

Evolution as a critical component of plankton dynamics

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Microevolution is typically ignored as a factor directly affecting ongoing population dynamics. We show here that density-dependent natural selection has a direct and measurable effect on a planktonic predator–prey interaction. We kept populations of *Brachionus calyciflorus*, a monogonont rotifer that exhibits cyclical parthenogenesis, in continuous flow-through cultures (chemostats) for more than 900 days. Initially, females frequently produced male offspring, especially at high population densities. We observed rapid evolution, however, towards low propensity to reproduce sexually, and by 750 days, reproduction had become entirely asexual. There was strong selection favouring asexual reproduction because, under the turbulent chemostat regime, males were unable to mate with females, produced no offspring, and so had zero fitness. In replicated chemostat experiments we found that this evolutionary process directly influenced the population dynamics. We observed very specific but reproducible plankton dynamics which are explained well by a mathematical model that explicitly includes evolution. This model accounts for both asexual and sexual reproduction and treats the propensity to reproduce sexually as a quantitative trait under selection. We suggest that a similar amalgam of ecological and evolutionary mechanisms may drive the dynamics of rapidly reproducing organisms in the wild.

Keywords: *Brachionus calyciflorus*; chemostat; clonal selection; cyclical parthenogenesis; evolutionary dynamics; rapid evolution

1. INTRODUCTION

Population dynamics and evolutionary dynamics are often treated as separate fields that require different approaches and methodologies both in experimental and theoretical studies. In particular, the two dynamic processes traditionally have been believed to operate on different time-scales, that is population dynamics are rapid while evolution's progress is slow (e.g. Khibnik & Kondrashov 1997). In many cases, however, evolutionary change can occur at rates comparable to the rates at which the abundances of populations and communities change and a growing number of studies have demonstrated rapid evolution at ecologically relevant time-scales (Thompson 1998; Hairston *et al.* 1999; Hendry *et al.* 2000; Cousyn *et al.* 2001; Reznick & Ghilambor 2001; Stockwell *et al.* 2003). In this study, we show how clonal selection operating on a fundamental life-history trait (the production of amictic versus mictic offspring) affects the dynamics of plankton populations. We report results from experimental populations of cyclically parthenogenetic rotifers whose dynamics can be accurately described by a mathematical model (Fussmann *et al.* 2000). These dynamics were shaped by evolutionary change, so much so that they could only be correctly predicted when evolution was explicitly included in the model.

Brachionus calyciflorus Pallas is a monogonont rotifer species that exhibits cyclical parthenogenesis in the wild; a population persists through time with phases of amictic (ameiotic) and mictic (sexual) reproduction following one another in a seasonal pattern (e.g. Serra & King 1999).

The life cycle begins when diploid amictic females hatch from diploid diapausing eggs. Rapid clonal propagation of these diploid females characterizes the amictic phase. The sexual phase is initiated when amictic (parthenogenetic) females produce mictic female offspring. Mictic females produce haploid eggs that develop into haploid males if unfertilized. Fertilized mictic eggs, however, develop into diapausing eggs that can undergo extended periods of dormancy. Amictic females hatching from diapausing eggs initiate another phase of parthenogenetic reproduction, and so on.

High population density of conspecifics has been experimentally established as an intrinsic stimulus for the production of mictic eggs in *B. calyciflorus* (Gilbert 1963, 2002). Gilbert's (1963) results suggest that the rate of mictic female production is proportional to the density of females and that the rotifers obtain information about the current population density via the concentration of one or several chemical substances that accumulate when rotifer density increases. This is consistent with observations of natural populations of rotifers where the onset of mixis is gradual and the population consists of mictic and amictic females coexisting in variable fractions (Aparici *et al.* 2001).

Sexual reproduction of monogonont rotifers results in the production of diapausing eggs, an essential adaptation that ensures survival in the time-varying environments the plankton inhabit. At the start of a new season, genetic variance—represented by different clones—is routinely re-established through emergence from the dormant egg bank (Gómez & Carvalho 2000). Recently, Gilbert (2002) performed crowding experiments with a strain of *B. calyciflorus* and detected significant clonal variation in the propensity to produce mictic females in response to

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the crowding stimulus. From these observations, and because planktonic rotifers typically undergo many generations of asexual reproduction during a seasonal cycle, we suspect clonal selection to have an important, naturally recurring impact on the population dynamics of these animals. In our experiments, mating of male and female rotifers was prevented by turbulence in the culture vessels. This extreme condition enabled us to study the effect of clonal selection against sex on the rotifer population dynamics without any confounding effects of recombination.

We found that, after *ca.* 2 years, the propensity to reproduce sexually had disappeared entirely from the rotifer population. The rate at which this happened was especially high when the chemostat cultures initially reached large population size (including male production by some genotypes), and then, as the algal resource was depleted, decreased rapidly, thus producing conditions for strong natural selection. We combined replicated community experiments and mathematical modelling to demonstrate that the observed dynamics were unlikely to result from population interaction alone but that evolution of the rotifer population towards asexual reproduction was a critical component of the dynamic process.

2. METHODS

(a) *Experiments and extra-experimental observations*

Females of the rotifer *B. calyciflorus* were collected from Milwaukee Harbor, Lake Michigan, USA (courtesy of M. Boraas) and axenic cultures were established using the unicellular green alga *Chlorella vulgaris* Beij. (UTEX no. 26) as food. We cultured these two species together in flow-through culture systems (380 ml glass chemostats) at 25 ± 0.3 °C, constant illumination at $120 \pm 20 \mu\text{E m}^{-2} \text{s}^{-1}$ (wide-spectrum fluorescent lamps) and used a culture medium that contained nitrogen at a concentration that limited algal growth ($80\text{--}514 \mu\text{mol l}^{-1}$), plus non-limiting concentrations of other nutrients and trace metals: P, S, B, Ca, K, Na, Mg, Cl, Fe, I, Li, V, Mn, Co, Cu, Zn, Se, Rb, Sr, Mo; we added vitamins B₁, B₁₂, and biotin to ensure the long-term survival of *B. calyciflorus*. Sterile air was bubbled through the chemostat vessels to prevent CO₂ limitation of the algae and to enhance mixing. It was this mixing that prevented mating of male and female *B. calyciflorus*. For more details on the set-up see Fussmann *et al.* (2000). We cultured the daughter generations of the initial *B. calyciflorus* founder population in consecutive chemostats for 901 days under constant conditions, except that we changed the dilution rate and nitrogen concentration of the culture medium for the purpose of experimentation, which we have described elsewhere (Fussmann *et al.* 2000). Cultures were sampled daily in duplicate using sterile syringes. Rotifer density and the occurrence of mictic individuals were determined by direct count under a stereo dissecting microscope. Algal density was established using a CASY I particle counter. The experiment pertinent to this study was run from days 0–30, i.e. at the beginning of the culturing period, when both mictic and amictic rotifers were present. We ran four simultaneous replicate trials, which were initiated by adding 50 *B. calyciflorus* to each of the four chemostats that contained steady-state cultures of *C. vulgaris*. We set the dilution rate of the chemostat cultures to $\delta = 0.44 \text{ d}^{-1}$ and nitrogen concentration in the inflow medium to $N_i = 514 \mu\text{mol l}^{-1}$.

(b) *Mictic and amictic rotifers*

For our study it was crucial to be able to distinguish between mictic and amictic *B. calyciflorus*. While male *B. calyciflorus* are considerably smaller and morphologically different from females, there are no morphological differences between mictic and amictic females. However, females produce any of three types of egg that can be easily distinguished during routine microscopic inspection: large, soft-cased subitaneous eggs (amictic eggs that hatch immediately, produced by amictic females), small soft-cased ‘male’ eggs (unfertilized mictic eggs that hatch immediately, produced by mictic females), or large, hard-cased diapausing eggs (fertilized mictic eggs that go through a period of dormancy, produced by mictic females). Thus, for all egg-carrying females it was straightforward to determine whether they were mictic or amictic. For those females in a sample that were not carrying eggs, we assumed the same ratio of mictic to amictic individuals as found for the egg-carrying females. The proportion of mictic females among all females π (‘mictic ratio’; table 1) was estimated as

$$\hat{\pi} = \frac{M_e + N_e[M_d/(M_e + A_e)]}{M_e + N_e + A_e}, \quad (2.1)$$

where M_e , A_e and N_e are the numbers of females with mictic, amictic, and no eggs in a sample, respectively.

(c) *Quantitative trait (QT) model for mixis evolution*

The QT model we propose here is an extension of a model (F2K model; Fussmann *et al.* 2000) we have previously used to predict successfully the dynamics of nutrients and interacting populations of algae and rotifers in the chemostat system. The F2K model is a double Monod model (Nisbet *et al.* 1983) that does not allow for mixis. It could thus be expected to produce quantitatively accurate predictions only for periods when rotifer reproduction was almost exclusively amictic. Because our experiment fell into a period of extensive mictic reproduction and because there was evidence in the data for selection against mixis, we amended the F2K model to include mictic females and evolution of a QT related to mixis (bold type indicates these amendments):

$$\begin{aligned} \frac{dN}{dt} &= \delta(N_i - N) - F_C(N)C, \\ \frac{dC}{dt} &= F_C(N)C - \frac{1}{\epsilon}F_B(C)(B + M) - \delta C, \\ \frac{dR}{dt} &= (1 - p)F_B(C)R - (\delta + m + \lambda)R, \\ \frac{dB}{dt} &= (1 - p)F_B(C)R - (\delta + m)B, \\ \frac{dM}{dt} &= pF_B(C)R - (\delta + m)M, \\ F_C(N) &= \frac{b_C N}{K_C + N}, \quad F_B(C) = \frac{b_B C}{K_B + C}. \end{aligned} \quad (2.2)$$

All state variables are modelled in units of moles of nitrogen. N and C denote the concentration of nitrogen and *Chlorella*, respectively; B denotes the density of amictic female *Brachionus*, and M is the density of mictic females. R is the density of females that lay subitaneous eggs, a subset of the amictic fraction. By using two state variables for amictic rotifers we incorporated age structure into the model: R denotes the amictic rotifer density corrected for the loss of fecundity with age, with λ being the rate at which fecundity decays. Note that, in the model, mictic females have the same consumption rate as amic-

Table 1. Parameters associated with mixis of *Brachionus*.

parameter	name	meaning	use
p	mixis allocation	fraction of subitaneous eggs that are bound to be mictic females when they mature	model (equation (2.2)); unknown for experimental data
π	mictic ratio	proportion of mictic females among all females	model and data; $\pi(t)$ is an estimate of $p(t)$, with an unknown time delay
$\hat{\pi}$	estimate of mictic ratio	as above	data (equation (2.1)); only direct measure of mixis available from experimental data
a	propensity to mixis	constant of proportionality relating p to <i>Brachionus</i> density (equation (2.3))	model (equation (2.4)); shown to be a QT under selection
\hat{a}	estimate of propensity to mixis	as above, but substituting $\hat{\pi}$ for p	data; value for QT derived from experimental data

tic females but do not reproduce, so we do not need to distinguish between older and younger amictic rotifers. This reflects the fact that mictic and amictic females have the same size, morphology and feeding behaviour; however, aeration kept the chemostat content perpetually turbulent, which effectively prevented rotifer mating and sexual reproduction of offspring other than males (we never observed any sexually produced diapausing eggs in chemostat samples). This means that mictic rotifers have zero fitness and the biomass and energy invested into them and their (male) eggs are eventually washed out of the chemostat vessel. Male rotifers and male eggs are not part of the model, as they make no contribution to growth of the female population in the chemostat and do not feed (Serra & Snell 1998). N_i is the nitrogen concentration in the inflow medium; δ is the dilution rate of the chemostats; $F_C(N)$ and $F_B(C)$ are the numerical response functions of *Chlorella* and *Brachionus*; b_C and b_B are the maximum birth rates of *Chlorella* and *Brachionus*; K_C and K_B are the half saturation constants of *Chlorella* and *Brachionus*; ε is the assimilation efficiency of *Brachionus*; and m is the *Brachionus* mortality rate.

The parameter p ('mixis allocation'; table 1) denotes the fraction of subitaneous eggs that are destined to become mictic females when they mature. We assume that p is a linear function of the current total density of rotifers,

$$p = \min(a \cdot (B + M), 1), \quad (2.3)$$

and wish to model the dynamics of a (the 'propensity for mixis'; table 1), considered as a QT under selection. We use the standard model in which the rate of change in trait value is proportional to the per capita fitness gradient (Lande 1976; Saloniemi 1993). For equations (2.2) and (2.3) this gives (with ν as the constant of proportionality):

$$\begin{aligned} \frac{da}{dt} &= \nu \frac{\partial}{\partial a} [(1-p)F_B(C)] = -\nu F_B(C) \frac{\partial p}{\partial a} \\ &= \begin{cases} -\nu F_B(C)(B+M) & \text{if } p < 1 \\ 0 & \text{if } p = 1 \end{cases} \end{aligned} \quad (2.4)$$

The fitness gradient is always non-positive, reflecting the fact that selection in the chemostat flow-through environment never favours mixis.

(d) Model parameter estimation

We estimated parameters by fitting the model to the four experimental chemostat runs. The model was fitted only to the *Chlorella* and *Brachionus* data, owing to the imprecision of the mixis indices based on the data (mixis allocation p itself is intrac-

Table 2. Fitted values for model parameters.

parameter	fitted value
ε	0.033 033
ν	$1.502 \times 10^{-4} \text{ ml}^2 \text{ female}^{-2}$
$a(0)$	0.072 51 ml female ⁻¹
N content <i>Chlorella vulgaris</i>	$2.054 \times 10^{-8} \mu\text{Mol}$
N content <i>Brachionus calyciflorus</i>	$3.295 \times 10^{-4} \mu\text{Mol}$

table in the experimental part of the study; mixis ratio π represents an estimate of p with an unknown time delay but has to be estimated in return by $\hat{\pi}$; equation (2.1)). This method has the advantage of providing an additional check on the model: does it produce patterns of mixis similar to the data, even though it was not fitted to those data?

We fitted five parameters (table 2): ε , ν , $a(0)$ and the scaling factors for *Chlorella* and *Brachionus* between model units (N concentration) and data units (individuals per ml). All other parameters were held fixed at the values in Fussmann *et al.* (2000). All parameters were assumed to be constant across chemostats and constant over time. In addition, initial conditions were fitted separately for *Chlorella* (model variable C) and *Brachionus* (model variable B) in each chemostat (i.e. it was not assumed that initial values were measured exactly, because survivorship of the *Brachionus* inoculum at t_0 may have varied among chemostats). Other initial conditions were assumed as follows:

- (i) $R(0) = B(0)$: experiments initialized with newly matured individuals,
- (ii) $M(0) = 0$: initially no mixis was occurring, based on the mixis indices from the data,
- (iii) $N(0) = 50 \mu\text{mol l}^{-1}$ based on measurements of total soluble nitrogen in the chemostats.

The fitting criterion was the sum of absolute errors on our usual plotting scale for sample counts (*Chlorella*: 10^6 cells ml⁻¹; *Brachionus*: females ml⁻¹). Fitting was programmed in R (Ihaka & Gentleman 1996). We used lsoda (in the ODESOLVE library) to compute numerical solutions of the model, and the fitting criterion was optimized using the Nelder–Mead algorithm (function OPTIM). To avoid spurious local minima we used a multistart procedure similar to that of Turchin & Ellner (2000).

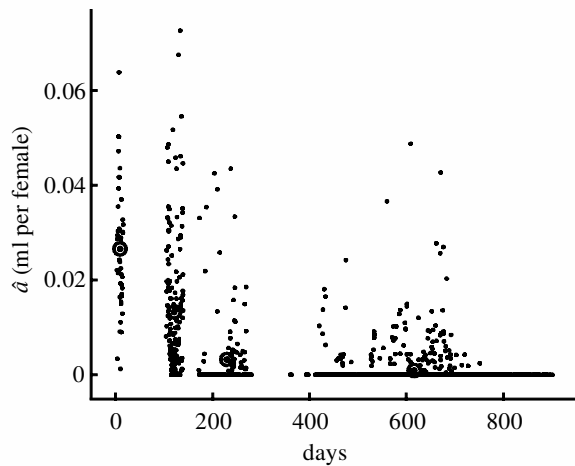


Figure 1. Long-term evolution of the QT a ('propensity for mixis' in *Brachionus*) in consecutive chemostat cultures over 901 days. Small dots ($n = 1374$) represent estimates (\hat{a}) of a calculated as $\hat{a} = \hat{\pi} (B + M)$ from separate chemostat samples (means of two replicate samples), where $(B + M)$ is the total density of female *Brachionus* rotifers (both amictic and mictic) and $\hat{\pi}$ is an estimate of the ratio between mictic and total female *Brachionus*. Only data from samples with $(B + M) > 5$ *Brachionus* ml^{-1} are plotted. Large symbols indicate the average \hat{a} for each of four distinct sampling periods. Note that accumulation of dots at $\hat{a} = 0$ results in a line-like appearance.

That is, randomly generated parameter choices were compared to identify good contenders, and optimization was repeated from multiple good contenders to confirm that the same 'best fit' was found repeatedly. We did not attempt to quantify the precision of parameter estimates because our focus is on comparing alternative models rather than parameter estimation. Estimates are presented only to verify that the data can be fitted with parameter values that are consistent with the available experimental evidence.

3. RESULTS

Over the more than 900 days that we monitored our chemostat cultures, the mictic reproduction of *B. calyciflorus* steadily declined (figure 1). The average mictic ratio decreased from $\bar{\pi} = 0.15$ during the experimental period (days 0–30) to $\bar{\pi} = 0.03$ for the post-experimental observation period (days 96–901). After day 751 we observed neither female *B. calyciflorus* carrying mictic eggs nor males, indicating that reproduction had become entirely amictic in the chemostat cultures. Accordingly, we found evidence in the data for long-term evolution of the QT a , the 'propensity for mixis'.

We observed very reproducible plankton dynamics in all four replicate chemostat trials (figure 2). The characteristics were: (i) a bimodal abundance pattern of female *Brachionus* followed by complete extinction; (ii) a maximum of males and mictic ratio falling temporally between the two maxima in female *Brachionus* abundance; and (iii) an almost steady decline of the algal population which decelerated between the two maxima of female *Brachionus* abundance. The timing of these characteristic events differed by a few days among replicates and the absolute values of abundance and mictic ratio varied slightly.

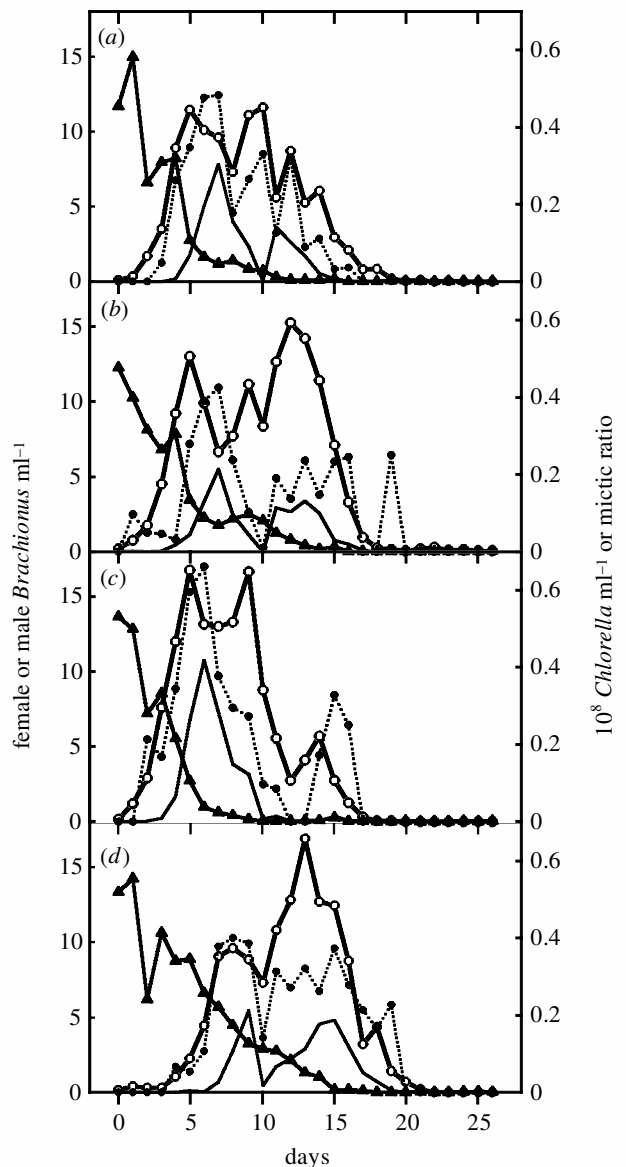


Figure 2. Dynamics of *Brachionus calyciflorus* (rotifers) and *Chlorella vulgaris* (green algae) in four replicate (a)–(d) chemostat trials (experiment; days 0–26). Open circles with solid line, *B. calyciflorus* females; solid line, *B. calyciflorus* males; black circles with dotted line, mictic ratio; triangles with solid line, *C. vulgaris*.

The concentration of female *Brachionus* during the experimental period could be used to predict how prevalent sexual reproduction was in the rotifer population. The mictic ratio $\hat{\pi}$ increased linearly with *Brachionus* density (figure 3). The mean value of the QT a , the propensity for mixis, can be estimated as the slope $\hat{a} = 0.0258$ ml per female from linear regression of $\hat{\pi}$ versus female rotifer density. Our experiments and long-term observations revealed, however, that the QT a was subject to evolutionary change. Selection against mixis in the chemostats was sufficiently strong to be detected even during the relatively short experimental period (days 0–30 of our experiment): \hat{a} , calculated from a moving regression of $\hat{\pi}$ versus female *Brachionus* concentration, declined in the first half of the experimental period (figure 4). The reversal of this trend after day 14 (figure 4) does not mark a reversal in the direction of evolution but is due to the fact that $\hat{\pi}$ is an

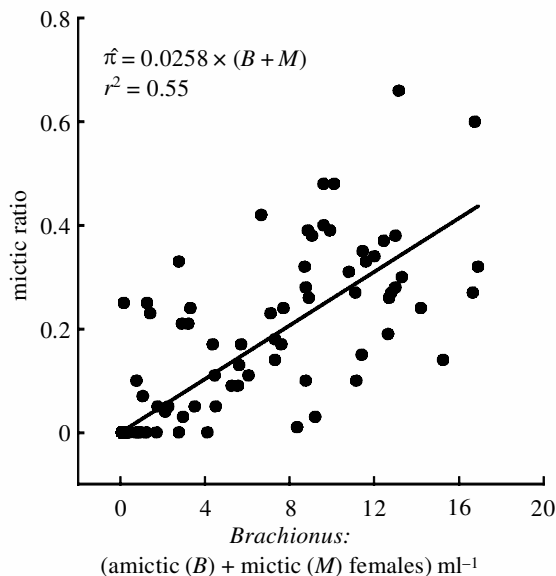


Figure 3. Linear regression of mictic ratio versus female *Brachionus calyciflorus* density for the first 30 days of *Brachionus* culture (experimental period; $n = 92$). Data from all samples with $(B + M) > 0$ *Brachionus* ml^{-1} are included. $\hat{\pi} = 0.0258 \times (B + M)$; $r^2 = 0.55$.

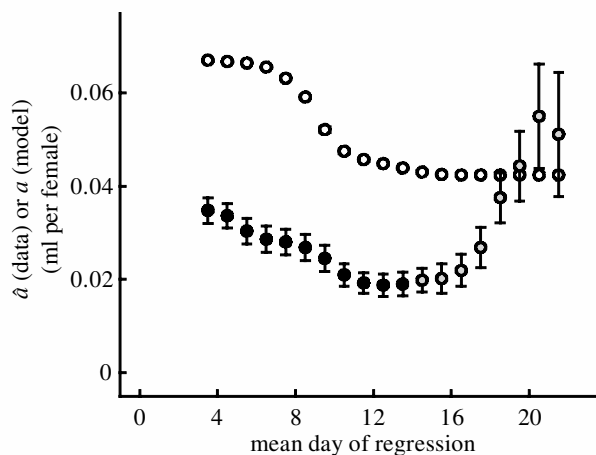


Figure 4. Short-term evolution during the first 30 days of *Brachionus* culture (experimental period). Each symbol represents the slope \hat{a} (data) or a (model) \pm s.e. of a linear regression, forced through the origin, for seven consecutive days of $\hat{\pi}$ (estimate of mictic ratio; data) or p (mixis allocation; model) versus total female *Brachionus* density $(B + M)$. Open symbols, a based on data generated by the mathematical model; black symbols, \hat{a} based on experimental data; grey symbols indicate unreliable estimates of \hat{a} (see text). See table 1 and figure 1 for other variable name definitions.

inappropriate estimator of the mixis allocation p when *Brachionus* females no longer reproduce (see § 4 and figure 5c).

Our model (equations (2.2), (2.3) and (2.4); table 2) matches the qualitative features of the population data from the experiment with reasonably good quantitative agreement (figures 2 and 5). It captures the essentials of the experimental dynamics: the double peaks of female *Brachionus* at about days 5 and 12 (figure 5a), the exploitation of the algal resource, retarded between days 5 and

12 (figure 5b), and a local maximum of the mictic ratio shortly after the first rotifer maximum, followed by equilibrating mictic ratios (figure 5c). We observed a similar plateau of mictic ratios in two of the four experimental runs (figure 2b,d and figure 5c) before total rotifer densities became too low for precise estimates. The model also predicts that the 'propensity for mixis' (i.e. the density response parameter a) was reduced but not eliminated over the course of the experiment. Estimates of \hat{a} derived from the experimental data evolve in parallel with the value of a predicted by the mathematical model during the first half of the experimental period (figure 4). The difference in absolute values between a and \hat{a} is due to the imperfect estimation of p (model) by $\hat{\pi}$ (data). For the second half of the experimental period, data and model prediction disagree (figure 4), reflecting the fact that the parameters underlying the computation of a , mixis ratio π and mixis allocation p , diverged drastically after the second maximum of *Brachionus* (figure 5c).

Mixis and evolution of the QT a are components of the model that are essential to describe correctly the population dynamics of the rotifers (figure 6). If we remove evolution from the QT model (by setting $da/dt = 0$ and $a = \text{constant} = a(0)$) *Brachionus* no longer drives the algal population to extinction and both species coexist at an equilibrium (figure 6b). An attempt to fit the experimental data without allowing for the possibility of mixis evolution leads to extinction of both rotifers and algae but the characteristic double peak of rotifer abundance in the original data is not recovered (figure 6c). Similarly, if both evolution and mixis are removed (the F2K model; see § 2(c)) the model predicts again a simple boom-and-crash cycle where the rotifers rapidly and completely exploit their algal resource followed by their own extinction (figure 6d).

4. DISCUSSION

The predator-prey dynamics between *Brachionus* and *Chlorella* in our laboratory chemostat cultures can be understood only by incorporating the evolution of one of the basic life-history characters of the predator, mixis. A simple model lacking evolution enabled us previously to predict key aspects of the dynamics, including transitions between equilibrium coexistence and stable limit cycles (Fussmann *et al.* 2000). However, this model proved to be incomplete when mictic females accounted for a considerable fraction of the rotifer population. Guided by our improved model we arrive at the following scenarios for the experimental dynamics.

At the beginning of our trials, rotifer populations increased from low initial numbers while algal density decreased. Mictic female production was density dependent, and this caused the mictic ratio and the numbers of male rotifers (with a delay) to rise together with female density. The mixis ratio reached *ca.* 50%, i.e. about half of the available resources were invested into mictic rotifers, a non-reproductive dead end in the chemostat setting. The rotifer population then began to decline when the amictic rotifer reproduction no longer compensated for the combined losses by mortality, decreased fecundity and chemostat dilution rate. Algal population decline decelerated during this period because, at lowered algal densities,

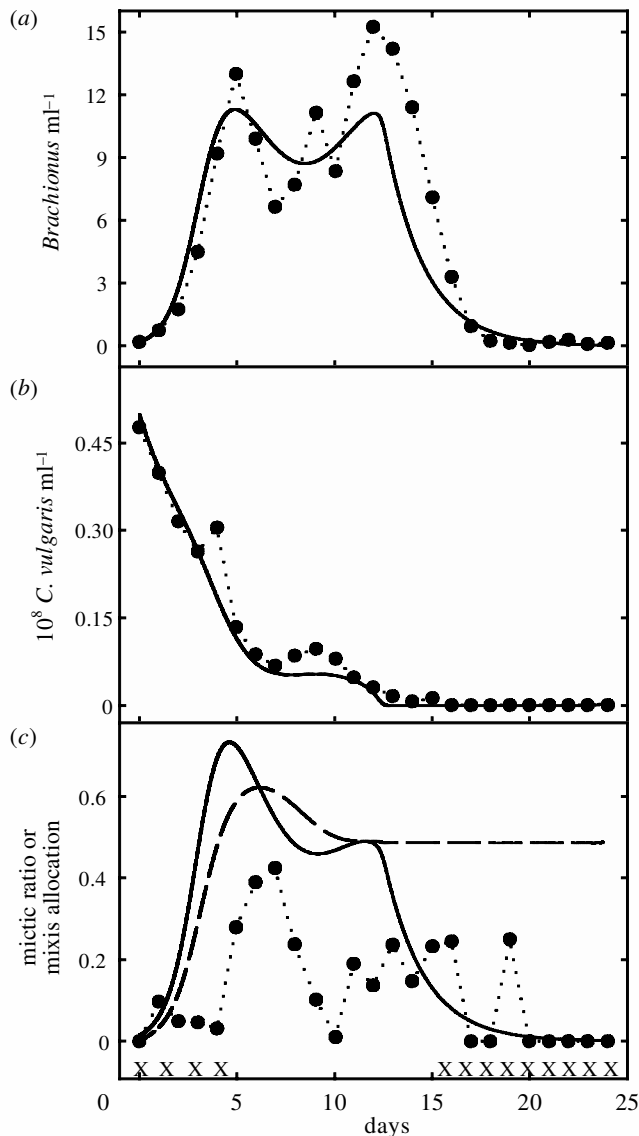


Figure 5. Comparison of model predictions (solid and dashed lines) with real, experimental data (dotted lines) for the chemostat trial shown in figure 2*b*. (a) *Brachionus calyciflorus* density, (b) *Chlorella vulgaris* density, and (c) mixis allocation p (solid line), mixis ratio π from model (dashed line), and estimated mixis ratio $\hat{\pi}$ from data (dotted line). X symbols above the x -axis indicate sampling dates with a female *B. calyciflorus* density of less than 5 ml^{-1} .

which resulted in elevated nutrient concentrations (data not shown), algal reproduction was able to compensate for the consumption by rotifers. As rotifer density declined, the fraction of amictically reproducing rotifers increased owing to the combined effects of reduced crowding and continued evolution of a reduced propensity for mixis. This allowed the rotifer population to rise again and to reach a second maximum. Although rotifer density was then as high as or higher than at the first maximum, both the mixis allocation and the mictic ratio were lower due to the evolution against mixis that had occurred up to this point.

Thus, in the experiment, two very different mechanisms acted together to produce the bimodal shape of the rotifer dynamics. The first maximum (and the subsequent decline of rotifers) occurred when a major fraction of the

available resources was channelled into the production of mictic, non-successfully mating female and male rotifers. A shift towards predominantly amictic rotifers caused the second maximum, after which rotifers died out because they had overexploited their resource.

Although the model performed best on the *Chlorella* and *Brachionus* abundance data to which it was fitted (figure 5*a,b*), it also predicted reasonably well the mixis patterns observed in the experiments (figure 5*c*). At the beginning and end of the experiments rotifer abundances were very low, which prevented reliable estimates of the mictic ratio. Mixis indices based on sample sizes of less than 5 rotifers ml^{-1} may show considerable stochastic fluctuations owing to the presence or absence of a single mictic female in a sample, and were therefore excluded from our interpretation. Also, the use of the observed mictic ratio $\hat{\pi}$ as a surrogate for the unobserved mixis allocation p is only valid prior to the second rotifer maximum. Beyond this point rotifers soon stop reproducing, so the ratio of amictic to mictic females remains nearly constant despite the decrease in rotifer abundance (which would lead to a decrease in p if reproduction were occurring). The result is a spurious increase in our estimates of the mixis propensity a (grey circles in figure 4).

Serra & King (1999) formulated a mathematical model for bisexually reproducing monogonont rotifers that produced dynamical patterns comparable to those we observed in our experiments and model simulations. Their model is considerably simpler than our QT model in that it contains neither resources as state variables (rotifer growth is instead density dependent) nor age structure, and mixis allocation (our p) was incorporated either as a constant intermediate value or as a switch where p was either zero or one depending on the current rotifer density, without any evolution of mixis. Despite its more abstract character, the Serra and King model with constant mixis allocation predicted rotifer population size to reach an equilibrium after a single maximum, similar to our simulations without evolution of mixis where rotifer density and mixis allocation reached a constant value after damped oscillations (figure 6*b*). When p is switched between zero and one in response to a density threshold, the Serra and King model predicts sustained population oscillations driven by the periodic change between amictic and mictic reproduction. The damped oscillations in our model without mixis evolution (figure 6*b*) are not due to density-dependent mixis, but rather are predator-prey oscillations. Our model incorporates the population dynamics of the resource species, *Chlorella*. If we instead supply food to the rotifers at a constant rate, we also observe rotifers reaching equilibrium after a single maximum in our model.

Mictic females accounted for more than 50% of total female abundance shortly after our cultures were started, but after *ca.* 750 days in continuous culture no signs of mixis (mictic females and males) remained. Boraas (1983) and Bennett & Boraas (1989) observed a similar loss of mictic reproduction over time in their *B. calyciflorus* flow-through cultures. Boraas (1983) found *ca.* 40% mictic females in newly established *Brachionus* chemostat cultures, but no longer observed mictic females and males after two to three months. Boraas (1983) concluded that selection against sexual reproduction was responsible for his observations, presumably because this was the most

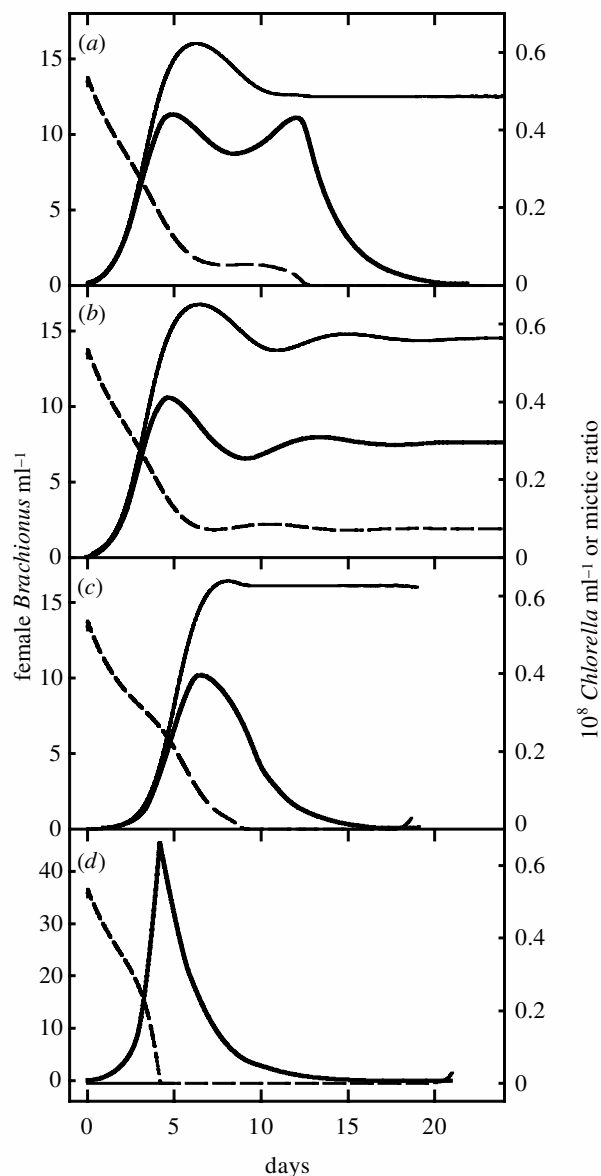


Figure 6. Dynamics predicted by four different versions of our model. (a) Full (QT) model with mixis and selection against mixis. (b and c) Models with mixis but no selection against mixis. (b) Simulation with the same parameter values as in the QT model ($\varepsilon = 0.033\ 033$; $a = 0.072\ 51$ ml per female). (c) Best fit of the experimental data to the model without selection ($\varepsilon = 0.0258$; $a = 0.075\ 13$ ml per female). (d) Model (F2K, see text) without mixis (and without selection). Thick solid lines, *Brachionus calyciflorus* females; dashed lines, *Chlorella vulgaris*; thin solid lines, mictic ratio π (model).

parsimonious explanation. We have shown that our own experimental data and modelling results are indeed consistent with rapid selection against *Brachionus* clones that are prone to reproduce sexually. Directional selection of a QT has been illustrated by 'climbing up an adaptive hill' of increasing fitness, gradually approaching a trait value that maximizes fitness (e.g. Futuyma 1998; Orr 2000). The rate of change of the trait decreases as the trait approaches its optimal value, and becomes zero when the optimal value is reached and selection becomes stabilizing. The observed long-term change of the mean propensity for mixis in our cultures (figure 1) is consistent with direc-

tional selection towards the optimum value in this environment ($a = 0$ because sexual reproduction yields no offspring). Starting with relatively high values of a at ca. 0.03 ml per female, a approached zero asymptotically, that is: very rapidly initially but at a decreasing rate with increasing culture time.

We hypothesize that the initial population of *Brachionus* collected from Lake Michigan consisted of a wide variety of genotypes constituting the additive genetic variance, on which directional selection could act. Gilbert (2002) experimentally established high clonal variation in the propensity to produce mictic females in *B. calyciflorus* whereas Gómez & Carvalho (2000) have used microsatellite analysis to reveal 'an unexpectedly high number of genotypes' in a field population of *B. plicatilis*. Gómez & Carvalho (2000) also detected a reduction of genotypic diversity of this population at the end of its parthenogenetic phase revealing the effects of clonal selection. Similar dynamics have been found for other cyclical parthenogens (e.g. *Daphnia*; Carvalho & Crisp 1987).

Although our findings are derived from an artificial and tightly controlled laboratory system, they may be of great relevance for understanding the dynamics of rotifers and other cyclical parthenogens in the wild. The fact that only amictic reproduction contributes to population growth, a key feature of the chemostat system by design, is also true at times for natural populations in lakes or temporary bodies of water where favourable and unfavourable conditions alternate seasonally. Sexual reproduction of both monogonont rotifers and cladocerans is coupled to the production of diapausing eggs that must undergo a period of dormancy before they may rejoin the active population. Dormancy typically lasts at least several months (but may last much longer) and thus exceeds by far the average generation time of parthenogenetically reproducing plankton populations (usually measured in days). Therefore, mictic females in the wild—just like their counterparts in the chemostats—will have zero fitness as participants in the 'real time' population dynamics. Selection against clones exhibiting a high propensity for mixis should be strong during the growing season. With the start of a new growing season, however, genetic variance becomes reinstated by the emergence of new, sexually recombined clones that went into diapause in previous seasons. As a result, rapid natural selection against mixis may act in natural populations over short, seasonal periods in much the same way as in the chemostat system. If so, plankton ecologists trying to capture the seasonal community dynamics in a lake or pond, but considering solely demographic parameters and trophic relationships (as is currently common practice) may not succeed because they neglect the evolutionary dimension of the process.

M. Boraas and C. Kearns provided advice on chemostat setup. R. Babcock, A. Holmes and A. Katholos helped sample and maintain the chemostats. This study was supported by a grant from the Andrew W. Mellon Foundation to S.P.E. and N.G.H.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.