

Antagonistic species interaction drives selection for sex in a predator–prey system

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Abstract

The evolutionary maintenance of sexual reproduction has long challenged biologists as the majority of species reproduce sexually despite inherent costs. Providing a general explanation for the evolutionary success of sex has thus proven difficult and resulted in numerous hypotheses. A leading hypothesis suggests that antagonistic species interaction can generate conditions selecting for increased sex due to the production of rare or novel genotypes that are beneficial for rapid adaptation to recurrent environmental change brought on by antagonism. To test this ecology-based hypothesis, we conducted experimental evolution in a predator (rotifer)–prey (algal) system by using continuous cultures to track predator–prey dynamics and in situ rates of sex in the prey over time and within replicated experimental populations. Overall, we found that predator-mediated fluctuating selection for competitive versus defended prey resulted in higher rates of genetic mixing in the prey. More specifically, our results showed that fluctuating population sizes of predator and prey, coupled with a trade-off in the prey, drove the sort of recurrent environmental change that could provide a benefit to sex in the prey, despite inherent costs. We end with a discussion of potential population genetic mechanisms underlying increased selection for sex in this system, based on our application of a general theoretical framework for measuring the effects of sex over time, and interpreting how these effects can lead to inferences about the conditions selecting for or against sexual reproduction in a system with antagonistic species interaction.

KEYWORDS

Chlamydomonas, effects of sex, evolution of sexual reproduction, experimental evolution, fluctuating selection, predator–prey, trade-off

1 | INTRODUCTION

Despite inherent costs (Bell, 1982; Maynard Smith, 1978; Williams, 1975), sexual reproduction has been shown to be advantageous in the face of selection by interacting or coevolving species

(Brockhurst et al., 2014; Busch, Neiman, & Koslow, 2004; Ellison, Cable, & Consuegra, 2011; Greeff & Schmid-Hempel, 2010; Haafke, Abou Chakra, & Becks, 2016; Jokela, Dybdahl, & Lively, 2009; Lively, Craddock, & Vrijenhoek, 1990; Lively & Morran, 2014; Morran, Schmidt, Gelarden, Parrish, & Lively, 2011; Vergara, Jokela,

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& Lively, 2014). If species interactions are antagonistic and recurrent, as may occur in predator–prey or host–parasite systems, the ensuing fluctuating environment can cause populations to be continuously moved away from local fitness optima where their genetic associations already match the current environmental conditions. Organisms must then rapidly adapt to the novel conditions which can provide a benefit to sex (Barton, 1995; Becks & Agrawal, 2010; Charlesworth, 1993; Haafke et al., 2016; Korol & Iliadi, 1994; Lynch & Deng, 1994; Otto & Nuismer, 2004; Peters & Lively, 1999).

This idea has been formalized into the Red Queen hypothesis (RQH). It suggests that coevolution of host and parasite can drive the evolution of sex through negative frequency-dependent selection where common genotypes evoke a genetic response in the antagonist, resulting in a fluctuating environment composed of continual biotic interaction (Bell, 1982; Jaenike, 1978). Related to the RQH are early population genetic models considering species interactions for the maintenance of sex, which suggest that interactions without coevolutionary change can also select for sex when fluctuations in population sizes of interacting species generate a continuously changing environment (Bell, 1982; Jaenike, 1978). Within a predator–prey system, changing predator densities and predation pressure can lead to fluctuations in selection for prey defence when predation pressure is high, and for prey competition when it is low, given the frequently observed trade-off in these two traits (Kasada, Yamamichi, & Yoshida, 2014; Lind et al., 2013; Yoshida, Hairston, & Ellner, 2004). Even though fluctuations in predator–prey population sizes are common in nature (Kendall, Prendergast, & Bjornstad, 1998), can lead to rapid adaptation in prey (Agrawal, Hastings, Johnson, Maron, & Salminen, 2012; Friman, Jousset, & Buckling, 2014) and to fluctuating selection depending on predator densities, there is still no empirical test for the hypothesis that cyclic predator–prey dynamics and selection can provide the conditions selecting for sex in the prey. Such a test would add to our understanding of the evolution of sex by considering antagonistic species interaction, but without victim–exploiter coevolution.

For considering species interaction and fluctuating population densities, there are at least two population genetic mechanisms that could drive selection for sex (Gandon & Otto, 2007). Theory predicts that changes in selection can lead to changes in the sign of fitness interaction (epistasis and dominance) followed by the generation of genetic associations of the same sign (Barton, 1995). These changes in fitness interaction and genetic association continuously result in mismatches between which genotypes are currently most fit and which are most common (i.e. a situation of rare advantage) (Lively & Morran, 2014). These conditions can select for sex through an immediate short-term benefit by increasing the frequency of fit genotypes, as well as through an intermittent long-term benefit by increasing variance in fitness (Barton, 1995). Under these conditions, we would expect to see cyclic changes in the mean fitness (short-term effect) and variance in fitness (long-term effect) of sexually versus asexually derived offspring from within the same population. The alternative population genetic mechanism is if epistasis is weak and/or invariable and there are sustained changes in directional selection; sex can

be beneficial for increasing fitness indirectly by accelerating the rate of response to selection (long-term benefit) (Barton & Otto, 2005; Maynard Smith, 1978). Despite theoretical predictions for short- and long-term effects of sex, there remains a dearth of empirical studies aiming to link current ecological and population genetic theory for the evolution of sex.

Herein, we tested the idea that predator–prey cycles can lead to fluctuating selection and that recurrent, antagonistic species interaction can select for increased rates of sex in the prey. We conducted an experimental evolution study using an obligate asexual predator (rotifer, *Brachionus calyciflorus*) and facultative sexual prey (unicellular alga, *Chlamydomonas reinhardtii*), both of which can be commonly found within freshwater plankton communities. Planktonic herbivores encounter a broad range of algal prey and can apply strong selective pressures on phytoplankton (Franks, 2001; George, Lonsdale, Merlo, & Gobler, 2015; Jones & Ellner, 2004; Lewis, Breckels, Steinke, & Codling, 2013; Muylaert et al., 2010; Reichwaldt, Wolf, & Stibor, 2004; Sommer, 1989; Sunda & Hardison, 2010). Both species are facultative sexual, but we used here rotifers from a monoclonal, obligate asexual laboratory stock to constrain predator (co)evolution, as we were only interested in investigating selection for sex in the prey. *C. reinhardtii* is heterothallic with two mating types, + and -, and although the frequency of sex in natural populations of *C. reinhardtii* is unknown, both mating types are found in nature (Harris, 2009).

We formulated and tested several predictions relevant to our system and experimental design. First, in accordance with previous results, we predict fluctuating selection for competitive versus defended prey (Becks & Agrawal, 2010; Becks, Ellner, Jones, & Hairston, 2010, 2012; Yoshida et al., 2004). Specifically, changes in traits determining defence and competition are expected to fluctuate over time, with prey defensiveness selected for and competitiveness selected against when predation pressure is maximized (Ellner & Becks, 2011). Prey defence is assessed via cell morphology and predator growth rates; increases in prey defence will lead to reduced predator growth rates (Becks, Ellner, Jones, & Hairston, 2010), as will the increased handling time of defended prey types that lose motility (Koch & Becks, 2016) or evolve to grow as colonies instead of single, motile cells (Becks, Ellner, Jones, & Hairston, 2012).

Second, we predict that fluctuating selection can drive increased rates of sex in the prey. This prediction is based on population genetic theory and modifier models that help explain the conditions under which sex (and recombination) can evolve by considering the dynamics at genes that modify reproduction mode and the frequency of crossover events (Gandon & Otto, 2007; Nei, 1967). We also predict changes in the rate of sex will lag behind changes in selection because it takes time for the prey to produce zygospores as a result of sexual reproduction (Harris, 2009). In *C. reinhardtii*, populations grow via asexual (vegetative) reproduction, whereas the switch to sexual reproduction is typically induced by nitrogen starvation (Harris, 2009). Previous studies using *C. reinhardtii* showed, however, that the switch from differentiating as a vegetative cell to a sexual cell can be altered by

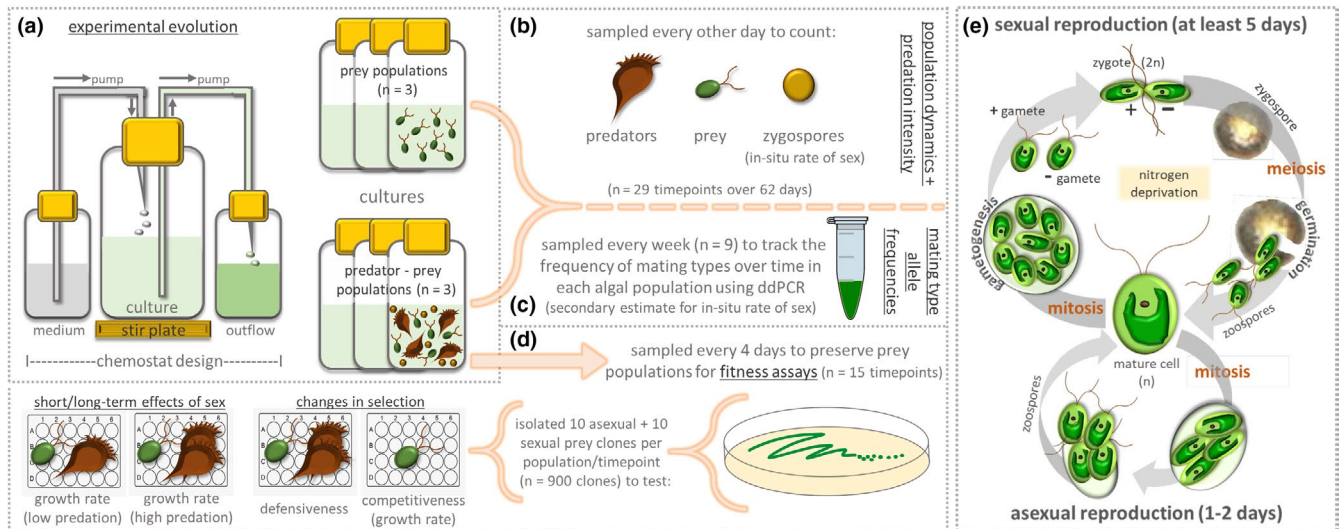


FIGURE 1 Experimental set-up and prey life cycle. (a) Replicate algal prey populations with and without rotifer predators were experimentally evolved over 62 days using continuous cultures (chemostats). (b) All chemostats ($n = 6$) were sampled every other day to track predator-prey population dynamics, predation intensity (predator/prey ratios) and the rate of sex in algal prey populations over time (predators were absent within controls and zygospores were never observed). (c) All replicate algal populations ($n = 6$) were sampled weekly to track the frequency of mating type alleles in each population over time, using ddPCR, which served as another estimate of the in situ rate of sex. (d) Prey populations ($n = 3$) from the predation treatment were sampled every 4 days (15 time points) to isolate replicate sexual and asexual algal clones for fitness assays. One set of assays tested for changes in selection for competitive and defended prey over time; competitiveness in the prey was measured using absolute growth rates, whereas predator growth rates were used as a proxy for prey defensiveness. The other set of assays tested the short- and long-term effects of sex by measuring fitness of sexually and asexually produced offspring in the environment that they were produced in; to account for fluctuations in selection for defence and competition, algal growth rates were measured under low or high predation pressure, which simulates the selection conditions that the clones evolved under (see Supplementary Methods). (e) An asexual generation in the algal prey herein lasts 1–2 days, whereas the sexual cycle requires at least 5 days, representing a significant cost of sex in this system

selection and occur when nitrogen is replete (Bell, 2005; Koch & Becks, 2016). Previous work also showed that the presence of rotifer predators alone was not sufficient to induce the switch to sex as spontaneous sexual reproduction occurred only in *C. reinhardtii* populations with predators when the costs of sex were experimentally removed (Koch & Becks, 2016).

Third, we expect that patterns for the effects of sex (short/long term) over time correlate with changes in selection over time, based on population genetic predictions (Barton, 1995; Barton & Otto, 2005; Gandon & Otto, 2007; Maynard Smith, 1978). For example, we would expect changes in the direction of selection to be in synchrony with changes in the mean (hereafter: short-term effect) and/or variance of fitness (hereafter: long-term effect) of sexually produced offspring when changes in selection lead to changes in the sign of fitness interaction. Finally, we present a discussion on inferring the underlying population genetic mechanism providing a benefit to sex under the conditions in the present experiment.

2 | METHODS

2.1 | Model system and experimental evolution

Our model system consisted of the haploid algal prey, *Chlamydomonas reinhardtii*, and rotifer predator, *Brachionus calyciflorus*. From the

Chlamydomonas Resource Center at the University of Minnesota (Laudon, 2013), we obtained isogenic *C. reinhardtii* strains for each mating type, CC-1010 (+ mating type) and CC-1009 (- mating type), which are complementary descendants of a single wild-collected zygote (Proschold, Harris, & Coleman, 2005). Rotifers were taken from an asexual, monoclonal laboratory stock culture. This culture descends from a single natural source that was shown to have substantial genetic variation in the strength of the stimulus needed to induce sex, but has subsequently lost the stimulus for sex and is now laboratory-adapted (Becks & Agarwal, 2010).

We conducted an experimental evolution study that used continuous cultures (chemostats) for evolving replicate ($n = 3$) algal populations with and without (control) predators in order to follow predator-prey dynamics, predation intensity (predator/prey ratios) and the rate of sex in algal prey populations over time (Figure 1a,b). Each chemostat had a volume of 850 ml, was continuously stirred, ventilated, and fresh media [80 μ M nitrogen] supplied at a constant flow through rate of 0.11 d^{-1} . Cultures were maintained at 26°C. To allow zygospore maturation (Harris, 2009), we set the cultures to an 8h L:16h D cycle. We inoculated all 6 chemostats with 1×10^6 cells of each mating type, followed by 300 rotifers in 3 of the chemostats 3 days later. Chemostat sampling began 4 days after rotifer inoculation.

The experimental evolution study lasted for 62 days, and each chemostat was subsampled (a) every other day (with a 9-day gap

between the last two sampled time points) by extracting 50 ml samples to enumerate predators, prey and zygospores using a Neubauer haemocytometer and compound microscope (Figure 1b), (b) every week to track the frequency of mating types over time in each algal population using ddPCR (Figure 1c) and (c) every 4 days by streaking out subsamples onto agar plates to isolate algal clones for subsequent fitness assays to test for changes in selection and the effects of sex (Figure 1d). These subsequent assays, however, could not be performed for the control group since sex was never observed in the algal populations without predators (see section 3). Zygospores are thickly walled zygotes with a distinct size and orange colour that makes them a clearly visible product of mating following the fusion of two (\pm) gametes (Harris, 2009). Herein, zygospore counts are used to estimate the in situ rate of sex in the prey.

C. reinhardtii is an isogamous species; therefore, the classic 'cost of males' does not exist here (Lehtonen, Jennions, & Kokko, 2012; Maynard Smith, 1971, 1978). Under the experimental conditions herein, an asexual generation of *C. reinhardtii* and *B. calyciflorus* takes 1–2 days, whereas the *C. reinhardtii* sexual cycle requires at least 5 days (Figure 1e). Thus, one major cost of sex in this system is the time necessary for completing the sexual cycle (population growth does not occur during the zygospore stage of the sexual cycle), in addition to the potential for recombination load.

2.2 | Assays measuring changes in selection and the effects of sex

The following assays were conducted for 15 time points, over 62 days, and each replicate chemostat ($n = 3$) of the predation treatment to test for changes in selection, as well as the effects of sex. First, we randomly isolated 10 asexual and 10 sexual clones to serve as a representative population subsample (see Supplementary Methods). Then, we measured the fitness (growth rate) of these 900 clones in the absence of predators (0 rotifers), under low predation (5 rotifers), under high predation (10 rotifers), as well as their defence against predation. The first and last measurements are used as proxies for traits related to competitiveness and defensiveness, respectively. The growth rate measurements under low and high predation are used as prey fitness measurements under the experimental conditions the algae experienced over time as rotifer population density, and thus predation intensity, fluctuated over time (see Supplementary Methods). A common garden experimental design was used for all assays, which were conducted at the same time and after several generations in the absence of predation (i.e. the time between sampling the last set of clones from the chemostats and assaying them was about a month, ~30–90 additional asexual generations). Thus, observed differences in all fitness assays are predicted to be heritable. We determined algal growth rates from optical density measurements, and predator fitness was used as a proxy for estimating algal defence (see Supplementary Methods).

We tested for changes in selection for competitive and defended prey using the absolute population growth rate (no predation) and

defence data. We also determined the frequency of defended phenotypes out of these 20 clones, based on their morphology and behaviour. Phenotypes that grow in colonies or lose motility increase prey handling time for the predator and lead to reduced predator growth rates (Figure S1) (Becks et al., 2010). They are thus considered defended, whereas motile single-celled phenotypes (wildtype) are considered undefended; undefended prey are more competitive (grow faster) compared to defended phenotypes (Becks et al., 2010; Yoshida et al., 2004).

We used the same set of sexual and asexual offspring ($n = 10$ each) to estimate the effects of sex by comparing the mean fitness (short-term effect) and variance in fitness (long-term effect) of offspring in the environment that they were produced in. Therefore, to account for fluctuations in selection for growth and defence, we used the growth rate data from the low (L) and high (H) predation environments, respectively, which simulates the selection conditions that the clones evolved under. We then matched each time point to either environment based on predator–prey ratios (see Supplementary Methods). We used these designations to determine from which assay environment (L, H) data would be plotted for the short- and long-term effects of sex.

To estimate selection differentials, we first plotted a linear regression of the phenotypic data (fitness: growth rate versus defence) for each of the 20 clones to obtain a slope for each time point. These selection differentials (absolute values) were then plotted over time to show the changes in selection for each replicate chemostat, along with the frequency of zygospores (in situ rate of sex).

2.3 | Wavelet analyses

We used wavelet analyses to estimate the period of cycles in the (a) algal prey populations, (b) predator–prey ratios, (c) changes in selection, and (d) short- and long-term effects of sex (WaveletComp package in R (Roesch & Schmidbauer, 2014)) (see Supplementary Methods). For all wavelet analyses, we followed standard time series analysis practices and used smoothed time series data (spline function in R) after detrending (pracma package (Borchers, 2015)). Generally, wavelet analysis decomposes a time series into time/frequency space simultaneously and provides information on both the amplitude of any periodic signal within the series and how this amplitude varies with time. Significances of periodicity were assessed by testing the null hypothesis that a period is not relevant at a certain time of the time series by using a simulation algorithm representing white noise (default methods in the WaveletComp package (Roesch & Schmidbauer, 2014)). We further tested for a significant relationship between oscillations in the predator–prey ratios and (a) population mean defence, (b) population mean growth (competitiveness), (c) the frequency of defended phenotypes and (d) the short- and long-term effects of sex. We did this by identifying the dominant and significant phase shifts between these time series. Next, we used wavelet coherence analyses to measure the local correlation between two series over a specific period (WaveletComp package in R (Roesch & Schmidbauer, 2014; Torrence

& Compo, 1998)). We extracted from these analyses the significant phase shifts, which are expressed in days and show the number of days the defence, competition, or frequency of defended types is lagging behind the predator–prey ratio.

2.4 | Estimating rates of sex

To quantify sex, we measured (a) in situ rates of sex, (b) allele frequencies of mating types and (c) conducted a test for evolutionary changes in the rate of sex. The first two measurements were done for all control and experimental populations ($n = 6$), whereas the last test was only conducted for the prey populations within the predation treatment. Zygospore counts and changes in the frequency of mating types over time were used as estimates of the in situ rate of sex, with the latter using the weekly population subsamples and digital droplet polymerase chain reaction (ddPCR) (see Supplementary Methods) (Koch, Jeschke, & Becks, 2015). We conducted an additional assay under standardized conditions testing for the time that is required for the production of a mating reaction (pellicle formation) comparing algal populations from day 62 to the ancestors (see Supplementary Methods). We used the time that is required for the production of a mating reaction (pellicle formation) based on the assumption that the ancestor switches only in a nitrogen-free environment or when all nitrogen is consumed. In contrast, the genotypes isolated from the predation treatment, which likely evolved to switch in the absence of the trigger, were predicted to switch earlier as compared to the ancestors. Pellicle assays can be used as an end-point detection method for quantifying a rate of sex in *Chlamydomonas* because gametes are too difficult to distinguish, visually, from vegetative cells (Harris, 2009). Pellicles are the result of aggregated zygotes forming a film on the air–water surface and are easily seen by the naked eye, which eliminates the need to continuously sample and disturb the mating reaction to look for gametes or zygotes. Rotifers were not included in this assay.

3 | RESULTS

3.1 | Predator–prey population sizes fluctuate over time

Over the course of 62 days, we found that prey densities (Figure 2d–f) and predation intensity (Figure 2g–i) fluctuated significantly over time in the predator–prey treatment (wavelet analysis (Figure S2): algal prey = 8.6 ± 0.6 days; predator–prey ratio = 7.8 ± 1 days). In contrast, algal populations were significantly more stable in the controls (Figure 2a–c; coefficient of variation for days 10–62: predation = 0.41 ± 0.047 , control = 0.17 ± 0.045 ; Kruskal–Wallis test: $\chi^2 = 3.86$, $df = 1$, $p < .05$) and maintained higher densities at carrying capacity (control vs. predation treatment: maximum population density, Wilcoxon–Mann–Whitney U test: $W = 7,535$, $p < .0001$).

3.2 | Predation pressure and selection changes over time

Across all prey populations, algal competitiveness (growth rates) and defence levels fluctuated continuously with a significant period of 9.3 ± 1 days, indicating that selection for competitive ability and defence changed over time (Figure 3a–f). Furthermore, we found that the predator–prey ratio (Figure 2g–i) and prey population mean defence (Figure 3a–c) cycled in-phase (i.e. the maximum in the prey defence trait and the predator–prey ratio occurred simultaneously) (phase shift: 0.5 ± 1.5 days, Figure 3d–f). We also found that changes in the predator–prey ratio and frequency of defended types oscillated in-phase (phase shift: 0.3 ± 1.7 days, Figure 3d–f). In contrast, the predator–prey ratio (Figure 2g–i) and prey competitiveness (mean population growth rate, Figure 3a–c) cycled in anti-phase where changes in the growth rate lagged behind changes in the predator–prey ratio by 6.1 ± 0.6 days (Figure 3d–f). Taken together, these results indicate that oscillations in predation pressure drove oscillations in selection for prey defence and competition with a period of 2–9 algal generations depending on the number of sexual and asexual reproduction cycles (9 asexual generations at the most and ~2 sexual generations at the least).

3.3 | Increased selection for sex in the algal prey populations

Zygosporos were never observed in the controls during the experimental evolution study (Figure 2a–c), whereas zygosporos, and thus sex, were observed at several time points in the predation treatment (Figure 2d–f). These changes in the in situ rate of sex lagged behind the changes in selection (Figure 5a–c). Using chemostat systems where fresh medium was supplied at a constant rate, *C. reinhardtii* did not experience nitrogen starvation in our study. Since *Chlamydomonas* did not experience the trigger of nitrogen starvation to switch to sexual reproduction, and since it was previously shown that the switch to sexual reproduction is not a stress-induced response (Koch & Becks, 2016), this observation suggests selection for sexual reproduction.

We also tracked mating type frequencies over time using ddPCR and found that one mating type quickly moved towards fixation across all three control populations (Figure 4a–c). We found a similar decline in one mating type for the populations from the predation treatment, but frequencies of the lower type started to increase to similar frequencies as the other type around week 4 (Figure 4d–f). Importantly, an almost equal frequency of the two mating types suggests high frequencies of sex; a germinating zygosporos releases an equal number of progeny based on mating type.

To test for heritable and evolutionary changes in the rate of sex, we compared the time required to form mating reactions between the ancestor and evolved populations from the end of the experiment. We found that sex occurred significantly faster in the

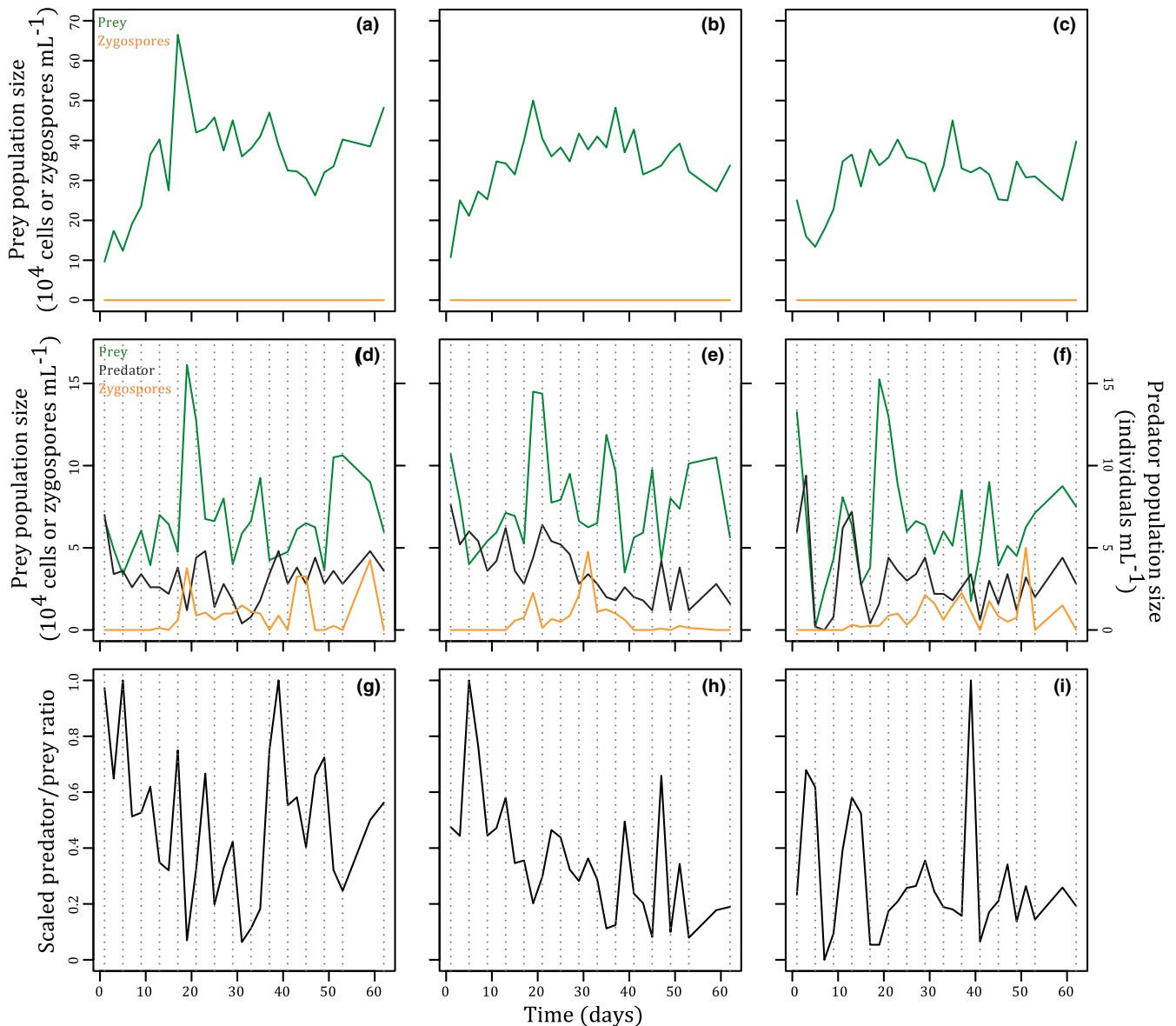


FIGURE 2 Predator–prey population dynamics and in situ rate of sex. Predator (black) and prey (green) population dynamics, and rate of sex (zygospores, orange) for replicate populations without (a–c, controls) and with predation (d–f). Predation intensity (g–i) calculated as the ratio of predator/prey densities and scaled to the maximum value for each corresponding population of the predation treatment (d–f). Dashed vertical lines (d–i) correspond to the 15 time points when prey populations were sampled for subsequent testing

evolved algal populations than in the ancestors, under the nitrogen conditions that the populations evolved in (80 μ M nitrogen; evolved, 315 ± 45 min; ancestors, 450 ± 0 min: Wilcoxon–Mann–Whitney U test: $W = 48$, $p < .001$) but not control conditions (0 μ M nitrogen; evolved, 180 ± 30 min; ancestors, 180 ± 0 min: Wilcoxon–Mann–Whitney U test: $W = 24$, $p = 1$; Figure 4g). Mating reactions from the last chemostat replicate (Figures 2f and 4f) produced zygotes, under both conditions (0 and 80 μ M nitrogen), but pellicles did not form. These populations were tested a second time, under the same conditions and densities as the previous mating reactions, and the same result was obtained. Again, we sampled the reactions and found zygotes, but they did not aggregate to form a pellicle. We confirmed the presence of both mating types in each mating reaction using PCR (data not shown; see Supplementary Methods). Thus, the

populations turned sexual, but because pellicles were not formed, an accurate end-point time could not be recorded, and this replicate was therefore dropped from the statistical analysis.

Finally, we used the data from Figure 3a–c (prey competition and defence) to calculate selection differentials for each time point and replicate population and correlated the estimates of selection strength with the change in the in situ rate of sex (frequency of zygospores, Figure 5a–c; see Supplementary Methods). We found a significant lag between the change in selection and change in the rate of sex of 11.2 ± 2.4 days (2.8 time points ± 0.6). This is consistent with our prediction that changes in selection drove selection for sex and also consistent with the biology of the organism, where a time lag would occur between when sex is selected for and when the products of sex (zygospores) can be observed.

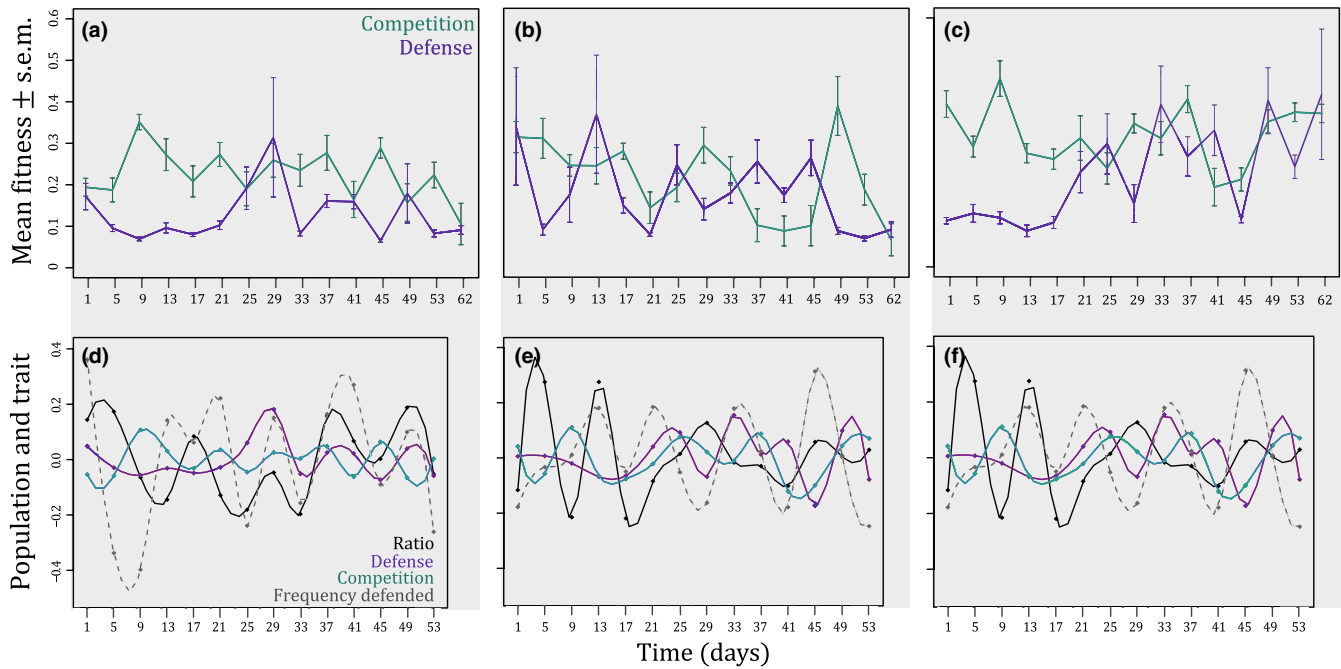


FIGURE 3 Changes in selection for competitive and defended prey. (a–c) Competitiveness (prey population mean growth rate without predators [day^{-1}], green) and defensiveness (predator population mean growth rate, where low predator growth rates indicate high prey defence levels [$1/\text{final predator number}$], purple) of 20 algal clones per time point for each replicate population of the predation treatment ($\pm \text{SEM}$). Across all replicate populations, competition and defence fluctuated significantly over time (9.3 ± 1 days, wavelet analysis). (d–f) Smoothed and detrended time series data to estimate periodicity and phase shifts between the predator–prey ratio (black) and changes in the trait value for prey population mean defence (purple), prey population mean competitiveness (green) and frequency of defended phenotypes (grey). The predator–prey ratio cycled in-phase with the mean defence trait and frequency of defended phenotypes, but cycled in anti-phase with mean competitiveness (see main text for wavelet statistics). Grey boxes indicate matching data sets. (a–c and d–f correspond to order of replicate populations in Figure 2d–f).

3.4 | Short- and long-term effects of sex fluctuate over time in the prey populations

We next compared the mean and variance in fitness of sexual to asexual offspring in their selected environment (i.e. high or low rotifer densities corresponding to the rotifer densities when algal cells were isolated) at 15 time points (see Section 2; Table S1). As we did not observe sexual reproduction in control populations without predators (Figure 2a–c), we did this only for the predation treatment. Our results showed that the short- and long-term effects of sex fluctuated as having costs and benefits over time with a significant period of ~ 9 days or 2–9 generations (Figure S3, short-term effect: 8.58 ± 2.4 days; long-term effect: 9.96 ± 3.8 days) (Figure 6).

4 | DISCUSSION

We tested the hypothesis that cyclic predator–prey dynamics can select for sex in the prey by comparing *Chlamydomonas* populations evolving in the presence and absence of predation. We found significant fluctuations in prey population density and predation intensity, over time, leading to significant fluctuations in selection for defended and competitive prey with a period of ~ 8 –9 days ($= 2$ –9 generations). We also found that sexual reproduction occurred in the prey

populations but never in the controls without predators. Indeed, when testing for heritable changes in the rate of sex, we found a faster switch to sexual reproduction in the evolved prey populations compared to the ancestors. Since this test was conducted after several asexual generations in the absence of predation (i.e. from stored populations), and in a controlled environment that also lacked predators, this result indicates selection for increased rates of genetic mixing in the prey populations. Herein, we further examined the effects of sex and found that the short- and long-term effects of sex fluctuated significantly over time and with a periodicity similar to that of the changes in predation intensity and selection (~ 9 days, or 2–9 generations). This shows that the fitness effects of sex not only changed over time, but with a consistent pattern, and continually led to time points where sex had a selective advantage depending on the direction of selection and likely underlying genetic associations.

We did consider, however, the possibility that the phenotypic changes in defence and competition were plastic. Although, in the time between when clones were sampled from the chemostats (Figure 2d–f, dashed vertical lines) and tested in the fitness assays (Figure 3a–c), which was more than 30 asexual generations, predators were absent. Additionally, in the chemostat environment where selection continuously changed, plastic responses lasting more generations than what occurred in that 9-day cycle would not be beneficial. Finally, sexual offspring, used in the assays measuring

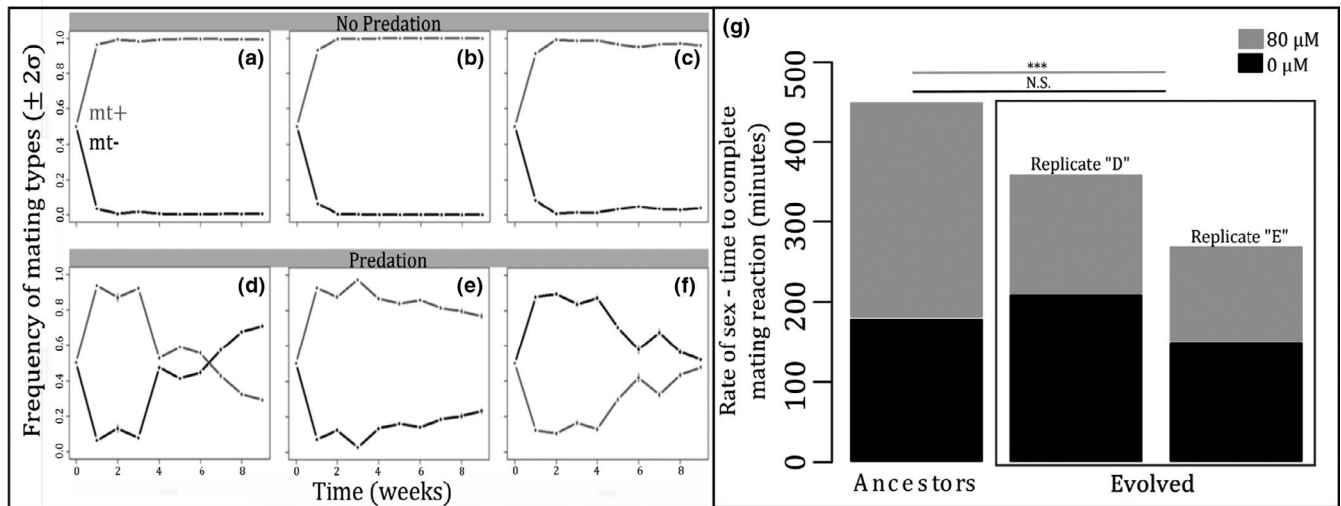


FIGURE 4 Selection for sex in the ancestors and evolved prey populations. (a–f) Changes in the frequencies of mating types in algal populations with and without predation over time measured using multiplex ddPCR. As an additional measure of selection for sex, we used ddPCR to track the frequencies ($\pm 2\sigma$) of both mating types in replicate populations without predation (a–c, controls) and with predation (d–f). In control populations, the (+) mating type (grey) rapidly moved towards fixation, essentially eliminating the (-) mating type (black) and potential for sex, whereas both mating types were maintained at more similar frequencies in the predation treatment. (g) The time (in minutes) to complete a mating reaction, in the absence of predators, in control ($0 \mu\text{M}$ nitrogen, black bars) and evolved conditions ($80 \mu\text{M}$ nitrogen, grey bars) for ancestors and replicate evolved populations of the predation treatment (d–e refer to chemostat replicates corresponding to Figure 2d–e, 3a–b, d–e; see Supplementary Methods). Sex occurred significantly faster ($***p < .0001$) in the evolved algal populations (from day 62) than in the ancestors under evolved conditions (grey bars), but there was no significant difference in sex rates of ancestors and evolved populations under control conditions (black bars; statistics in text). There was no variation among technical replicates; therefore, error bars are imperceptible. Replicate 'F' was dropped because a pellicle did not form (see main text)

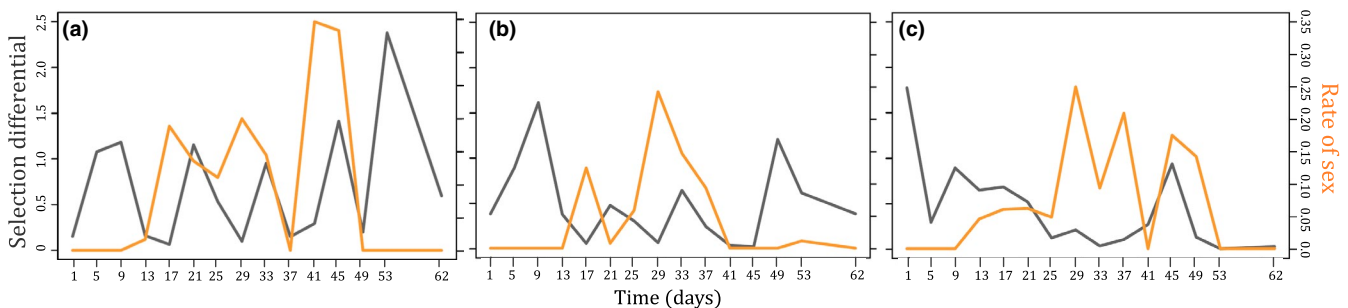


FIGURE 5 Selection differentials. (a–c) Selection differentials (absolute values, grey) of 20 clones per time point for each replicate population of the predation treatment, showing changes in selection for competitive and defended prey over time, and the in situ rate of sex (frequency of zygospores, orange; see Supplementary Methods). Across replicate populations, there was a significant lag between the change in selection and change in rate of sex of 2.8 ± 0.6 time points. (a–c correspond to order of replicate populations in Figure 2d–f)

the short- and long-term effects of sex (Figure 6), were propagated under standardized conditions and forced to undergo an additional round of sex (see Supplementary Methods). Together, these points indicate that all measured phenotypic differences were heritable.

4.1 | Rates of sex

Although rates of sex were low overall in the prey populations exposed to predators, this is attributable to the strength of selection for sex mainly depending on the net benefit of sex, as well as the costs of sex in the system. Here, the major costs of sex are the time needed to complete the sexual cycle (at least 5 days), which is

substantial compared to an asexual cycle (1–2 days), as well as the inhibition of population growth during the sexual cycle. These costs still have to be overcome, and although short-term benefits of sex appear to exist, they are intermittent (cyclic).

Regarding the mating type allele frequencies, it is important to note that our ddPCR results are consistent with previous work on intraspecific competition in *C. reinhardtii* populations composed of both mating types where the loss of one mating type, via natural selection, occurred during asexual reproduction; ultimately, these populations became sexually sterilized (Bell, 2005; Collins, 1993). In the literature on the evolution of sex and geographic parthenogenesis, it has been argued that for many heterothallic, facultative sexual populations, the loss of sex—or the loss of the potential for

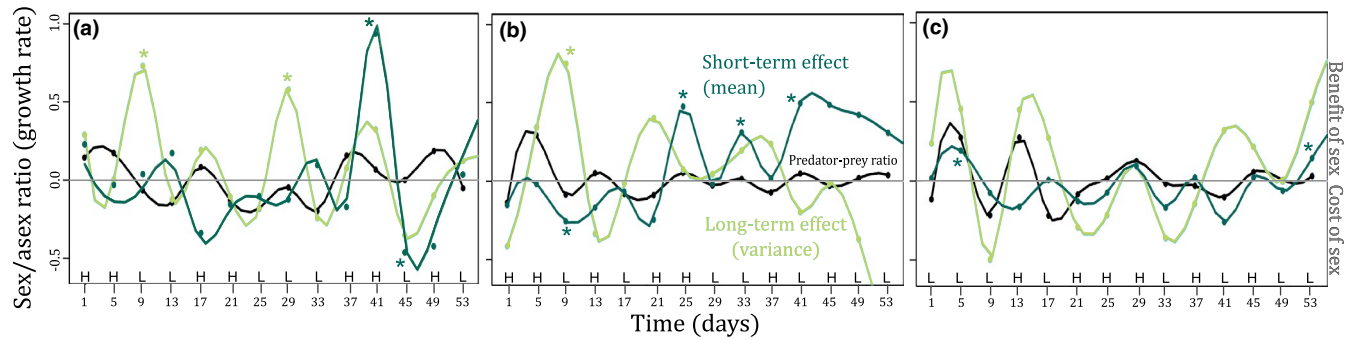


FIGURE 6 Fluctuations in the short- and long-term effects of sex under fluctuating predation pressure. (a–c) The effects of sex fluctuated significantly over time (statistics in main text; Fig. S3). Log₁₀-transformed ratios of sexual to asexual offspring fitness (growth rate, day⁻¹) from replicate populations of the predation treatment were used to determine the short-term (mean fitness, dark green) and long-term (variance in fitness, light green) effects of sex over time in their selected environments (Low or High predation, see Supplementary Methods and Table S2). Equal fitness is represented by the grey line at '0', with a benefit of sex indicated above the line and cost of sex below it. Day 1 represents the first day of the experiment, and thus the cross of ancestral isolates. Asterisks indicate significant differences between asexual and sexual offspring (Table S1). (a–c correspond to order of replicate populations in Figure 2d–f, 3a–c)

sex—may occur frequently, as a result of the loss of one mating type allele (Collins, 1993; Schön, Martens, & Dijk, 2009). Here, we have seen this for populations without predation (Figures 2a–c and 4a–c). Whether fixation of the same mating type (+) across control populations was due to stochastic (e.g. random mutation conferring a fitness advantage), or deterministic causes (e.g. an inherent competitive advantage), is however unknown; the (+) mating type did not dominate in all experimental populations (Figure 4f). Regardless, the loss of a mating type independently across all control populations precluded the ability to test for evolutionary changes in the rate of sex between ancestors and end-point clones.

4.2 | Potential underlying population genetic mechanisms selecting for sex

In a landmark paper, Barton (1995) outlined the contributions of short- and long-term effects to the evolution of sex in a general theoretical framework. This framework can be used to evaluate the population genetic mechanisms by which sex is favoured under different hypotheses. For considering species interaction via fluctuating population densities (or coevolution), there are at least two population genetic mechanisms that could drive selection for sex, fluctuating epistasis and directional selection (Gandon & Otto, 2007). For the fluctuating epistasis hypothesis, theory predicts that changes in selection can lead to changes in the sign of epistasis, followed by the generation of genetic associations (i.e. 'linkage disequilibria', in haploids) of the same sign (i.e. positive or negative) (Barton, 1995). Since it takes time to build genetic associations, a lag between epistasis and linkage disequilibria occurs, leading to opposing signs and a mismatch at points in time between which genotypes are currently most fit and which are most common (Barton, 1995). This is crucial, because these particular conditions can select for sex through an immediate short-term benefit by increasing the frequency of fit genotypes or decreasing the frequency of unfit genotypes, as well as

through an intermittent long-term benefit by increasing variance in fitness (Barton, 1995). As selection continues to change, so will the underlying state of fitness interaction and genetic association, leading to changes in selection for or against sex over time. Therefore, the expectation is that if fluctuating epistasis is operating, it would be evidenced by fluctuations in the short- and long-term effects of sex over time, which is indeed what we find here (Figure 6) (Gandon & Otto, 2007; Otto & Lenormand, 2002; Peters & Lively, 1999, 2007; Salathé, Kouyos, & Bonhoeffer, 2009).

This hypothesis requires that selection changes rapidly enough so that the sign of epistasis changes with a period of 4–10 generations and combinations of alleles advantageous in one generation quickly become disadvantageous in the following generation and so on (Barton, 1995; Maynard Smith, 1978; Peters & Lively, 1999). It has been shown, mathematically in haploid models, that high rates of recombination can also evolve with shorter periods (i.e. 2–7 or 2–9 generations depending on how tightly linked the modifier locus is), which determine higher rates of genetic mixing (Gandon & Otto, 2007). Our data match the theoretical prediction where the effects of sex fluctuated over time with a significant period of ~9 days (i.e. 2–9 generations) (Figure S3). This timing is also consistent with our data showing that selection changed every 4–5 days (~9-day period), followed by a change in the rate of sex ~9–13 days later (Figure 5). Furthermore, under these conditions, changes in the short-term effect are expected to occur in-phase with the changes in the predator–prey ratio and selection. Indeed, we found that changes in the short-term effect were immediate and in-phase with changes in the predator–prey ratio (i.e. they cycled with similar timing) (0.05 ± 0.12 of a period; 0.43 ± 1 days), but changes in the long-term effect of sex lagged behind the predator–prey ratio (0.31 ± 0.22 of a period; 2.5 ± 1.8 days).

Alternatively, slower fluctuations in selection will create sustained directional selection, causing changes in the mean phenotype over time and selection for sex if there is weak negative epistasis, and if fitness costs are high (Barton & Otto, 2005; Maynard Smith, 1978). In the case of very rapid fluctuations, there would be little sustained directional selection and sex would not be advantageous. Moreover,

directional selection only selects for sex when negative genetic associations (disequilibria) are present (i.e. beneficial alleles tend to be found with unfavourable ones more often than expected) because fitness variance is low. In this case, sex is beneficial for increasing fitness indirectly by accelerating the rate of response to selection by increasing genetic variance (long-term benefit) (Barton & Otto, 2005; Maynard Smith, 1978). Under a scenario of selection for sex via sustained directional selection, we would not expect to see fluctuations in the effects of sex, but instead the presence of only long-term benefits, which is not what we find here.

Although the patterns observed for the effects of sex, the changes in selection, as well as the population dynamics, appear inconsistent with alternative hypotheses, we cannot exclude them entirely. Biotic fluctuations imposed by antagonistic coevolution can also lead to fluctuations in epistasis and genetic associations of the type necessary to continuously favour sex (i.e. RQH). Under this scenario, one would also expect to observe fluctuations in the short- and long-term effects of sex (Gandon & Otto, 2007; Peters & Lively, 1999). While we argue that biotic fluctuations are, indeed, a source of fluctuating selection in our experiment, we exclude predator coevolution here, which distinguishes our study from other tests of the RQH. Predator populations were monoclonal (no initial standing genetic variation) at the start of the experiment and had relatively small population sizes (2000 individuals on average) and only ~30 asexual generations, which greatly reduces the potential for novel mutations and, thus, coevolution.

Another set of hypotheses considers the conditions that may generate negative genetic associations (disequilibria) and long-term benefits of sex. A moving fitness optimum, where the optimal phenotype constantly changes and leads to sustained directional selection in the population, can repeatedly lead to negative disequilibria and therefore provide an advantage to sex via a long-term benefit (Barton, 1995; Maynard Smith, 1978). This mechanism requires, however, that there is relatively little variation in the sign and magnitude of epistasis and that the strength of epistasis is weak (Barton, 1995; Otto & Feldman, 1997). Genetic drift and selection can also generate negative disequilibria and lead to the evolution of sex via a long-term benefit (Barton & Otto, 2005; Hill & Robertson, 1966). It has been suggested that although a drift-based advantage to sex is more likely in finite populations (Gandon & Otto, 2007), it can occur even in very large populations if they are spatially structured (Martin, Otto, & Lenormand, 2006) and/or selection acts on a large number of loci (Iles, Walters, & Cannings, 2003). However, with either scenario of directional selection combined with negative epistasis or drift, we would not expect to observe an advantage of sex through a short-term effect or fluctuations in the effects of sex over time.

5 | CONCLUSION

Herein, we used a general theoretical framework to test predictions made by early population genetic models considering species

interaction for the evolution and maintenance of sexual reproduction, which suggest that interactions without coevolutionary change can also select for sex when fluctuations in population sizes of interacting species generate a continuously changing environment (Bell, 1982; Jaenike, 1978). Taken together, our results suggest that predator-mediated fluctuating selection can generate conditions leading to selection for sex in prey populations. We propose that selection for competitive versus defended prey changed every few generations, leading to changes in the sign of epistasis and recurring situations where prey were no longer matched to their environment because an excess of previously advantageous but now disadvantageous genotypes existed. Sex could then be selected for (intermittently) as recombination offered an immediate fitness benefit by increasing the frequency of advantageous genotypes that were disproportionately rare. Even though epistasis has been implicated as an essential factor in theoretical models of the evolution and maintenance of sex, there remains a dearth of experimental evidence. Therefore, in light of recent advancements in sequencing technology and genomic analyses (e.g. McDonald, Rice, & Desai, 2016), alternative approaches to studying the maintenance of sex and epistasis still need to be tested (e.g. Kouyos, Silander, & Bonhoeffer, 2007), and as we have done here using the short- and long-term effects of sex (e.g. Becks & Agrawal, 2011). Finally, our results further underline the importance of investigating the role of predator–prey interaction in driving the evolution and maintenance of sexual reproduction. Although we observed an increase in sex in the prey populations, it was only to low rates which is attributable to the costs of sex and biology of the organism, but it could also indicate that the selection dynamics within this system are insufficient to observe the evolution of higher rates. Furthermore, additional testing—and on a finer timescale—is necessary to determine whether conditions of fluctuating population sizes and selection are strong enough to maintain sex over the long term.

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CONFLICT OF INTERESTS

The authors declare no financial interests.

DATA AVAILABILITY STATEMENT

The relevant data sets will be deposited in Dryad. Dryad DOI: doi:10.5061/dryad.gtht76hj4

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SUPPORTING INFORMATION

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