Maternal age and spine development in a rotifer: ecological implications and evolution

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Abstract. *Brachionus calyciflorus* typically develops long, defensive spines only in response to a kairomone from the predatory rotifer, *Asplanchna*. However, in the absence of any environmental induction, females of some clones produce daughters with increasingly long spines as they age; late-born individuals can have posterolateral spines as long as those induced by *Asplanchna*: up to 50% or more of body length. Here, we construct a model using data from life-table and predator–prey experiments to assess how this maternal-age effect can influence the distribution of spine lengths in reproducing populations and provide defense against *Asplanchna* predation. When *Asplanchna* is absent, the frequency of individuals with late birth orders rapidly becomes extremely low; thus, any cost associated with the production of long-spined individuals is minimal. When *Asplanchna* is present at densities too low for spine induction, and preys selectively on individuals with no or short posterolateral spines, the frequency of long-spined individuals rapidly increases until a stable birth-order structure is reached. As a result, mortality from *Asplanchna* predation is greatly reduced. The pronounced and novel birth-order effect in this rotifer appears to be an effective bet-hedging strategy to limit predation by *Asplanchna* when its kairomone induces no or less than maximal spine development.

Key words: bet hedging; birth order; defense; predation; rotifers; spine development.

INTRODUCTION

Many planktonic rotifers exhibit pronounced phenotypic plasticity (reviewed by Gilbert [1999, 2013]). The plastic traits typically are the length of spines extending from the anterior, and especially posterior, ends of the body's integumentary skeleton. This plasticity and its control have been most extensively studied in *Brachionus calyciflorus*, where a pair of posterolateral spines may be absent or vary continuously in length from just detectable to half of the body length or more (Gilbert 1966, 1967, 2011a, Halbach 1970a, Schröder and Gilbert 2009). As these spines increase in length, so do the two pairs of anterior spines and the pair of posteromedian spines (Fig. 1). The spine-development response of an individual occurs within the maternal body cavity before the oocyte from which it hatches is oviposited and begins cleavage divisions (Gilbert 1967, 1999). Thus, an individual's phenotype is fixed for life well before its birth.

Several studies with different geographic strains of *B. calyciflorus* have shown that three environmental factors can influence spine length. Short posterolateral spines can be induced by low temperature and low food concentration (Gilbert 1967, Halbach 1970a, Stemberger 1990), and very long ones can be induced by a kairomone from the predatory rotifer *Asplanchna* (Fig.

1; Gilbert 1967, 1980, 2011a, Halbach 1970a, Stemberger 1990). The effects of these factors are additive until spines reach some maximum length (Halbach 1970a).

The pronounced spine-development response of *B. calyciflorus* to *Asplanchna* kairomone greatly decreases its susceptibility to *Asplanchna* predation, since the long spines can make the rotifer very difficult to capture and ingest. The much more modest spine-development responses of *B. calyciflorus* to low temperature and low food availability are less clearly beneficial, but may increase fitness by reducing the rotifer's sinking rate (Stemberger 1990, Gilbert 2011b).

Pronounced spine development in certain strains of *B. calyciflorus* also can occur without any environmental inducer. In some clones, spine length is controlled endogenously via maternal age; during a female's reproductive period, offspring spine length increases progressively with birth order until maximal spine development occurs (Schröder and Gilbert 2009). Late-born offspring of some clones have posterolateral spines as long as those induced by *Asplanchna*. The effects of maternal age and *Asplanchna* kairomone are additive. When cultured for life in the absence of *Asplanchna* kairomone, females born with *Asplanchna*-induced spines contain residual inducer and produced a series of daughters with increasingly long spines that were consistently longer than those of control females without such spines (Gilbert 2012).

This maternal-age effect in *B. calyciflorus* appears to be unique in the animal kingdom; we know of no other
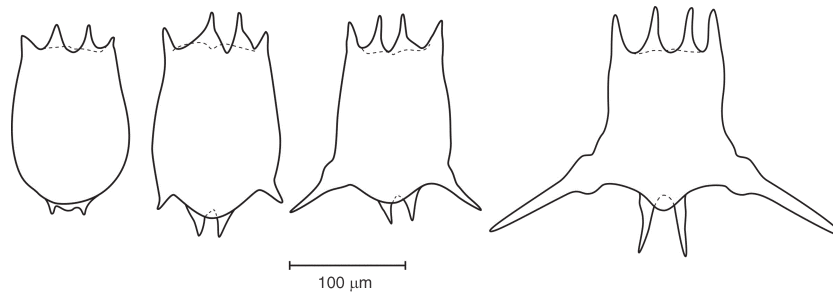
FIG. 1.   Continuous spine-length variation in a Lake Washington clone of *Brachionus calyciflorus* (redrawn from Gilbert [1967]). Individuals are juveniles. Spine development involves elongation of anteromedian, anterolateral, posteromedian, and, especially, posterolateral spines.

case where the age of a mother has a major effect on the morphology of her offspring. Maternal age can have a small effect on wing size in *Drosophila* (Røgilds et al. 2005). Also, it can affect survival and fecundity in a weevil (Opit and Throne 2007), and in rotifers cultured for several orthoclone generations in which parental age is kept constant (King 1969).

The maternal-age effect on spine development in *B. calyciflorus* may be a bet-hedging strategy to assure that some individuals in a population always are protected against *Asplanchna* predation (Schröder and Gilbert 2009). We elaborate on this hypothesis and suggest that clones with the maternal-age effect have an advantage both when *Asplanchna* populations begin to develop, and also whenever *Asplanchna* is at densities too low to induce maximal spine development. When *Asplanchna* first appears in a community, no individuals in the *B. calyciflorus* population would have *Asplanchna*-induced spines (remember that spine length is set in the embryo stage). However, clones with the maternal-age effect would have produced some late-born, long-spined offspring that are resistant to *Asplanchna* predation. Probably more importantly, *Asplanchna* may be a significant predator whenever the concentration of its kairomone is too low to induce maximal spine development. Under these conditions, and since the maternal-age and kairomone effects are additive, late-born offspring of clones with the maternal effect would have longer spines than those of clones without the maternal-age effect. Thus, *Asplanchna* predation may impose strong natural selection among genotypes that express different levels of this maternal-age effect.

Here, we develop a model to address several questions regarding the ecological and selective significance of the maternal-age effect on spine development in *B. calyciflorus*. In the absence of *Asplanchna*, what is the expected distribution of birth orders, and hence spine lengths? If there were allocation or ecological costs associated with the development and maintenance of long spines, producing some long-spined offspring could decrease fitness. If *Asplanchna* is present but not sufficiently abundant to induce spine development, how can it affect the distribution of birth orders and spine lengths, and

how effective is the maternal effect in reducing mortality from *Asplanchna* predation? This study appears to be the first to consider birth-order distribution as an important population parameter.

METHODS

The relationship between birth order and spine length was obtained from a previously published life-table experiment with a clone of *B. calyciflorus* (clone 2 derived from Florida clone FL82; [Schröder and Gilbert 2009]). The ratio of spine length to body length for successive offspring increased with birth order, being zero for the first-born and then gradually increasing to a maximum of about 0.43 by the 11th born (Table 1). These ratios were determined for adult individuals and are lower than they would be for newborns, since spine length increases at a lower rate than body length during postnatal growth (Gilbert 1967).

TABLE 1.   Effect of maternal age on offspring production and posterolateral spine length in a Florida clone of *Brachionus calyciflorus*.

Maternal age (d)	Maternal survival	Offspring	
		Birth order	Spine length : body length
1.35	1	1	0
1.74	1	2	0.027 (0.018)
2.22	1	3	0.045 (0.021)
2.51	1	4	0.145 (0.034)
2.85	1	5	0.183 (0.022)
3.23	1	6	0.240 (0.047)
3.58	1	7	0.265 (0.040)
4.08	1	8	0.325 (0.035)
4.45	1	9	0.357 (0.042)
4.83	1	10	0.372 (0.030)
5.30	1	11	0.423 (0.020)
5.67	1	12	0.438 (0.019)
5.88	1	13	0.432 (0.016)
6.33	1	14	0.427 (0.027)
6.98	0.83	15	0.422 (0.025)
7.50	0.83	16	0.437 (0.028)

Notes: Data are from a life-table experiment conducted at 20°C and with *Cryptomonas erosa* food (2×10^4 cells/mL; Schröder and Gilbert 2009; J. J. Gilbert, *unpublished data*). maternal age and ratio of spine length to body length are means; values in parentheses are SE.

TABLE 2. Survival of *Brachionus calyciflorus* following attack by *Asplanchna* of different species and body lengths.

Asplanchna and *B. calyciflorus* body length (μm)	*B. calyciflorus* posterolateral spine length	Probability of surviving *Asplanchna* attack	Reference
A. girodi (~750)			Gilbert (1967)
~150	absent	0.91	
	long	1.00	
A. brightwelli (~750)			Halbach (1971)
~150	absent	0.05	
	short	0.10	
	long	0.94	
~225	absent	0.20	
	short	0.75	
	long	1.00	
A. sieboldi (~560)			Gilbert (1967)
~150	absent	0.11	
	long	0.86	
A. sieboldi (~870)			Gilbert (1967)
~200	absent	0	
	long	0.25	
A. sieboldi (~870)			Gilbert (1980)
~150	very short	0	
	long	0.98	
~200	very short	0.65	
	long	1.00	

Notes: Results are from direct observations of encounters. *Asplanchna* species body lengths are in parentheses. Long posterolateral spines are at least half the body length. For details see references.

In the model, the formal relationship between birth order and the ratio of spine length to body length was determined from a nonlinear regression analysis using all data points in this experiment between birth orders 1 and 16 (see Fig. 3 [clone 2] in Schröder and Gilbert 2009). We fit the following logistic function:

$$\text{SL} = \frac{a(\text{BO} + c)}{b + \text{BO} + c}$$

where SL is spine length, BO is birth order, and a, b, and c are parameters of the model. This model was fit to the data using the NLIN procedure of SAS v. 9.3 (SAS Institute 2011). The resulting parameters were used to assign spine lengths to individuals based on their birth order in the model.

Details from the same life-table experiment (J. J. Gilbert, *unpublished data*) were used to determine maternal survival and the relationship between maternal age and the birth order of her daughters (Table 1). Age-specific survival and fecundity were assumed to be unaffected by birth order and spine development. This seems reasonable, since several laboratory experiments with *B. calyciflorus* showed that possession of long, *Asplanchna*-induced spines did not affect reproductive rate or timing between consecutive reproductive bouts (Gilbert 1980, 2012).

We used several different scenarios in the model to determine the effect of *Asplanchna* predation on the survival of *B. calyciflorus* with spines of different length.

Studies in which interactions between *B. calyciflorus* and *Asplanchna* have been directly observed clearly show that long posterolateral spines provide a very effective defense against capture and ingestion (Gilbert 1967, 1980, Halbach 1971). However, the relationship is complicated, because predation risk varies not only with spine length but also with the species of *Asplanchna* and with the body size of the *Asplanchna* and *Brachionus* (Table 2). For example, in one study (Halbach 1971), the probability of *B. calyciflorus* with no spines and long spines surviving attacks by adult *Asplanchna brightwelli* was, respectively, 0.05 and 0.94 for small *Brachionus* and 0.2 and 1.0 for large ones. In another study (Gilbert 1980), the probabilities for *B. calyciflorus* with very short spines and long spines surviving attacks by adult *Asplanchna sieboldi* were 0 and 0.98 for small *Brachionus* and 0.65 and 1.0 for large ones.

The model

We formulated a stage/age structure projection model of population dynamics based on the methods of Caswell (2012). In this model, birth order is considered a fixed stage to which an individual is assigned for its entire life. Adult *B. calyciflorus* individuals produce a single egg approximately every 12 hours at 20°C (Table 1), and so the transition matrix was constructed assuming that each iteration corresponds to this 12 hour interval. Thus, age is scaled to 12-hour intervals, and all transition probabilities and fecundities are scaled

to these time intervals. The life cycle consisted of three juvenile ages, followed by 16 reproductive ages. An individual could survive for a maximum of these 16 reproductive bouts (i.e., all individuals are assumed to die after their reproductive bout 16).

Survival values in the matrix were determined by the posterolateral-spine lengths associated with birth order. We assumed a conservative, linear relationship between the probability of surviving one iteration of the model and spine length. We specified a minimum survival for individuals of birth order 1, and a maximum to individuals at the asymptote of the spine length/birth order function, and we interpolated survival values between these based on the linear relationship between spine length and survival. For simplicity, and because spine development is set upon hatching, we assumed that survival probabilities at all ages (juvenile and adult) were identical and based only on spine length (i.e., birth order).

We analyzed the model using the standard techniques for age/stage structured matrix models (Caswell 2001). The projected population growth rate (i.e., λ) is used here as the measure of fitness for a given spine production strategy and is calculated as the dominant eigenvalue extracted from the stage-age transition matrix. The quantitative values of projected population growth depend critically on the specific survival values used. Because we do not have specific estimates of *Asplanchna* predation on *B. calyciflorus* having different spine lengths from the field to parameterize the model, we do not evaluate population growth rates directly. Our main interest is in exploring the relative fitness of different spine production strategies when *Asplanchna* abundances are too low to induce spine development but still can impose mortality on *B. calyciflorus* (i.e., relative fitness differences among genotypes that produce spine lengths to different degrees across birth order). Thus, we are interested not in the absolute values of projected population growth rates, but rather the relative fitness differences among different strategies. Other demographic features of the population (e.g., reproductive values, stable stage/age distribution) also scale with the ratio of the minimum and maximum survival values used. The stable stage distribution of birth orders/spine lengths was calculated using the eigenvector associated with the dominant eigenvalue (Caswell 2001). Thus, we can make quantitative statements about population demographic features based on proportional changes in minimum and maximum survival due to spine lengths. Specifically, we compared situations of no selective *Asplanchna* predation (where the minimum and maximum survival values based on spine length/birth order are the same) to various scenarios in which spine length afforded individuals some increase in survival when faced with *Asplanchna* predators. These survival values defined the probabilities of surviving each 12-hour age interval throughout the life cycle.

RESULTS

Results from the model for various survival schedules based on spine lengths are shown in Fig. 2. When there is no, or no selective, *Asplanchna* predation, when minimal and maximal survivals are the same, the frequency of individuals with high birth orders soon becomes extremely low. The low frequency of these individuals occurs after only one generation of parthenogenetic reproduction and is not further reduced when a stable stage (birth order) structure is attained a few generations later (Fig. 2A, top panel). About 60% of the individuals in the population are first or second born, and the frequency of individuals with birth orders >8 is extremely small (~5%). Clearly, early-born females undergo much reproduction before their mothers produce their 16th daughter. This result has a great effect on the distribution of spine lengths in the population, since there are very few late-born individuals with long spines (Fig. 2A, bottom panel). About 60% of the individuals have essentially no posterolateral spines, and only about 10% of the individuals have spine length to body length ratios greater than 0.25 (birth orders >6).

Mortality imposed by *Asplanchna* can appreciably alter spine-length distributions by preferentially eliminating spineless or short-spined individuals and thus increasing the frequency of later-born females with longer spines. Fig. 2B–D illustrates two conclusions. First, the frequency of later birth orders with longer spines increases as the survival of individuals with the longest spines (those with maximal survival probability) increases relative to that of those with no spines (those with minimal survival probability). Second, for any combination in which *Asplanchna* selects for long-spined individuals, this frequency increases in successive generations until a stable stage (birth-order) distribution is reached. When minimal and maximal survivals are set at 0.1 and 0.8 (Fig. 2D), there are essentially no individuals without spines, and the frequency of individuals with spine to body length ratios >0.35 (birth orders >8) is about 90% when a stable birth-order structure is reached.

Selective predation by *Asplanchna* on early-born individuals with no or short defensive spines increases the frequency of long-spined individuals in populations and thus subsequently reduces the susceptibility of populations to *Asplanchna* predation. The effect of various combinations of minimal and maximal survivals on projected population growth rate (λ), and thus relative fitness, is shown in Table 3. As the survival of long-spined individuals (maximal survival) relative to the survival of spineless individuals (minimal survival) increases, so does the fitness of these types relative to types that do not produce spines with higher birth order. The relative increase in fitness depends only on the ratio of minimal and maximal survivals and is independent of absolute survivals.
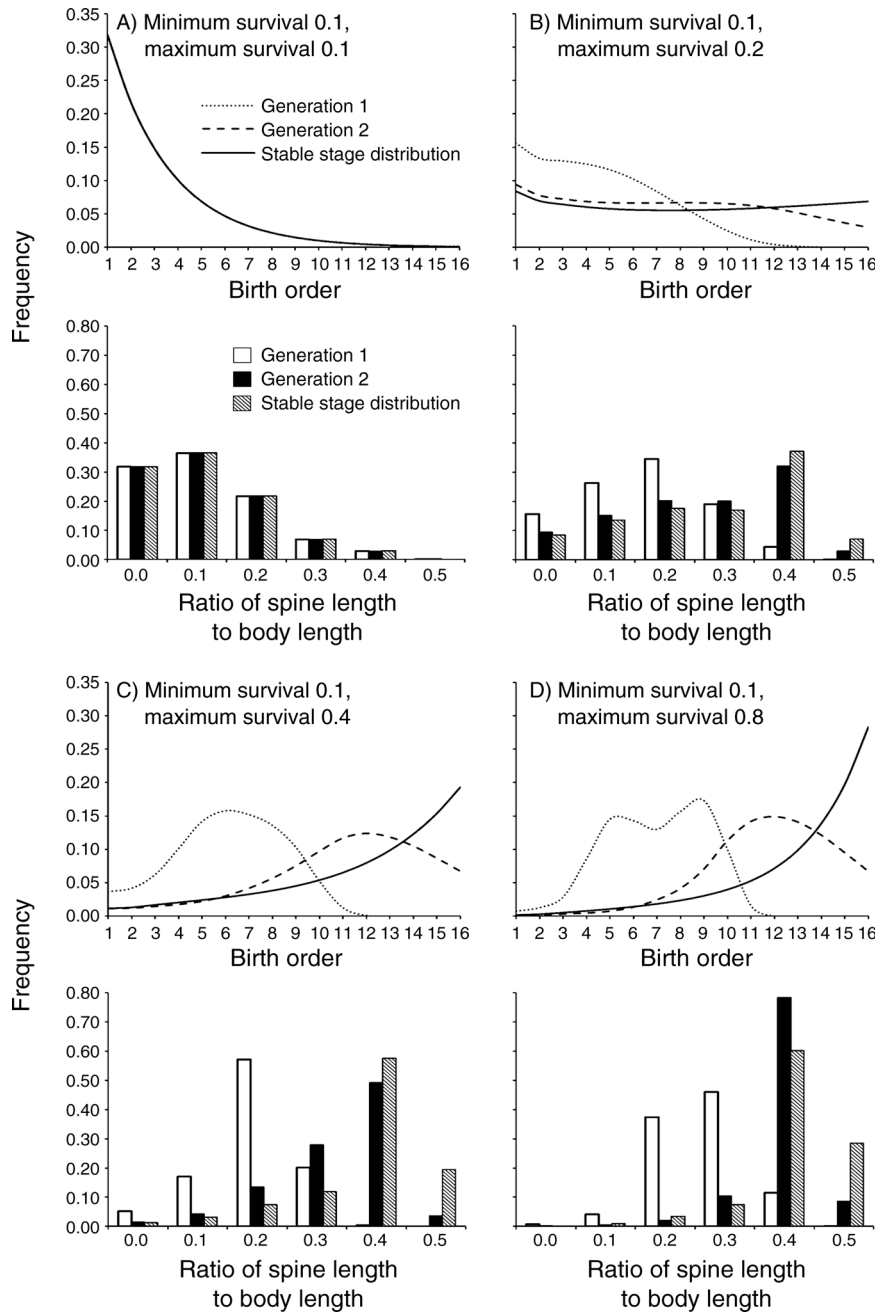
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FIG. 2. Distributions of birth orders and spine-length : body-length ratios (mid-point values) in populations of *Brachionus calyciflorus* under different simulated conditions of *Asplanchna* predation. Each panel shows the results for simulations of a specific scenario of minimum and maximum survival based on spine length. In each panel, the top graph shows the frequency of individuals of each birth order (and thus spine length) after an initial cohort of same-aged individuals completes one life cycle (i.e., after 19 12-hour time intervals, which is identified as one generation here; dotted line), two such generations (i.e., 38 12-hour time intervals; dashed line), and a stable stage distribution (solid line). The bottom graph in each panel shows a frequency histogram of spine-length : body-length ratios for these same time points. In these histograms, we characterize the data as though one were collecting rotifers from a population and measuring spine and body lengths without any knowledge of birth orders. When minimum survival (survival of individuals with no posterolateral spines) and maximum survival (survival of individuals with longest posterolateral spines) are the same (i.e., panel A), there is no, or no selective, *Asplanchna* predation. As maximum survival increases relative to minimum survival (i.e., panels B–D), individuals with the longest spines are increasingly well defended against *Asplanchna* predation.

DISCUSSION

The model results provide strong support for the hypothesis that the maternal-age effect on spine development in *B. calyciflorus* increases fitness whenever *Asplanchna* densities are too low for its kairomone to induce spine development but high enough to impose significant predation. In populations of clones with this trait, selective predation by *Asplanchna* on individuals with no or short posterolateral spines rapidly increases the frequency of late-born offspring with long defensive spines. This, in turn, decreases mortality from *Asplanchna* predation.

The extent to which clones with the maternal-age trait have a fitness advantage over clones without it is determined only by the relative survival of individuals with the longest spines (maximal survival) and ones with the shortest spines (minimal survival); it is independent of actual survival values. For example, the model results show that clones with the maternal effect have a 4.5-fold greater fitness (λ) than those without it whenever maximal survivals (late-born individuals with the longest spines in clones with the maternal trait) are 8 times greater than minimal survivals (first-born individuals of clones with the maternal trait and all individuals of clones without the trait) (Table 3).

In actual laboratory or natural systems, the ratio of maximal and minimal survival values would vary, and absolute survival values would be determined by environmental factors, especially by the abundance of *Asplanchna*. For example, the mortality rate likely would increase with *Asplanchna* density and temperature and decrease with *B. calyciflorus* density. In some small rock pools and ponds, *Asplanchna* and *B. calyciflorus* densities ranged from 0 to 5 $\times 10^3$ and from 0 to 10^4 individuals/L, respectively, and tended to vary inversely (Halbach 1970*a*, *b*).

A very important fact is that the effects of maternal age and *Asplanchna* kairomone on spine development in *B. calyciflorus* are additive. Thus, clones with the maternal trait should have a fitness advantage over clones without it not only when the concentration of *Asplanchna* kairomone is too low to induce any spine development (as in our model results) but also whenever it induces sub-maximal spine development.

In natural communities, *B. calyciflorus* may frequently co-occur with *Asplanchna* when this predator induces no or sub-maximal spine development but still can inflict significant mortality. Under laboratory conditions, the spine-development response of *B. calyciflorus* is very sensitive to *Asplanchna* density, with half-maximal and maximal spine development occurring at *Asplanchna* densities of about 1 and 8 individuals/L, or 1.7 and 11 µg dry mass/L, respectively (Gilbert 2011*a*). However, the probably proteinaceous *Asplanchna* kairomone is very labile (Gilbert 1967); under laboratory conditions at 25° and 15°C, it has a half-life of about 1.5 and 2.5 days, respectively (Halbach 1970*a*). In natural systems, threshold *Asplanchna* densities for spine development

TABLE 3. Model results showing how the fitness of a given survival schedule (as measured by projected population growth rate λ) of *Brachionus calyciflorus* in the face of *Asplanchna* predation is affected by increasing the survival of late-born individuals with maximally long posterolateral spines (maximal survival) relative to that of early-born individuals with no such spines (minimal survival).

Survival probability		Projected population growth rate (λ)	Fitness relative to clone with no spines
Minimal	Maximal		
0.1	0.1	0.147	1.00
0.1	0.2	0.201	1.37
0.1	0.4	0.362	2.47
0.1	0.6	0.515	3.51
0.1	0.8	0.662	4.52
0.01	0.01	0.0147	1.00
0.01	0.02	0.0201	1.37
0.01	0.04	0.0362	2.47
0.01	0.06	0.0515	3.51
0.01	0.08	0.0662	4.52
0.6	0.6	0.879	1.00
0.6	0.7	0.901	1.02
0.6	0.8	0.932	1.06
0.6	0.9	0.980	1.11
0.6	0.95	1.012	1.15
0.6	0.99	1.041	1.18

may be higher than in the laboratory, perhaps because more diverse and abundant microbial communities reduce the half-life of its kairomone.

This possibility is supported by examination of Halbach's (1970*a*) detailed field observations on the relationship between *Asplanchna* density and *B. calyciflorus* spine development. Typically, half-maximal and maximal spine development occurred when *Asplanchna* densities were 2–10 and 6–30 individuals/L, respectively. In one water body, newborn rotifers first developed maximally long spines only when the *Asplanchna* density reached about 10^2 individuals/L; in another water body, this happened when the *Asplanchna* density was almost 10^3 individuals/L. These field data indicate that *Asplanchna* frequently could prey extensively on *B. calyciflorus* before it reaches densities high enough to induce maximal spine development. At such times, clones with the maternal-age effect would have a fitness advantage over those without it.

While the maternal-age effect on spine development in *B. calyciflorus* clearly seems advantageous at times when *Asplanchna* is present but at densities too low for its kairomone to induce pronounced spine development, spine development in the absence of *Asplanchna* could have a cost. However, for several reasons this cost probably is minimal. First, our model results show that, in the absence of *Asplanchna*, the frequency of long-spined individuals decreases to extremely low values after only one generation of female parthenogenesis. Secondly, individuals with long, *Asplanchna*-induced spines can reproduce at the same rate as those with no posterolateral spines (Gilbert 1980, 2012). However, while there seems to be no fixed allocation cost associated with spine development in this rotifer,

individuals with long spines may be less fit in some conditions or biological interactions (Gilbert 2013).

In conclusion, our model results support the hypothesis that the maternal-age effect on spine development in *B. calyciflorus* is a bet-hedging strategy to defend against *Asplanchna* predation both before this predator can induce spine development, and also whenever its kairomone is not sufficiently concentrated to induce maximal spine development. Production of long-spined offspring by older females assures that some individuals in the population always will be well protected against *Asplanchna* predation, and that the frequency of long-spined individuals will rapidly increase and effectively reduce mortality if *Asplanchna* predation does occur.

While all clones of *B. calyciflorus* tested have the potential to develop long spines in the presence of *Asplanchna* (Gilbert 2013), not all clones of a strain exhibit the maternal-age effect on spine development. For example, of eight clones of a strain from Georgia, four exhibited no maternal-age effect, all offspring having no (three clones) or very short (one clone) posterolateral spines, and four showed a pronounced maternal age effect (Schröder and Gilbert 2009). We know nothing about the distribution or frequency of clones with this maternal-age trait in natural populations, but suspect that there may be selection for them whenever *Asplanchna* occurs intermittently and at densities too low for its kairomone to induce maximal spine development.

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