Hydrobiologia (2007) 593:151–157
DOI 10.1007/s10750-007-9065-6

ADVANCES IN ROTIFER RESEARCH

Mate choice and sexual conflict in *Brachionus plicatilis* (Rotifera)

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Abstract Male mate choice in *Brachionus plicatilis* is based on information from coronal receptors and contact chemoreception of a glycoprotein signal on the body surface of females. Male mating behavior is modulated by mating signal quality and strength, which determines male mating persistence and circling intensity. We probe the sensory abilities of males by better resolving their discrimination of female age. Males preferentially initiate mating with young females, but not too young. Males circle 0.2 h old females just hatched from resting eggs only about 1/2 as frequently as 12 h old females. Males are even more discriminating of females with whom they copulate, preferring 3 h old females significantly more than 0.2 h or 6 h old females. In contrast, males cannot distinguish between virgin females and those who have already copulated. Similarly, males circled and copulated with females hatching from resting eggs with equal frequency as those hatching from amictic eggs. The counterpoint to male mate choice is female resistance to mating. When males contact females, the females respond with one of four behaviors: no response, acceleration, foot flipping, or coronal retraction. In 65% of male–female encounters, there was no initial response by females. However, when males began circling females, females accelerated 11.1 times more often than when males were absent. The second type of evasive female behavior was foot-flipping, which tends to knock off males attempting to circle the female. In the presence of circling males, females performed foot-flipping behavior three times more often than in the absence of males. Coronal retraction, where they stop swimming and withdraw their corona, was observed less frequently than acceleration or foot-flipping, and there was no difference in the presence or absence of males. These data are interpreted in the context of sexual conflict, where the behaviors that optimize male and female fitness differ.

Keywords Mate choice · Sexual conflict · Males · Mating · Age · Sex · Swimming · Foot

Introduction

Sexual reproduction in the rotifer *Brachionus plicatilis* is induced by the accumulation of a mixis induction protein produced by the rotifers themselves (Snell et al., 2006). Unfertilized mictic (sexual)

Guest editors: S. S. S. Sarma, R. D. Gulati, R. L. Wallace, S. Nandini, H. J. Dumont & R. Rico-Martínez
Advances in Rotifer Research

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females produce males who have a sex-specific swimming pattern and engage in stereotypical mating behavior upon contacting young, conspecific females (Snell, 1989; Snell et al., 1995; Gómez & Serra, 1996). Males have keen sensory capabilities and are able to discriminate females based on species, sex, age, and reproductive status. Male mate choice is based on information from coronal receptors and contact chemoreception of a glycoprotein signal on the body surface of females (Snell et al., 1995). Male mating behavior is modulated by mating signal quality and strength, which determines male mating persistence and circling intensity. Mate selection for males is therefore non-random and based on preference for specific female traits.

In contrast to males, females do not exhibit clear mate choice. The role of females in mating is more passive, with no searching for mates or signaling them from a distance. Females elicit male mating behavior through signals on their body surface, but males can only detect these upon contact. Other than this chemical signaling, females seem to have little influence on the decision of males to mate. However, once male contact occurs, females could either facilitate mating or resist. Evidence of female resistance to mating was first provided by Gómez & Serra (1995), who reported that females often contracted their coronas, flipped their foot, or accelerated upon contact with males. This behavior was interpreted as evasive, making it more difficult for males to continue circling. Similar evasive female behavior was reported in *B. calyciflorus* by Gilbert & Walsh (2005).

In this paper we further probe the sensory abilities of males by better resolving their discrimination of female age, examining male ability to distinguish between virgin females and those who have already copulated, and to differentiate between amictic and resting egg hatchlings. Female age is an important characteristic to males because older females are not fertilizable (Snell & Childress, 1987). Mating with females who have already copulated could lower male mating success because of first sperm precedence in fertilizing resting eggs. Resting egg hatchlings are always amictic females, so male insemination of these females would never produce resting eggs.

We also examine the possibility of cryptic female mate choice through resistance to mating by recording female responses to male contact. These data are interpreted in the context of sexual conflict (Arnqvist & Rowe, 2005), where the behaviors that optimize male and female fitness differ. Several sources of sexual conflict in rotifers are identified resulting from their reproductive physiology.

Materials and methods

Rotifers used in these experiments were hatched from resting eggs of *Brachionus plicatilis* Russian strain, which is a member of the Manjavacas clade (Gómez et al., 2002). Resting eggs of this population have been maintained in lab since 1983, and periodically produced in mass cultures. Resting eggs were hatched at 25°C in fluorescent light of 2,000 lux in 15 ppt artificial seawater (ASW) prepared from deionized water and Instant Ocean salts. Rotifers were fed the green alga *Tetraselmis suecica* cultured at 25°C in F medium (Guillard, 1983) at 15 ppt salinity. All experiments were conducted at 25°C in 15 ppt ASW.

Resting eggs were hatched by incubating in ASW for 24 h. The hatching dish was frequently checked so that the age of all experimental females was determined ±10 min. In a few experiments, females hatched from amictic eggs were utilized. In these cases, ovigerous females were isolated in a small volume and checked every 10 min for hatchlings. Hatchlings were removed and held in 250 µl ASW in a multi-well plate without feeding until they reached a certain age and were tested in a mating bioassay. Males used in experiments were isolated by filtration from log-phase 200 ml mass cultures. Females were retained by a 90 µm filter, but males passed through and were collected on a 53 µm filter and resuspended in fresh ASW. Males were of mixed ages, but only fast swimming males were utilized in the bioassay. We were careful to match ASW salinity with mass culture salinity because males had reduced propensity to mate when transferred into medium that differed by more than 3–4 ppt.

The mating bioassay was modified from that described by Snell & Hawkinson (1983) and was conducted in round-bottom wells of a 96-well plate. Seven males were transferred to the well and the volume was reduced to about 20 µl by removing ASW with a micropipette. The test female was introduced by transfer of a small volume with a micropipette. Male and female behavior was observed for 5 min under a dissecting microscope at 12× magnification and simultaneously videotaped. The number of male–female encounters, circlings, and copulations were recorded for eight replicate females of each age for a total of 64 mating bioassays (eight ages × eight replicates). Percent circling and copulation was calculated as the number of circlings or copulations divided by the total number of encounters.

The responses of 10 females of age 3 h to males were scored from the videotapes made during mating bioassays. For each female, an observation period ranging from 28 to 140 s was identified when males were circling the female. For the same female, a 83–190 s period, where males were absent was also identified. For each of these observation periods, we calculated the rate per minute of female responses when males were circling and when males were absent.

The responses to male–female encounters were classified as follows. When males contacted females with their coronas it was counted as an encounter. It was scored as circling when males moved at least two revolutions around the female while maintaining coronal contact. Copulation was recorded when males lost coronal contact and attached to females by their penis, typically at the female's corona. Female responses to male contact were scored as no response when females maintained their swimming speed and direction. Acceleration was recorded when females were observed to clearly increase their swimming speed after male contact or during circling. Acceleration was quantified afterwards using a motion analysis system (Motion Analysis Corporation, http://www.motionanalysis.com) that estimated swimming speed frame by frame during the acceleration event. Foot-flipping was recorded when females moved their foot toward and away from their body in a series of thrashing motions. The foot acted as a lever causing a female's body to jerk erratically from side to side. Corona retraction was scored when females pulled their corona back within their lorica, halting swimming, and increasing hydrostatic pressure in the pseudocoelom.

Significant effects of female age on male circling and copulation were determined by one-way analysis of variance using eight female ages as the independent variable, each with eight replicates. Analysis of variance was performed with arcsine transformed percent circling and copulation, but nearly identical results as the untransformed data were obtained, so the latter are presented. Means comparison was performed with a Tukey–Kramer HSD analysis. Significance of female acceleration, foot-flipping and coronal retraction in the presence and absence of males was determined using a *t*-test. All calculations were made with the statistical program JMP (SAS Institute, http://www.jmp.com) on a G5 iMAC computer.

Results

Male *B. plicatilis* preferentially initiate mating with young females, but not too young (Fig. 1). Males circle 0.2 h old females just hatched from resting eggs only about ½, as frequently as 12 h old females. Similarly, males initiate mating with 12 h old females about twice as often as 24 h old females (Table 1). Even though males prefer younger females, they will still circle 72 h old females. Male *B. plicatilis* are even more discriminating of females with whom they copulate, preferring females that differ in age by only a few hours (Fig. 1). Three hour old females elicited significantly more male copulations than 0.2 h or 6 h old females (Table 1).

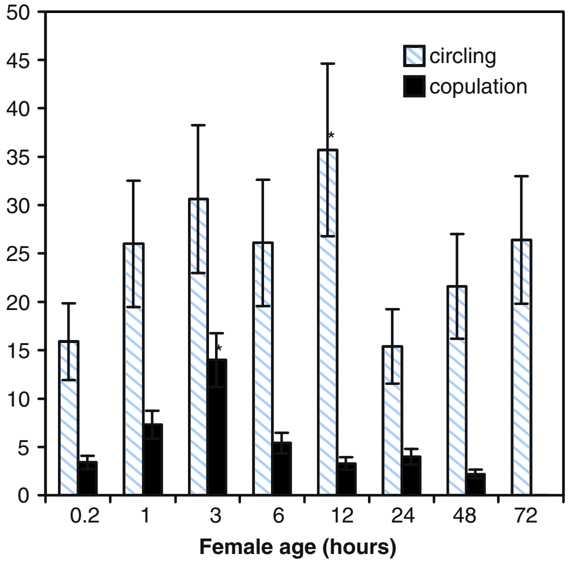

Fig. 1 Effect of female age on male circling and copulation. Asterisks indicate significance differences in male circling or copulation with females. Vertical lines indicate standard errors

Table 1 One-way ANOVA and Tukey–Kramer HSD for male circling and copulation with females of various ages

Circling					
Source	DF	Sum of squares	Mean square	F ratio	Prob > F
Female age	7	3370.4	481.5	3.046	0.0073
Error	72	11380	158.1		
Total	79	14751			
Copulation					
Female age	7	1190.9	170.1	7.901	<0.0001
Error	72	1550.4	21.53		
Total	79	2741.3			
Tukey–Kramer HSD					
Fem age (h)	Group	Mean % circling	Fem age (h)	Group	Mean % cop
12	A	35.7	3	A	13.6
3	AB	30.6	1	AB	7.3
72	AB	26.4	6	BC	5.4
6	AB	26.1	24	BC	4.0
1	AB	26.0	0.2	BC	3.4
48	AB	21.6	12	BC	3.3
0.2	B	15.9	48	BC	2.1
24	B	15.4	72	C	0

Although male discrimination of mates by female age is well-developed, males cannot distinguish between virgin females and those who have already copulated with another male. Virgin females experienced 11.9 ± 1.9 male encounters before copulation as compared to 12.0 ± 2.3 encounters for females who had previously copulated. Likewise, males circled and copulated with females hatching from resting eggs with equal frequency as those hatching from amictic eggs (circling: 24.8% ± 4.8 vs. 24.7% ± 5.6, copulation: 5.7% ± 1.6 vs. 6.1% ± 4.8).

The counterpoint to male mate choice is female resistance to mating. When males contact females, the females respond with one of four behaviors: no response, acceleration, foot flipping, or coronal retraction. In 65% of male–female encounters, there was no initial response by females. No evasive behaviors were performed and swimming speed and trajectory remained steady. However, when males began circling females, females accelerated 11.1 times more often than when males were absent (Fig. 2). This difference is significant by *t*-test (df = 10, $t = -4.16$, $P = 0.002$). Females swimming at 0.3–1 mm/s accelerated swimming 3–18 times faster, topping out at 5.5 mm/s. Some females accelerated at first contact with males and others accelerated after the males began circling.

The second type of evasive behavior exhibited by females was foot-flipping. Foot-flipping tends to knock off males attempting to circle the female. In *B. plicatilis* the foot is relatively large, representing 15% of body area and 69% of body length. In the presence of circling males, females performed foot-flipping behavior three times more often than in the absence of males (Fig. 2). This difference if significant by *t*-test (df = 12, $t = -6.42$, $P < 0.0001$). A third type of evasive behavior by females is coronal retraction, where they stop swimming and withdraw their corona. We observed this behavior less frequently than acceleration or foot-flipping, and there was no difference in the rate of coronal retraction in the presence or absence of males (df = 18, $t = 1.27$, $P = 0.222$).

Discussion

Male *B. plicatilis* clearly exhibit mate choice, discriminating species and geographical populations (Snell & Hawkinson, 1983; Gómez & Serra, 1995; Rico-Martinez & Snell, 1995; Gómez & Snell, 1996; Ortells et al., 2000), males versus females, young versus old females (Gómez & Serra, 1996), and to a limited extent mictic versus amictic females (Gómez & Serra, 1996). In this paper we showed that males discriminate among

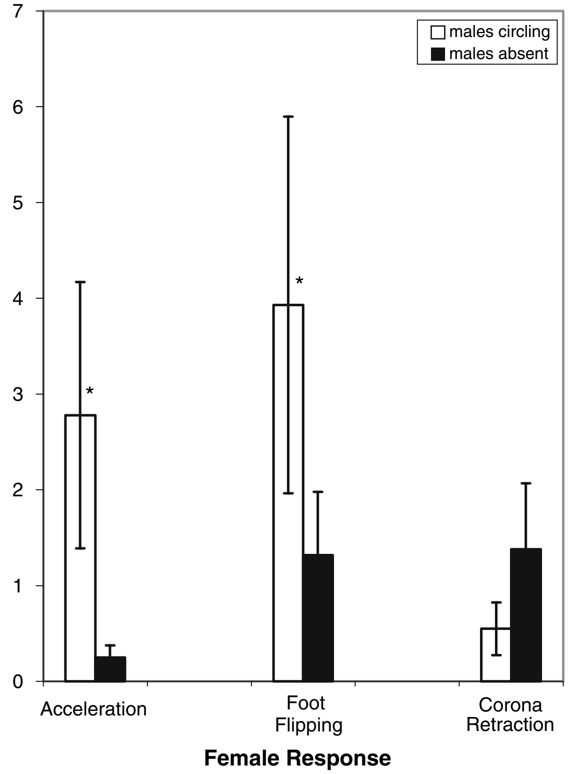

Fig. 2 Female resistance to male mating attempts. Asterisks indicate significance differences in female behavior when males circle compared to when males are absent. Vertical lines indicate standard error

females that differ in age by only a few hours and preferentially copulate with 3 h old females. We further showed that males cannot discriminate virgin females from those who have already copulated, nor can they discriminate resting egg hatchlings (always amictic females) from amictic egg hatchlings (amictic or mictic females). Similar male mate choice has been described for other monogonont species including *B. calyciflorus* (Gilbert, 1963; Gilbert & Walsh, 2005), *Asplanchna brightwelli* (Aloia & Moretti, 1973), and *Epiphanes senta* (Schröder, 2003).

Male fitness is not only determined by their chemosensory abilities to discriminate potential mates, but also determined by the persistence of male mating behavior. Those males best able to overcome the evasive behaviors of females are the ones most likely to successfully copulate. This includes persisting in circling as females foot-flip or accelerate and localizing the corona to insert the penis for insemination.

Female resistance to mating has been clearly demonstrated by their acceleration and foot-flipping behavior. This creates a trade-off between attraction versus resistance. Females put considerable effort into chemical signals that they use to elicit male mating behavior. Yet, when males contact females to initiate mating, females actively evade male mating attempts instead of facilitating them. Two hypotheses have been put forth to explain female resistance to mating. The first suggests that mating has deleterious effects on females (Arnqvist & Rowe, 2005). This may be the case with brachionids with their hypodermic insemination (Gilbert, 1988) that may be traumatic for females. Insertion of the penis through the coronal membrane may lead to loss of pseudocoelomic fluid and/or the introduction of pathogens. The second hypothesis suggests that female resistance is a barrier to screen out weak males (Eberhard, 2002; Eberhard & Cordero, 2003). It is supposed that female foot-flipping and acceleration make it more difficult for males to maintain circling behavior around females as they attempt to find the corona for insemination. Only the strongest, most persistent males will be able to inseminate in the face of female resistance. More research is needed to determine, which of these hypotheses best accounts for mating resistance by female rotifers.

In this study we found that female foot-flipping and acceleration were the two most common means of female resistance. Coronal retraction occurred, but less frequently and not differentially in the presence of males. Coronal retraction during *B. plicatilis* male circling has been noted by Gómez & Serra (1995). They described it as female mating resistance, but it was not quantified. Gilbert & Walsh (2005) also reported coronal retraction by female *B. calyciflorus* during male circling and that it was differential among geographical populations. Females from Texas and Australia populations often retracted their coronas when circled by males from a Florida population, but rarely did so when circled by males of their own population. These observations suggest that females can discriminate males from their own population and increase their level of resistance to unfamiliar males.

Sexual conflict arises when different behavior maximizes fitness for each sex (Chapman et al., 2003; Arnqvist & Rowe, 2005). Rotifer reproductive physiology provides the basis for sexual conflict in a

Table 2 Causes of sexual conflict in monogonont rotifers.

Males	Females
• Multiple matings (∼10), polygamy	• Single insemination (2–3 resting eggs)
• Sperm precedence?	• Sperm competition?
• No parental care	• Limited parental care (carry eggs)
• Fitness limited by access to female gametes	• Fitness limited by quality of male gametes
• High variance in mating rate	• Mating rate consistent
• Search costs high	• Do not search for mate
• Male persistence—circling intensity, insemination rate, mate choice	• Female resistance to circling—acceleration, foot flipping
• Mating costs—increased predation, expenditure of limited energy	• Mating costs—increased predation, reduced foraging, traumatic insemination

variety of traits (Table 2). For example, newborn rotifer males have about 30 sperm (Snell & Hoff, 1987) and deliver about three sperm per copulation (Snell & Childress, 1987). This enables them to perform about 10 inseminations before their sperm are exhausted. Males are therefore polygamous and they do not contribute to parental care. In their short lifespan, male fitness is usually limited by access to female gametes, so their fitness is maximized by finding and inseminating as many young mictic females as possible. Population densities during mixis in natural *B. plicatilis* populations are such that males typically encounter enough females so that a choice strategy is favored by selection (Gómez & Serra, 1996). This means that males will commonly exhaust their sperm during their reproductive lifetime. Males therefore maximize their fitness by choosing young females for copulation. In contrast, fertilized mictic females produce only 2–3 resting eggs (Snell & Childress, 1987), which are likely fertilized by a single male. With their entire resting egg production based on one fertilization, optimal fitness for females is also to be choosy and discriminate male quality. One way to achieve this is by female resistance in mating through foot-flipping and acceleration. This allows only the highest fitness males to overcome this barrier and successfully inseminate. However, whether male screening for genetic benefits better explains rotifer female resistance than direct selection to avoid male imposed mating costs (injury, infection) remains to be determined (Chapman et al., 2003).

There are a number of intriguing questions remaining. Do females adjust their level of resistance according to their perception of male fitness? How do females sense and evaluate male fitness? Can males discriminate high fitness females, those with high fecundity or the ability to produce high quality resting eggs? Do males adjust their level of persistence to match their perception of female fitness? How do they sense and evaluate female fitness? Answering these questions will provide insight into rotifer male and female strategies for mating and the selection pressures directing their evolution.

Acknowledgment This work was supported by the National Science Foundation grants BE/GenEn MCB-0412674 to TWS.

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