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Population size, not density, serves as a cue for sex ratio adjustments in polychaete worms

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Running title: Sex ratio and local mate competition

36 Abstract

37 Local Mate Competition theory (LMC) predicts female-biased offspring sex-ratios when mate
38 competition occurs locally between siblings. The mating system of marine polychaete worm
39 *Dinophilus gyrociliatus* apparently fits LMC theory well. Females lay egg-capsules with 3-6 large
40 eggs (which produce females), and 1-2 small eggs (which produce males), resulting in female-
41 biased sex-ratios. However, mating occurs between larvae, inside egg-capsules and brothers fertilize
42 sisters, possibly preventing any competition between unrelated males and obviating the need for
43 sex-ratio adjustments to local population size. Therefore, we tested whether mothers adjusted their
44 offspring sex-ratio to local population size, controlling for density. As predicted by LMC theory,
45 sex ratios of single females were less male-biased than those of populations of multiple females,
46 suggesting that males also compete with unrelated males. Sex ratio adjustments occurred
47 irrespective of density, revealing sophisticated perception of the social environment in these worms.

48

49

50 Key words: local mate competition, *Dinophilus gyrociliatus*, polychaete worm, sex ratio, perceptual
51 ability

52

53 Introduction

54

55 Sex allocation theory predicts conditions in which organisms are expected to adjust their
56 relative allocation to male and female offspring (Charnov 1982; West 2009). Generally, the
57 proportion of males to females is predicted to be equal (1:1) in panmictic populations of gonochoric
58 species (as a result of frequency-dependent selection), if parents pay similar costs for sons and
59 daughters (Fisher 1930; Charnov 1982). If costs differ, allocation of resources to sons and daughters
60 should respond to the relative gains possible through either sex (Charnov 1982). Indeed, there are
61 taxa where sex ratios are strongly biased towards one sex (Hamilton 1967; Clark 1978). Local mate
62 competition theory (LMC) explains biased sex-ratio when siblings compete for mating (Hamilton
63 1967). LMC theory suggests that natural selection favors female-biased sex ratios when matings
64 occur within patches and brothers compete for mating. Sex ratios become less female-biased as
65 competition between brothers decreases and chances of mating with unrelated females increase
66 (Charnov 1982; Werren 1983; Hardy 2002). By producing relatively fewer sons and more
67 daughters, mothers reduces the competition among brothers for mates, but also increase their fitness
68 return because by producing more daughters, they produce more mates for their sons (Taylor 1981,
69 West 2009).

70 LMC theory has been tested in different organisms (e.g. Hamilton 1979; Waage 1982;
71 Yamaguchi 1985; West et al. 2005; Sato & Saito 2007). For example, wasps increase the number of
72 sons as the number of females that lay in the patch increases (Charnov 1982; Kinoshita et al. 2002;
73 Shuker & West 2004; Shuker et al. 2006).

74 The marine polychaete worm *Dinophilus gyrociliatus* is a diploid organism with
75 chromosomal sex determination (the male sex is heterogametic), egg dimorphism and a special
76 mating system (Charnov 1987; Simonini et al. 2003; Sella 2006). Females lay transparent egg-
77 capsules which contain relatively very small eggs (~ 40 µm) and develop into dwarf males, and
78 relatively very large eggs (80-100 µm) which develop into females (Åkesson & Costlow 1991). The

79 sex ratio of the eggs in the capsule is female-biased (e.g. 1:3), as well as the parental investment in
80 daughters. Quoting Charnov (1987, p. 224), "... While the *Dinophilus* sex ratio is mildly female-
81 biased, note that the egg dimorphism means that the resources allocated to daughters are vastly
82 greater than allocated to sons. A simplistic calculation might go as follows: if the resources
83 allocated per egg scale with egg volume (which scales with a linear dimension cubed), then each
84 female egg gets ≈ 8 times the resources of each male egg. With a sex ratio of 3:1, this means a
85 resource ratio of 24 units to daughters: 1 unit to sons; about 96% of the reproductive resources go to
86 daughters."

87 Mating occurs between newly-hatched, related, larvae inside capsules, setting the stage for
88 high local mate competition. Females grow larger than males and outlive them (males do not even
89 have a digestive system). Sex ratio is plastic in these worms in response to diet, temperature,
90 salinity and age. Females produce eggs with more male-biased sex-ratios when they are fed less
91 proteinaceous diet, get older, or are reared under stressful temperature or salinity conditions
92 (Prevedelli & Simonini 2000; Simonini 2001; Simonini & Prevedelli 2003). However, if mating
93 occurs strictly within egg-capsules, this should prevent any possibility for competition between
94 unrelated males and thus completely obviate the need for sex-ratio adjustments to local population
95 size. Therefore, we tested whether *D. gyrocoliatius* females adjusted their offspring sex-ratio to local
96 population size. We also controlled for potential confounding effects of population density
97 (accumulation of catabolites, oxygen consumption, etc.).

98

99 Material and methods

100

101 Study species

102 *Dinophilus gyrocoliatius* is a small, interstitial, marine polychaete worm, commonly found in
103 harbors and shallow waters all around the world. Populations are highly dispersed, but local
104 densities fluctuate (Prevedelli et al. 2005). This species is gonochoric, sexually dimorphic and

105 reproduces iteroparously. Males are only 50 μm long, their coelom is almost entirely occupied by
106 the testis and, although detailed studies are lacking, they usually die shortly after fertilizing their
107 sisters inside egg capsules (Prevedelli & Simonini 2000). Females are approximately 1000 μm long.
108 They emerge from capsules 5-6 days after spawning and produce the free-living population.
109 Females begin spawning when they are 10-14 days old (Simonini et al. 2003).

110

111 Experimental procedure

112 We used a laboratory population established from females collected in Sicily (Italy) in 2008.
113 We isolated 200 females into Petri dishes and used their same-age offspring for the experiment.
114 After each female laid at least one egg capsule, she was removed, and her offspring was reared to
115 sexual maturity and randomly assigned to one of the following treatments, which differed by female
116 population-size and/or by enclosure size:

117 single females in small enclosures: 1 female in 4 ml of water (density: 0.25 females/ml; n =
118 29 replicates);

119 single females in large enclosures: 1 female in 16 ml of water (density: 0.0625 females/ml; n
120 = 34 replicates).

121 multiple females in small enclosures: 10 female in 4 ml of water (density: 2.5 females/ml; n =
122 6 replicates);

123 multiple females in large enclosures: 10 female in 16 ml of water (density: 0.625 females/ml;
124 n = 3 replicates).

125 To avoid pseudoreplication, multiple females were not sisters. Because counting eggs (and
126 especially the tiny male eggs) within egg-capsules is difficult and time-consuming, the number of
127 replicates for the large population size was small (multiple females, where we expected many egg-
128 capsules). As a result, the number of replicates was unbalanced among treatments. The experiment
129 started the same day for all groups and lasted 16 days. Every other day, we noted the number of

130 newly laid egg-capsules and the number of male and female eggs per capsule (stereomicroscope, 40
131 x magnification). Egg capsules were then removed to avoid variation in social conditions.

132 Worms were reared in filtered marine-water (density: 1024 g/m³) and fed TetraMin Baby[®] *ad*
133 *libitum*. Petri dishes were kept in thermostatic chambers (20°C). Sea water was not renewed during
134 the experiment.

135

136 Statistical analyses

137 We calculated the sex ratio of egg capsules in each replicate as the number of male eggs divided by
138 the sum of male and female eggs.

139 Following Wilson & Hardy (2002), we used a generalized linear model (GZLM) with
140 binomial distribution and logit link function (dependent variables: number of male eggs and total
141 number of eggs; categorical predictor variables: female population-size and enclosure size). We
142 removed non-significant interaction term (IBM SPSS 20.0.0 statistical package).

143

144 Results

145

146 Overall, females produced 2474 egg capsules, which contained a total of 9812 female eggs
147 and 4707 male eggs.

148 Female population-size was a significant predictor of the sex ratio of the eggs, whereas
149 enclosure size was not (GZLM, female population-size: Wald $\chi^2 = 12.927$, df = 1, P = 0.0003;
150 enclosure size: $\chi^2 = 0.367$, df = 1, P = 0.545). There was a 0.277-fold increase in the sex ratios of
151 the eggs of multiple females compared to those of single female. Therefore, sex ratios shifted
152 significantly towards more male-biased values when multiple females were reared together,
153 compared to single females (Fig. 1).

154 The production of egg capsules by females was not affected by group size or enclosure size
155 (GLM, female population-size: $F_{1,69} = 0.522$, $P = 0.473$; enclosure size: $F_{1,69} = 2.749$, $P = 0.102$). On
156 average, each female produced 15.47 ± 0.525 egg capsules during the 16-day long experiment.

157

158 Discussion

159 These results showed that *D. gyrociliatus* females adjusted the sex ratio of their offspring in
160 response to the local population size; sex ratios (proportions of males) increased as the number of
161 mothers in the local population increased. This result is in accordance with the expectations of
162 LMC theory (Hamilton 1967). This was not an effect of population density (e.g., accumulation of
163 catabolites); by varying enclosure size, we controlled for these unwanted effects and found that sex
164 ratio adjustments occurred irrespective of density.

165 According to LMC theory (Hamilton 1967), the offspring sex-ratio increases in subdivided
166 populations when multiple mothers lay eggs in the same patch, so that competition for mating
167 diminishes. In *D. gyrociliatus* egg capsules subdivide the populations and brothers compete for
168 mating with females inside the capsule, apparently preventing any possibilities for competition
169 between unrelated males. Sex-ratio adjustments would promote mothers' reproductive success by
170 reducing competition between brothers and increasing the number of mates for their sons (Taylor
171 1981; West 2009). When local population size is large, competition between brothers would
172 decrease if males could leave their capsules and mate with unrelated mates. According to Schmidt
173 & Westheide (1972) males live long enough (1-2 weeks) to make it possible. However, such a long
174 lifespan in organisms that do not even have a digestive system seems surprising. Indeed, we usually
175 do not find males around in our populations, which are composed exclusively of females. However,
176 during the present experiment, males were occasionally seen leaving their capsules and moving
177 briefly around neighboring capsules (C.M.) as also reported by Traut (1966). Further studies are
178 necessary to test male lifespan. Mothers might favor male mating opportunities also by laying their
179 egg capsules close to each other, which might give dwarf short-lived males some chance to meet

180 other mates within short distance (G.S., unpublished data). Future studies will test whether female
181 adjustments include variations in the relative size of male and female eggs in response to local
182 population size, as such variations might also affect the lifespan of these dwarf males.

183 We do not know which cues mothers use to sense how many other mothers (or egg capsules)
184 are around. However, marine worms use waterborne chemical cues to find their mates (e.g., Ram et
185 al. 2008) or to sense how many partners are around (Schleicherová et al. 2006, 2010) and adjust
186 their sex allocation accordingly (Sella & Lorenzi 2003; Lorenzi et al. 2005, 2006, 2008). Similarly,
187 *D. gyrociliatus* mothers could perceive chemical cues produced by other mothers (or by egg
188 capsules) and adjust their offspring sex ratio appropriately.

189

190

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193

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- 273

274 Legend

275

276 Fig. 1. The mean sex ratio (\pm s.d.) in egg capsules (proportion of male eggs/total number of eggs)
277 by female population-size and enclosure size. Pooling data from small and large enclosures, single
278 females ($n = 63$ replicates) produced on average (\pm s.e.) 4.33 ± 0.14 female eggs and 1.74 ± 0.05
279 male eggs per egg-capsule ($n = 963$ egg capsules). Multiple females ($n = 9$ replicates of 10 females
280 each) produced on average 3.91 ± 0.26 female eggs and 2.09 ± 0.16 male eggs per egg-capsule ($n =$
281 1511 egg capsules).