

食物浓度和品系对萼花臂尾轮虫生殖期 历时和混交雌体形成的影响

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摘要:应用单个体培养方法比较研究了藻类食物浓度(2.0、5.0、8.0 和 11.0×10^6 cells/mL)和品系对采自广州、青岛和芜湖等地的萼花臂尾轮虫生殖期历时、后代数和后代中混交雌体百分率的影响。结果表明,食物浓度对轮虫的生殖期历时和轮虫全部后代中的混交雌体百分率有显著的影响,但对轮虫的总后代数无显著的影响。品系对轮虫的生殖期历时、总后代数和全部后代中的混交雌体百分率均有显著影响。3 品系间,广州品系轮虫的生殖期历时最长,为 (4.53 ± 0.19) d;总后代数最多达 (18.75 ± 0.26) 个;全部后代中的混交雌体百分率最低,仅为 $4.32\% \pm 0.51\%$ 。而青岛品系轮虫全部后代中的混交雌体百分率最高,达 $49.90\% \pm 2.15\%$;其生殖期历时和总后代数与芜湖品系间均无显著的差异。食物浓度和品系间的交互作用仅对轮虫全部后代中的混交雌体百分率有显著影响。3 品系中,仅芜湖品系轮虫的生殖期历时、总后代数和全部后代中的混交雌体百分率受食物浓度的显著影响;母体年龄对轮虫每天所产后代中的混交雌体百分率的影响因轮虫品系的不同和培养时所用的食物浓度的不同而异。

关键词:萼花臂尾轮虫;品系;食物浓度;生殖期历时;混交雌体百分率

文章编号:1000-0933(2005)08-1831-07 中图分类号:Q959.181 文献标识码:A

Effects of food concentration on the duration of reproductive period and the formation of mictic female in different strains of freshwater rotifer *Brachionus calyciflorus*

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Abstract: Strain is one of the most important internal factors which determine the mictic female formation and resting egg production in rotifers. Studies on the differences of mictic female formation among different rotifer strains not only can accumulate material for demonstrating the role of genetic factors in determining mictic female formation but also are important for selecting suitable rotifer strains for the mass production of resting eggs. In this study, the effect of food concentration (2.0, 5.0, 8.0 and 11.0×10^6 cells/mL) of algae *Scenedesmus obliquus* on the duration of reproductive period, the number of offspring and the percentage of mictic females in the offspring of three *Brachionus calyciflorus* strains collected from Guangzhou, Wuhu and Qingdao of China respectively was studied with replicated individual cultures at $(25 \pm 1)^\circ\text{C}$. The results showed that the effect of food concentration on the duration of reproductive period and the percentage of mictic females in all the offspring were significant, but there was no effect on the number of total offspring. Among the four food concentrations, the reproductive period of the rotifer cultured at 8.0×10^6 cells/mL of *Scenedesmus* was the longest (4.19 days), whereas the differences in the

基金项目:国家自然科学基金资助项目(39870158);安徽省优秀青年基金资助项目(04043050);安徽省自然科学基金资助项目(00042416)

收稿日期:2004-04-20; **修订日期:**2005-02-24

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Foundation item: National Natural Science Foundation of China (No. 39870158); Excellent Youth Foundation (No. 04043050) and Natural Science Foundation of Anhui Province, China (No. 00042416)

Received date: 2004-04-20; **Accepted date:** 2005-02-24

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other three food concentrations were not significant. The percentage of mictic females in all the offspring produced by the rotifer in her lifetime at the food concentration of 5.0×10^6 cells/mL was the highest (43.35%), and that at 11.0×10^6 cells/mL was the lowest (16.87%). Strain influenced significantly the duration of reproductive period, the number of total offspring and the percentage of mictic females in all the offspring. Among the three strains, Guangzhou strain had the longest reproductive period (4.53 days), produced the largest number of offspring (18.75 individuals) in her lifetime, and had the lowest percentage of mictic females (4.32%) in all the offspring. Qingdao strain had the highest percentage of mictic female (49.90%) in all the offspring, but its duration of reproductive period and number of offspring produced in her lifetime were both similar to those of Wuhu strain. The interaction between food concentration and strain influenced markedly only on the percentage of mictic females in all the offspring. Among the three strains, the duration of reproductive period, the number of total offspring and the percentage of mictic females in all the offspring were significantly influenced by food concentration only for Wuhu strain. The effect of the age of rotifer mother on the percentage of mictic females in the offspring produced daily by her differed with both rotifer strain and food concentration, and only the percentage of mictic females in the offspring produced daily by Wuhu and Qingdao strains at both 5.0×10^6 and 8.0×10^6 cells/mL of food concentrations lowered significantly with their ages.

Key words: *Brachionus calyciflorus*; strains; food concentration; duration of reproductive period; percentage of mictic females

混交雌体的产生是轮虫由孤雌生殖向有性生殖转变及休眠卵形成的第一步,受众多外源性和内源性因素的影响和制约^[1, 2]。有关外源性因素对轮虫混交雌体形成的影响研究已有较多报道。目前已知,生育酚对西氏晶囊轮虫(*Asplanchna sieboldi*)的混交雌体形成有间接的诱导作用,并能与种群密度协同作用使混交雌体百分率明显升高^[3];连续光照(L:D=24:0)或较长时间的光照(L:D=15:9)可促进龙大椎轮虫(*Notommata copeus*)和微趾椎轮虫(*N. codonella*)以及鼠异尾轮虫(*Trichocerca rattus*)混交雌体的形成^[4, 5];几种臂尾轮虫混交雌体的形成与其种群密度间具有明显的正相关^[6]。此外,急剧降温、培养液 pH 值或盐度、食物的质和量均能显著地影响萼花臂尾轮虫(*Brachionus calyciflorus*)和褶皱臂尾轮虫(*B. plicatilis*)后代中的混交雌体百分率^[7~12]。而有关内源性因素如母体年龄、孤雌生殖的累积世代数和品系等对轮虫混交雌体形成的影响研究相对较少^[13~17],其中对轮虫品系间混交雌体形成的比较研究更不多见^[14, 16, 18]。为此本文研究了食物浓度对 3 品系萼花臂尾轮虫生殖期历时、后代数和后代中混交雌体百分率的影响,旨在为轮虫休眠卵形成机理的进一步研究积累资料,同时为轮虫的批量培养或休眠卵的规模化生产提供参考。

1 材料与方法

1.1 轮虫的来源与培养

实验用萼花臂尾轮虫分别由我国不同水系的水体沉积物中的休眠卵孵化而得。其中,广州品系(GZ Strain)的轮虫采于广州市华南师范大学校园内一池塘,属珠江水系;芜湖品系(WH Strain)的轮虫采于芜湖市镜湖,属长江水系;青岛品系(QD Strain)的轮虫采于青岛市中山公园内一池塘,属黄河水系。所用的轮虫培养液采用 Gilbert 的配方^[19],所用的饵料系由 HB-4 培养基^[20]培养的、处于种群指数增长期的斜生栅藻(*Scenedesmus obliquus*);斜生栅藻经离心浓缩后,用轮虫培养液配制成所需浓度后使用。

1.2 预培养

将三品系轮虫分别置于 4 种食物浓度(2.0 、 5.0 、 8.0 和 11.0×10^6 cells/mL)下进行预培养,所用培养器皿为 10mL 的玻璃试管,预培养在(25 ± 1)℃、自然光照(光照强度约 120lx;L:D=16:8)下进行。预培养过程中,通过每天去除一部分个体而使轮虫种群被始终控制在指数增长期。预培养时间在 7d 以上。

1.3 单个体培养

预培养结束时,分别从各试管中随机吸取带卵的非混交雌体约 100 个置于培养皿中继续培养,培养条件与预培养相同。之后从中吸取龄长约 2h 的幼体 48 个置于两块特制的带孔塑料板(每板 24 孔,每孔容积约 0.6mL)中继续培养。每孔加入约 0.4mL 轮虫培养液,培养条件与预培养相同。培养过程中每间隔 8h 观察 1 次,记录每孔内孵化出的轮虫幼体数,并把幼体分别吸出进行单个体培养至产卵以便根据卵的大小和形态确定该带卵雌体的类型。

1.4 雌体类型的鉴别

非混交雌体 产非混交卵。非混交卵无色、透明,可见胚胎发育过程,尤其在胚胎发育早期可见咀嚼器的几丁质板;其长、宽一般为 $100 \sim 110 \mu\text{m}$, $75 \sim 85 \mu\text{m}$ 。

混交雌体 单个体培养状态下的混交雌体为产雄卵的混交雌体,其所产的雄卵数目一般在 5 个以上,卵体积约为非混交卵

的 2/3。

1.5 参数的定义及计算方法

生殖期(产卵期)历时:指从第 1 枚卵产出到最后 1 枚卵产出所经历的时间。

混交雌体百分率=混交雌体数/(混交雌体数+非混交雌体数)。

2 结果与分析

2.1 轮虫的生殖期历时

各食物浓度下 3 品系轮虫的生殖期历时如图 1 所示。双因素方差分析结果表明,食物浓度和品系均对轮虫的生殖期历时有显著影响($p<0.05$),但两者间无显著的交互作用($p>0.05$)。4 种食物浓度下,食物浓度为 $8.0\times10^6\text{cells/mL}$ 时轮虫的生殖期最长,其余 3 种食物浓度间无显著差异。3 品系间,广州品系轮虫的生殖期最长,芜湖和青岛品系间无显著的差异。

芜湖品系轮虫的生殖期在食物浓度为 $8.0\times10^6\text{cells/mL}$ 时显著长于食物浓度为 $2.0\times10^6\text{cells/mL}$ 时,其他各浓度间无显著的差异;而青岛和广州品系轮虫的生殖期历时不受食物浓度的显著影响。

2.2 轮虫的后代数

不同食物浓度下 3 品系轮虫一生中所产的全部后代数目(总后代数)如图 2 所示。双因素方差分析结果表明,食物浓度对轮虫的总后代数无显著影响($p>0.05$),但品系却对其有显著影响($p<0.05$),食物浓度和品系间的交互作用对其也无显著的影响($p>0.05$)。3 品系间,广州品系轮虫的总后代数最多,而青岛和芜湖品系间无显著差异。

芜湖品系轮虫的总后代数在食物浓度为 $2.0\times10^6\text{cells/mL}$ 时最少,其他 3 个浓度间无显著的差异;而青岛和广州品系轮虫的总后代数不受食物浓度的显著影响。

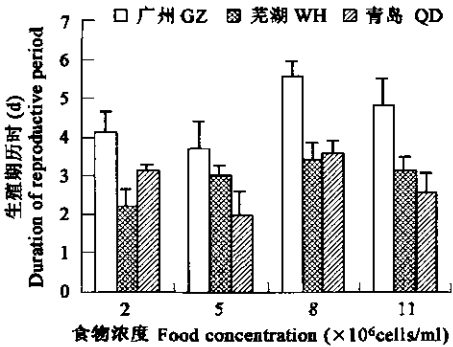


图 1 不同食物浓度下 3 品系萼花臂尾轮虫的生殖期历时

Fig. 1 The duration of reproductive period of three *B. calyciflorus* strains fed on different concentrations of diets

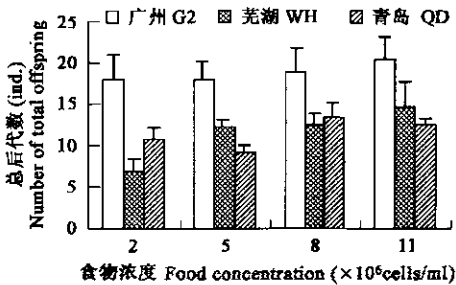


图 2 不同食物浓度下 3 品系萼花臂尾轮虫的总后代数

Fig. 2 The number of total offspring of three *B. calyciflorus* strains fed on different concentrations of diets

图 3 显示了各食物浓度下 3 品系轮虫每天所产的后代数。回归分析结果表明,各食物浓度下 3 品系轮虫每天所产的后代数均与母体的年龄呈曲线相关(表 1)。

表 1 不同食物浓度下 3 品系萼花臂尾轮虫雌体年龄(X,d)与其每天所产的后代数(Y,ind.)之间的关系

Table 1 The relationships between the age of females(X,d) and the number of offspring(Y,ind.) produced daily by three *B. calyciflorus* strains fed on different concentrations of diets

食物浓度 Food concentration	品系 Strains	回归方程 Regression equation	显著性检验 Significant test
$2.0\times10^6\text{cells/mL}$	广州 GZ	$Y=0.051X^2-1.121X+6.047$	$R^2=0.504, p<0.01$
	芜湖 WH	$Y=0.350X^2-3.233X+7.140$	$R^2=0.630, p<0.01$
	青岛 QD	$Y=0.123X^2-1.725X+5.970$	$R^2=0.512, p<0.01$
$5.0\times10^6\text{cells/mL}$	广州 GZ	$Y=-0.019X^2-1.035X+6.631$	$R^2=0.517, p<0.01$
	芜湖 WH	$Y=0.460X^2-4.340X+10.400$	$R^2=0.757, p<0.01$
	青岛 QD	$Y=0.432X^2-4.150X+9.560$	$R^2=0.767, p<0.01$
$8.0\times10^6\text{cells/mL}$	广州 GZ	$Y=0.050X^2-1.081X+5.902$	$R^2=0.527, p<0.01$
	芜湖 WU	$Y=-0.000X^2-1.346X+6.655$	$R^2=0.694, p<0.01$
	青岛 QD	$Y=0.200X^2-2.512X+7.834$	$R^2=0.631, p<0.01$
$11.0\times10^6\text{cells/mL}$	广州 GZ	$Y=0.089X^2-1.732X+8.324$	$R^2=0.641, p<0.01$
	芜湖 WH	$Y=0.297X^2-3.470X+10.052$	$R^2=0.547, p<0.01$
	青岛 QD	$Y=0.546X^2-5.091X+11.500$	$R^2=0.836, p<0.01$

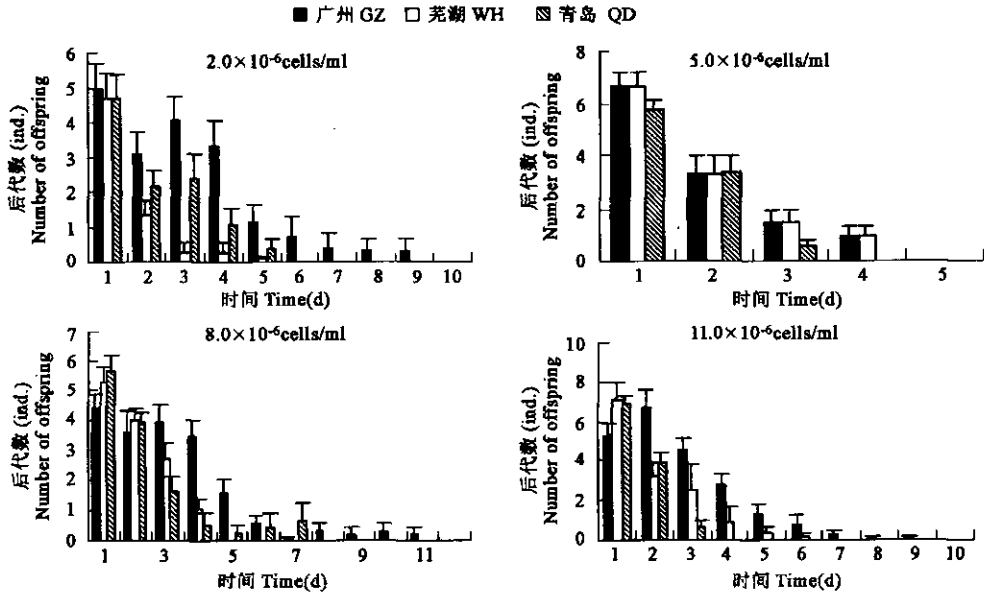


图3 不同食物浓度下3品系萼花臂尾轮虫雌体每天所产的后代数(母体的年龄从第1个幼体孵出时开始计算)

Fig. 3 The number of offspring produced daily by three *B. calyciflorus* strains fed on different concentrations of diets (The age of females was supposed as zero when the first neonate was hatched)

2.3 轮虫所产全部后代中的混交雌体百分率

图4显示了各食物浓度下3品系轮虫所产的全部后代中的混交雌体百分率。双因素方差分析结果表明,食物浓度和品系以及两者间的交互作用均对轮虫全部后代中的混交雌体百分率有显著影响($p < 0.05$)。4种食物浓度下,食物浓度为 5.0×10^6 cells/mL时轮虫全部后代中的混交雌体百分率最高,食物浓度为 11.0×10^6 cells/mL时最低。3品系间,广州品系轮虫全部后代中的混交雌体百分率最低,而青岛品系最高。

芜湖品系轮虫所产全部后代中的混交雌体百分率在食物浓度为 5.0×10^6 cells/mL时最高, 11.0×10^6 cells/mL时最低, 2.0×10^6 cells/mL和 8.0×10^6 cells/mL间无显著的差异;而青岛和广州品系轮虫所产全部后代中的混交雌体百分率不受食物浓度的显著影响。

回归分析结果表明,芜湖品系轮虫所产全部后代中的混交雌体百分率(Y)与食物浓度($X, \times 10^6$ cells/mL)间呈曲线相关,回归方程为:

$$Y = -0.0126X^2 + 0.1283X + 0.1807 \quad (R^2 = 0.589, p < 0.01)$$

2.4 母体在不同年龄时所产后代中的混交雌体百分率

各食物浓度下3品系轮虫每天所产后代中的混交雌体百分率如图5所示。回归分析结果表明,芜湖品系和青岛品系轮虫均在食物浓度为 5.0 和 8.0×10^6 cells/mL时每天所产后代中的混交雌体百分率随母体年龄的增大而降低(表2)。

3 讨论

3.1 食物浓度和品系与轮虫的生殖期历时

已有研究结果表明,食物浓度对轮虫非混交雌体生殖期历时的影响常因轮虫种类的不同而异。低浓度的藻类食物对中吻轮虫(*Enicetruncus linnhei*)和褶皱臂尾轮虫的生殖期历时无显著的影响^[21],但使大肚须足轮虫(*Euchlanus dilatata*)和红臂尾轮虫(*B. rubens*)的生殖期缩短^[22,23];高浓度的食物使壶状臂尾轮虫(*B. urceolaris*)的生殖期显著缩短^[24]。本研究中,作者发现食物浓度对轮虫非混交雌体生殖期历时的影响还因轮虫品系的不同而异。3品系萼花臂尾轮虫中,只有芜湖品系轮虫的生殖期历时显著地受食物浓度的影响,表现为在食物浓度为 8.0×10^6 cells/mL时显著长于食物浓度为 2.0×10^6 cells/mL时,其他各浓度

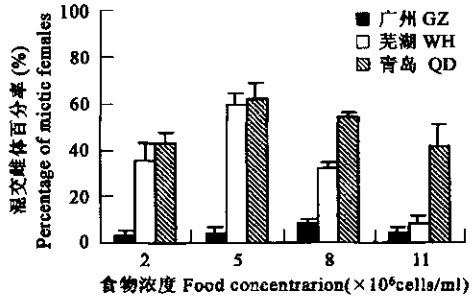


图4 不同食物浓度下3品系萼花臂尾轮虫全部后代中的混交雌体百分率

Fig. 4 The percentage of mictic females in all the offspring of three *B. calyciflorus* strains fed on different concentrations of diets

间无显著的差异;此与 King 对大肚须足轮虫的研究结果^[22]相似。

表 2 不同食物浓度下 3 品系萼花臂尾轮虫雌体年龄与其每天所产后代中的混交雌体百分率间的关系
Table 2 The relationships between the age of females and the percentages of mictic females in the offspring produced daily by three *B. calyciflorus* strains fed on different concentrations of diets

食物浓度 Food concentration	品系 Strains	回归方程 Regression equation	显著性检验 Significant test
5.0×10 ⁶ cells/mL	芜湖 WH	$Y=0.024X^2-0.319X+0.999$	$R^2=0.523, p<0.01$
	青岛 QD	$Y=0.024X^2-0.355X+1.034$	$R^2=0.477, p<0.01$
8.0×10 ⁶ cells/mL	芜湖 WH	$Y=0.032X^2-0.305X+0.749$	$R^2=0.436, p<0.01$
	青岛 QD	$Y=0.030X^2-0.360X+1.040$	$R^2=0.631, p<0.01$

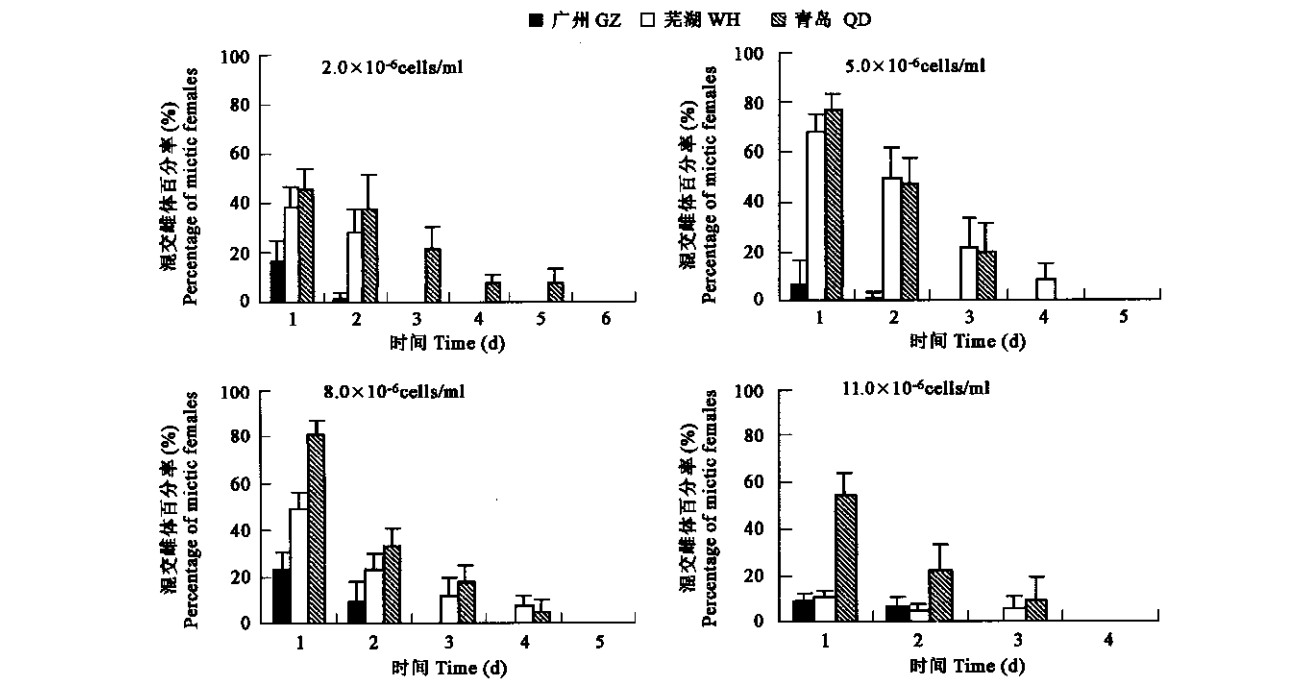


图 5 不同食物浓度下 3 品系萼花臂尾轮虫每天所产后代中的混交雌体百分率(母体的年龄从第 1 个幼体孵出时开始计算)
Fig.5 The percentage of mictic females in the offspring produced daily by three *B. calyciflorus* strains fed on different concentrations of diets(The age of females was supposed as zero when the first neonate was hatched)

3.2 食物浓度和品系与轮虫最大生殖潜力的可实现程度

一些研究结果表明,轮虫的最大生殖潜力是一定的,不同种类的轮虫具有不同的最大生殖潜力。3 品系萼花臂尾轮虫间,广州品系轮虫的总后代数最多,而青岛和芜湖品系间无显著差异。可见,轮虫的最大生殖潜力也因品系的不同而存在着差异。

轮虫最大生殖潜力的可实现程度受环境因子的影响,其中食物的可得性和丰度是轮虫实现其最大生殖潜力的首要条件^[25]。芜湖品系轮虫的总后代数在食物浓度为 2.0 × 10⁶cells/mL 时最少,其他 3 个浓度间无显著的差异;而青岛和广州品系轮虫的总后代数不受食物浓度的显著影响。考虑到所使用的斜生栅藻的最大浓度已达 11.0 × 10⁶cells/mL,预培养过程中当斜生栅藻浓度达 14.0 × 10⁶cells/mL 时已不利于轮虫的种群增长,因此可以认为,单个体培养状态下萼花臂尾轮虫实现其最大生殖潜力所需的最低食物浓度在不同品系间也存在着差异;芜湖品系萼花臂尾轮虫实现其最大生殖潜力所需的最低食物浓度为 5.0 × 10⁶ cells/mL,而青岛和广州品系轮虫实现其最大生殖潜力所需的最低食物浓度为 2.0 × 10⁶cells/mL。

3.3 食物浓度和品系与轮虫所产全部后代中的混交雌体百分率

有研究发现,以适宜浓度(1.0~2.0 × 10⁶cells/mL)的蛋白核小球藻(*Chlorella pyrenoidosa*)为食物培养萼花臂尾轮虫时,其后代中混交雌体百分率最低,高于或低于该浓度皆可明显提高混交雌体百分率^[13];但也有研究发现,一定范围内的食物浓度对轮虫后代中的混交雌体百分率无显著影响^[26]。本研究结果表明,广州品系轮虫全部后代中的混交雌体百分率不受食物浓度的影响,与 Snell 的结论^[26]一致;但是芜湖和青岛品系轮虫全部后代中的混交雌体百分率均在中等食物浓度(5.0、8.0 × 10⁶cells/mL)时最高。因此,本作者推测前人研究结果间所存在的不一致性可能与其所使用的食物浓度以及轮虫的品系有关。

品系是影响轮虫混交雌体产生(有性生殖发生)的重要内源性因素之一。研究结果表明,3 品系萼花臂尾轮虫中,广州品系轮虫全部后代中的混交雌体百分率最低,青岛品系最高,从而再次证实了上述观点。

3.4 母体年龄与轮虫每天所产后代中的混交雌体百分率

已有研究发现,萼花臂尾轮虫母体的年龄影响其后代的混交雌体百分率,表现为年轻的雌体可产生较多的混交雌体后代^[14, 17, 18]。结果表明,3 品系中仅芜湖品系和青岛品系轮虫均在食物浓度为 5.0 和 8.0×10^6 cells/mL 时每天所产后代中的混交雌体百分率随母体年龄的增大而降低。可见,母体年龄对轮虫每天所产后代中的混交雌体百分率的影响还因轮虫品系的不同和培养时所用的食物浓度的不同而异。

3.5 本研究的实践意义

当前,轮虫被有效地应用于水产经济动物的苗种生产可通过两个途径得以实现。其一是以收获轮虫为目的的轮虫规模化培养,在此过程中人们希望轮虫种群中的混交雌体百分率被尽可能地降低以获得较高的轮虫生产量;其二是以收获轮虫休眠卵为目的的休眠卵批量生产,在此过程中人们希望能够持续收获大量的休眠卵,因此在维持轮虫种群适度增长的同时保持种群中相对较高的混交雌体百分率将是至关重要的^[27]。生产过程中,人们常通过调节外源性因素来调节轮虫的繁殖方式,或通过选择较适宜的轮虫品系以最终达到上述目的。就本研究结果而言,3 品系萼花臂尾轮虫中,广州品系轮虫的总后代数最多,其所产的全部后代中混交雌体百分率最低;青岛品系与之相反。因此,若以收获轮虫为目的,则应选择广州品系轮虫为培养对象;若以收获轮虫休眠卵为目的,则宜选择青岛品系轮虫为培养对象。

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