural habitat, we found that the fecundity of the asexual lineages during one year was on average similar to the reproductive rate of the 10% best sexual lineages (Figure 2) implying that after accounting for the cost of males, the per-capita growth rate of even the best sexual lineages should be distinctly lower than that of the asexual lineages under field conditions. As we did not compete lineages, we are measuring the growth potential of lineages. Such comparison of growth potential of sexual and asexual lineages has proven to be an informative way to assess the potential differences among lineages in absence of other factors (Wolinska and Lively 2008; Crummett and Wayne 2009; Stelzer 2011; Tabata et al. 2016). Nevertheless, the growth potential should also be referenced against potential ecological factors that can counterbalance the cost of sex (Wolinska and Lively 2008; Jokela et al. 2009; Morran et al. 2011; Stelzer 2012).

We expected that if the ecological factors that we excluded from the experiment were not important, the fecundity of the clones we measured in the enclosures would be a good predictor of clonal frequencies in the natural population. This expectation was not met by our data (Figure 2): the frequencies of the clones in the wild population did not positively correlate with the reproductive rates measured in the cage enclosures. Clonal structure in *Potamopyrgus antipodarum* populations is such that there are usually a few common and many rare clones (Dybdahl and Lively 1995a, b; Fox et al. 1996; Jokela et al. 1999, 2009; Paczesniak et al. 2014). In an earlier laboratory study, we found that many of the rare clones had low fitness relative to other clones, and many clones did not even reproduce at all (Jokela et al. 2003). Here, in a more natural setting, we did not find a significant difference in the reproductive output of common and rare clones (Figure 2). By contrast, we did find large variation in the reproductive potential of the clones (Figure 2). For example, the two most common clones (535 and 47) did not have the highest reproductive output in our experiment, and the range in the number of offspring varied almost four-fold among the common clones (Figure 2). This result suggests that there are other important selective factors than intrinsic reproductive rate contributing to the observed variation in genotype frequencies of sexuals and asexuals in the natural population of Lake Alexandrina.

We should note that, as we started the snail enclosures with a single female, the results should reflect the genotype-specific propensity for growth in conditions where competitive interactions are significantly reduced. We also expected that the majority of sexual females would carry sufficient sperm so that sperm limitation would not reduce the fecundity in the first generation. Indeed, sperm storage is a common feature in gastropods, including up to 18 months in laboratory cultures of *Potamopyrgus* (Wallace 1992). Even so, it is possible that some sexual lineages were limited by sperm availability, which is why we focus on the top 10% of sexual lineages.

With the laboratory infection experiments, our goal was to assess how parasite resistance contributes to lineage success in the natural population. While all common clones expressed relatively high fitness when measured with basic life-history traits (Figure 2), parasite resistance turned out to be a strong predictor of clonal frequency in the field (Figure 3). We conclude from these results that, in order to become a "successful clone," a productive suite of life-history traits is important, but resistance to local parasites selects the winners. Coevolutionary theory predicts that a common resistant genotype "invites" a co-evolutionary response from the parasite. Our results show that baseline variation in life-history traits has the potential to produce large variation in frequencies of snail clones over a short number of generations, and that lineages that become successful in Lake Alexandrina population are relatively resistant to co-existing parasites. We found that parasite resistance was a more important predictor of local genotype frequencies than the baseline variation in key life-history traits (Figure 3). In such a co-evolutionary scenario, the lineage success is expected to be transitory, defined by the co-evolutionary dynamics of the parasite adaptation. This result, coupled with earlier studies showing frequency-dependent fluctuations in frequencies of common *P. antipodarum* clones associated with parasite adaptation (Lively 1987; Dybdahl and Lively 1998; Jokela et al. 2009), support the hypothesis that parasite coevolution is important for maintenance of clonal and sexual diversity in these snail populations.

The results of this study suggest that reproductive output of many of the common clones is high enough to successfully outcompete the sexual population (Jokela et al. 1997). It thus appears that without strong ecological interactions (e.g. parasitism, intraclonal and/or interclonal competition) in natural populations, the clones would have the ability to replace the coexisting sexual lineages over few generations. In natural habitats, the infection risk by trematode parasites experienced by *P. antipodarum* is not constant (Jokela and Lively 1995b). Instead, the likelihood of infection varies spatially, at least in part due to the density of the transmission stages, which for the parasite species that use multiple hosts in their life cycle may also reflect the density of definitive host. Our study demonstrates cost of resistance for *P. antipodarum*, which in part may contribute to genetic heterogeneity we observed in rather small spatial scales (e.g. between habitats) in natural populations (Fox et al. 1996; Jokela et al. 1999; Paczesniak et al. 2014). It appears that the most productive clones are also more susceptible to parasites (Figure 3), while the clones that are most common are among the most resistant and still competitive against the average sexual lineages. This result suggests that the fitness ranking of the "best clones" is subject to infection risk, but it also implies that conditions for sexual lineages to win might be ecologically narrow. The sexuals are between a rock and a hard place - even in environments with high infection risk, some resistant and productive clones may have a transitory competitive advantage before adaptation by the parasites breaks the resistance advantage of the clones (Jokela et al. 2009).

Our study aimed to understand the relative contribution of fitness components to the success of asexual clones in a natural population of coexisting sexual and asexual snails. In conclusion, our results demonstrated that parasite resistance is a better predictor of the frequency clones than high performance in basic life history traits (survival, reproduction). In our enclosure experiment we measured reproductive rate of *P. antipodarum* clones in conditions that excluded the majority of ecological interactions (parasitism, intra-specific competition). We found that the mixed population of *Potamopyrgus* snails in Lake Alexandrina consists of many clones that have the reproductive potential to be able to outcompete the sexual lineages. Indeed, our results suggest that the conditions in which the sexuals are favoured in Lake Alexandrina require a large advantage in ecological interactions. This is in accordance with laboratory competition experiments where asexual lineages have been shown to increase in frequency in the absence of parasite pressure (Gibson et al. 2017; Jokela et al. 1997). While we were able to explain the frequencies of clones in the population with infection experiments that revealed the genotype specificity of parasite resistance, such an experiment is not sufficient to address longer term advantage of sexual lineages under variable parasite pressure. Indeed, parasite-mediated selection for sex has been shown to fluctuate in Lake Alexandrina (Gibson et al. 2018). What our study suggests is that strong ecological interactions are required in order to explain why sexual lineages are successful in this population. In Lake Alexandrina the sexual population is most abundant in the shallow regions of the lake, where parasite risk is highest (Jokela and Lively 1995a). Studies including a larger geographic scale support the view that parasite risk is likely to be an important contributor to the success of sex in many of these snail populations (Vergara et al. 2013; Gibson et al. 2016).

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