

Delayed mixis in rotifers: an adaptive response to the effects of density-dependent sex on population growth

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In most cyclically parthenogenetic life cycles, sex is needed to produce resting stages. In several species of cyclically parthenogenetic rotifers, some generations of clones are not responsive to a density-dependent signal that triggers sexual female production. These unresponsive rotifers hatch from resting eggs and typically pass 8–12 generations of female parthenogenesis before becoming receptive to the mixis signal. We addressed the selection for mixis delay using a simulation model. A delay of sexual reproduction could increase population growth through parthenogenesis and thus the number of resting eggs ultimately produced. In a monomorphic population without mixis delay, we determined the optimal ratio of mictic to amictic females (mixis ratio) to be 45%, and the optimal population density threshold for induction of mictic females (mixis threshold) to be 82 rotifers L^{-1} . This mixis pattern, however, was not an evolutionarily stable strategy. A mixis ratio of 14% and threshold of 70 rotifers L^{-1} proved to be resistant to invasion by other mixis patterns. When we gave this phenotype a mixis delay of 8–10 days, it could invade a population with the same mixis pattern, but lacking a mixis delay. The advantage of delaying mixis was relatively small, suggesting that a polymorphism is possible.

INTRODUCTION

Cyclically parthenogenetic life cycles are not common among animals, but they are useful in exploring questions about the evolution of sex (Hebert, 1987; Simon *et al.*, 2002). In many cyclical parthenogens, an association exists between sexual reproduction and resting stage production, which causes a short-term selective pressure for the maintenance of sex. Because of their theoretical importance, these life cycles have been thoroughly investigated and the interactions among various life history parameters are fairly well understood. Rotifers are one of the prime examples of cyclical parthenogens, and their evolutionary potential and constraints are well characterized (Serra *et al.*, 2003). Recently, a new phenomenon in the rotifer life cycle has been reported by Gilbert (Gilbert, 2002) for a Florida strain of the freshwater monogonont rotifer *Brachionus calyciflorus*. He described phenotypes that were not responsive to a density-dependent chemical signal that triggers mictic (sexual) female production. These unresponsive rotifers hatched from resting eggs and

passed 8–12 generations before becoming fully receptive to the mixis signal. This is an extraordinarily long maternal effect and moreover was puzzling since it was previously believed that rotifers hatching from resting eggs immediately became competent to respond to a mixis signal present in the environment. The discovery of delayed mixis has raised questions about how it could be advantageous over a pattern of no mixis delay (Gilbert, 2003b).

Mixis delay has now been shown to occur in several other but not all rotifer species (T. Schröder and J. J. Gilbert, submitted for publication). Significantly decreased responses to crowding in early generations from resting egg hatching occur in a strain of *Brachionus angularis* from Argentina, in a strain of *Rhinoglena frontalis* from a habitat near the Oder River, Germany, and in two strains of *Epiphanes senta* from habitats near this river. Mixis delay was especially pronounced in *E. senta*. In both *E. senta* strains, no mictic females were produced in crowded populations initiated by females of the first

and fifth generations from resting eggs, while a low but significantly higher percentage of mictic daughters was produced by females of the seventh generation. In a separate experiment with one of these strains, the percentage of mictic females produced increased from zero for females of the first generation to ~5% for females of the sixth generation and to ~20% for females of the thirteenth generation. Each of these increases was significant. On the other hand, two rotifer strains showed no mixis delay. In both a strain of *B. calyciflorus* from Georgia and one of *R. frontalis* from a habitat near the Oder River, crowded populations initiated by stem females hatching from resting eggs had just as high a percentage of mictic daughters as those initiated by females from the eighth and tenth generations respectively. Thus, the mixis-delay phenomenon probably is widespread but not universal.

The purpose of the present paper is to develop a model to test the hypothesis that mixis delay can be an advantageous trait that can invade a population. First, we explore demographic explanations for why delayed mixis might provide a selective advantage. Next, we use simulation to determine the optimal ratio of mictic to amictic females (mixis ratio) and the optimal population density threshold for induction of mictic females (mixis threshold), in a monomorphic population without mixis delay. Finally, we determine whether this population can be invaded by another phenotype with a different mixis ratio or threshold or by another phenotype with the same mixis ratio and threshold but with a mixis delay.

METHOD

The model

We simulated the dynamics of a population growing in an environment where favorable and unfavorable conditions alternate. Favorable conditions were considered to be the population growth season (Fig. 1), which was followed by a period during which the population was absent from the water column. The length of the population growth season was assumed to be randomly varying and population growth was assumed to be density-dependent. Typically, growth season was a more or less predictable period each year. The population was conceptualized as being comprised of clone cohorts. A clone cohort is a cluster of clones hatching from many resting eggs at the same time and is a set of individuals having the same pattern for sexual reproduction (mixis pattern). We employ this concept because we want to study the evolution of traits (mixis ratio, mixis threshold and mixis delay) that (i) are shared by clones hatched on different days and (ii) vary among clones hatched at the same time. For the invasibility analysis, we need to know how

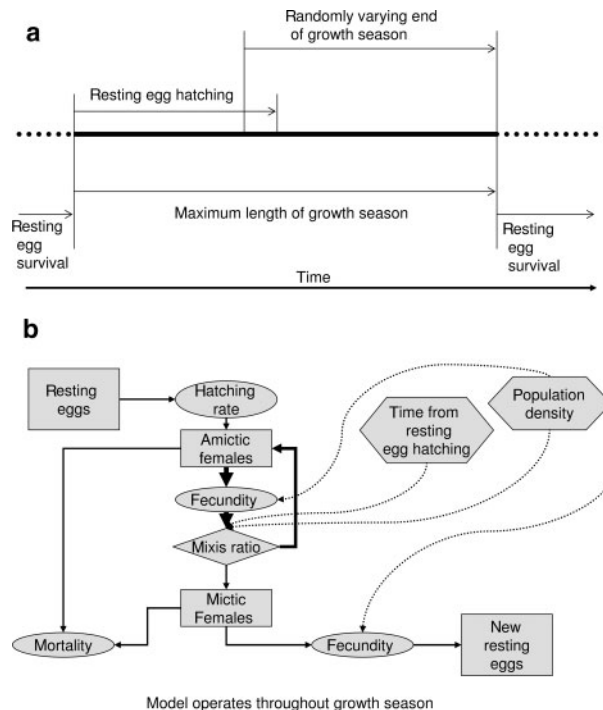


Fig. 1. (a) Gross phenology of a seasonal cycle of rotifer population growth. Resting eggs produced in the previous year do not hatch synchronically, but at a constant rate during a specified time window. The growth season may end with uniform probability anytime during the indicated period. This implies random variation in the growth season length. (b) Flowchart showing the population growth model employed. Thick arrow loop shows the parthenogenetic reproduction cycle. Recruitment of amictic females from resting eggs only occurs during a defined period of the growth season. Fecundity declines linearly with population density. Mixis pattern is the result of three traits: density threshold for mixis initiation (mixis threshold), delay in mixis response to density after resting egg hatching (mixis delay) and the proportion of mictic daughters produced after mixis is induced (mixis ratio). Note that individuals having the same mixis pattern will be clustered in several clone cohorts (same age after resting egg hatching) and that individuals with a different mixis pattern may be present in the population.

frequent a pattern is in the set clones hatching on a particular day. Therefore, the concept of clone cohorts is imposed by our modeling approach.

The *i*th mixis pattern (or reproductive strategy) is then a set of three values: T_i (the threshold population density for mixis initiation), D_i (the days of mixis delay after clone hatching from a resting egg) and m_i (the mixis ratio, after mixis initiation). For the clone cohort with the *i*th mixis pattern and hatching time *j* ($j = 0, 1, 2, \dots$), these parameters determine the current mixis ratio [$m_{ij}(N, t)$; i.e. the fraction of eggs produced by an amictic female that develop into mictic daughters] as follows

$$m_{ij}(N, t) = m_i, \quad \text{if } N \geq T_i \text{ and } t \geq (D_i + j) \quad (1)$$

$$m_{ij}(N, t) = 0, \quad \text{if } N < T_i \text{ or } t < (D_i + j)$$

where N is the total population density and $t = 0$ when the first resting egg hatchlings occur in the growth season.

Population dynamics within a growth season are described by a set of equations (Fig. 1), which is a generalization for several clone cohorts of the equations developed by Serra and King (Serra and King, 1999), except for the mictic ratio function. The equations are

$$\frac{dA_{ij}}{dt} = b(N)(1 - m_{ij}(N,t))A_{ij} - qA_{ij} \quad (2a)$$

$$\frac{dM_{ij}}{dt} = b(N)m_{ij}(N,t)A_{ij} - qM_{ij} \quad (2b)$$

where A_{ij} and M_{ij} are, respectively, the density of amictic and mictic females in clones with mixis pattern i and hatching time j , q is the mortality rate and $b(N)$ is the birth rate, which is a function of the population density [notice that $N = \sum_{ij} (A_{ij} + M_{ij})$]. Competition (density-dependent growth) is assumed to affect birth rate, providing logistic growth (i.e. per capita growth rate declines linearly with density). Therefore,

$$b(N) = b_{\max} - (b_{\max} - q) \left(\frac{N}{K} \right) \quad (3)$$

where b_{\max} is the maximum birth rate, K is the carrying capacity and N is the total population density per litre in the water column. Notice that $b(N)$ tends to b_{\max} when N tends to 0 and that $b(N) = q$ when $N = K$. This implies that the birth rate of amictic females equals mortality rate. We assumed that mortality rate is density independent, because one of the strategies described in starved rotifers was to stop reproduction, which allows adults to resist relatively long periods (Kirk, 1997).

The dynamics are described for a clone cohort by equations (2) in a growth season starting when resting eggs hatch (e.g. spring in temperate environments). Therefore,

$$\begin{aligned} M_{ij} &= A_{ij} = 0, & \text{if } j < t \\ M_{ij} &= 0 \text{ and } A_{ij} = \frac{R_i}{H}, & \text{if } j = t \end{aligned} \quad (4)$$

In these equations, R_i is the density of resting eggs having the i th mixis pattern that was produced in the previous growth season (Fig. 1). The resting eggs are assumed to hatch from time 0 to H at a constant rate.

Population growth season ends abruptly at a time between E_α and E_ω , which is randomly varying and uniformly distributed. The end of growth season may occur before all resting eggs have hatched, and it is

assumed that the unhatched eggs do not survive until the next growth season due to predation, deterioration or because they become buried in the sediment.

The clone cohorts produce new resting eggs, which have to be sexual, after mixis initiation. These new resting eggs (R'_i) go immediately into diapause until the next growth season. It is assumed that resting egg production rate is density dependent, and that the cost of a resting egg relative to the cost of an amictic egg is c^{-1} . This extra cost accounts for both costs because males need to be produced for resting egg production (assumed to be two-fold because of sex ratio theory) and costs because more resources are allocated in resting egg than in amictic egg. It follows that $c < 0.5$. This results in the following rate for resting egg production:

$$\frac{dR'_i}{dt} = \sum_j [cb(N)M_{ij}] \quad (5)$$

Note that resting eggs inherit the mixis pattern of their maternal clone. In this way we assume a simple genetic determination of diapausing strategies (Ellner, 1997; Spencer *et al.*, 2001). Notice that resting eggs have no memory of the hatching time of their maternal clone. Even though resting eggs diapause in the sediment, their densities can be expressed per water column volume, where they are produced and where the resting egg hatchlings will become distributed.

The dynamics described to this point account for a single growth season. In order to model a series of growth seasons, between-year dynamics have to be described. We assumed that the resting egg pool has a maximum constant size designated as B_{\max} . If the number of resting eggs produced in a growth season was higher than B_{\max} , the phenotype-specific resting egg density was proportionally decreased. If this adjustment was not performed, an exceptionally large resting egg pool might accumulate, which could cause an unrealistically high recruitment rate of hatchlings from the sediment. Such extraordinarily high recruitment (i.e. faster than exponential growth at the onset of the growth season) has not been observed in natural rotifer populations (personal observations).

Mortality and spatial dispersal can be thought of as factors for resting egg pool reduction. Therefore, the between growth season dynamics are

$$R_i(y+1) = R'_i(y) \left[\frac{B_{\max}}{B(y)} \right], \quad \text{if } B(y) \geq B_{\max} \quad (6)$$

$$R_i(y+1) = R'_i(y), \quad \text{if } B(y) < B_{\max}$$

where $B(y) = \sum_i R'_i(y)$ and y and $y+1$ are two consecutive growth seasons.

Simulations

The model was parameterized and simulated in order to investigate the evolution of mixis patterns. Parameter values (Table I) are based on literature values or on reasonable assumptions, if needed. A wide range for several of the model parameter values has been reported (Snell *et al.*, 1999), but we chose to use values for *Brachionus*. Moreover, we have made several realistic assumptions where necessary (i.e. non-synchronous resting egg hatching, a strong competition period and uncertainty in growth season length).

In the simulations, initial resting egg pool was assumed to be of size B_{max} , and several mictic patterns (i.e. mictic ratio, m , mixis threshold, T , and mixis delay, D) were explored. Each simulation consisted of a series of 40 growth seasons, and simulations with the same parameter values were repeated in order to compute the fate of a population. Two types of simulations were performed. In the first, we assumed a monomorphic population composed of a single mictic pattern. The long-term fitness of each mictic pattern was estimated as the cumulative number of resting eggs

produced (i.e. $\sum_i R'_i(y)$, where y ranges from 1 to 40). In the second type of simulation, populations were assumed to be composed of two phenotypes with differing mixis patterns. One phenotype (resident) was initially more frequent (95% of the population) than the other (5%, invader). This approach allowed us to compute the invasibility capacity of a rare mixis pattern. The rationale behind an invasibility analysis is explained, for instance, in the book by Bulmer (Bulmer, 1994).

RESULTS

Population dynamics with and without mixis delay

Our hypothesis is that delaying mixis can provide an advantage, but what exactly are the effects on population dynamics that provide this advantage? We compared the growth dynamics of genotypes with and without mixis delay through a single season in Fig. 2. Without delay, mixis is controlled only by the mixis threshold; as soon as

Table I: Parameter values for the model, their description and rationale. Values for the genus Brachionus have been favored

Parameter	Description	Value	Rationale
b_{max}	Intrinsic birth rate (d^{-1})	1.3	Maximum per capita growth rates (i.e. $b_{max} - q$) for laboratory <i>Brachionus</i> populations are in the range 0.25–2.2 d^{-1} , frequent values being around 1 d^{-1} (Miracle and Serra, 1989). This and the next value were chosen so that $b_{max} - q = 0.8 d^{-1}$, considered a conservative value for field populations.
q	Intrinsic mortality rate (d^{-1})	0.5	q was 0.22 d^{-1} in a laboratory <i>Brachionus</i> population (Serra, 1987). An approximately two-fold higher value was assumed in order to get realistic densities in simulations; the higher value is associated with additional mortality expected in the field (Serra and Carmona, 1993).
K	Carrying capacity (individuals L^{-1}). This carrying capacity can be achieved only if no mixis occurs.	500	<i>Brachionus</i> population peaks may be transiently as high as 10 000 L^{-1} . However, density plateaus are usually observed between 100 and 1000 L^{-1} (Carmona <i>et al.</i> , 1995; Ortells, 2002)
c	Ratio between amictic female egg cost and inclusive resting egg cost	0.2	In <i>Brachionus</i> , resting egg producing mictic females lay ~ 3 eggs, while amictic females lay ~ 15 eggs (Snell and Garman, 1986; Serra, 1987); only a fraction of mictic females produce resting eggs, while the remaining produce males.
B_{max}	Maximum size of resting egg pool (resting eggs L^{-1} of water column)	1	This value is an assumption, empirically chosen because it produced the expected exponential population growth at the onset of the growth season.
H	Period for resting egg hatching (from 0 to H) (days)	31	This value is an assumption, empirically chosen to cause (i) overlap between the resting egg hatching period and strong competition (i.e. growth cessation in the absence of mixis) and (ii) the end of growth season may occur while resting eggs are still hatching.
E_{α}	Minimum growth season length (days)	10	This value and the next were empirically chosen to cause an average growth season of ~ 1 month, with a large uncertainty in growth season length.
E_{ω}	Maximum growth season length (days)	51	

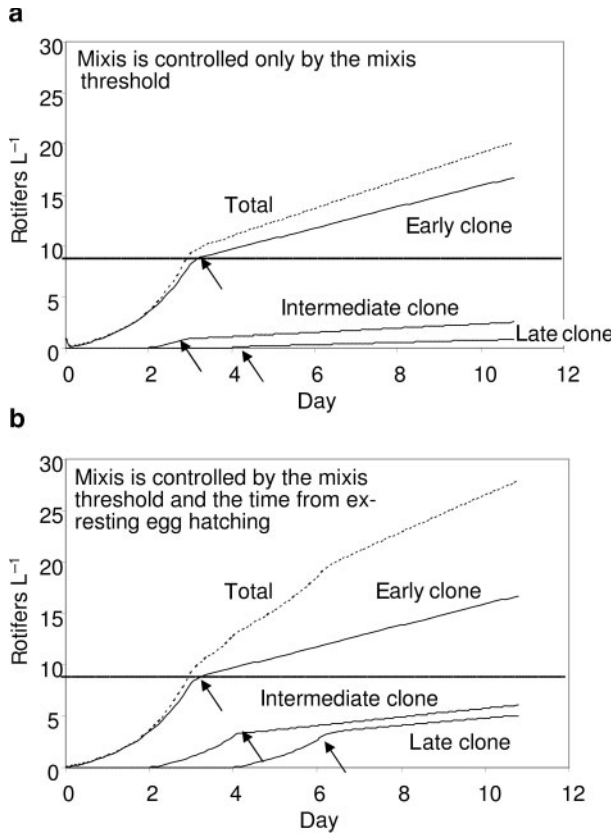


Fig. 2. Dynamics (clone and total densities) of three clone cohorts founded at different times from resting egg hatching. Two patterns for investment in mixis are shown, without mixis delay (**a**) and with mixis delay (**b**). The horizontal line shows the mixis density threshold. The arrows indicate the time for mixis initiation for each clone cohort. The dynamics were generated by applying the model with $m = 0.5$, $T = 9$ ind. L^{-1} , and $D = 2$ d, if applicable (other parameters, if applicable, as in Table I).

this density is reached, mictic females are produced. Mictic females produce only males or resting eggs, neither of which contribute to current population growth. Late hatching clones hatch into an environment where the mixis signal is already above threshold. These females will begin producing mictic daughters immediately, before they can utilize parthenogenesis to rapidly enlarge their population size. Because they prematurely initiate mixis, intermediate and late hatching clones are unable to contribute much to population growth. In other words, a cost of mixis response to density may be incurred via late hatching because of their low contribution to population growth rate (Fig. 2a). Notice that hatching time is assumed not to be completely under intrinsic control, which implies that it would have an unavoidable proportion of late hatching resting eggs for any mixis strategy.

In contrast, late hatching clones, which delay mixis by remaining unreceptive to the mixis signal for several

generations after resting egg hatching, make a significant contribution to population growth (Fig. 2b). With a mixis delay, late hatching clones emerge from resting eggs and grow parthenogenetically for several generations since their mixis is controlled by mixis threshold and the number of generations from resting egg hatching. They reach large population size before mixis is triggered and their population growth is slowed. This also results in a larger number of resting eggs being produced.

Determining whether delayed mixis is an evolutionarily stable strategy

First we explored the effect of mixis ratio and mixis threshold on cumulative resting egg production in a monomorphic rotifer population without mixis delay (Fig. 3). In this first exploration, we used cumulative resting egg production $[\sum_i R_i'(y)]$ as an optimization criterion because several mixis patterns might produce the same or very similar final resting egg production. This could happen in spite of systematic differences in yearly resting egg production due to our assumption in equation (6), which implies an upper limit for the resting egg pool founding a growth season. Mixis ratios of 20–70% and mixis thresholds of 20–100 rotifers L^{-1} were investigated. The maximum cumulative number of resting eggs produced in 40 growth seasons was $\sim 150\,000$ at a mixis ratio of 45% and a mixis threshold of 82 females L^{-1} . This provided the optimum mixis pattern in a monomorphic population and we proceeded to investigate whether this could be improved upon by an invading phenotype with a different mixis pattern.

In the first set of invasibility analyses, we examined phenotypes without mixis delay. After some preliminary explorations of mixis ratios of 10–45% and thresholds of

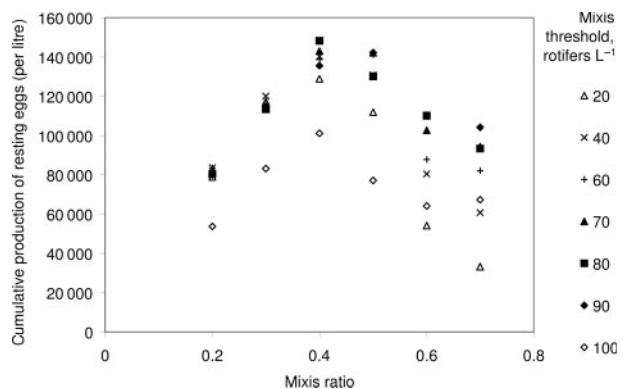


Fig. 3. Combined effect of mixis ratio and mixis threshold on resting egg production in a monomorphic population. The population density threshold for mixis is shown in the legend. No mixis delay was assumed. Cumulative production of resting eggs is the total number of resting eggs produced per litre in 40 growth seasons (average of 20 samples).

20–110 females L^{-1} , we discovered that phenotypes with decreased mixis were able to invade resident populations, with the optimum mixis pattern of a 45% ratio and 82 L^{-1} threshold. We then explored resident populations initiating mixis at thresholds of 65–85 females L^{-1} with mixis ratios of 10–22% (Fig. 4). Invaders possessed a similar range of mixis thresholds and mixis ratios of 10–20%. We were searching for an evolutionarily stable mixis strategy (ESS)—one that could invade a population, but could not itself be invaded. Because there are stochastic elements in the model (i.e. growth season length), there is some variability in outcome. However, it can be seen that a resident population with a 14% mixis ratio and 70 female L^{-1} threshold is most resistant to invasion (most gray boxes in Fig. 4, columns). Similarly, an invader with a 14% mixis ratio and 70 female L^{-1} threshold is most successful at invading (most black boxes in Fig. 4, rows). Consequently, a phenotype with this mixis pattern

should be able to invade another population with a different mixis pattern, but able to resist invasion by a phenotype with another mixis pattern.

Our next step was to examine whether a phenotype with a 14% mixis ratio, threshold of 70 females L^{-1} and delayed mixis could invade a resident population with a similar mixis ratio and threshold, but with no mixis delay. The fate of an invader with various mixis delays is illustrated in Fig. 5. Beginning from an initial frequency of 0.05, invader frequency after 40 growth seasons is plotted for several mixis delays. Delaying mixis does not allow the invader to increase relative to the resident phenotype until about a 7-day delay. The optimum mixis delay is 9 days and the benefit of delaying mixis is rapidly lost after an 11-day delay. This shows that phenotypes delaying mixis by 8–11 days after resting egg hatching should be able to invade a resident phenotype without mixis delay. Invasibility of a resident

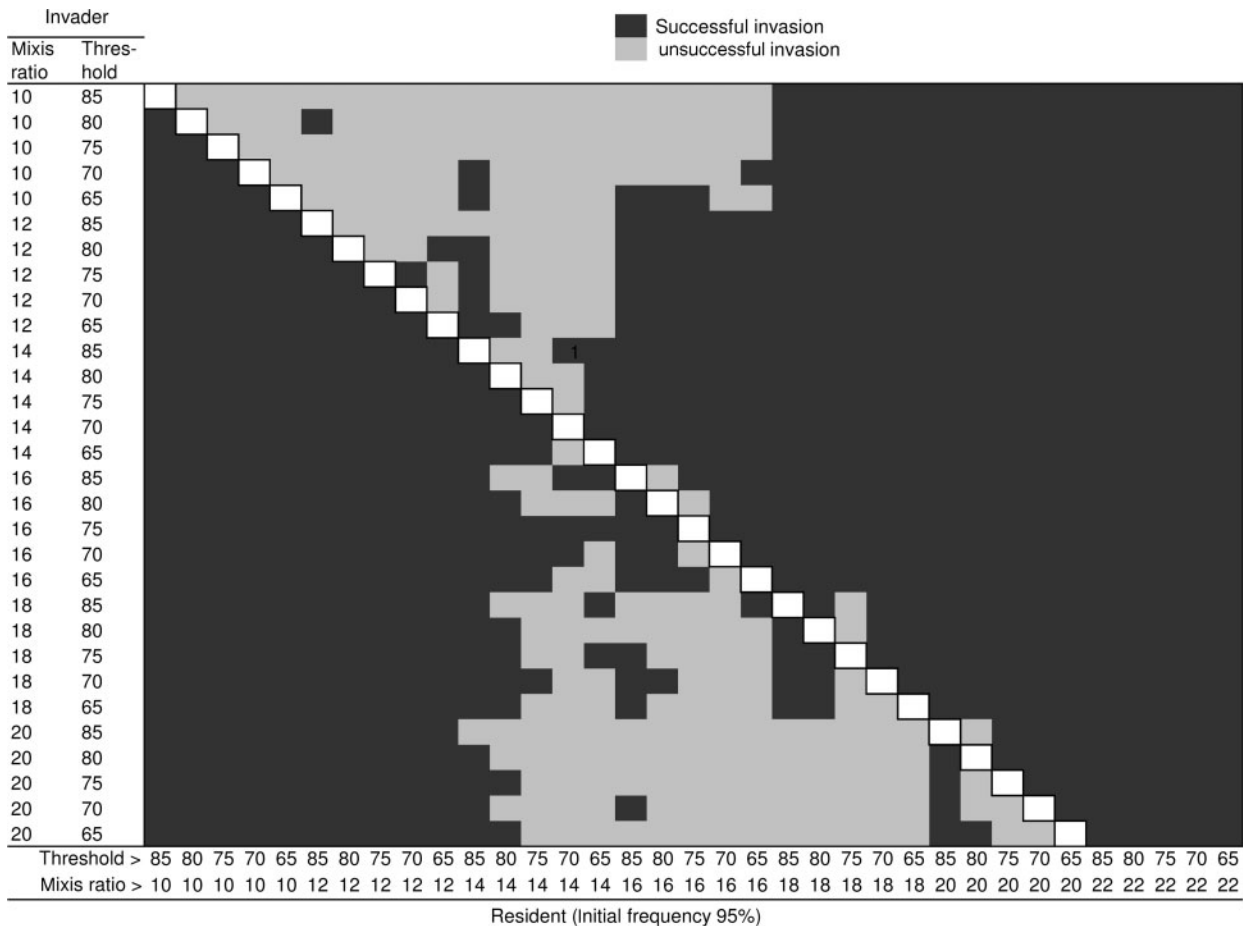


Fig. 4. Invasibility analysis for different mixis patterns with no delay in mixis after resting egg hatching. Invasion was assessed as successful if the initial frequency of invader (0.05) increased after 40 growth seasons. Final invader frequency is the average of 20 invasions. Black boxes indicate successful invasion, gray boxes represent unsuccessful invasions after 40 seasons ($n = 20$). White boxes on the diagonal represent conditions where resident and invader have identical mixis patterns. The closest mixis pattern to an evolutionarily stable mixis strategy (ESS) is a mixis ratio of 14% and a threshold of 70 L^{-1} .

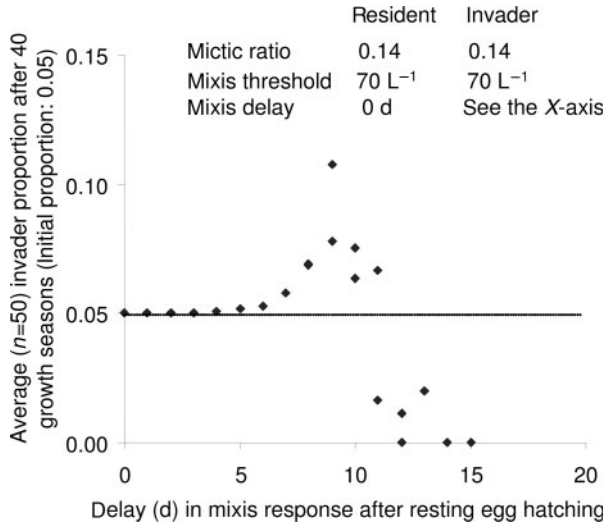


Fig. 5. Invasibility analysis for phenotypes with a mixis delay.

population by a phenotype delaying mixis is explored in more detail in Table II. Mixis ratios of 12–18% and thresholds of 65–80 females L⁻¹ are examined for delays of 8–12 days. The interaction of these three parameters is clearly seen, as is the variety of combinations that could successfully invade. The advantage of delaying mixis, however, is always relatively small, so that even

after 40 growth seasons, phenotypes with the optimal mixis delay of 9 days have typically no more than doubled their initial frequency of 0.05. This suggests that a stable polymorphism may be possible, with approximately selectively equivalent phenotypes, some delaying mixis and some not.

DISCUSSION

Our results showed that phenotypes with the ability to delay mixis initiated sex at larger population sizes and produced more resting eggs. As a result, they could consistently invade populations without mixis delay. This is because mictic female production is a considerable drag on population growth (Serra and King, 1999). Mictic females produce either males or resting eggs, neither of which contribute to current population growth. In contrast, amictic females produce daughters parthenogenetically, driving population growth to very large sizes. A phenotype initiating mixis at large population size will produce many more resting eggs than a phenotype initiating mixis at a smaller size (Gilbert, 2002). Encounters between males and young, fertilizable mictic females would be more likely in populations initiating mixis at higher densities, and therefore larger numbers of resting eggs would be deposited in the sediment egg bank. In

Table II: Invasibility of a resident mixis pattern with a mixis ratio of 0.14, mixis threshold of 70 L⁻¹ and no delay in mixis response after resting egg hatching

Mixis ratio	Mixis threshold	Mixis delay (days)				
		8	9	10	11	12
0.12	65	0.0435	0.0479	0.0579	0.0332	0.0124
	70	0.0490	0.0427	0.0294	0.0438	0.0019
	75	0.0406	0.0547	0.0478	0.0365	0.0273
	80	0.0370	0.0393	0.0581	0.0100	0.0079
0.14	65	0.0669	0.1046	0.0978	0.0253	0.0085
	70	0.0684	0.0780	0.0753	0.0166	0.0110
	75	0.0703	0.1116	0.1780	0.0688	0.0484
	80	0.0702	0.0999	0.0717	0.0826	0.0372
0.16	65	0.0748	0.1041	0.0642	0.1241	0.0355
	70	0.0727	0.1140	0.1610	0.0396	0.0393
	75	0.0710	0.0875	0.1056	0.0847	0.0005
	80	0.0641	0.0891	0.1495	0.1134	0.0356
0.18	65	0.0525	0.1069	0.1081	0.1212	0.0000
	70	0.0554	0.0953	0.1368	0.0532	0.0372
	75	0.0642	0.1014	0.1141	0.1300	0.0641
	80	0.0547	0.0973	0.1055	0.0605	0.0106

Invader mixis pattern is shown (initial frequency of the invader is 0.05). These values are invader frequencies after 40 growth seasons (years; sample size, 50). Successful invasions are in bold.

rotifers such as *Brachionus* and *Epiplanes*, where the environmental signal for mictic female production is crowding, mixis delay should reinforce the crowding response and ensure that sexual reproduction occurs at high population densities. Mixis delay would be especially important in these rotifers if the crowding cue was not species-specific (Gilbert, 2002, 2003a). Ultimately, the number of resting eggs produced is the best measure of long-term fitness in rotifer populations (Serra *et al.*, 2003). Phenotypes delaying mixis by remaining unresponsive to the mictic signal for the first several generations after resting egg hatching are growing to larger sizes before mixis is initiated and producing more resting eggs.

Mixis delay may be most important at the clonal level (Gilbert, 2002), even if the mixis signal is very specific, as in *B. calyciflorus* (Gilbert, 2003a). If resting eggs hatch from the egg bank at different times, later-hatching clones could be at very low population densities when the early-hatching clones are sufficiently crowded to trigger mictic female production. Mixis delay would ensure that the late-hatching clones would not respond to the crowding cue and could increase in abundance for some generations via parthenogenesis before committing to sexual reproduction.

Why is there such a large difference between the monomorphic population mixis optimum (45% mictic ratio and 82 L^{-1} mictic threshold) and the invading population optimum (14% ratio and 70 L^{-1} threshold)? A likely explanation is based on the effects of mixis on clonal competition. Maximization of resting egg production in a monomorphic population occurs without competition from asexual phenotypes. Then, maximization requires a heavy investment in sexual reproduction at the expense of asexual population growth. The scenario is very different when heritable variation in mixis patterns is considered, since a mixis pattern producing a lot of resting eggs (the maximum possible) would leave the clone vulnerable to invasion by a phenotype investing less in mixis and more in asexual population growth. Note in equation (2) that mixis ratio decreases the birth rate of amictic females, which are the source of population growth. It follows that the lower the mixis ratio, the higher the short-term growth rate, which results in a competitive advantage. The invading population optimum, therefore, represents a compromise between asexual competitive ability and resting egg production, whereas a monomorphic resident population simply maximizes resting egg production without regard for competitive ability.

The mechanism for mixis delay is not understood. The mixis response to crowding appears to be triggered by a soluble signal molecule secreted by the rotifers themselves (Stelzer and Snell, 2003). This molecule should contain enough information so that closely related species can

discriminate their own species-specific signals (Gilbert, 2003a); however, nothing is known about the chemical nature of this signal. The mictic signal may interact with a receptor on parental females or their oocytes (Gilbert, 2003b), but the mechanism is unknown. Transduction pathways of environmental signals are understood in some model invertebrates (Krieger and Breer, 1999), and this could provide guidance about how rotifer sensory systems are organized.

Mixis could be delayed by an inhibitor that prevents expression of the mictic female phenotype (Gilbert, 2002). While amictic females produce diploid eggs (one mitotic maturation division) that develop parthenogenetically into females, mictic females produce haploid eggs (equational and reductional meiotic maturation divisions). If the haploid eggs are not fertilized, they develop parthenogenetically into males. If they are fertilized, they develop into diapausing, encysted embryos which can later resume development into amictic females. Amictic and mictic females are morphologically similar and differ primarily in the ploidy of their eggs. A factor inhibiting embryonic development into mictic females may be produced in the vitellarium of fertilized mictic females and then transferred to her resting eggs. This inhibitor could assure that stem females hatching from resting eggs are amictic and also could be passed on, in decreasing amounts, for a number of successive parthenogenetic generations. Thus, mixis delay may be due to a gradual release from this inhibition.

The obvious advantage of delayed mixis to late-hatching clones is consistent with the prevalence of the trait in many populations (T. Schröder and J. J. Gilbert, submitted for publication). However, it is inconsistent with the surprisingly low invasiveness of the trait in our simulations (Fig. 5). For example, it took an invading phenotype 40 growth seasons to double in frequency from 0.05 to 0.1. There are large variations in mixis patterns in nature (Snell *et al.*, 1999), and these patterns are dependent on the demographic environment (Serra and King, 1999; Serra *et al.*, 2003). Nevertheless, our results are not out of range. For instance, it has been found (Carmona *et al.*, 1995) that a population of *Brachionus plicatilis* sensu stricto in Torreblanca Marsh, Spain, had an average mixis ratio of 12.4% and a mixis threshold of 6.6 L^{-1} (30 L^{-1} for a mixis ratio $>5\%$). For a co-occurring *Brachionus rotundiformis* population, these values were 26% mixis ratio and threshold of 23 L^{-1} (90 L^{-1} for a mixis ratio $>5\%$). The absolute values of the mixis optima that we found by simulation are probably not so informative, or so general, since there are strong interactions among life history parameters. The results of our analysis should be viewed as qualitative, demonstrating that delayed mixis is adaptive and potentially responsive to selection. The exact optimum depends

on several parameters (growth season length, carrying capacity and relative cost of resting eggs), which are known to vary among natural rotifer populations (Gilbert, 2002), and these are still poorly characterized. Until these are better characterized, we can expect only qualitative results from the simulations.

This study suggests that many different combinations of mixis ratio, threshold and delay are approximately equivalent. We expect that many delayed mixis phenotypes could invade, with the fate of each being determined by chance or alternately being favored, then disfavored by fluctuating selection. Under these conditions, a stable polymorphism is quite possible, with some phenotypes possessing a mixis delay and some not. We expect that the environmental conditions favoring mixis delay are ones where competition among clones is regularly strong and the cost of resting egg production is high. Since delayed mixis has been observed in several natural rotifer populations (T. Schröder and J. J. Gilbert, submitted for publication), these environmental conditions may be common.

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