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Do stable environments select against phenotypic plasticity in hermaphroditic sex allocation?

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Abstract

Phenotypic plasticity is the environment-induced change in the phenotype of an organism. Natural selection operates for the ability of individuals to adjust their phenotype to the current environmental conditions when environmental conditions fluctuate. Simultaneous hermaphrodites may exhibit plasticity in sex allocation according to the availability of mates at any particular time. The plasticity in sex allocation has probably evolved under fluctuating mating opportunities, which are usually low but increase when hermaphrodites incur sudden demographic expansion. Here we compare the plasticity in sex allocation in two different populations of the hermaphroditic polychaete worm *Ophryotrocha diadema* - a laboratory population and a wild one. Worms from the laboratory population were kept under constant crowded conditions for about 200 generations (i. e. they were exposed to high mating opportunities). Worms from the wild population were kept under crowded conditions for 20 generations only. Worms from the laboratory population showed significantly less plasticity in sex allocation than worms from the wild population. Although we cannot rule out the hypotheses that genetic drift or local adaptation played a role in the differences between the two populations, the most likely explanation for our results is that worms of the laboratory population underwent a loss of plasticity in sex allocation because they were kept under

constant mating opportunities. In fact, the sensory and regulatory machinery that worms use for exhibiting plastic sex allocation responses is likely to be the same as the machinery that is required for mate searching and sex role synchronization between mating partners. The need to maintain this machinery can explain why worms from the laboratory population diminished their plasticity in sex allocation but did not lose it completely. Therefore our results give some clues as to how plasticity in sex allocation evolves or is constrained.

Keywords: *Simultaneous hermaphrodites*, *sex allocation*, *mating opportunities*

Introduction

When the environment is heterogeneous and unpredictable in time and/or space, there is no predominant selection for a given environment-sensitive trait value and natural selection operates for the ability of individuals to adjust their phenotypes to the environmental conditions they are facing. The resulting adaptation represents a phenotypic plasticity - i.e. an environment-induced change in the phenotype of an organism (Pigliucci 2005). This implies that the same genotype codes for alternative phenotypes or for reaction norms depending on environmental conditions. Hence, the plasticity of a given trait may be itself a trait under selection and may be favoured by selective pressures (on that trait) that often change their direction (Pigliucci 2005). The evolution of phenotypic plasticity is the object of much theoretical and empirical research, mainly in developmental biology (e.g. Ernande & Dieckmann 2004; Fusco & Minelli 2010; Marty et al. 2011).

Sex allocation is the allocation of resources to male versus female reproduction in sexual species (Charnov 1982; West 2009). In simultaneous hermaphrodites, sex allocation deals with how hermaphrodites partition their reproductive resources between the male and female

functions. Many selective pressures, including sexual selection, operate to favor the optimal distribution of reproductive resources between the two sexual functions in hermaphrodites (Charnov 1982; Lorenzi & Sella 2008; Schärer 2009). When mating opportunities are rare (i.e., when mating group size is small, according to Charnov, 1982), selection favours hermaphrodites that express a relatively female-biased sex allocation. When mating opportunities are high, selection favors hermaphrodites that express a relatively more male-biased sex allocation. The changing pressures exerted by varying mating opportunities counter the evolution of a fixed optimum for sex allocation. As a consequence, hermaphrodites should be selected to adjust their sex allocation plastically and opportunistically in response to the mating opportunities they are facing. By adjusting their sex allocation to these mating opportunities, hermaphrodites have more chances to produce offspring through the potentially more rewarding sex function (the preferred sex role) (Leonard 2005, 2006; Anthes et al. 2006, 2010; Lorenzi & Sella 2008; Schärer 2009; Di Bona et al. 2010; Hart et al. 2011). The evolution of plastic sex allocation in hermaphrodites is a key subject in sex allocation theory, but empirical tests are rare, as far as we know. In most cases, we do not know the prevailing levels of mating opportunities that hermaphroditic organisms face in the wild but social conditions predict the number of mates (e.g., Pongratz & Michiels 2003; Janicke & Schärer 2009). Generally, hermaphrodites are expected to live at low densities and meet their mates rarely (Ghiselin 1969; Westheide 1984; Sella & Ramella 1999; Puurtinen & Kaitala 2002). However, many species exhibit some degrees of plasticity in sex allocation (e.g., Tan et. al. 2004; Baeza 2007; Brauer et al. 2007; Hart et al. 2011; Hoch & Levinton 2012). This suggests that the opportunities for mating fluctuate in the wild. Indeed, environmental heterogeneity is the main factor for the evolution of a plastic trait (Pigliucci 2005; Fusco & Minelli 2010).

106 What happens to a plastic trait if the environment becomes constant and stable for
107 generations? Costly traits are selected against when the selective pressures that favor their
108 maintenance in a population are relaxed (e.g. Lahti et al. 2009). Hence, if plasticity in sex
109 allocation is a costly trait, we expect a loss in plasticity when the environment is
110 homogeneous for a sufficiently long time-period (DeWitt et al. 1998; Auld et al. 2010).
111 The simultaneous hermaphrodite *Ophryotrocha diadema* is a small polychaete marine worm
112 that flexibly adjusts its sex allocation to the mating opportunities they face (Lorenzi et al.
113 2005). Worms spend two thirds of their lifetime as mature hermaphrodites. When adults,
114 worms estimate the presence and number of potential mates through the perception of
115 waterborne cues (Schleicherová et al. 2006, 2010). They react to variation in such cues by
116 changing their sex allocation in as little as 5 days (Lorenzi et al. 2008). Such a quick
117 response suggests that sex allocation plasticity in *O. diadema* worms evolved in
118 environments where mating opportunities fluctuated. Indeed, *O. diadema* worms rarely have
119 been found in field collections (Åkesson 1976; Simonini et al. 2009). They are likely to live
120 at extremely low population densities (Westheide 1984; Sella & Ramella 1999; Simonini
121 pers. comm. to M. C. L.), where mating opportunities are scarce. However, *Ophryotrocha*
122 worms experience demographic explosions from time to time (Prevedelli et al. 2005).
123 Therefore, worms occasionally have high opportunities for mating. These fluctuations are
124 precisely the conditions that should favor the evolution of the plasticity in sex allocation.
125 In our laboratory cultures, worms have been kept in crowded mass cultures (on average 200
126 worms in 60 ml) for more than three decades and for as many as approximately 200
127 generations. Therefore, they live under crowded conditions, where the opportunities for
128 mating are stably high. We are also rearing a population recently captured from the field that
129 has been kept in mass cultures (as above, on average 200 worms in 60 ml) for less than 2

130 years (i. e. 20 generations). We tested whether these two populations differ in their plasticity
131 in sex allocation.
132
133 **Materials and methods**
134 *The wild and the laboratory population*
135 *O. diadema* polychaete worms are known only from a site in the Pacific Coast (Long Beach
136 harbor, Los Angeles, California, Åkesson 1976) and a site in the Mediterranean Sea (Porto
137 Empedocle, Sicily, Simonini et al. 2009). The Pacific Coast population comes from a 1989
138 collection of approx. 50 worms (Åkesson, pers. comm. to G. S.). Loss of genetic diversity in
139 closed populations (such as laboratory populations) depends on the number of founders and
140 the number of generations. Following Frankham et al. (2002), the number of founders of the
141 worm population from the Pacific Coast was large enough to retain 98% of the
142 heterozygosity of the source population. Generally, closed populations (with founder
143 number = 50) should retain approximately 20% of their original genetic variation after 200
144 generations of captive life (Frankham et al. 2002). However, the worm population
145 originating from the Pacific Coast was replicated and spread among scientific laboratories
146 all over the world, including our laboratory at the University of Turin. In our laboratory, the
147 population was maintained in 4-8 replicates. To limit inbreeding depression and loss of
148 genetic variation, we mixed population replicates with each other about twice a year.
149 Additionally, every 5-7 years, we mixed our laboratory population replicates with worms
150 coming from the B. Åkesson laboratory, Goteborg University and R. Simonini laboratory,
151 Modena and Reggio Emilia University. Therefore, we cannot estimate the actual level of
152 heterozygosity in our population replicates from the Pacific Coast but we expect that it is
153 higher than the heterozygosity predicted for closed populations after 200 generations (see

154 above). We define the population origination from the Pacific Coast as the 'laboratory'
155 population.
156 The Mediterranean population comes from a 2008 collection of 56 individuals in the
157 Mediterranean Sea (Simonini et al. 2009). Therefore, as for the number of founders, the
158 initial level of heterozygosity should be nearly 98% and after 20 generations, the loss of
159 heterozygosity should be < 15% (Frankham et al. 2002). Therefore, we call this population
160 the 'wild' population.
161 In both populations we identify focal worms (and their eggs) from their mates by means
162 of a genetic natural marker: worms have alternative phenotypes of body and egg colour
163 (yellow-egg or white-egg phenotypes).
164
165 O. diadema *biology*
166 *O. diadema* worms live in mussel clusters, in nutrient-rich harbour-waters. Their life cycle
167 consists in a protandrous phase followed by a simultaneously hermaphroditic phase. During
168 the protandrous phase, worms produce sperm and fertilize eggs, but cannot produce eggs
169 yet. During the hermaphroditic phase, worms repeatedly develop eggs and sperm. In pairs,
170 hermaphrodites regularly alternate their sexual roles. Each partner lays a jelly cocoon of 25
171 eggs on average every third day (Sella 1985). In large groups, where the opportunities for
172 mating are higher, worms exhibit a relatively less female-biased sex allocation than worms
173 reared in pairs (Lorenzi et al. 2005). In this species plasticity in sex allocation is easily
174 measured as the variation in allocation to the female function (Lorenzi et al. 2005). In
175 contrast, variation in the male function is not easily measured. Worms compete for
176 fertilizations (Lorenzi et al. 2006) – but produce strikingly few aflagellate sperm (Morrow
177 2004). Variation in the male function in high *vs*. low mating opportunities is therefore
178 mainly visible through difficult and time-consuming behavioural observation under the

179 microscope, rather than through variations in sperm production (Lorenzi et al. 2005, 2006).
180 If mating opportunities change within the hermaphrodite lifespan (of approximately 3
181 months), worms appropriately redistribute their reproductive resources to the two sexual
182 functions, tracing current mating opportunities within a few days (Lorenzi et al. 2008).
183
184 *Standard rearing conditions*
185 In laboratory, worms are reared in filtered sea-water (35‰ salinity) in glass bowls, in
186 thermostatic cabinets at 20°C. They are fed with frozen spinach once a week. They are kept
187 in similar conditions and in similarly crowded mass cultures in the other laboratories in the
188 world.
189
190 *Experimental procedure*
191 We tested the plasticity in sex allocation by exposing worms of the wild and laboratory
192 populations to either low or high opportunities for mating. We obtained egg-cocoons from
193 paired worms and reared sibling larvae together until they had mature male and female
194 functions. Then, non-siblings, same-age, virgin worms with yellow-egg phenotype
195 (hereafter, focal worms) were randomly assigned to either low or high opportunities for
196 mating. Under low mating opportunities, each focal worm was kept with a white-egg
197 phenotype worm; under high mating opportunities, each focal worm was kept with 11 white-
198 egg phenotype worms (36 replicates per mating opportunity level and per population). All
199 worms were sexually mature and virgin. Within replicates, worms came from the same
200 population (either laboratory or wild population). They were kept in 10-ml glass bowls.
201 We measured the female allocation of focal worms as the number of eggs they laid during
202 a three-week experimental time. We inspected bowls twice a week to check for the focals'
203 egg-cocoons. We measured female allocation by counting the eggs laid by focal worms.

Then we removed all the eggs to keep mating opportunities constant (juveniles compete for fertilizations with mature hermaphrodites, Sella & Lorenzi 2003). At the end of the experiment we also measured worm body-size as the number of chaetigerous segments.

Statistical analysis

We tested the variation in female allocation by population and by mating opportunity using a Generalized Linear Model for normal distribution with identity link function (with female allocation as the dependent variable and population and mating opportunities as the fixed factors). Generalized linear models relax the requirement for the homogeneity of variances that is required for testing hypotheses using linear models. We added body size as a covariate to the model because female fecundity is often related to body size. Data analysis were performed on focal worms, which were alive at the end of the experiment (see Table I). Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The plasticity in female allocation differed significantly between the wild and laboratory populations (interaction population*mating opportunity: Wald $\chi^2 = 8.528$, $P = 0.003$) (Figure 1, Table I). Female allocation also differed significantly between mating opportunities (Wald $\chi^2 = 82.535$; $P < 0.0001$) and between populations (Wald $\chi^2 = 23.233$, $P < 0.0001$), controlling for body size (that had no effect on female allocation: Wald $\chi^2 = 2.741$, $P = 0.098$). Worms from the wild population exhibited a steeper decrease in egg production than worms from the laboratory population when mating opportunities increased.

[Insert Figure 1 about here]

[Insert Table 1 about here]

Discussion

Worms from the laboratory population had significantly less plasticity in sex allocation than worms from the wild population. This is shown statistically in the significant interaction term population*mating opportunity and it is shown graphically by the non-parallel responses to the opportunities for mating by worms from the wild and laboratory populations (Figure 1).

There are three possible explanations for these results – namely, genetic drift, local adaptation and the evolution of plasticity in sex allocation. We cannot rule out any of these explanations based on available data. First, genetic drift allows isolated populations to be differentiated for a given trait value. However, to counter the effect of genetic drift and inbreeding, we have been mixing our laboratory population replicates with new worms inside of and between laboratories since we began rearing them in our laboratory. Moreover, we kept the size of every population-replicate around 200 individuals, so that the overall size of the laboratory-population was approximately 800 – 1600 individuals. With such population sizes, it is unlikely that the divergence in sex-allocation plasticity between the wild and the laboratory populations is the effect of genetic drift and inbreeding (Frankham et al. 2002).

Second, local adaptation is possibly the main cause of the difference between the wild and laboratory population. According to the local adaptation hypothesis, the Mediterranean population (the founders of our wild population) should have experienced more variable mating opportunities than the Pacific population (the founders of our laboratory population) for many generations. Unfortunately, we do not know much about the density of Pacific and Mediterranean populations of *O. diadema*. However, 2 and 50 individuals were collected in the Pacific Coast (in 1972 and 1989, respectively, sampling effort unknown; pers. comm. by B. Åkesson to G. S.) and 0.1-6.6 individuals per kg^{-1} of mussel clusters were collected in

2006, 2007 and 2008 in the Mediterranean Sea (Simonini, pers. comm. to M. C. L.). Therefore, there are no data in favor of the hypothesis that the Mediterranean population had mating opportunities that fluctuated more often than those of the Pacific population. In other words, there is no evidence that local adaptation explain our results.

Third, the evolution of plasticity in sex allocation remains as a potential explanation. The differences in the plasticity in sex allocation between populations might be explained as consequences of the loss of sex allocation plasticity in the laboratory population following a lowering of the main selective pressure for plasticity i.e. the stochasticity in mating opportunities. In the laboratory, worms have been exposed to constantly high opportunities for mating for as many as c. 200 generations. Under stable mating opportunities, plasticity in sex allocation is no longer adaptive. Actually, we would have expected that worms had lost all their plasticity in sex allocation after 200 generations of constantly high mating opportunities. Instead, worms in the laboratory population retained some plasticity in sex allocation.

The maintenance of traits that are no longer adaptive is not rare (e.g. Rydell et al. 2000; Peer et al. 2007; Lahti et al. 2009), possibly due to the limited costs of such traits.

The costs of plasticity consist in both production and maintenance costs (DeWitt et al. 1998; Auld et al. 2010). The production costs of plasticity in sex allocation could be paid by plastic hermaphrodites if changes in sex allocation require costly resources that a fixed sex allocation does not require (such production costs were not found in *O. diadema*, Lorenzi et al. 2008). The maintenance costs of plasticity in sex allocation could be paid by plastic hermaphrodites if opportunistic sex allocation requires the maintenance of sensory and regulatory machinery that a fixed sex allocation does not require. Simultaneous hermaphroditic worms adjust their sex allocation by means of a sensory machinery that uses chemical cues to inform on the number of potential mates (Schleicherová et al. 2006). The

same sensory machinery is most likely also vital for mate searching, as worms respond differentially to different concentrations of the chemical cues (Schleicherová et al. 2010). Therefore, worms seem to use the same sensory machinery for both searching for mates and adjusting their sex allocation. The loss of such sensory machinery is not compatible with reproduction, if it is used also for mate searching. This could explain why we did not observe a complete loss of phenotypic plasticity in sex allocation in the laboratory population, even if plastic sex allocation was no longer advantageous in the stable laboratory environment.

Conclusive evidence that the stable laboratory environment selected against phenotypic plasticity in worm sex allocation will require further tests. Tests will include checking sex allocation plasticity in multiple worm-populations in the wild, and relate the observed plasticity to local fluctuations in population density.

There are very few experimental studies on the ecological factors that favor the fixation or maintenance of phenotypic plasticity (Pigliucci 2005; Hallsson & Bijorklund 2012). Hence, within this context, our results can serve to give some clues as to how plasticity in sex allocation evolves or how it is constrained.

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405

406 **Figure legends**

407

408 Figure 1. The female function (as measured by the individual values of egg production) in
409 the wild and laboratory populations at two levels of mating opportunities. Lines are drawn
410 from mean egg production values and highlight the significant interaction between mating
411 opportunities and populations.