Eco-evolutionary feedback promotes Red Queen dynamics and selects for sex in predator populations

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Running title: Eco-evolutionary dynamics and sex

Keywords: **eco-evolutionary feedback, Brachionus, Chlamydomonas, experimental evolution, Red Queen, maintenance of sex**

Type of article: **Original Article**

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Abstract

Although numerous hypotheses exist to explain the overwhelming presence of sexual reproduction across the tree of life, we still cannot explain its prevalence when considering all inherent costs involved. The Red Queen hypothesis states that sex is maintained because it can create novel genotypes with a selective advantage. This occurs when the interactions between species induce frequent environmental change. Here we investigate whether coevolution and eco-evolutionary feedback dynamics in a predator-prey system allows for indirect selection and maintenance of sexual reproduction in the predator. Combining models and chemostat experiments of a rotifer-algae system we show a continuous feedback between population and trait change along with recurrent shifts from selection by predation and competition for a limited resource. We found that a high propensity for sex was indirectly selected and was maintained in rotifer populations within environments containing these eco-evolutionary dynamics; whereas within environments under constant conditions, predators evolved rapidly to lower levels of sex. Thus, our results indicate that the influence of eco-evolutionary feedback dynamics on the overall evolutionary change has been underestimated.

Introduction

Sexual reproduction is almost universal while its inherent costs have made its maintenance difficult to explain (Maynard Smith 1978; Bell 1982). One major hypothesis for the evolution of sex suggests that the benefits of sex outweigh its costs when populations are adapting to novel environments (Weismann 1889; Maynard Smith 1988; Charlesworth 1993). Empirical studies testing this hypothesis showed however that when conditions are not frequently changing, the advantage of sex or outcrossing is brief on an evolutionary time scale. For example, sex or outcrossing rates increased during adaptation to novel environmental conditions but then declined when populations were close to a new fitness plateau after 10-30 generations (Morran et al. 2011; Becks and Agrawal 2012). Thus frequent environmental changes have been suggested as one key factor for the maintenance of sex on longer time scales. Indeed, major hypotheses on the evolution of sex and many of the pluralistic approaches focus on changing biotic or abiotic environments over space (Agrawal 2009b; Becks and Agrawal 2010) or time (Van Valen 1973; Hamilton 1980; Bell 1982; Otto and Nuismer 2004).

One particular hypothesis (Red Queen Hypothesis) suggests that coevolution of species can drive the evolution of sex through negative frequency dependent selection (Van Valen 1973; Jaenike 1978; Bell 1982). With recurrent environmental change stemming from Red Queen dynamics, populations are frequently moved away from fitness optima and must adapt to novel environmental conditions. Sexual reproduction is then maintained under these conditions because a modifier locus, that determines higher genetic mixing rates (i.e., the rate of sex, selfing and/or recombination; (Nei 1967)) hitchhikes with alleles under positive selection. To date, however, almost all theoretical and all empirical studies on the Red Queen hypothesis have focused on host-parasite interactions; minimal to no consideration has been given to other victim-exploiter system, such as predator-prey (but see Jaenike

1978; Bell 1982). Predator-prey interactions are a key ecological process often leading to fluctuating environments. Fluctuations from edible to inedible prey as the result of selection by predation, and back to edible prey, are well documented e.g. in aquatic systems (e.g., Hairston et al. 1999; Walsh and Post 2011). Thus, exploring other victim-exploiter systems should be important for a broader understanding of the maintenance of sexual reproduction.

Herein, we explore the role of predator-prey interactions for the maintenance of sex. We propose that eco-evolutionary feedback dynamics can create the recurrent environmental changes that select indirectly for the maintenance of sex in a predator population. Previous work with predator-prey systems showed that eco-evolutionary feedback dynamics occur for prey populations with intraspecific trait variation (Abrams and Matsuda 1997; Yoshida et al. 2003; Becks et al. 2012). This variation is observed as a trade-off between their competitive ability for nutrients and defensive ability against predation (Fig. 1A). As a result the predators experience a fluctuating environment of defended and undefended prey: an increase in the predator population select for increases in the frequency of the defended prey. The high frequency of the defended prey in return leads to decreasing predators, which selects for faster growing but undefended prey through competition for resources, and so on (Fig. 1 B,C). Note that the evolutionary change considered here and elsewhere (e.g., Yoshida et al. 2003) is the change in frequency of two genotypes due to selection. It is thus the switch between selection by predation and competition for a limited resource that drives the changes in the predators' environment (Fig. 1C). They occur without, as well as with, coevolutionary change in the predator (Fig. 1D-F). Thus these conditions differ from other coevolutionary and epidemiological models for species interactions (e.g. Lively 2010). Hence, providing a distinct, but so far unconsidered scenario under which the Red-Queen conditions could select for sex.

Here, we test whether sexual reproduction of predators is beneficial when the environment changes repeatedly from one prey type to the other. We use a series of model simulations and experiments with rotifer-algal systems with the rotifer *Brachionus calyciflorus* as predator and the green algae *Chlamydomonas reinhardtii* as prey. Previous experiments with this rotifer-algal system showed eco-evolutionary feedback dynamics for genetically variable algal population (Jones et al. 2009; Becks et al. 2010; Becks et al. 2012). The prey population consisted of a *colonial* algae clone, which pose a defense against grazing by the rotifer, and a *single-celled* algae clone, which is undefended but faster growing compared to the *colonial* algae. Previously, the rotifer populations were genetically homogeneous and obligately asexual (Fussmann et al. 2003) without a possibility to adapt to the fluctuations in the prey types. Here, we use a genetically diverse and facultative sexual rotifer population allowing for coevolution as well as for sexual reproduction. *B. calyciflorus* are cyclic parthenogenetic and reproduce predominantly by ameiotic parthenogenesis (Gilbert 1963). Sexual reproduction (mixis) in these rotifers is density dependent, with heritable variation in the response to various densities (Becks and Agrawal 2010). Asexual (amictic) females produce diploid eggs, whereas, sexual females produce haploid males, and diploid resting eggs after fertilization. Previous experiments with the rotifers *Brachionus calyciflorus* showed that directional selection during adaptation to novel conditions result indeed in indirect selection for higher rates of sex within populations (Becks and Agrawal 2012) despite the costs associated with sex. An asexual generation of this *B. calyciflorus* population requires about 1.5 days and a sexual about 4.5 days (+ 1.5 days for an additional asexual generation). Thus one major cost for sexual reproduction in these rotifers is the significantly longer time for a reproduction cycle.

For a comprehensive study, we use both theoretical and empirical methods. We designed agent based models to test i) whether recurrent change of different prey (algae) types select indirectly for the maintenance of sex in a predator population and ii) whether eco-evolutionary feedback dynamics with coevolution of predator and prey can create these recurrent changes and select indirectly for the longer maintenance of sex in the predator. We then ran chemostat experiments using the *Brachionus-Chlamydomonas* system to test the model results. Our experimental setup involves conditions where rotifers and algae are coevolving (hereafter: coevolving) and where coevolution was suppressed but rotifers were free to evolve (hereafter: non-coevolving). From the chemostat experiments, we recorded changes in the propensity for sex — the rate of sex within a population. We finally discuss alternative explanations to the Red Queen hypothesis that could be at work here, namely differences in the number of niches (Tangled Bank Theory (Bell 1982), the Hill Robertson effect (Hill and Robertson 1966; Felsenstein 1974), selection for dormancy rather than sexual reproduction, and population size differences.

Methods

Eco-evolutionary feedback model. We build on a model where eco-evolutionary feedback dynamics in one predator and evolving prey system result in unique dynamics of predator and prey populations compared to classical predator-prey systems (Yoshida et al. 2003; Becks et al. 2010; Becks et al. 2012). The 'classical' system is without evolution in the prey and results in short cycles with a phase shift of a quarter of a period between predator and prey. On the other hand this `eco-evo’ model results in increased cycle length and predator and prey cycle almost out of phase. The `eco-evo’ model was parameterized for the *Brachionus-Chlamydomonas* system (Becks et al. 2010) and describes the dynamics of nitrogen N, two algae types A_i (i =1,2), two rotifer types R_j (j=1,2) and senescent rotifers S_j in a chemostat system (with only one predator present: j =1). The prey types are assumed to be two genotypes within a single species, differing in their ‘palatability’, p_i^j that determines their relative risk of being attacked and consumed by specific predator types, and their ability to compete for nitrogen, K_A^i. We considered two cases, either one predator or two predator types present in the system. For the case with one predator type, we modeled the two prey types in such a way that one prey type is the superior competitor but not defended against the predator ('single-celled algae'; p_1^1 = 1 and K_A^1= 8) and the other prey type is the inferior competitor but defended ('colonial algae'; p_2^1 = 0.1 and K_A^2= 2.2). For the case with two predators, parameter estimates are based on experimental data, that one predator type is adapted to one prey type ('colonial algae') and not so much to the other prey type ('single-celled algae'; p_1^1 = 0.1 and p_2^1 = 1), and vice versa ('colonial algae': p_1^2 = 0.1 and p_2^2 = 1). Our model can be represented by the following system of equations:

$$\frac{dN}{dt} = \delta(N_{stock} - N) - \sum_i \frac{p_i^j A_i N}{K_A^i + N}$$

$$\frac{dA_i}{dt} = A_i \left[\frac{\mathrm{X}_A p_i N}{K_A^i + N} \quad \frac{p_i^j G(R_j + S_j)}{K_R^j + \max(Q^j, Q^{j*})} - \delta\right]$$

$$\frac{dR_j}{dt} = R_j \left(\frac{\mathrm{X}_R G Q^j}{K_R^j + \max(Q^j, Q^{j*})} - m - \delta - \lambda\right)$$

$$\frac{dS_j}{dt} = \lambda R_j - (\delta + m) S_j$$

where X_R is the rotifer conversion, K_R^j is the rotifer half saturation constants and G is the rotifer grazing parameter. $Q^j = p_1^j A_1 + p_2^j A_2$ and defines the total amount of 'prey quality' as perceived by the rotifer j with p_i^j as the weights for the respective prey types. The critical level $Q^{j}*$ determines when the rotifer functional response changes from linear to type II. Results from our model simulations are presented in Figure 1 and parameter values in Table 1.

Evolution of sex - model. Agent based stochastic model where each rotifer is defined by a food locus (f) and a sex modifier locus (τ). The f locus determines the rotifer's ability to catch (or select) a particular algae type. The τ locus determines if the rotifer will enter a sexual or asexual cycle: a rotifer switches from producing only asexual (amictic) individuals to sexual (mictic) ones when stimulated with respect to the population density (τ defines the density threshold in proportion to a set maximum population size R_{max}). Each day rotifers caught prey depending on their genotype and the prey present at that time point. In order to ensure the effects observed are driven by sex we simulated under no or low mutation rates - all offspring genotypes were subjected to low mutations with probability μ at each locus. In our simulations we assumed two alleles for the food locus to keep the model as minimal as possible while representing the food web described above (Fig. 1D). For the sex modifier locus, we assumed five alleles. This reflects the modifier approach (Nei 1967) where intermediate rates of sex are possible beside obligate asexual and sexual reproduction and is based on initial parameter exploration (we chose 5 alleles for the modifier locus to capture the observation seen in Fig S3). Rotifers are diploid organisms and thus the variation results from these allelic combinations. For simplicity we assumed that allele interaction is driven by dominance – heterozygotes exhibit the same phenotype as the homozygote dominant genotype. For example, for the food locus with 2 alleles we consider f_1 to dominate f_2 so individuals with f_1f_2 would behave the same as f_1f_1. We also allow for random assortment between the food and sex modifier loci during gamete production. Simulations were initialized with a mixed population of size R (population size was not fixed), composed of only asexual individuals and we simulated 1000 days (>70 generations). We considered environmental conditions altered exogenously (Fig. 2A-B) and through eco-evolutionary feedback dynamics (Fig. 2C-D).

Exogenous switching occurred by alternating algae densities in intervals of σ, whereas eco-evolutionary feedback dynamics occurred when the frequency of the algae types could change directly from predator-prey interactions, such as grazing and competition. The amount of algae caught per rotifer was determined using the following equation:

$$\Delta a_i = a_{i\_max}\,[\frac{a_i}{K_A^i + max(a_i,\ a_T)}\ ]$$

Algae types varied in defensive a_{i_max} and competitive C_i traits; this is necessary in order to capture the observed trade-off between their competitiveness (doubling rate) and defensive ability against predation. Each algal type was describe by two parameters ($a_{\_max}$, C_i), and these determined how much the rotifer can feed and how fast each algal type can reproduce (in this model we defined competiveness as a measure of reproductive rate). The algal population also depended on the parameters a_T and K^i_A, the threshold of the population and the half saturation constant, respectively. Thus, using these equations the algae population evolved as a result of selective pressure from competition between the types and rotifer grazing. This simple set up allows us to coevolve these two populations and test the effects of algal defense and competition on the maintenance of sex in rotifers.

Chemostat experiments. All experiments were carried out in chemostats with a dilution (flow-through) rate of 0.3 d^{-1}, i.e. 30% of the populations in the chemostat including nutrients, algae, rotifers and eggs were replaced each day by fresh medium (Fig. S2; (Fussmann et al. 2000; Becks et al. 2010). The coevolution environments were established in one-stage chemostat systems, containing both the rotifers and the *single-celled* and *colonial* algae clones. Chemostats were inoculated with two strains of the algae *Chlamydomonas reinhardtii*; one strain grows mostly as single cells or small colonies of 2-7 cells (University of Texas Culture Collection UTEX no. 1009), hereafter *single-celled* algae (*Chlamydomonas* usually undergoes two mitotic divisions before daughter cells are released). The second strain grows in colonies of 8-138 cells (hereafter: *colonial* algae), which was isolated from a culture where the UTEX 1009 strain grew together with the rotifer for 6 months. The non-coevolution environments were established in two-stage chemostats to separate algal and rotifer growth as *Chlamydomonas* consistently evolves some level of defense after 1-4 weeks in the presence of rotifers (Becks et al. 2010; Woltermann and Becks *unpublished data* 2014). Therefore, algae grew to steady state densities in first stage chemostats with the same resource levels as in the coevolution treatment and rotifers in second stage chemostats received a constant amount of alga. The second stage chemostats did not receive any additional nutrients. Thus, algal growth and evolution were constrained in the presence of rotifers, maintaining a constant trait in the algae population.

We used *Brachionus calyciflorus* from stock cultures to inoculate both the one stage and second stage chemostats. Our *B. calyciflorus* stock was derived from field-collected resting eggs (Becks and Agrawal 2011). Whereas resting eggs of *B. calyciflorus* usually undergo dormancy and hatching from resting eggs is delayed significantly for days and weeks, this rotifer stock is atypical as asexual females hatch within a few days after laying. This rotifer stock was kept at low densities with low amounts of sex and large population sizes (>500 individuals), refreshed in regular intervals by resting eggs (every 4-8 weeks; stored at 4°C), and fed regularly with single-celled *Chlamydomonas*. The laboratory stock population exhibited considerable genetic variation for the mixis stimulus (Fig. S3) and for fitness-associated traits (measured as lifetime reproduction per female) on *single-celled* and *colonial* algae (Fig. S5) when measured at the start of the experiment (Fig. S4). Densities of rotifers (females and males), number of asexually produced and sexually produced eggs (resting eggs), algae density and colony size (number of cells per colony) were determined daily using subsamples taken from the chemostats. For estimating the cycle length, we counted the days between maxima (insufficient cycles for a statistical analysis of the cycle length). Chemostats were sampled daily for 9 weeks. To test for the long-term maintenance of sex, chemostats ran for 6 additional weeks, with sampling in weeks 12-14. Rotifers in one of the one-stage chemostat became extinct within the first two weeks and thus data collection was halted. A second set of non-coevolution chemostats was started at a later time point from the same rotifer stock, this time using the *colonial* algae strain in the first stage chemostats. Within each environment, we started with five replicates, a total of 15 chemostats. For an overview of the experiments and evolution assays, see Figure S6.

Evolutionary dynamics

Rotifer fitness. Every second week, 24 clones were isolated from each population, transferred individually to test plates containing 1 ml *single-celled* algae and then lifetime reproduction (fitness) of 1-4 neonates of the 3rd generation after isolation was measured with *single-celled* algae and 1-4 neonates of the same isolated genotype on *colonial* algae (all algae concentrations: 150,000 cells/ml). Rotifer genotypes that had a higher fitness on *single-celled* algae were regarded as being better adapted to *single-celled* algae.

Frequency of sex. Switching to sexual reproduction (mixis) in *B. calyciflorus* is density dependent with heritable variation for the density, which induces mixis. We used this genetic variation in the sensitivity to switching as a measure for the rate of sex (= propensity for sex). 42 individuals were isolated and one neonate of the surviving 3rd generation after isolation was individually transferred to a single well. Density of rotifers were monitored every ~24h until the first male occurred. This density was used as an estimate for propensity of sexual reproduction (Aparici et al. 2001; Becks and Agrawal 2013). A clone that starts producing males at a lower density is considered to have a higher propensity for sex.

Data analysis

Statistical analyses were performed in R (Team 2010) using the lme4 package (Bates and Maechler 2010). We used a linear model to test for a change in average number of cells/colony in the non-coevolution treatment (day>10). Amplitudes of the average number of cells/colony (maximum - minimum) from non-coevolution and coevolution treatments were compared using a Welch Two Sample t-tests. We applied linear mixed models with food (*single-celled* or *colonial* algae), environment (non-coevolution or coevolution) and week as fixed, and clone nested within replicated population as random effect to analyse the lifetime reproduction data. To test for differential adaptation on *single-celled* or *colonial* algae within the non-coevolution and coevolution environment, we analysed the data also using environment specific generalized mixed models with week and food as fixed effects and clone nested within replicated population as random. As not all tested clones survived the first two generations before measuring lifetime reproduction, we also analysed survival of isolated clones till the 3rd generation. Therefore, a generalized linear model was used to test for the effects of treatment and week using the number of surviving clones out of all clones (GLM with proportion data). Differences among environments in the mixis inducing female densities were tested using GLMMs (with Poisson error distribution) with environment, week and their interaction as fixed and replicate nested within environment as random factor. For the comparison of the in-situ sex rates (percentage of resting eggs out of all diploid eggs), we used environment (non-coevolution or coevolution) as fixed and replicated population within environment as random factor. Based on the fitness data suggesting that non-coevolution populations were adapted after 3 weeks, we analyzed the data for days 1-24 and days 25-66, as well as days 88-103 separately. We used cross-correlations for identification of significant time lags between change in the rotifers' inducibility threshold and the algal defense trait. The time lag was used to determine for which the cross-correlation between the two data sets is maximized and significant at the 5% level (coevolving: day 2; non-evolving: n.s.). We then assumed a delay of 2 days to test for a correlation of evolutionary change in prey populations (change in the prey defense trait) with later changes in the in-situ sex rates (GLMM with replicated population as random factor). We applied the same procedure for the correlation between the in-situ rate of sex and population growth rate. We did not find significant time lag for the coevolution environment (GLMM: $\chi2=0.37$, df= 1, p=0.544). We used linear models, to test for the correlation between fraction of rotifers adapted to single-celled algae and differences between the two environments.

Results

Evolution of sex - model. We used an agent based model specific to the rotifer life cycle that reflects the actual costs associated with sexual reproduction. We explored the conditions that select for the maintenance of sex in the predator population with two algae types differing in their defense against predation and competitive ability. We illustrate how different model and parameter settings affect the maintenance of sex by showing the frequency of simulations in which sex was maintained until the end (Fig. 2). Simulations show that under constant environmental conditions do not maintain sexual reproduction in the rotifer populations (Fig. 2B, orange point). Furthermore, varying the maximum population size of the rotifer with constant food environments had a negative effect; sexual reproduction was lost increasingly faster with smaller population sizes (average last day sex observed: population size 10: day 0; 100: day 25; 1000: day 42). However, with two prey types oscillating driven by extrinsic forces, we found that sexual females were maintained more frequently in the population with increasing switch intervals σ. When switches were too rare, sexual reproduction was more often lost. Additionally we tested whether it is the change in the frequency of the two algae types or just the fluctuations in the population size of one algae type that allows for the maintenance of sex in the rotifers. For this we ran simulations with a single algae type, where its population size fluctuated with intervals σ at 80% and 20% densities. In such a scenario, sex was only found for small σ (Fig. 2B); for larger intervals σ sexual reproduction was not observed at all. In another set of simulations we tested if the presence of two algae types, but without fluctuations allows the maintenance of sex in the rotifers. We found that sex was not maintained when there was no temporal change in the frequency of the algae types (Fig. S15).

In the next step, we removed the exogenous switch of the algae types and allowed for a change in algae frequency directly through grazing and competition. For this, each algal type was described by two traits - a_{i_max} (level of defense) and C_i, (maximum growth rate) - to account for the trade-off between defense and competitiveness that allow for eco-evolutionary feedback dynamics. With eco-evolutionary dynamics, the frequency of simulations where sex was favored and maintained were higher when the competition between the prey types was high ($C_1 < 2.5$). As the cycle length of predator and the prey types depends on the trade-off in the prey population, we observed an increase in the selection of sex for $C_1 < 2.5$. Because our simulations are stochastic we compare outcomes we can only infer if the frequency of sex increased (more often) or decreased (less often) from random. The abrupt drop in the simulations with sex being maintained more often for $C_1 > 2.5$ results from the break down of the eco-evolutionary feedback dynamics when the defense becomes cheaper (Jones and Ellner 2004, 2007). Thus, eco-evolutionary dynamics in predator-prey systems can select for sexual reproduction in the predator given that there is a strong trade-off between competitiveness for limiting resources and defense in the prey population. Note that sex is maintained here through its linkage to selected food locus (f) given that the sex modifier locus has no direct fitness effect.

Eco-evolutionary dynamics - experiments. We tested the model predictions on eco-evolutionary dynamics with coevolution in replicated chemostat experiments and we found that, similar to the model predictions (Fig. 1E), predator and prey cycled out of phase (Figs. 3E,F, S7, S9), indicating eco-evolutionary feedback dynamics (Hiltunen et al. 2014). We also observed changes in the frequency of the two algae types here measured as the average number of cells per colony (defense trait, Figs. 3, S7, S9, S11) with a cycle length of ~25 days (=6-17 rotifer generations). Furthermore, we observed the cyclic (time-lag) relation between changes in the defense trait and the predator density (Fig. 3F).

In contrast and as by experimental design, the algae defense trait did not change in the non-coevolution environments with *single-celled* algae (Figs. 3, S8, S10, S12; linear model for colony size~time: F =1.612, df =1, p=0.205) and without any correlation between the defense trait and the predator-population sizes. Under these experimental conditions, we also observed different population dynamics compared to the coevolving environments; short cycles in all replicates at the beginning of the experiment and after week ~5 some populations were more stable (Figs. 3B, S8, S10) while others showed continuous oscillations (Figs. 3B, S8, S10).

Rotifer evolution. To test for evolutionary change in rotifers over time, we isolated individuals from each population at five time points and tested their fitness when grown on *single-celled* or *colonial* algae independent of the environment they evolved in. To minimize environmentally induced effects rather then heritable changes, we measured fitness two generations after isolation and maintenance in standardized conditions. Overall, rotifers adapted differentially in both environments and on the different food types over time (LME: food*week*environment: $\chi2=37.50$, df= 3, $p=3.6*e^{-8}$; environment: $\chi2=55.68$, df= 4, $p=2.34*e^{-11}$; food: $\chi2=71.23$, df= 4, $p=1.28*e^{-14}$; week: $\chi2=43.57$, df= 4, $p=7.87*e^{-9}$) and a new fitness optimum was reached after 3-5 weeks. Within the non-coevolution environment, the averaged estimates of individual rotifer fitness showed a significant increase over time when grown on *single-celled* algae in the coevolution but not on *colonial* algae (Fig. 4B,E; LME: week*food: χ2=8.53, df= 1, p=0.0025; food: χ2=66.84, df= 2, p=3.07*e^{-15}; week: χ2=9.69, df= 2, p=0.008). Rotifers from the coevolution environments had similar fitness increases over time on both *colonial* and *single-celled* algae (Fig. 4B,E; LME: week*food: χ2=0.015, df= 1, p=0.90; food: χ2=0.006, df= 1, p=0.94; week: χ2=33.89, df= 1, p=5.84*e^{-9}). We observed, however, fluctuations in the frequency of rotifers having a higher fitness on *single-celled* algae over time. Furthermore, the fraction of *single-celled* algae correlated with rotifers having a higher fitness on *single-celled* algae in the coevolving environments, but not in the non-coevolving environments (LM: non-coevolution vs. coevolution: F=22.7, df=1, p=4.5*e^{-5}; Fraction *single-celled* algae vs. frequency rotifers adapted to *single celled* algae in the coevolution environments: F=7.53, df=1,p=0.01; Figs. S9,S10). We also tested for survival as a fitness component and found no difference between environments but that survival increased over time (GLM: week*environment: χ2=1.36 df= 1, p=0.24; environment: χ2=0.34, df= 1, p=0.56; week: χ2=49.16, df= 1, p=2.35*e^{-12}).

Evolution of propensity for sex - experiments. The rotifers experienced fluctuating environments with an average cycle lengths of ~25 days in the coevolution environment as a result of the eco-evolutionary feedback (Figs. 3E,F, 4F, S7,S9). The agent based model (Fig. 2) predicts that these changes in the prey environment maintain a higher propensity for sex in the predator population in comparison to constant environments (non-coevolution environment). We found that the *in-situ* rate of sex remained at high levels in the coevolution environment where the algae changed from *colonial* to *single-celled* and vice versa. However, these levels dropped significantly in the non-coevolution environment (Fig. 5A; GLMM coevolution versus non-coevolution: days 1-24: χ2=2.39, df=1, p=0.12; days 25-66: χ2=5.63, df=1, p=0.018; days 88-103: χ2=10.69, df=1, p=0.001). Furthermore, we estimated the propensity for sex by recording the density at which individual clones switched to sexual reproduction under standardized conditions. Again, significantly higher propensities for sex occurred in the coevolution environment compared to the non-coevolution environment (GLMM: environment * week: χ2=79.01 df=2, p=2.2*e $^{-16}$; week: χ2=85.41 df=3, p=2.2*e $^{-16}$; environment: χ2=238.45 df=4, p=2.2*e^{-16}; Fig. 5B). Additionally, we found a positive correlation between the evolutionary changes in the prey defense trait (change in mean clump size) and the changes in the *in-situ* sex rate in the coevolving environment (GLM: χ2=10.34, df=1, p =0.00134). However, such a correlation was not apparent for the non-coevolution environment (GLM: χ2=0.38, df=1, p =0.5641).

As rotifers used for the experiments came from stocks with *single-celled* algae, the introduction of the *colonial* algae could lead to different rates of adaptation between the coevolution and non-coevolution environments. Generally, selection for genetic mixing is most advantageous under high rates of adaptation. However, close to fitness peaks, it is negative or negligible depending on the costs (Morran et al. 2011; Becks and Agrawal 2012). We found a high propensity for sex during adaptation in the coevolution and non-coevolution environments with *single-celled* algae (GLMM: weeks 1-4 non-significant; Fig. 5B; Table S1). They were maintained, however, only in the coevolution environments, even after the populations' average fitness equilibrated after weeks 3-5 (Fig. 4F). If adapting to *colonial* algae would drive the maintenance of sex, the propensity for sex would have decreased after a new fitness peak on *colonial* algae was reached (Becks and Agrawal 2012). Nevertheless, to test if adaptation to just *colonial* algae results in a higher propensity for sex in the coevolution environments we considered a second non-coevolution environment. This was started 4 month after the other two treatments with the *colonial* algae in the first stage chemostats (see Methods). Under this condition, the propensity for sex rapidly declined (Fig. 5B; GLMM: χ^2=34.58 df=4, p=5.67*e $^{-7}$), by 5-6 weeks, sex induction was no longer observed

(Fig. 5B, Table S1). Thus the observed high level of sex in the coevolving environments is unlikely the result of slower adaptation to the *colonial* algae (but see discussion below).

Discussion

A key finding of our study is the maintenance of sexual reproduction - measured as the propensity for sex - in the predator population as a result of eco-evolutionary feedback dynamics and fluctuations in the presence of two prey types. There are strong indications that the Red Queen is at work here, as there is a positive correlation between the prey defense traits and the rate of sex. Furthermore, our data show coevolution between algae and rotifers as predicted by Red Queen dynamics: the average fitness of the population stayed constant over time after initial adaptation by the rotifer populations (Fig. 4E), while the frequencies of genotypes (here estimated as algae phenotypes and rotifers being better adapted to *single-celled* algae) fluctuated over time (Figs. 4F, S9). It is worth considering the enormous costs of sex attributed to the time a sexual cycle requires in comparison to an asexual. This underlines the strong selection for sexual reproduction in the rotifer populations under these conditions.

The Red-Queen hypothesis proposes that coevolution of species imposes negative frequency dependent selection, which can drive the maintenance of sex (Jaenike 1978; Hamilton 1980). Here, the evolution of an algal defense (i.e., increase in frequency of the *colonial* algae) is followed by an evolutionary change in the rotifers (adaptation to *colonial* algae). In return, these changes in the predator population drove the evolutionary change in the algal prey (increase in *single-celled* algae) and so on. A key mechanism is that the shift in the prey genotypes depends on the feedback between the trade-off in the prey and the predator population dynamics; the advantage of being rare in the algae population became only important after the evolution in the algae itself changed the ecological dynamics (predator densities), causing simultaneously an evolutionary change in the predator.

In contrast to the Red Queen Hypothesis, specific variants of the Tangled Bank Theory (Bell 1982) propose that sex is beneficial because sexually produced offspring can use different ecological niches (here the *colonial* and *single-celled* algae), thus reducing sib competition. Such mechanism may be operating if one observes a positive correlation between the genetic mixing rate and offspring number (Burt and Bell 1987); since increasing offspring number also increases competition, and thus, selection for sex. We tested for this relation, by comparing the in-situ rate of sex (Fig. 5A) with the growth rates from the rotifer populations (Fig. 3), and found no correlation (GLMM: coevolution: $\chi 2=0.36$, df=1, p =0.5444). Hence, we can reject that the Tangled Bank is in effect. Furthermore, additional conditions (Maynard Smith 1976) such as high resource competition, high costs of sex (e.g. an asexual cycle takes only 1.5 days, a sexual 4.5 days) and the lack of reproductive isolation between asexual and sexual rotifers render the Tangled Bank Theory as unlikely in our system. We observed rapid loss of sexual reproduction in the non-coevolving environments with *colonial* algae and we thus refute the idea that slower adaptation to *colonial* algae maintains sex with coevolution. Although our data suggests that the starting populations were similar with respect to fitness related diversity and the propensity for sex, it is important to recognize that the experiment with *colonial* algae was started 4 month after the other two treatments and there could have been differences in the initial rotifer diversity. Our rotifer populations adapted, however, quickly to the *colonial* algae (initially low densities increased within 2-3 weeks to equal densities as in the non-coevolution environments with *single-celled* algae; Fig. S13) and the propensity for sex followed the same trend when comparing with the two non-coevolving with *single-celled* algae and the coevolving environments (lower in week 1 but was similar in week 2; Table S1).

Another possible mechanism maintaining higher propensities for sex in the coevolving environments is the Hill-Robertson effect. In finite populations and with selection acting simultaneously at more than one locus, recombination can break down unfavourable linkage between sites under selection (e.g. negative linkage disequilibrium where beneficial and detrimental are in linkage) (Hill and Robertson 1966; Felsenstein 1974) and increase the efficacy of natural selection. Our agent based model (Fig. 2) assumes only one locus that determines rotifer fitness and there is no information of the genetic basis for adaptation in rotifers. The role of the Hill-Robertson effect for our finding is thus not clear. It is also possible that the presence of the two prey types selects for sex through mechanisms other than antagonistic coevolution (Dolgin and Otto 2003; Roze 2014). However, our model simulations show that sex is only maintained when the two food types fluctuate in their frequency either through external forcing (Fig. 2A) or the eco-evolutionary feedback (Fig. 2B). Nonetheless, future work is necessary to identify the exact underlying mechanism.

Selection for dormancy and differences in population sizes are two other potential mechanisms that could explain the observed differences in the rate of sex. Sexual reproduction in rotifers results in the production of resting stages and thus the differences in the maintenance of sex could alternatively be explained by selection for dormancy. We can reject this alternative explanation, as experiments were conducted in chemostats with an exchange rate of 30% per day. Dormancy would not be advantageous because more than 96% of the resting eggs are washed out within ten days when assuming no hatching from the resting eggs. A cycle in the trait takes ~25 days, thus waiting till the times when conditions are favorable again could not be adaptive under these conditions. The dilution does however add substantially to the cost of sexual reproduction, as delayed hatching can increase the probability resting eggs will be washed out. In *Brachionus*, the switch to sexual reproduction is density dependent and rotifer densities were on average higher in both non-coevolving environments. Selection for lower propensities for sex and thus higher rotifer densities required for inducing sex could also evolve through selection against induction at low densities in the non-coevolving environments. Our agent based model (Fig. 2a) shows, however, that as population size decreases in constant environments, sex is lost even quicker. This result needs, however, to be tested experimentally to fully reject this alternative mechanism.

As with all other experimental studies on the maintenance of sex, it is not possible to predict whether the higher rates of sex in the coevolving environments would be maintained on the long-term. We observed however, that high rates of sex were maintained even after the initial adaptation in the coevolving environment (weeks 5-6), and were still high when testing again at week 14. Similar to most theories on the maintenance of sex, the proposed mechanism of continuous adaptation of rotifers to the changes in the prey types would break down, as soon as there would be two asexual predator clones specialized on either prey type.

The agent based model used here, is clearly simplified as it considers only one locus for the adaptation to the different food types and considers only the effects of segregation and recombination between the food and sex modifier locus. However, it is important to note that our model allowed us to disentangle multiple factors such as population size, single or coevolving populations, fluctuating environments, and competition. We also did not fix population size and rotifer populations could go extinct. While this stochasticity increased complexity it also added a natural element to the outcome. We refrained from strong forms of dominance (under- or over-dominance), which might change selection for sex (Agrawal and Otto 2006; Agrawal 2009a). Future work will be needed to test whether the results for selection of sex are the same when adding other fitness related loci and disentangling the effects of segregation and recombination.

Our study shows that rotifers coevolved with the algal prey over time and the frequency of rotifer clones better adapted to *single-celled* algae fluctuated over time similar to our model predictions (Figs. 1c,d). We found that the average fitness estimates were initially low in both environments and on both algae (Fig. S14 a,f; week 1). Average fitness increased rapidly when measured on *single-celled* algae in both environments. Average fitness on *colonial* algae stayed low in the non-coevolution environments (Fig. S14 g-h), whereas in the coevolution environments it increased and remained high (Fig. S14 b-e). The initial low fitness of rotifers in both environments is most likely due to genetic mixing (rates of sex are high during the first three weeks in both environments; Fig. 5), adaptation to the chemostat environment, and adaptation to the *colonial* algae in the coevolution environment. In well-adapted populations, genetic mixing can recreate bad combinations of alleles that have been eliminated by past selection, reducing mean fitness (Lynch and Deng 1994). This genetic slippage is most likely also responsible for the reduction in variance of fitness (most noticeable after week 3; Fig. S14). Although, identifying the mechanisms of adaptation in the rotifers was not the aim of this study, we considered potential pathways. For instance, adaptations to *colonial* algae may be behavioral, e.g. a reduction in handling time, or adaptations to food quality, e.g. changes in the carbon to nitrogen ratios (5.1 +/- 0.19 and 8.37 +/- 1.94 for *single-celled* and *colonial* algae respectively; n=3). On the other hand, morphological changes, such as widening of the mouth opening, are unlikely since rotifers are eutelic. Future work will be needed to identify the specific adaptation.

Conclusion

The Red Queen hypothesis is a prominent explanation for the prevalence of sexual reproduction. However, until now experimental tests for the maintenance of sex (outcrossing, selfing, or recombination) have been limited to host-parasite systems. We show here that antagonistic coevolution can select for sex in predator-prey systems, specifically showing the evolution of sex in the exploiter population. The process we have illuminated here is distinct from other studies in that eco-evolutionary feedback, i.e., the recurrent switch from selection by predation and competition for scarce resources, determines the maintenance of sex. Furthermore, sex evolving in the predator shows that the Red Queen is not restricted to the victim population (see also Howard and Lively 2002). Thus our study broadens the conditions under which antagonistic coevolution can explain the maintenance of sex while also highlighting the importance of the ecological context in which genetic mixing evolves. Indirect selection by eco-evolutionary feedback dynamics plays a major role for evolutionary processes.

Acknowledgments: We are grateful to Noemi Wolterman, Guilherme Alexandre Stecher Justiniano Pinto, Nadja Gogrefe and Sarah Frehse for helping us with the data collection, Christian Hilbe for discussion of the model, and Maarten Boersma for running the C/N analysis. We thank Aneil F. Agrawal, Ursula Gaedke, Martha Valiadi, Arne Traulsen and Manfred Milinski for comments on an earlier version of the manuscript.

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Figure 1: Simulations of eco-evolutionary dynamics in a predator-prey system without (A-C) and with coevolution (D-E); scaled population sizes of the total prey (green line), total predator (red line), prey trait (frequency of prey type 1; blue dashed line) and predator type 2 (black dashed line). (C,E) Show the frequency of the prey type 2 as a function of predator density. For model description see Material and Methods and Table 1. A-C) For predator prey systems with evolving prey (here: change in frequency of two prey genotypes), eco-evolutionary feedback dynamics occur when we observe predator and prey cycling out of phase. B) These dynamics are also characterized by a time lag between changes in the predator density and the defense trait (major ecological and evolutionary drivers at different time points). D-F) With coevolution (change in frequency of two prey and two predator genotype), we found that the qualitative dynamics of predator and prey did not change; total prey and predator are cycling out of phase but now with varying cycle length and amplitude. Again, we found a time-lag relation between the changes in the predator density and the frequency of the prey type. The changes in the total predator population size are accompanied by changes in the frequency of the two predator types, but the overall dynamics are driven by the feedback between the changes in total predator density and the trade-off in the prey population.

Figure 2: Maintenance of sexual reproduction in rotifer populations with recurrent changes in their prey environment driven by extrinsic changes (top) or eco-evolutionary feedback dynamics with coevolution (bottom). A,B) We used an individual based model specific to the rotifer life cycle with two algae types differing in their defense against predation and competitive ability to test for the conditions that select for sex, here shown as the frequency of simulations where sex was maintained for 1000 days. Extrinsically driven changes between to food types (filled circles) and changes in food density (high=80% and low 20%, open circles; parameters: f=2, τ=5, N=10^2 N_{max}=10^4 days=10^3, lifespan=14 days, μ =0.001; see main text and Methods). Orange point is without any change in the environment for maximum rotifer population sizes of 10, 100, 1000 individuals. C,D)

With eco-evolutionary dynamics (C_1 for prey A_1, varied, while remaining constant for prey A_2, C_2=8; other parameters: f=2, τ=5, N_A=2x10^5, N_r=30 N_{R_max}=10^7, days=10^3, lifespan=14 days, μ =0.0001, K_A = 8x10^4, a_T = 10^4, a_{1_max} = 4x10^4, a_{2_max} = 5x10^4, C_2 = 8). Frequency of simulations where sex was maintained for 700, 800, 900, 1000 days (light -> dark grey).

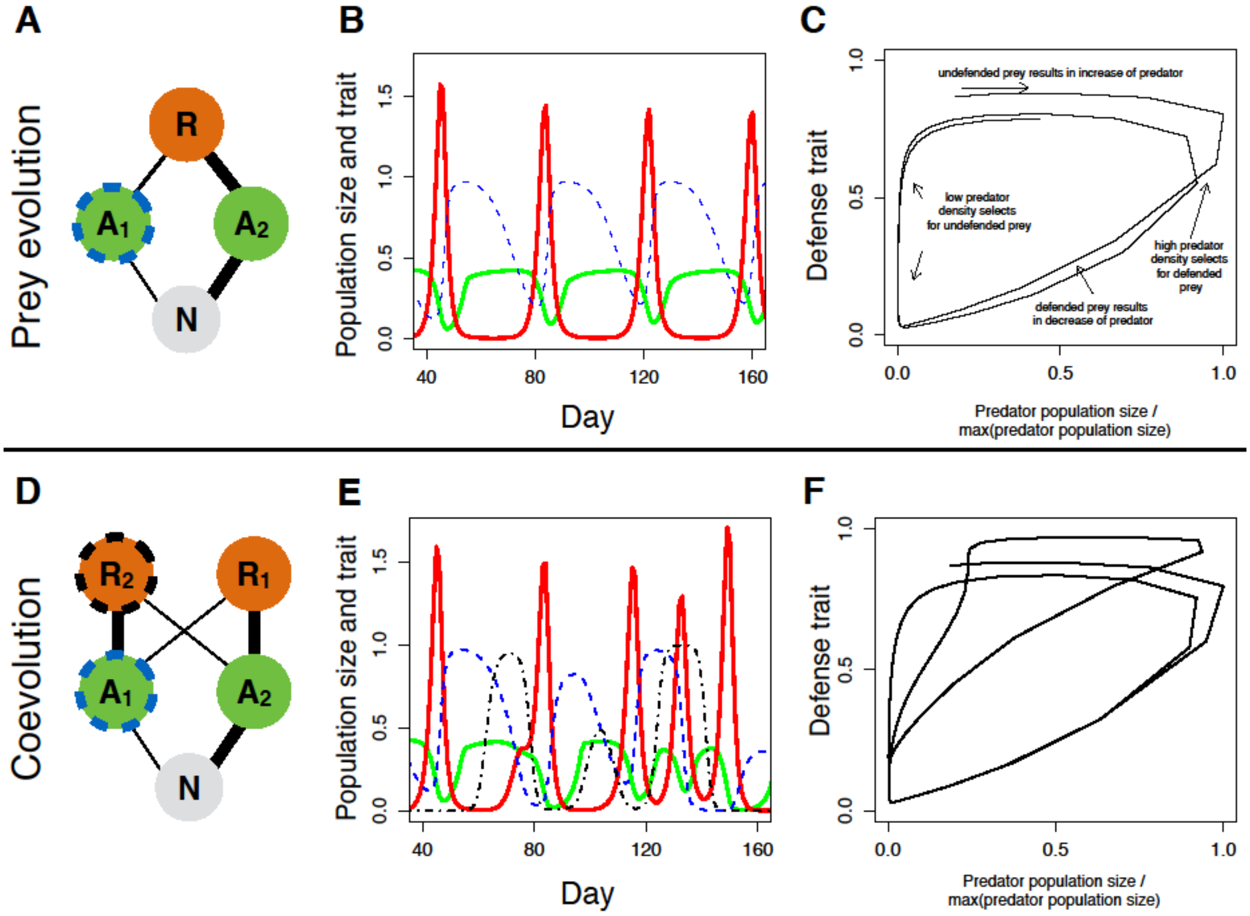
Figure 3: Representative dynamics of rotifer and algal populations in predator-prey chemostat experiments in non-coevolving (top) and coevolving environments (bottom) measured daily. Predator density (*Brachionus* 10 *ind/ml; red circles); prey density (*Chlamydomonas* 10^5 * cells/ml; green circles). The plotted curves are smooths of the data using cubic local polynomial regression with plugin bandwidth selection (Cabrera 2007). Blue lines show the mean defense trait in the algae (average number of cells/colony). Solid horizontal black lines mark days when males were present. A-C) Non-coevolving environments with constant supply of non-evolving *single-celled* algae (undefended; see Materials and Methods). D-F) Coevolving environments with eco-evolutionary feedback (c.f. Fig. 1D-F). B,C,E,F are examples, for other replicates see Fig. S7, S8.

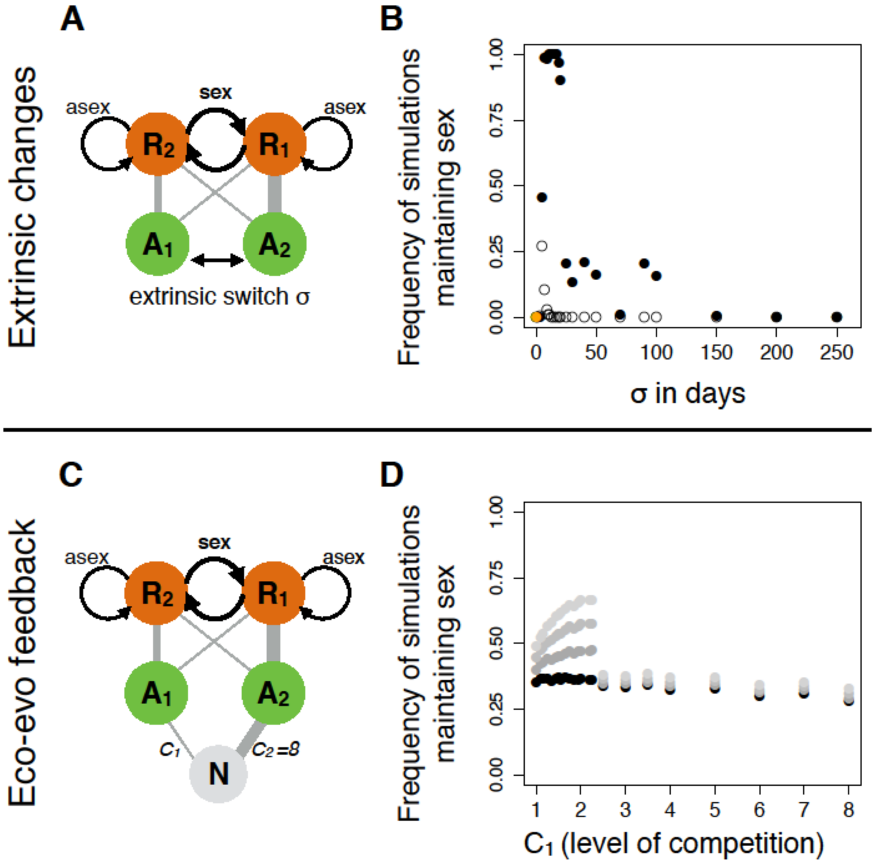
Figure 4: Adaptation of rotifer populations in non-coevolving (A-C) and coevolving environments with *single-celled* algae (D-F). B) Average fitness of rotifers from non-coevolution environments grown on *single-celled* algae (circle), and grown on *colonial* algae (triangles; n=5), C) Representative dynamics of frequencies of *single-celled* algae and rotifer densities (both scaled to their maximum), and rotifer clones better adapted to *single-celled* algae in the non-coevolving (same data set as in Fig. 3B top, other replicates Fig. S10). E) Average fitness of rotifers from the coevolution environment: grown on *single-celled* algae (diamonds), grown on *colonial* algae (squares) (n=4). F) Representative dynamics of frequencies of *single-celled* algae and rotifer densities (both scaled to their maximum), and rotifer clones better adapted to *single-celled* algae in the non-coevolving (same data set as in Fig. 3E bottom, other replicates Fig. S9).

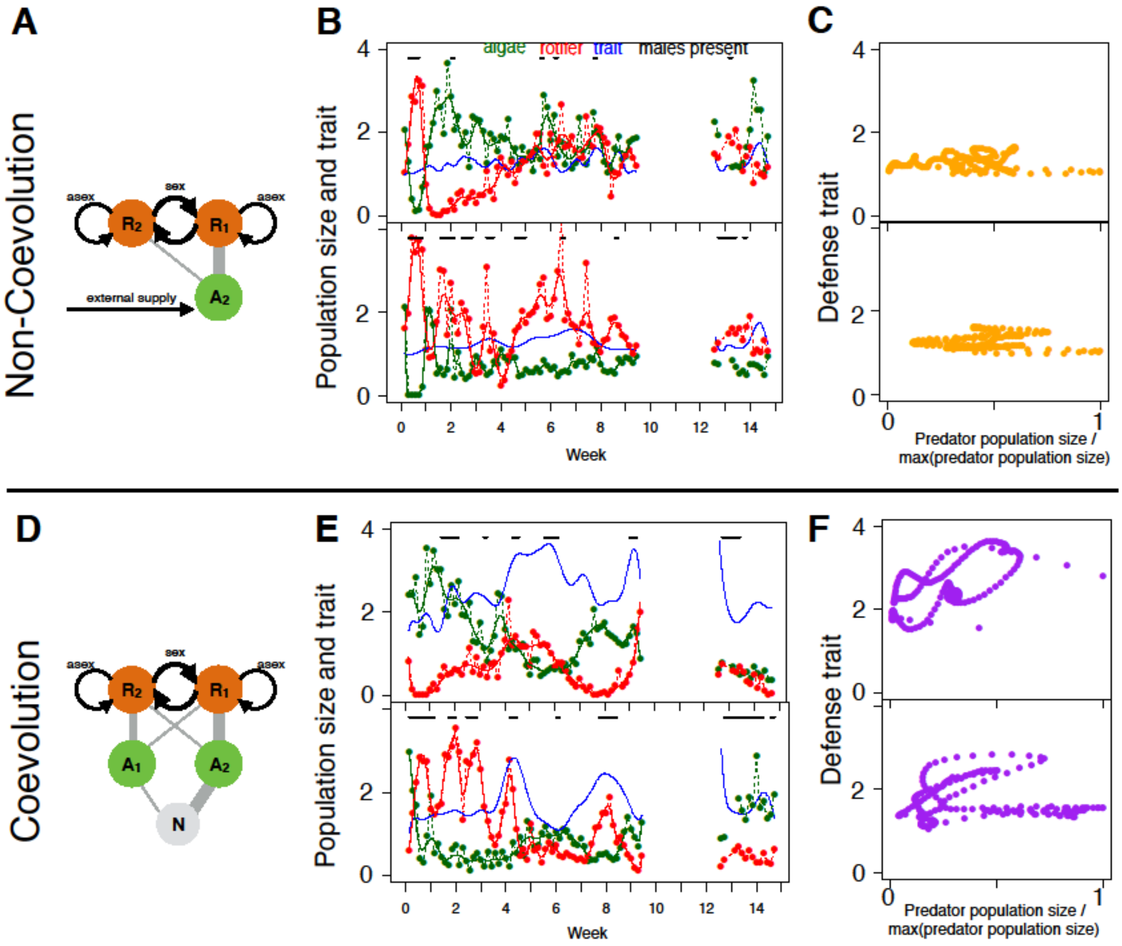
Figure 5: Evolution of the rate of sex in *B. calyciflorus* populations in non-coevolving and coevolving environments. A) Fraction of sexually derived offspring (=resting eggs) out of total diploid offspring. Error bars: SD. Right panel: average fractions for the time periods 1-3 shown on top of the left panel. B) Propensity for sex measured under common assay conditions as the threshold density required for inducing sexual reproduction (3rd generation after isolation). The non-coevolution with *colonial* algae environments was started only after the other two; Error bars, 2 SEM (*: p<0.05; **: p<0.01; for the comparisons non-coevolution *single-celled* algae and coevolution environment; for statistics see Table S1).

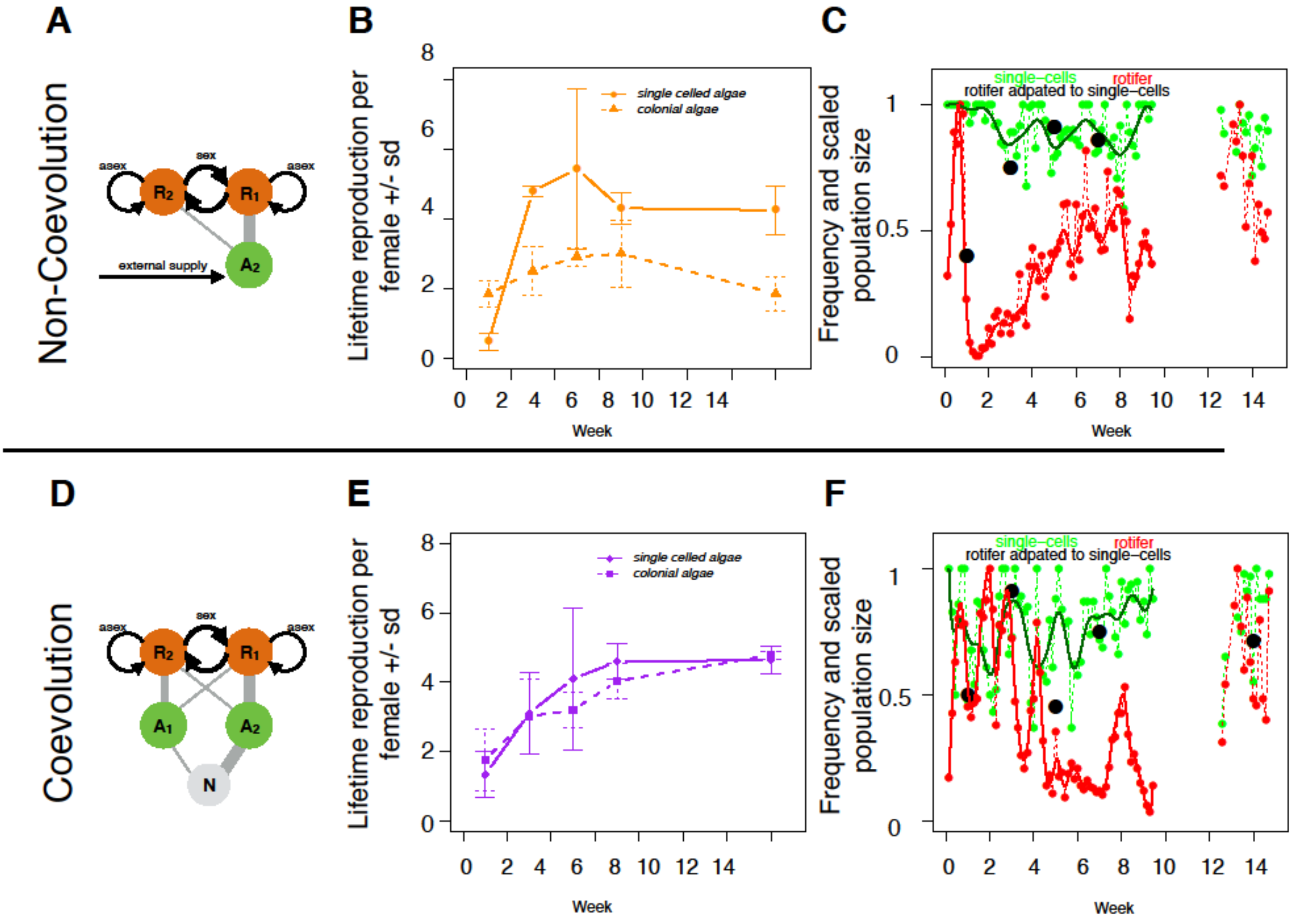
Table 1: Summary of model parameters and values.

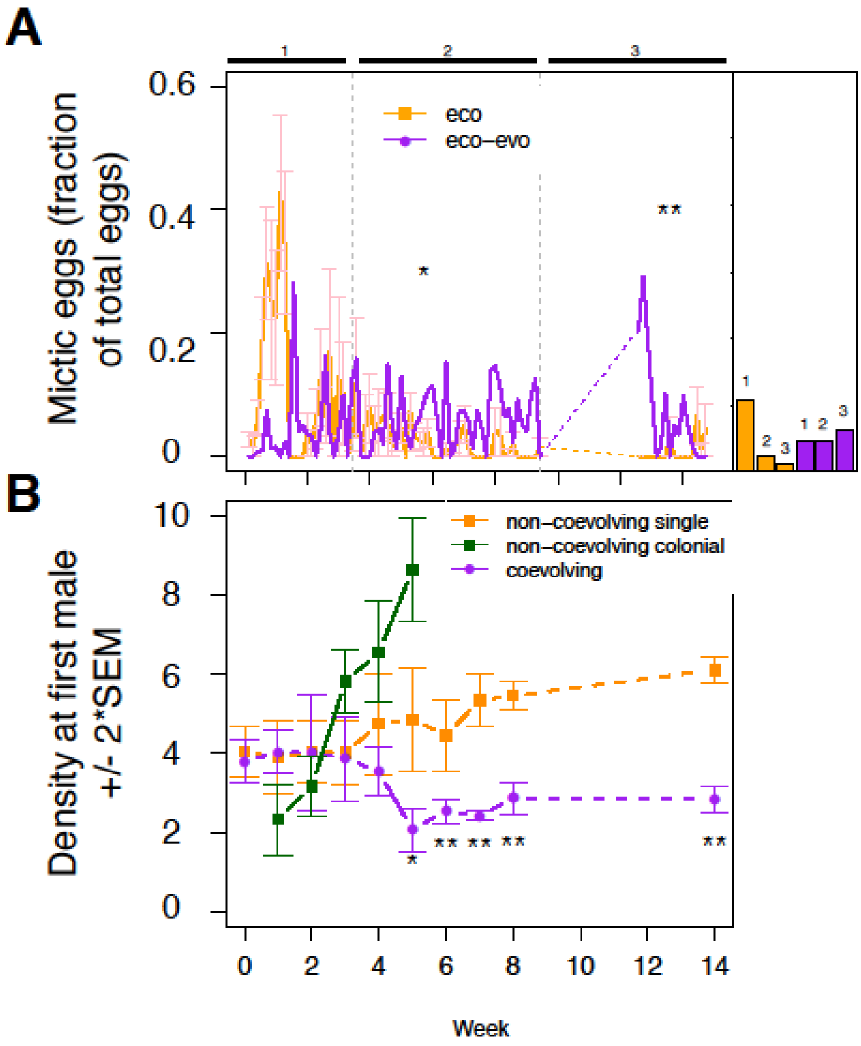
Parameter and value	**Description**
$\delta = 0.3\, d^{-1}$	dilution rate of chemostat
$N_{stock} = 160\, \mu\, mol\, N/l$	limiting nutrient in supplied medium
$0 < p_i^j < 1$	predator specific prey palatability
$K_A^1 = 8;\ K_A^2 = 2.2$	algae half saturation constant
$K_R^j = 0.15$	rotifer half saturation constant
$Q^{j*} = 50000$	critical prey density for rotifer clearance
$X_A = 0.0027$	algal conversion
$G = 0.011\, ml/rotifer/day$	rotifer grazing rate parameter
$m = 0.055\, d^{-1}$	rotifer mortality
$\lambda = 0.4\, d^{-1}$	rotifer senescence rate
$X_R = 170$	rotifer conversion


A
Prey evolution
R
A1
A2
N
B
Population size and trait
0.0
0.5
1.0
1.5
40
80
120
160
Day
C
Defense trait
0.0
0.5
1.0
undefended prey results in increase of predator
low predator density selects for undefended prey
high predator density selects for defended prey
defended prey results in decrease of predator
Predator population size / max(predator population size)
D
Coevolution
R2
R1
A1
A2
N
E
Population size and trait
Day
F
Defense trait
Predator population size / max(predator population size)


A
Extrinsic changes
asex
sex
asex
R₂
R₁
A₁
A₂
extrinsic switch σ
B
Frequency of simulations maintaining sex
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
σ in days
C
Eco-evo feedback
asex
sex
asex
R₂
R₁
A₁
A₂
C₁
C₂ =8
N
D
Frequency of simulations maintaining sex
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
C₁ (level of competition)


A
B
C
Non-Coevolution
asex
sex
asex
R₂
R₁
external supply
A₂
algae
rotifer
trait
males present
Population size and trait
0
2
4
Week
0
2
4
6
8
10
12
14
Defense trait
Predator population size / max(predator population size)
0
1
D
E
F
Coevolution
asex
sex
asex
R₂
R₁
A₁
A₂
N


A
Non-Coevolution
asex
sex
asex
R₂
R₁
A₂
external supply
B
Lifetime reproduction per female +/- sd
single celled algae
colonial algae
0
2
4
6
8
Week
C
Frequency and scaled population size
single−cells
rotifer
rotifer adpated to single−cells
0
0.5
1
Week
D
Coevolution
asex
sex
asex
R₂
R₁
A₁
A₂
N
E
Lifetime reproduction per female +/- sd
single celled algae
colonial algae
Week
F
Frequency and scaled population size
single−cells
rotifer
rotifer adpated to single−cells
Week
0
2
4
6
8
10
12
14


A
1
2
3
Mictic eggs (fraction of total eggs)
0.6
0.4
0.2
0
eco
eco–evo
*
* *
1
2
3
1
2
3
B
Density at first male +/- 2*SEM
10
8
6
4
2
0
non–coevolving single
non–coevolving colonial
coevolving
* *
* *
* *
*
* *
0
2
4
6
8
10
12
14
Week

692
693

694