Timing the initiation of sex: Delay mechanisms alter fitness outcomes in a rotifer population model

Bethany L. F. Stevens*
Department of Ecology, Evolution, and Marine Biology, University of California Santa Barbara, Santa Barbara, CA, 93106, USA

Silke van Daalen
Wageningen Marine Research
1976 CP, IJmuiden, Netherlands

Tirzah J. Blomquist
Biology Department, MS 34
Woods Hole Oceanographic Institution
Woods Hole MA 02543 USA

Kristin E. Gribble
Josephine Bay Paul Center for
Comparative Molecular Biology and Evolution
Marine Biological Laboratory
Woods Hole MA 02543 USA

Michael G. Neubert
Biology Department, MS 34
Woods Hole Oceanographic Institution
Woods Hole MA 02543 USA

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*Corresponding author

Abstract

Species that inhabit variable environments have complex mechanisms to precisely time their life-history transitions as conditions change. One such mechanism in rotifers is a block on sexual reproduction that extends across multiple asexual generations after emergence from diapause. It has been hypothesized that this delay is advantageous in competitive and stochastic environments. Here, we develop a model of cyclically parthenogenic rotifer populations with a novel formulation of a "mictic block" that prevents sexual reproduction by females that are not sufficiently distant, genealogically, from a stem ancestor that was produced sexually. We find that mictic blocks are indeed adaptive but that the most successful phenotypes have shorter blocks than previously reported and that the success of different delay phenotypes is highly dependent on the duration of the growing season. For a fixed environmental regime, a stable polymorphism is possible, wherein one strain performs better in years with an average-length growing season, and the other strain performs better in years with an "extreme" growing season, whether short or long. Our model provides an eco-evolutionary framework for the study of *Brachionus* rotifers, a model system for non-genetic maternal effects and the evolution of sex.

1 Introduction

For species that live in variable environments, the timing of life history transitions is often subject to strong selection pressure. Mistiming activities such as metamorphosis, reproduction, or emergence from dormancy relative to changes in the environment can significantly reduce fitness. As a result, species living in variable environments have evolved precise, sometimes elaborate, mechanisms to control the timing of life-history transitions. Many species rely on a combination of external cues, such as photoperiod or food availability (Zhang and Baer, 2000; Koch et al., 2009), and internal timing mechanisms, or biological clocks (Simon et al., 2001). Although external cues may provide information about imminent changes in conditions, internal clocks can protect populations from unreliable cues or abrupt fluctuations.

Brachionus rotifers that live in ephemeral ponds are examples of organisms with carefully timed life-history transitions. At the beginning of the growing season, resting eggs in the sediment hatch into asexual (amictic) females that live in the water column (Fig. 1). After a period of relatively rapid population growth fueled by asexual reproduction, amictic females begin producing mictic female offspring capable of sexual reproduction along with amictic female offspring. In the absence of fertilization by males, mictic females produce eggs that develop into males. Those males fertilize mictic females, who then produce resting eggs—stress-resistant, dormant embryos that can survive when the pond dries or freezes between growing seasons (García-Roger et al., 2019).

This reproductive strategy, in which several rounds of clonal reproduction are followed by a sexual event, is called *cyclical parthenogenesis*. In animal species, cyclical parthenogenesis commonly occurs in trematode parasites, cladocerans (e.g., *Daphnia* spp.), aphids, cecidomyiids (gall midges) and cynipids (gall wasps), in addition to rotifers (Neiman et al., 2014). The strategy strikes a delicate balance between the contrasting benefits of sexual and asexual reproduction (Bogdan and Gilbert, 1982; Gilbert, 2020). Critical to the success of cyclical parthenogenesis is the *mictic delay*: the time between the start of each growing season and the initiation of mixis, which determines how large the population becomes before sexual reproduction and the production of resting eggs begin.

Empirical work has identified both external and internal timing mechanisms that influence the length of the mictic delay in rotifer populations. Within the genus *Brachionus*, mixis is initiated in response to a density-

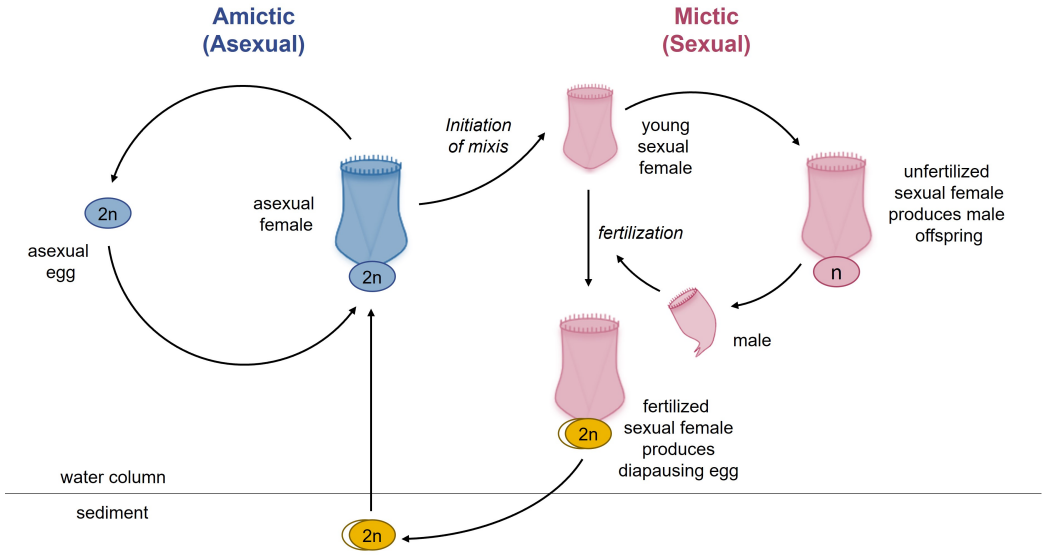

Figure 1: Life cycle of the rotifer *B. manjavacas*. Left (blue), the asexual (amictic) cycle, in which a female produces clonal diploid eggs by mitosis. Right (pink), the sexual (mictic) cycle, in which crowding conditions prompt a portion of females in the population to become mictic, producing haploid gametes via meiosis. If haploid gametes are not fertilized, they hatch into diminutive haploid males. Fertilized gametes develop into dormant resting eggs, able to desiccate and overwinter in the sediments. Upon hatching, resting egg restore the asexual cycle. Induction of the sexual phase is blocked for asexual females who are not sufficiently removed from their last sexual ancestor. Figure and caption adapted from Gribble (2021).

dependent chemical signal (Carmona et al., 1993; Stelzer and Snell, 2003). In this case, the length of the mictic delay will depend on the time it takes for the rotifer population to exceed a given density, the *mixis threshold* (Gilbert, 2017). Delays allow the population to reach higher densities before the onset of mixis, leading to more frequent male-female interaction and more successful sexual reproduction. Additionally, Gilbert (2002, 2003) showed that some strains exhibit a secondary, internal delay mechanism. In these populations, individuals do not become receptive to the density-dependent signal until many (8-12) generations after hatching from a resting egg. We refer to this second delay mechanism as a *mictic block*. Internally-timed mictic blocks are hypothesized to be particularly evolutionarily advantageous when a strain is

in competition with others that might interfere with its density-dependent chemical signal (Gilbert, 2017, 2020).

Serra et al. (2005) tested this hypothesis with a model of a cyclically parthenogenic rotifer population in a stochastic environment. In their model, females hatch from resting eggs and reproduce asexually until both a mictic threshold has been met and a minimum number of days have passed since the resting eggs hatched. Serra et al. (2005) used this model to demonstrate that a rotifer strain with a mictic block could successfully invade a resident strain without such a block, by growing asexually to larger population sizes before transitioning to mixis.

Notably, there is a distinction between a block that lasts a fixed number of days (*temporal block*), as Serra et al. (2005) explored, and a block that lasts a fixed number of generations (*generational block*), as suggested by more recent observations (Colinas, unpublished; Fig. S1). In the first case, a single individual could become sensitive to the mixis signal over its lifespan, which is inconsistent with current understanding of the rotifer life cycle. Because the timing of the mictic delay is critical to fitness in this system, we must carefully consider how the mictic block is implemented, both biologically and in model formulations.

Here, we investigate how this difference in block implementation alters the fitness and eco-evolutionary dynamics of a cyclically parthenogenic species. We first present a simplified model comparison that highlights the mathematical difference between temporal and generational block formulations. Then, inspired by Serra et al. (2005), we construct a model of a rotifer community with the capacity to delay mixis, in our case for a fixed number of generations. We explore how this new model alters the conclusions of the previous work, and identify optimal and evolutionarily stable strategies for timing the onset of mixis.

2 Distinguishing between two block implementations

Before introducing and analyzing the complete rotifer community model (with eggs, juveniles, mictic and amictic adults of different phenotypes), we first present a simplified example to illustrate the difference between the effect of a temporal block on resting egg production and a generational block

of the same duration, τ.

Consider a population of adults that may either be of the "stem" generation (those that initiate the population at time $t = 0$), $x_0(t)$, or of subsequent generations, $x_1(t)$. Let $x(t)$ denote the total population of adults such that $x(t) = x_0(t) + x_1(t)$.

All individuals in the population take τ days to mature to adulthood, are subject to mortality at the per capita rate q, and once mature, produce offspring at the rate 1 per time. If the initial size of the stem adult population is $x_0(0) = X_0$, then their population size thereafter decays exponentially:

$$x_0(t) = X_0 e^{-qt} \text{ for } t \geq 0. \tag{1}$$

Meanwhile, the population of adults of subsequent ("non-stem") generations changes according to

$$\frac{dx_1}{dt} = e^{-q\tau}\, x(t-\tau) - q x_1(t), \text{ for } t \geq \tau, \tag{2}$$

with

$$x_1(t) = 0, \text{ for } t < \tau. \tag{3}$$

The first term on the right-hand side of equation (2) represents the maturation of individuals that survive to adulthood (born to stem or non-stem parents) and the second term describes the mortality of non-stem adults.

Now consider two scenarios in both of which this population generates resting eggs at a per capita rate $r > 0$. In the first scenario, resting eggs are produced only by non-stem adults. This is a generational block of length 1 generation. In this case, the cumulative number of eggs produced by the population up to time t will be

$$\tilde{y}(t) = \int_0^t r x_1(s) ds. \tag{4}$$

Because no non-stem individuals reach adulthood before time τ, we also have

$$\tilde{y}(t) = \int_\tau^t r x_1(s) ds. \tag{5}$$

In the second scenario, all adults in the population produce resting eggs after a temporal block equal in length to the maturation time τ. In this second case, the number of resting eggs produced will be

$$y(t) = \int_\tau^t r x(s) ds = \int_\tau^t r(x_1(s) + x_0(s)) ds. \tag{6}$$

Substituting equation (1) for $x_0(s)$, equation (6) can be rewritten as

$$y(t) = \int_{\tau}^{t} r(x_1(s) + X_0 e^{-qs}) ds \tag{7a}$$

$$= \tilde{y}(t) + X_0 \frac{r}{q}(e^{-q\tau} - e^{-qt}). \tag{7b}$$

The second term in equation (7b) is positive for all $t > \tau$, so $y(t) > \tilde{y}(t)$. That is, a temporal block produces more resting eggs than a comparable generational block for all t (Fig. 2A,B). In both scenarios, no resting eggs are produced until $t = \tau$, when either the length of the temporal block has passed or the first non-stem adults mature. Yet, the temporal block results in a faster rate of production once the block is lifted. The difference between $y(t)$ and $\tilde{y}(t)$ will vary with the initial size of the stem generation, X_0, the egg production rate, r, and the duration of the block, τ. If resting eggs form the initial population for subsequent growing seasons, egg production by a time-blocked population becomes exponentially larger than egg production by a generation-blocked population over consecutive seasons (Fig. 2C). These differences led us to construct a rotifer population growth model with a generational mictic block (described next), the outputs of which we compare with the outputs of the temporal block model of Serra et al. (2005).

3 A rotifer model with a generational mictic block

Following Serra and King (1999) and Serra et al. (2005), we model the dynamics of a population of rotifers over the course of sequential growing seasons of variable length (Fig. 3). We take the growing season to be the period of time in which a vernal pool provides viable habitat in the water column, after which the pool dries or freezes; only resting eggs survive to the following growing season. We model the changes in the population densities of females at each life stage over the course of each growing season as follows.

We begin with an initial density of resting eggs in the sediment that hatch daily in clutches of equal size at times $t = 0, 1, \ldots, H$ (Serra et al., 2005). Let ϕ_i be the density of eggs of each phenotype i divided by $H+1$, such that every day in this hatching period, ϕ_i stem juvenile females of phenotype i are introduced into the water column. Assume that individuals die at rate q

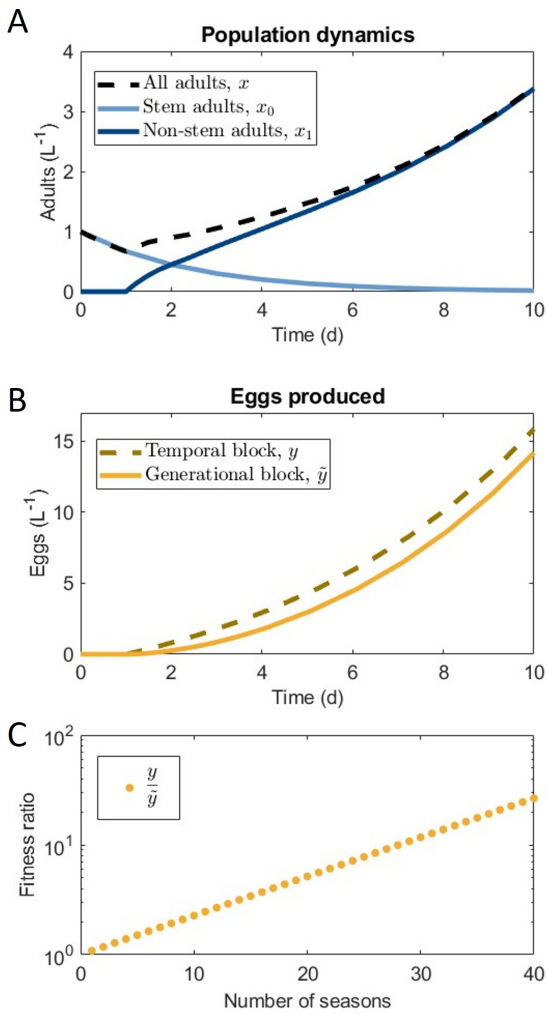

Figure 2: Difference in egg production between strains with a temporal block and a generational block. (A) The number of adults in the population changes according to the model described in equations (1-3). (B) The number of eggs produced differs between the two different block implementations and depends on the initial size of the stem generation, the egg production rate, and the length of the block; however, the number of eggs produced in the temporal block scenario (dashed) is always greater than that in the generational block scenario (solid). (C) The fitness ratio between the two strategies increases exponentially with the number of growing seasons. Parameter values for these simulations are $X_0 = 1, \tau = 1$, and $q = 0.4$. Season length is 10 days.

and that juveniles mature into adults after τ days. The population density of stem juveniles of phenotype i at time t, or $J_{i,0}(t)$, therefore changes as

$$\frac{\mathrm{d}J_{i,0}}{\mathrm{d}t} = \left[\sum_{k=0}^{H} \delta(t-k)\phi_i - \delta(t-k-\tau)\phi_i e^{-q\tau}\right] - qJ_{i,0}, \tag{8}$$

where t is time measured in days since the start of the growing season and $\delta(t)$ is the Dirac delta function. The first term within the brackets describes the introduction of clutches that emerge from diapause, while the second term accounts for the maturation of individuals that survive to adulthood τ days after they emerge. Maturation leads to the production of mature, amictic stem females of phenotype i, which also die at rate q:

$$\frac{\mathrm{d}A_{i,0}}{\mathrm{d}t} = \left[\sum_{k=0}^{H} \delta(t-k-\tau)\phi_i e^{-q\tau}\right] - qA_{i,0}. \tag{9}$$

Juvenile females of subsequent generations ($j > 0$) are produced by amictic adults. In the model, all offspring inherit the phenotype of their mother. Let $A_{i,j}(t)$ be the population density at time t of mature amictic females, with phenotype i, j generations removed from a stem female (for $j > 0$). These individuals will produce juveniles of generation $j+1$ at a birth rate, b, which is a function of total population density in the water column, $N(t)$:

$$b(N) = b_{max} - (b_{max} - q)\left(\frac{N}{K}\right), \tag{10}$$

where

$$N(t) = \sum_i \left\{ M_i(t) + \sum_j \left[A_{i,j}(t) + J_{i,j}(t)\right] \right\}. \tag{11}$$

Here, b_{max} is the intrinsic birth rate, K is the community-wide carrying capacity, and $M_i(t)$ is the population density at time t of mature mictic females of phenotype i.

The proportion of female juveniles produced by amictic adults that will mature into mictic adults, $m_{i,j}(N)$, is a function of the individual's generation, the population density at the time of reproduction (not at the time of maturation), and the individual's phenotype:

$$m_{i,j}(N) = \begin{cases} \overline{m_i}, & \text{if } N \geq T_i \text{ and } j \geq G_i \\ 0, & \text{otherwise.} \end{cases} \tag{12}$$

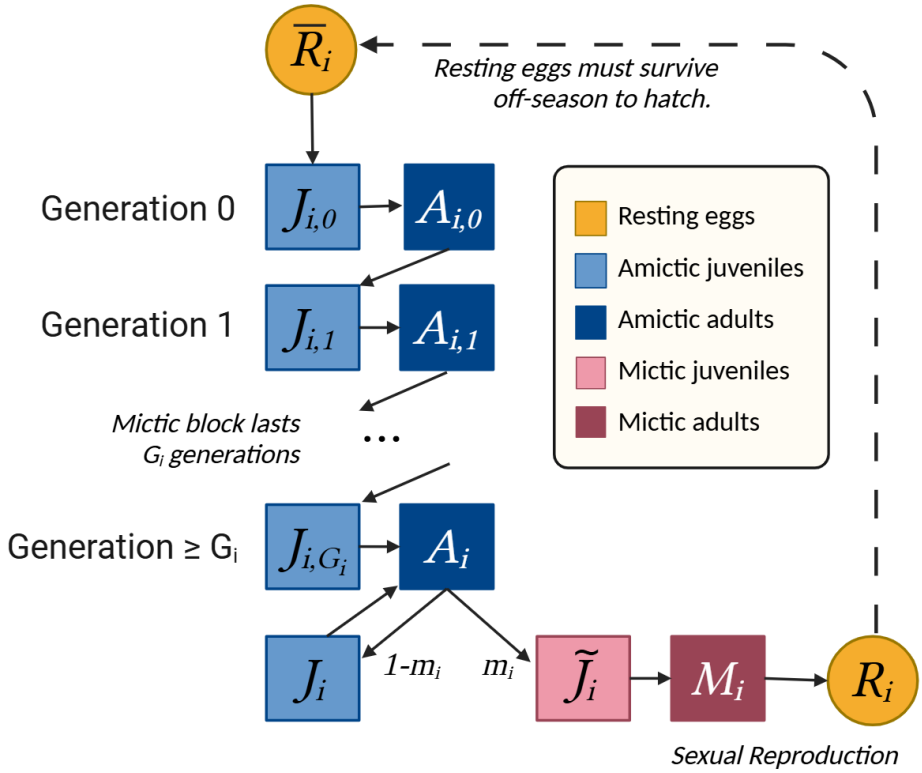

Figure 3: Schematic of cyclically parthenogenic life cycle model with generational block. Resting eggs can only produce stem juveniles (generation 0). Juveniles become adults of the same generation. Amictic adults can subsequently reproduce asexually to create juveniles in the next generation. G_i is the first generation which might produce mictic offspring if other conditions for mixis are met (see equation 7 in text). In this diagram, $G_i > 1$, but it could be any nonnegative integer (Fig. S2). Mictic adults only produce males (not included in the model) or resting eggs that will not hatch until the following season.

While $m_{i,j}$ and $\overline{m_i}$ are strictly proportions ($0 < m_{i,j} < 1$), we refer to these terms as mixis ratios, in keeping with the rotifer literature (e.g., Seudre et al., 2020). The phenotype of an individual comprises its mixis ratio, $\overline{m_i}$, mixis threshold, T_i, and the number of generations before mixis can occur, G_i.

The dynamics of juvenile, amictic adult and mictic adult females of all generations greater than 0 are then:

$$\frac{\mathrm{d}J_{i,j}}{\mathrm{d}t} = b(N(t))A_{i,j-1}(t) - b(N(t-\tau))A_{i,j-1}(t-\tau)e^{-q\tau} - qJ_{i,j} \quad (13)$$

$$\frac{\mathrm{d}A_{i,j}}{\mathrm{d}t} = b(N(t-\tau))A_{i,j-1}(t-\tau)e^{-q\tau}[1 - m_{i,j}(N(t-\tau))] - qA_{i,j} \quad (14)$$

$$\frac{\mathrm{d}M_i}{\mathrm{d}t} = \left[\sum_j b(N(t-\tau))A_{i,j-1}(t-\tau)e^{-q\tau}m_{i,j}(N(t-\tau))\right] - qM_i. \quad (15)$$

The three terms in equation (13) account for juvenile births, maturation of juveniles that survive their juvenile period, and mortality. The first terms in equations (14) and (15) represent the maturation of juveniles into amictic and mictic adults, respectively. We do not distinguish between generations of mictic adults, so the production of mictic adults by all generations is summed.

Finally, we simulate the number of resting eggs produced by mictic females over the course of each growing season. Let $R_i(t)$ be the population density of the resting eggs produced during the current growing season by phenotype i at time t. Resting eggs are produced at a reduced rate relative to the rate that amictic females produce juveniles:

$$\frac{\mathrm{d}R_i}{\mathrm{d}t} = cb(N(t))M_i. \quad (16)$$

$c < 1$, reflecting the costs of sexual reproduction, including the production of males and increased resource allocation to resting eggs.

As in Serra et al. (2005), we take the length of each season (S) to be a uniformly distributed random variable between S_α and S_ω. At the end of each growing season, all state variables reset to 0 (no individuals in the water column survive the period between growing seasons), and there is a maximum density, B_{max}, of surviving resting eggs across all phenotypes. If S is the length in days of the last growing season, $R_i(S)$ is the density of eggs of phenotype i that had been produced by the end of that season. Let $\bar{R}_i$

Symbol	Description	Value
State variables		
$J_{i,j}$	Juvenile females L^{-1} of phenotype i and generation j	-
$A_{i,j}$	Amictic adult females L^{-1} of phenotype i and generation j	-
M_i	Amictic adult females L^{-1} of phenotype i	-
R_i	Resting eggs L^{-1} of phenotype i	-
Environmental parameters		
S_α	Minimum growing season length (d)	10
S_ω	Maximum growing season length (d)	51
H	End of period for resting egg hatching (d)	31
Biological parameters for all rotifers		
q	Intrinsic mortality rate (d^{-1})	0.5
b_{max}	Intrinsic birth rate (d^{-1})	2.1
c	Cost of sexual reproduction	0.2
τ	†Maturation time (d)	1
K	Water column carrying capacity (individuals L^{-1})	500
B_{max}	Maximum size of resting egg pool (resting eggs L^{-1})	1
Strain phenotype parameters		
$\bar{m}_i$	mixis ratio	0-1
T_i	mixis threshold (individuals L^{-1})	0-100
G_i	†First generation that may produce mictic offspring	0-10

Table 1: Model state variables and parameters. Daggers in descriptions indicate variables that were added to our model relative to Serra et al. (2005). All parameter values were chosen to match those in previous work, except b_{max} which was increased to exactly adjust for the lost reproduction from individuals that do not survive their maturation step in our model.

denote the density of eggs of phenotype i that survive to hatch in the next growing season. The between-season dynamics are then defined by

$$\bar{R}_i = \begin{cases} B_{max}\frac{R_i(S)}{\sum_i R_i(S)}, & \text{if } \sum_i R_i(S) \geq B_{max} \\ R_i(S), & \text{otherwise.} \end{cases} \tag{17}$$

The first hatching in the next season will produce $\phi_i = \frac{\bar{R}_i}{H+1}$ individuals at time $t = 0$.

4 Analysis

4.1 Monomorphic Populations

We begin our analysis by exploring the dynamics of populations with a single shared phenotype. For these populations, our model produces qualitatively similar dynamics to those of Serra et al. (2005) (Fig. 4 A). Population growth starts slowly as resting eggs gradually hatch to produce stem juveniles and subsequently stem adults. The most rapid growth is achieved while these adults reproduce entirely asexually. Once their phenotype's mixis threshold, T_i, is reached, all adults that are of generation G_i or greater will begin producing some mictic adults which create resting eggs. The net growth of amictic individuals in the water column declines and the density of resting eggs increases.

In general, our simulated populations produce resting eggs more slowly than those presented in Serra et al. (2005). The maturation time included in our model results in a delay before resting eggs can be produced even in the absence of a mictic block, and, as demonstrated in the previous section, the rate of resting egg production at the onset of mixis is also lower. This exacerbates the risks and reduces the benefits of waiting to initiate mixis. We therefore expect the optimal threshold and block traits to be lower in our analysis compared to those reported in the previous work.

To test this hypothesis, we analyze our model primarily for the same environmental regime as explored in Serra et al. (2005). That is, we run simulations for 40 consecutive seasons wherein each season length is randomly chosen from a uniform distribution between 10 and 51 days. To compare the success of distinct phenotypes, we consider the average egg production per season across 40 experimental replicates. In the absence of a generational block, we find that the phenotype that maximizes expected egg production per season is a mixis ratio of 25% and a threshold value of 0.2 rotifers L^{-1} (Fig. 5A). This contrasts with the values of 45% and 82 rotifers L^{-1} reported in Serra et al. (2005). For long seasons, we see fitness patterns similar to those presented in Serra et al. (2005) (Fig. 6C). Because of the slower egg production in our model, however, strains with high threshold values fail to produce any resting eggs when seasons are short (Fig. 6A), which leads to zero fitness. Selection pressure therefore favors much lower threshold values than previously reported for the same environmental regime.

Phenotypes with low threshold values are more successful when paired

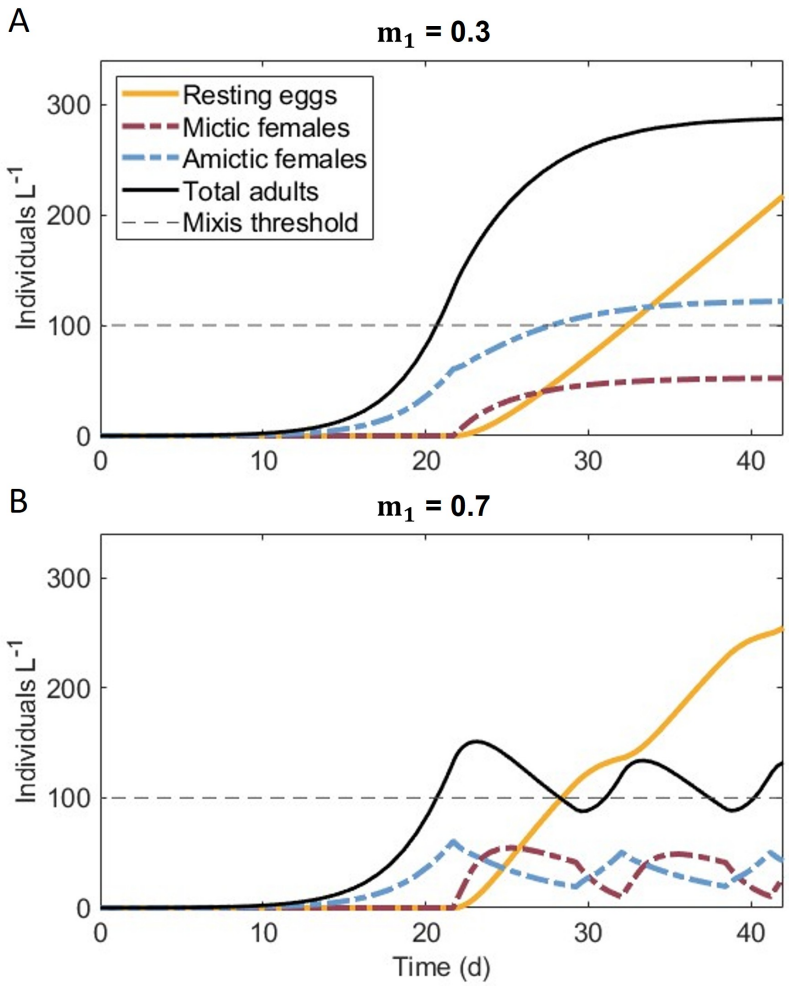

Figure 4: Dynamics within a single season for populations with no generational block ($G_i = 0$). Parameter values for these simulations are those indicated in Table 1, with initial resting egg density, B_{max}, mixis ratio (m_1) phenotypes indicated in the title to each panel, and mixis threshold indicated by the horizontal dashed line ($T_1 = 100$). When the mixis ratio is high enough to lead to population decline (B), the total population can oscillate around the mixis threshold.

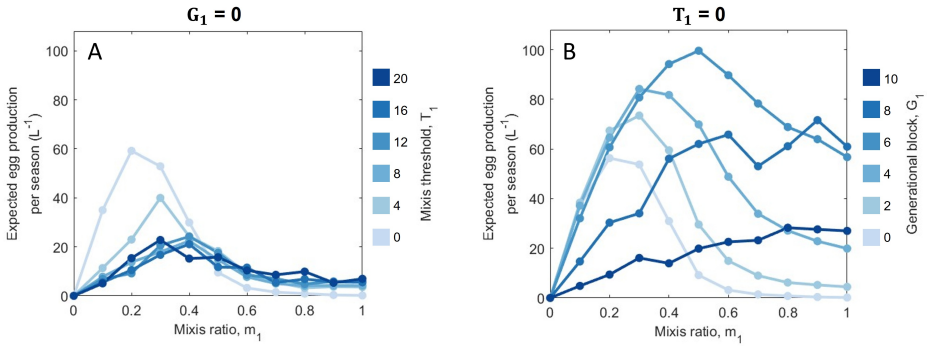

Figure 5: Expected egg production per season by monomorphic populations (A) without a generational mictic block and (B) without a mixis density threshold. Values plotted are the average eggs produced in 40 consecutive seasons of stochastic length (between 10 and 51 days) across 40 replicates with initial resting egg density, B_{max}. Generational mictic blocks of up to 6 generations generally increase the expected number of eggs produced in each season. In the absence of a mictic block, phenotypes with low mixis ratios and low mixis thresholds perform best on average. All parameters not indicated within the figure have values shown in Table 1.

with lower mixis ratios (Fig. 5A, Fig. 6C). A strain with a low threshold and mixis ratio will have an early onset of mixis and low rates of sexual reproduction throughout the season. This strategy is successful in short seasons but will produce fewer resting eggs than will strains with high mixis thresholds in long seasons. Without a generational block, surviving short seasons comes at the cost of reduced fitness in long seasons.

Generational blocks evidently reduce the trade-off between survival in short seasons and high fecundity in long seasons (Fig. 6). Compared to mixis density thresholds, which apply to all cohorts equally, generational blocks are more forgiving in very short seasons. The first cohort of eggs to hatch will lead to individuals of generation G_i after G_i days, such that some resting eggs may be produced at that time, even if the cohort is small. On the other end of the spectrum, generational blocks are also successful at producing large numbers of resting eggs in long seasons. Strains with generational blocks and low mixis thresholds generate roughly the same number of resting eggs as those with higher mixis thresholds (Fig. 6E). We expect this insensitivity to total population density to be particularly advantageous in a competitive context when the abundance of a competing strain would interfere with the density-dependent timing cue.

4.2 Competition and Invasion

We now expand our analysis to consider the competitive dynamics between rotifer strains with distinct mixis phenotypes. As in Serra et al. (2005), we introduce an invader phenotype into a monomorphic resident population at a frequency of 0.05 and simulate for 40 consecutive growing seasons as described above. We consider an invasion successful if invader frequency increases on average over 20 experiments.

We begin by considering only strains without a mictic block ($G_i = 0$) or mixis threshold ($T_i = 0$). For this phenotype, a mixis ratio of 0.2 was most successful in monomorphic populations within our standardized environmental conditions (Fig. 5, S3A). In contrast, we find a mixis ratio of 0.11 is most successful in competition (Fig. 7A). That is, in the absence of a mictic block, a mixis ratio of 0.11 can successfully invade and resist invasion by strains with any other mixis ratio.

Similarly, the most successful mixis threshold is lower in competition than in monomorphic populations. While a mixis threshold of 0.2 L^{-1} maximizes fitness for some phenotypes in isolation (e.g. $G_i = 0$, $m_i = 0.5$; Fig. S3),

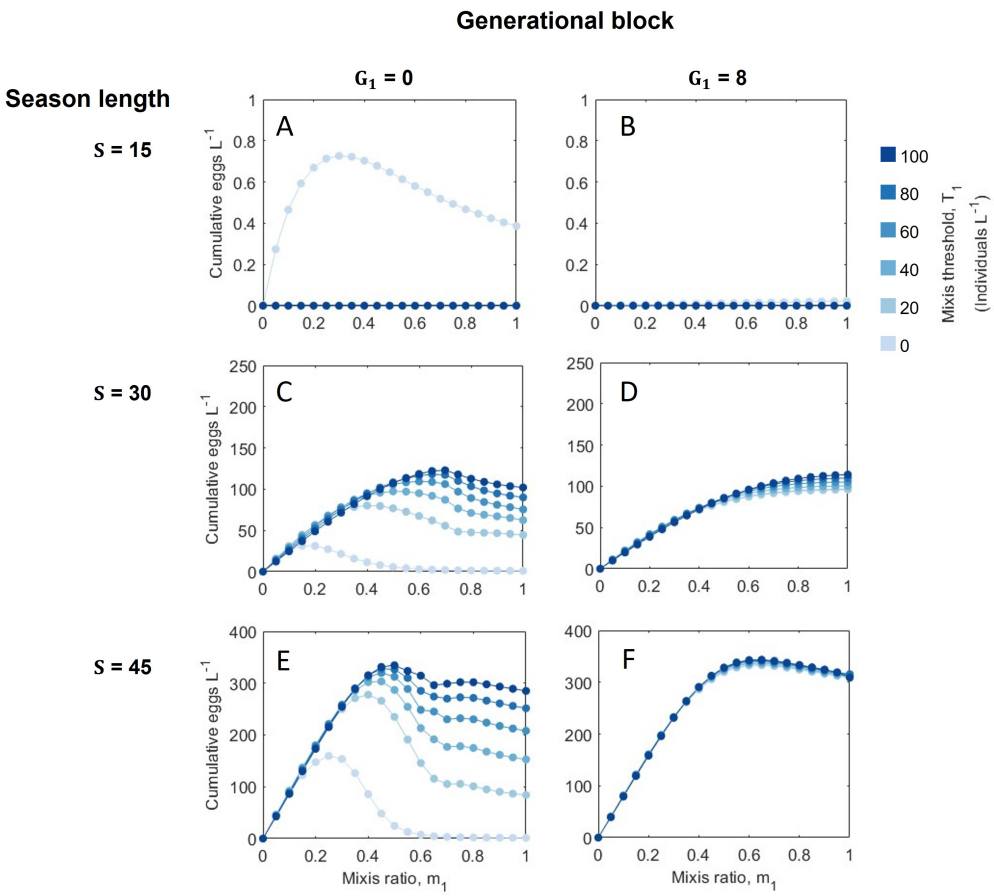

Figure 6: Egg production by monomorphic populations within short (A-B), medium (C-D) and long (E-F) seasons of fixed length. Phenotypes with low mixis thresholds perform better in short seasons, and those with high thresholds perform better in long seasons. A long generational block (right column) does poorly in short seasons but can improve performance of populations with low thresholds in long seasons. Resting egg production in (B) is low but nonzero ($\sim 0.01\ L^{-1}$) for strains with $T_i = 0$. Note that the y-axis scale changes between rows. Simulations were run with parameter values indicated in Table 1 and initial resting egg density, B_{max}.

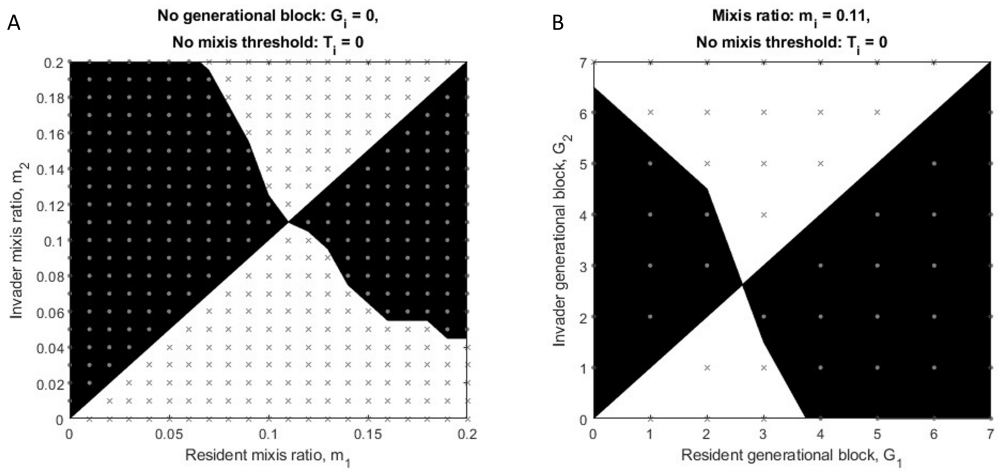

Figure 7: Pairwise invasibility plot for rotifers with A) variable mixis ratio given no generational block or mixis threshold ($G_i = 0, T_i = 0$). Invasion was deemed successful if, out of 20 simulations of 40 growing seasons each, the invader frequency increased on average from its initial frequency of 0.05. Gray crosses indicate unsuccessful invasions; circles indicate successful invasions. Black and white regions are interpolated regions of success and failure, respectively. Without a mixis bock, a mixis ratio of 0.11 is the best invader and most resistant to invasion. B) Pairwise invasibility plot for rotifers with variable generational blocks given a mixis ratio of 0.11 and no mixis threshold. Strains with $G_i = 3$ are most resistant to invasion and can successfully invade all others. All parameters not indicated within the figure have values shown in Table 1.

we find that resident strains can generally be invaded by strains with lower mixis thresholds (Fig. S4). Strains with no mixis threshold are the most successful invaders within our experiments, whether or not the residents have a mixis block (Fig. S4). Our results are qualitatively comparable to those reported in Serra et al. (2005), in that the optimal mixis ratio and threshold values are lower in competition than in isolation. They also align with the work of Montero-Pau and Serra (2011), which highlights the invasibility of residents that invest in sexual reproduction as a mechanism for maintaining coexistence.

We next ask whether a rotifer strain that delays mixis through a generational block can invade a resident population with no block and a mixis

ratio of 0.11 (Fig. 7B). We find that strains with blocks that last 2 or 3 generations can regularly invade and replace strains without a block (Fig. 8). The success of strains with blocks of 4 generations is highly sensitive to the stochasticity in realized season length, and blocks longer than 5 generations are mostly unsuccessful (Fig. 8). This result contrasts quantitatively with the findings of Serra et al. (2005) which identified the optimal invader block timing as 8-12 days, substantially longer than the three days it would take to produce adults of generation 2.

The difference between the most successful block length in our model and that identified in the previous work is in line with our expectations described in Section 2. The temporal block of Serra et al. (2005) results in a higher per capita rate of egg production after the block is lifted, artificially favoring a longer delay and a higher mixis ratio. In contrast, our generational block implementation favors a shorter mictic delay to make up for the slower production of resting eggs. Even with the more realistic generational block model formulation, we find that a block phenotype is adaptive, validating the conclusions of Serra et al. (2005). While this result is reassuring, we note that the success of any specific block length depends critically on the environmental regime and the relative chances of experiencing a short or a long season.

4.3 A Stable Polymorphism

We will now use the model we have developed to explore whether the generational mictic block is an evolutionarily stable strategy. This analysis extends beyond that of Serra et al. (2005), considering competition between strains with differences in any of the three distinct mixis traits (G_i, T_i, and m_i).

We first use an iterative optimization approach to find highly competitive phenotypes. Holding the mixis ratio and threshold values constant at 0.11 and 0, respectively, we find that a strain with $G_i = 3$ is the least likely to be invaded (Fig. 7B). Of strains with $G_i = 3$, we find that the best competitor has mixis ratio equal to 0.17 and threshold 0 (Fig. S4B, S5B). We next conduct a series of invasion experiments in which residents and invaders vary in both their values of G_i and m_i. We find that for all phenotypes within the range of $0 \leq G_i \leq 5$ and $0.05 \leq m_i \leq 0.6$, two combinations of traits produce particularly successful phenotypes. The first is that which we identified by varying each trait in isolation: $G_i = 3, m_i = 0.17$. The second successful phenotype has a longer delay and a higher mixis ratio, $G_i = 4, m_i = 0.23$.

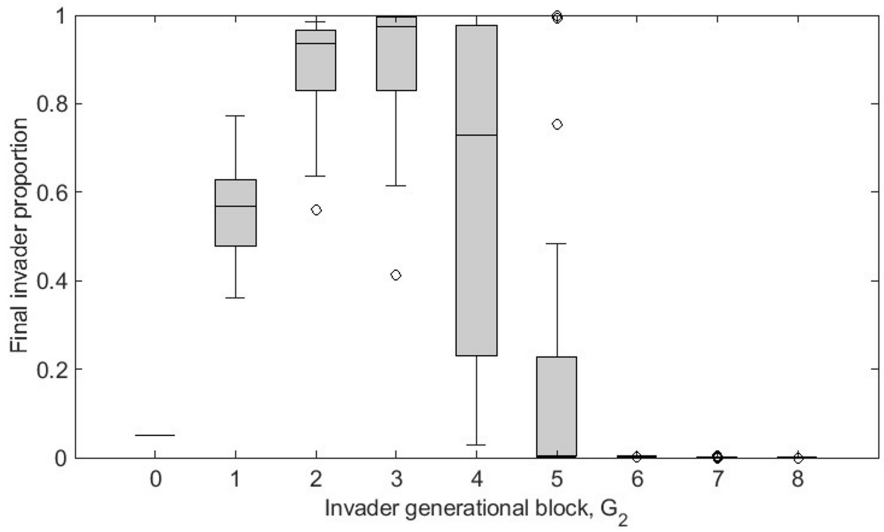

Figure 8: Invasibility outcomes for rotifers with the most competitive mixis ratio and threshold phenotype in the absence of a mictic block ($m_i = 0.11, T_i = 0$). Strains with this mixis strategy and generational blocks of 2-4 generations can regularly invade and replace a resident without a block. All other parameters for these simulations are those indicated in Table 1. Each box indicates the median and interquartile range (IQR) of 20 simulations per phenotype. Circles indicate values more than 1.5∗IQR away from the nearest box edge.

Given the fixed environmental parameters on which we have focused in this work, each of these two strains is able to invade the other when introduced at low abundances, indicating the potential for a stable polymorphism.

To understand the competitive dynamics between these strains, it helps to consider the trajectories of the two populations within a single growing season (Fig. 9). The strain with the shorter generational block initiates mixis quickly and generates more resting eggs at the start of the season. The higher mixis ratio of the second population leads to a rapid increase in the density of mictic individuals, such that, after day 15, the population with the longer delay outpaces the first in the production of resting eggs. However, the high mixis ratio in this population ultimately leads to a decline in the total population size, particularly given the high density of the competing strain. As a result, the first phenotype, that does well in very short seasons (< 15 d), is also the superior competitor in very long seasons (> 40 d). The realized variability in season length within our simulations leads to the sustained coexistence of these two strains over time.

5 Discussion

Cyclic parthenogenesis occurs in at least seven groups of animals, including the best studied cases of rotifers, cladocerans, and aphids (Hebert, 1987). In these systems, there is strong selection pressure on the timing of sex, such that the onset of sex has evolved to be influenced by both internal and external cues (Simon et al., 2002; Zhang and Baer, 2000; Koch et al., 2009). Here, we have developed a model of the generational block observed in rotifers that delays the onset of mixis. This model allows us to investigate the fitness outcomes of rotifer strains with distinct combinations of internal and external timing mechanisms, addressing questions about when different reproductive strategies are most advantageous.

We found that a phenotype with a generational mictic block can successfully invade a resident population that would be evolutionarily stable in the absence of a block (Fig. 7). This result supports the widespread hypothesis that generational blocks are adaptive and confirms the conclusions of Serra et al. (2005), despite the difference in the formulation of the mictic block between our model and theirs (highlighted in Section 2). Generational blocks increase the cumulative number of resting eggs produced in long seasons while still allowing for a minimal number of eggs in short seasons.

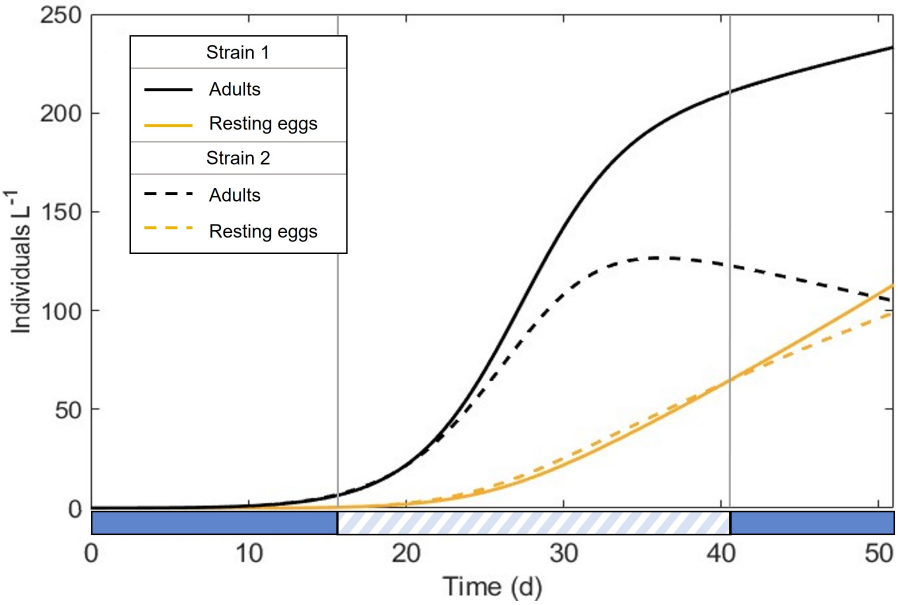

Figure 9: Population trajectories for strains that can coexist within our standard environmental regime (uniformly distributed season lengths between 10 and 51 days). Solid lines represent the strain with shorter mictic block ($G_1 = 3$) and lower mixis ratio ($m_1 = 0.17$). Dashed lines represent a strain with longer mictic block ($G_2 = 4$) and higher mixis ratio ($m_2 = 0.23$). The abundance of the two phenotypes are equal ($\frac{1}{2}B_{max}$) at the start of the simulation. Vertical gray lines and shaded bars along the x-axis indicate the two points in time when the density of resting eggs for the two strategies intersect. Mixis threshold, T_i, is 0 and all other parameter values in these simulations are those in Table 1.

We found the optimal length of the mictic block to be shorter than that reported in Serra et al. (2005), but we note that the success of phenotypes with generational blocks depends sensitively on the distribution of season lengths. Long generational blocks offer a greater benefit in longer growing seasons and have a fitness cost when seasons are short. This is consistent with patterns in block phenotypes observed in natural populations, although there is variability among species (Schröder and Gilbert, 2004). Generational blocks are not evident in populations in the Chihuahuan desert, for example, where vernal ponds only last a few days (Schröder et al., 2007). Across eastern Spain, Colinas et al. (2023) found that ponds with longer hydroperiods are home to rotifer strains with longer generational blocks. Those authors and others have attributed the success of block phenotypes to the increased growth of late-hatching clones, though our model indicates that early-hatching clones also benefit from a mictic block in long seasons (Fig. S6). Strains with high mixis ratios and a long mictic delay produce more eggs due to the prolonged period of asexual population growth.

Rotifer populations can exhibit a prolonged mictic delay as a result of either a high mixis threshold or a generational block. In our model, the fitness of a strain with a long generational block is relatively insensitive to its mixis threshold (Fig. 6), suggesting that either one of the two delay mechanisms is sufficient for high egg production in long seasons. Compared to phenotypes with high mixis thresholds, those with generational blocks are stronger competitors and are more successful across a range of season lengths. Given these advantages, we expect generational blocks to be a more prevalent mechanism for timing the initiation of sex, yet empirical work has reported a wide range of mixis thresholds (2.3 females L^{-1} (King and Snell, 1980); 6.6-22.9 individuals L^{-1} (Carmona et al., 1995); 147 individuals L^{-1} (Snell and Boyer, 1988)).

The prevalence of these mixis density thresholds suggests that they have benefits in addition to the associated delays in the initiation of sex. For example, high population densities may lead to more frequent encounters between males and females, and thus increase the likelihood of sexual reproduction; alternatively, high densities may anticipate impending resource limitation (Gilbert, 1967).

Interestingly, Seudre et al. (2020) found that naturally occurring genotypes with the lowest mixis thresholds also had the highest mixis ratios. This trend is the opposite of what we would expect if the mixis threshold controlled only the time of onset of sex (Fig. 6C). This observation reinforces

the hypothesis that high mixis thresholds are not only a strategy for initiating sex later, but reflect a difference in the overall investment of a strain in sexual versus asexual reproduction. While our model does not include any benefits of mixis density thresholds other than the resulting mictic delay, such benefits could be incorporated in future models.

Because demographic and environmental parameters vary between specific rotifer strains and habitats, we consider the results of our analysis to be qualitative rather than quantitative. That said, our model produces realistic within-season trajectories of both population size and the proportions of mictic females expected in the population. We note that for certain parameter values, our model exhibits oscillatory dynamics that were not evident in previous model formulations (Fig. 4B). When birth rates are low and the mixis ratio is high, the initiation of mixis will lead to population decline. If the population then drops below the mixis threshold, it will oscillate around T_i, toggling between producing mictic offspring and not. Evidence for such an oscillation has been reported in at least one natural population. While monitoring individual strains over time, Carmona et al. (1995) saw that the proportion of mictic females decreased during the growing season after an initial increase, leading to population growth and a second peak in population density in the latter half of the season. Our model indicates that this dynamic could be the result of the time lag between the conditions experienced by a female, which determine the mixis ratio of her offspring, and the contribution of those offspring to population growth once they mature. This is a non-genetic maternal effect, a phenomenon for which rotifers have become an important study system (Seudre et al., 2020; Liguori et al., 2024). Because our model tracks overlapping generations in the rotifer population, we expect it to be a valuable tool for future work on multigenerational maternal effects in this system.

Finally, we have used our model to validate the conjecture of Serra et al. (2005) that a stable polymorphism is possible within the environmental regime considered in their work. We find that two combinations of mixis traits produce phenotypes that can mutually invade each other and that their coexistence depends crucially on environmental conditions. A phenotype with a shorter mictic block and a lower mixis ratio does well both in short seasons, due to its early transition to sexual reproduction, and in long seasons, due to its sustained high population densities. In contrast, a strain with a longer mictic block and higher mixis ratio can out-compete the first strain in seasons of roughly average length. Competitive outcomes, there-

fore, depend on the distribution of the duration of the growing seasons. This may have important consequences for the sustained diversity of freshwater communities in the face of ongoing climate change. Different strains of rotifers have been observed to have different temporal mixis patterns within the same habitats, including some that are more continuous and others that are more periodic (Carmona et al., 1995). A change in the distribution of season lengths, as would result from changes in rainfall or evaporation, may lead to loss of this diversity. For the ecological scenario we analyzed, a shift toward shorter or longer growing seasons would not necessarily favor one strain over the other, but a change in the relative frequency of "extreme" years in either direction would.

We have argued in this manuscript for the careful consideration of the mechanisms by which life history transitions are timed. For cyclically parthenogenic rotifers, the success of a mictic delay strategy depends not only on the length of the delay, but also on how it is implemented in a population. We and Serra et al. (2005) have explored two such implementations, but some species may exhibit a mixture of timing mechanisms, including generational blocks as well as effects of birth order or time within the season. We have shown that a generational mictic block can be a robust and effective strategy for timing sexual reproduction in variable environments.

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Supplementary Figures

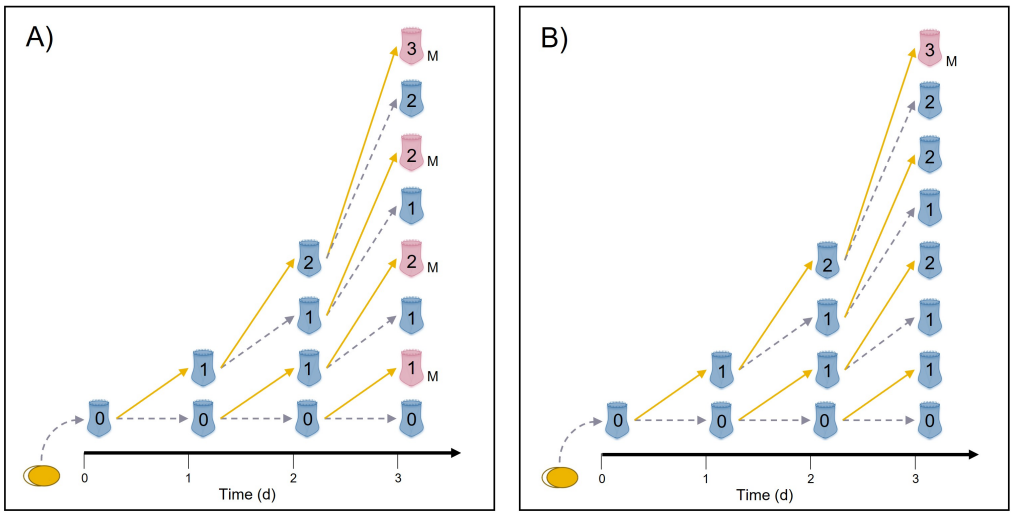

Figure S1: Example population where all rotifers produce one offspring each day demonstrating the difference between (A) a temporal block that lasts two days and (B) a generational block that lasts two generations. An initial resting egg hatches from diapause at $t = 0$. Gray dashed arrows indicate survival and yellow solid arrows indicate reproduction. The number overlaid on each individual indicates how many generations it is removed from the resting egg. For the sake of demonstration, we assume no maturation time, no mixis threshold, and a mixis ratio of 1. In case (A) the block ends at $t = 2$ and all subsequently produced rotifers are mictic, indicated with an "M". In (B) only amictic individuals of generation 2 can produce mictic offspring. At day three, there are four mictic individuals in (A) and only one in (B).

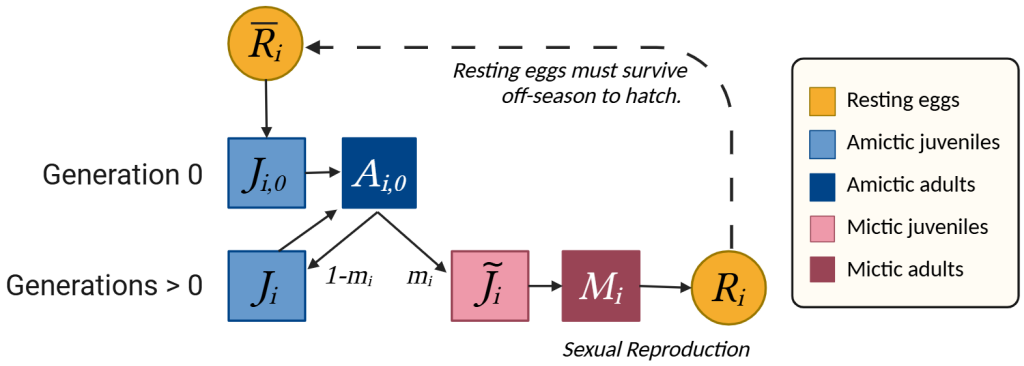

Figure S2: Version of model schematic in Fig., 3, but with $G_i = 0$. This is the case where the rotifer population has no mictic block. Note that there is still a mictic delay between resting egg hatching and new resting egg production that consists of the maturation time of stem and then mictic juveniles.

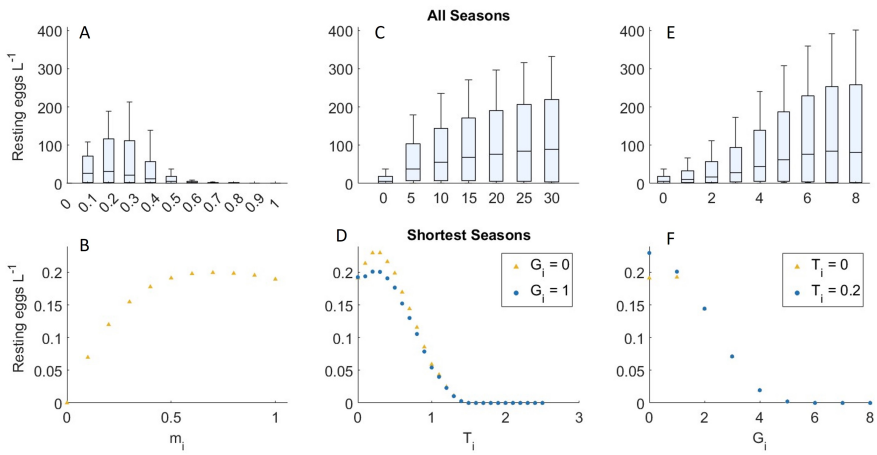

Figure S3: Distribution of resting egg production across range of possible season lengths (10-51 d) for strains with a range of (A-B) mixis ratios, (C-D) mixis thresholds, and (E-F) generational blocks. Upper panels show how longer delays and low mixis ratios increase the upper end of the distribution, i.e. success of strains in long seasons. Lower panels show that higher mixis ratios and shorter delays are most successful in the shortest seasons (length 10 d). In these shortest seasons, very small delays are optimal, either in the form of a low mixis threshold (D; $T_i = 0.2L^{-1}$) or one-generation block (F; $G_i = 1$). In all simulations represented here, starting egg density was b_{max} and parameters values were those indicated in Table 1. Phenotypes are $m_i = 0.5, T_i = 0, G_i = 0$, except where otherwise indicated.

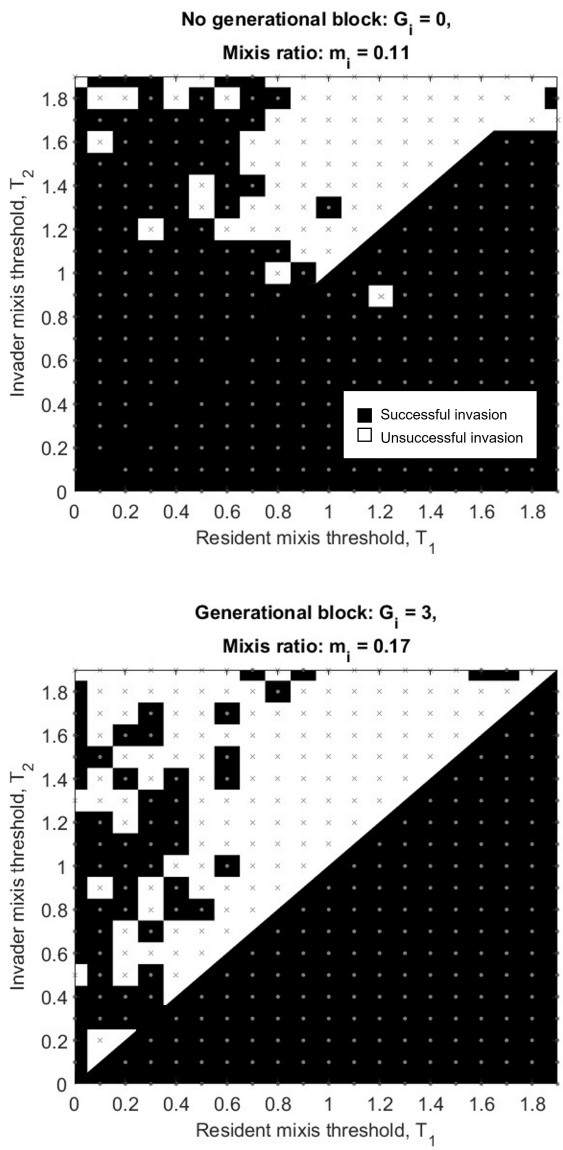

Figure S4: Pairwise invasibility plot for rotifer strains with variable mixis thresholds. As in Fig. 7, invasion was deemed successful if, out of 20 simulations of 40 growing seasons each, the invader frequency increased on average from its initial frequency of 0.05. White squares and cross marks indicate unsuccessful invasions, black sqaures and circle marks indicate successful invasion. Generally strains with lower mixis thresholds are more likely to successfully invade, and strains with no mixis threshold ($T_i = 0$) are able to invade all others. All parameters not indicated within the figure have values shown in Table 1.

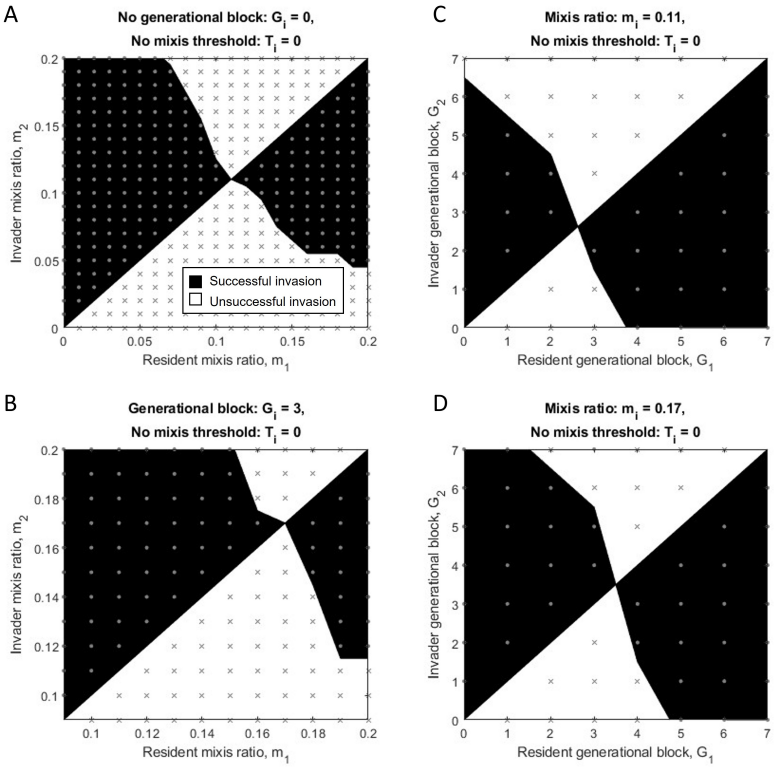

Figure S5: Pairwise invasibility plots for rotifers with A-B) variable mixis ratio and C-D) variable generational block. Panels A and C are exactly replicated from Fig. 7. As before, invasion was deemed successful if, out of 20 simulations of 40 growing seasons each, the invader frequency increased on average from its initial frequency of 0.05. Gray crosses indicate unsuccessful invasions; circles indicate successful invasions. Black and white regions are interpolated regions of success and failure, respectively. All parameters not indicated within the figure have values shown in Table 1.

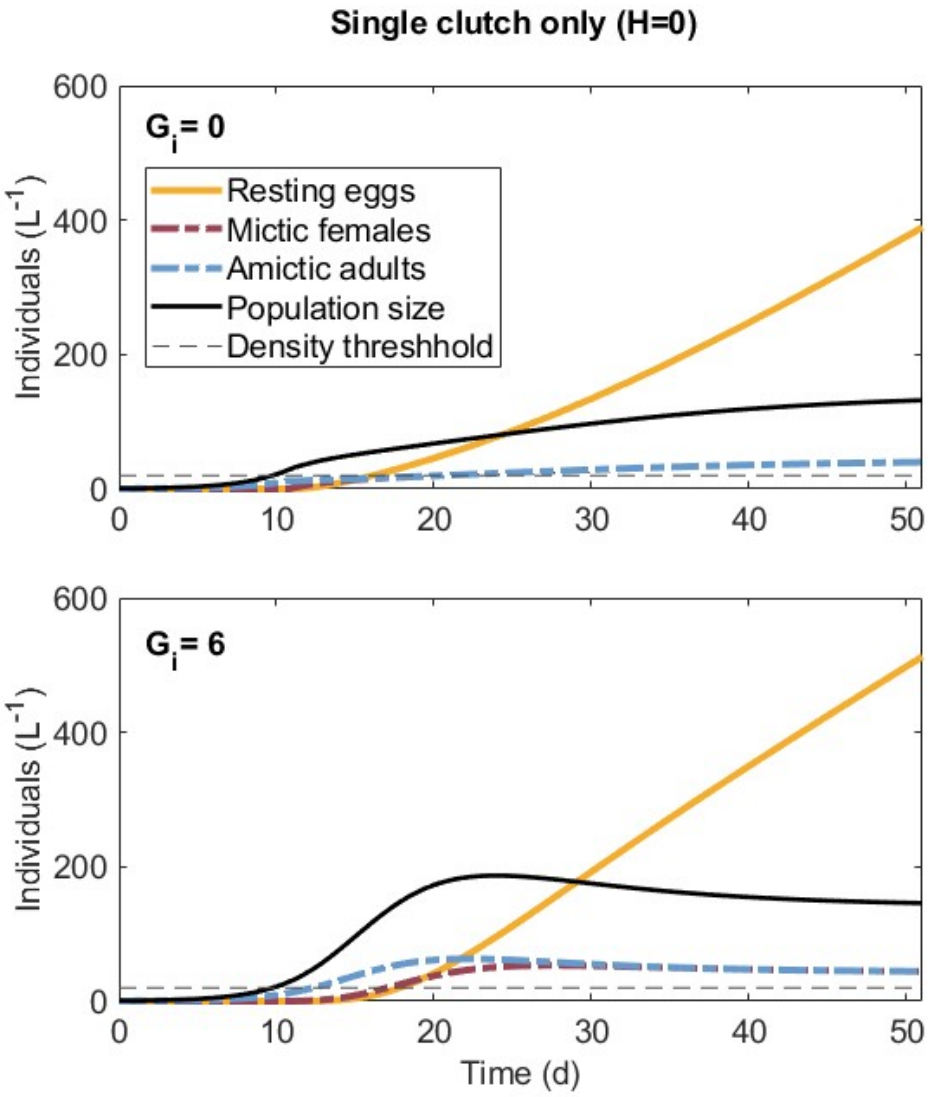

Figure S6: Population trajectories for monomorphic populations when all resting eggs hatch in a single clutch at t=0. Both strains have a mixis ratio and threshold of 0.5 and 20 individual L^{-1}, respectively. Strains with $G_i = 6$ (bottom) produce more resting eggs in a long season than those with no generational block (top). Benefits of generational block are not limited to the growth of late hatching clones.