IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Does the cost of a function affect its degree of plasticity? A test on plastic sex allocation in three closely related species of hermaphrodites

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/142727 since 2016-01-04T14:43:34Z

Published version:

DOI:10.1016/j.jembe.2014.01.010

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

12 October 2024

UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:
Questa è la versione dell'autore dell'opera:
Journal of Experimental Marine Biology and Ecology, 453, 2014,
doi:10.1016/j.jembe.2014.01.010

The definitive version is available at:
La versione definitiva è disponibile alla URL:
http://dx.doi.org/10.1016/j.jembe.2014.01.010

Does the cost of a function affect its degree of plasticity? A test on plastic sex allocation in three closely related species of hermaphrodites

D. Schleicherová [c], G. Sella [a], S. Meconcelli [a], R. Simonini [b], M.P.Martino [b], P. Cervella [a], M.C. Lorenzi [a]

[a] Department of Life Sciences and Systems Biology, Università di Torino, Via Accademia Albertina 13, 10123 Turin, Italy
[b] Department of Animal Biology, Università di Modena e Reggio Emilia, Via Campi 213/D, 41125 Modena, Italy
[c] Institute of Animal Ecology and Evolution, University of Hannover, Buenteweg 17d,30559 Hannover, Germany

ABSTRACT

Mating opportunities fluctuate in the wild and hermaphrodites have the chance of partitioning reproductive resources between their two sexual functions accordingly, i.e., they have a plastic sex allocation. Plasticity is usually promoted by environmental fluctuations butmay be affected by species-specific factors, which may be revealed by comparisons between related species.We testedwhether polychaeteworms of three related species of simultaneous hermaphrodites, *Ophryotrocha diadema*, *Ophryotrocha adherens* and *Ophryotrocha gracilis*, had plastic male and female allocation. We measured the costs of the female function and investigated whether the costs might affect the magnitude of plasticity in this function. To these aims, we exposed adult worms to three levels of mating opportunities and measured their female and male functions. In our experimental conditions, there was no adjustment in the male function, the cheapest function, whereas the three species differed in how they adjusted their allocation into the female function to mating opportunities. *O. diadema* and *O. adherens* worms exhibited highly plastic female allocation, and plasticitywas consistent across three measures of female function. In contrast, *O. gracilis* worms had a fixed female allocation, irrespective of mating opportunities. Additionally, when the sexual functions were relatively costly, their plasticity was greater than when they were relatively cheap. However, the magnitude of the plasticity did not depend solely on species-specific costs of the function, but also on the features of the mating system of each species.

1. Introduction

Mating opportunities vary in thewild. In separate-sex species,males respond to such variations by opportunistically and adaptively adjusting their sperm production, ejaculate size and/or courtship behavior (e.g. Barbosa, 2012; Candolin and Reynolds, 2002; Scaggiante et al., 2005; Shine et al., 2003; Wedell et al., 2002). Similarly, females adjust the number of matings or their brood sex-ratio to mating opportunities (e.g., Gage, 1995; Richardson and Burke, 2001; Shuker et al., 2006).

In simultaneous hermaphrodites, individuals invest their resources into two sexual functions at the same time, the male and female functions, and can respond to changes in mating opportunities by strategically expanding or reducing their resource allocation to one sex at the expense or in favor of the other. Sex allocation theory predicts a tradeoff between sex functions (Charnov, 1982; Schärer, 2009). When the size of the mating group is low, hermaphrodites are expected to invest fewresources into themale function (e.g., they are expected to produce just enough sperm to fertilize their partners' eggs) and devote their remaining resources to the female function (e.g., to egg production). As the size of the mating group increases, hermaphrodites should increase the amount of resources invested into themale function and decrease the amount invested into the female function. Empirical tests of these predictions often investigated the effect of the social group, rather than that of the mating group, because the latter is difficult to manipulate. By varying the size of the social group, itwas often found that adult hermaphrodites make plastic adjustments of their sex allocation as they sense a change in their social group, i.e., in mating opportunities (Brauer et al., 2007; Hart et al., 2011; Lorenzi et al., 2005). However, the way hermaphrodites adjust their sex allocation to mating opportunities varies widely among species. For example, in response to variation in the size of the social group, some species showa plasticmale allocation, but do not change their female allocation (e.g. Tan et al., 2004 in leeches; Baeza, 2007 in shrimps; Hoch and Levinton, 2012 in barnacles; Schärer and Ladurner, 2003 in flatworms). Conversely, other species show a plastic female allocation and an almost fixed male allocation (Lorenzi et al., 2005; Schleicherová et al., 2006, 2010 in polychaete worms). In contrast to the above animals, which mate through behavioral interactions, sessile colonial invertebrates may withhold female investment pending sperm reception (Hughes et al., 2002 in bryozoans). The diversity in the patterns of phenotypic plasticity in sex allocation could be the result of the variety of the model species used in this kind of research and of the experimental conditions in which plasticity in sex allocation was tested in different species and laboratories, but other factors may play a role too (Schärer, 2009).

More generally, we still know very little on how phenotypic plasticity evolves, both froma theoretical and an empirical point of view(Auld et al., 2010). Plasticity in sex allocation is central to evolutionary studies. Indeed, plasticity in sex allocation is a requisite in the models which describe the evolutionary transition between hermaphroditism and gonochorism (e.g., Delph and Wolf, 2005) and is tested in organisms whose breeding systems are under scrutiny as potentially intermediate steps in this transition (Lorenzi and Sella, 2013).

Although sex allocation plasticity involves demographic costs (Lorenzi et al., in press-a), it is not costly per se (Lorenzi et al., 2008). Therefore we hypothesized that it might be the relative costs of the female vs the male function that could influence the magnitude of phenotypic plasticity in sex allocation. We expected that plasticity in sex allocation reduced the risk of producing resource-consuming eggs when high mating opportunities enhanced the chances of reproducing via the cheaper male function. Assuming that plasticity in costly traits would help reducing the risk of wasting resources

when the environment changes,we tested the hypothesis that plasticity should be particularly strong both in the relativelymore expensive sex function (i.e., the female function) and in species where the costly sex function is especially resource consuming (e.g. where eggs are the largest, relatively to body size). In these cases, the penalties for a mismatch between allocation to the costly sex function and opportunities of mating via that function should be the most severe. Introducing a comparative perspective, i.e. comparing species which invest relatively different amounts of resources in their sex functions, may help us to understand whether the costs of sex functions play a role in shaping the magnitude of plasticity in sex allocation. We tested plasticity in female and male allocation in three species of simultaneously hermaphroditic worms of the genus *Ophryotrocha* - *Ophryotrocha diadema*, *Ophryotrocha adherens* and *Ophryotrocha gracilis*. The three species are closely related (Dahlgren et al., 2001; Thornhill et al., 2009), have similar morphology and reproductive biology, and reproduce over extended time periods, but differ in the amounts of resources invested in the female function; they produce eggs of different sizes and have largely different egg-laying rates (Paavo et al., 2000; Sella and Ramella, 1999). In contrast, the three species invest small amount of resources in sperm by producing few, aflagellate sperm (Morrow, 2004). We then expected that *O. diadema* and *O. adherens* (which make the largest investment in their female function) would show larger plasticity than *O. gracilis* in their female function.We measured plasticity inmale and female allocation by exposing worms to three levels of mating opportunities.

2. Materials and methods

2.1. Common general features of the study models

O. diadema, *O. adherens* and *O. gracilis* are small (<5 mm), transparent marine polychaete worms. Their life-cycle consists of a protandrous phase followed by a simultaneously hermaphroditic phase. During the protandrous phase,worms produce sperm. In the subsequent hermaphroditic phase, worms produce both sperm and eggs. In *Ophryotrocha* worms, gonads are very simple andmale and female gametes complete their development at each reproductive bout as free germ cells in the worm's coelom. Worms reproduce iteroparously for 5–13 weeks after they are sexually mature (Åkesson, 1976; Paavo et al., 2000; Sella, 1985). They produce aflagellate sperm (less costly than flagellate sperm, Morrow, 2004), in small numbers (e.g., 50 sperm per egg, Sella, 1990). Hermaphroditic Ophryotrocha worms are obligatorily outcrossing and mate through pseudocopulation, a very special form of external fertilization. During pseudocopulation, two worms stay in close contact with each other, until one worm releases eggs in a transparent jelly cocoon and the other releases its aflagellate sperm and deposit them on the eggs, inside the jelly cocoon (Paavo et al., 2000; Westheide, 1984). Because fertilization is external, there is no storage of allosperm, but multiple worms in the male role can fertilize the same cocoon (Lorenzi et al., in press-b). Owing to their small body size and their interstitial habitat, it is very difficult to study the behavioral ecology of *Ophryotrocha* worms in the field.

2.2. *O. diadema*

O. diadema worms were found in clusters of mussels growing in nutrient-rich waters of Californian harbors (Åkesson, 1976) but they may be more widespread (Simonini et al., 2009, 2010). In pairs, each hermaphrodite lays a large cocoon of about 30 eggs every third day (Premoli and Sella, 1995). These worms are egg-traders and regularly alternate sexual roles in successive reproductive bouts when they are in groups of 2 worms (Sella, 1985). Reproductive resources are also largely invested in mate competition when they are in groups N2 worms. In this condition,worms compete formating and plastically adjust their sex allocation (Lorenzi et al., 2005; Sella and Lorenzi, 2000, 2003). They become significantly more aggressive towards other hermaphrodites but do not change their low sperm count, which suggest that the resources they spare from the female function are invested in behavioral competition for mating in the male role (Lorenzi et al., 2006). These adjustments occur as a response to cues that inform worms on the number of potential mates and/or rivals, irrespective of other density factors such as metabolite accumulation, and oxygen consumption. (Lorenzi et al., 2005). Indeed, these worms sense the number of potential mates through waterborne chemical cues and adjust their male and female allocation appropriately, whereas encounter probability has no effect on sex allocation (Schleicherová et al., 2006). The effect of these chemical cues is so strong that we can manipulate the sex allocation ofworms by simply varying the amount of cues rather than varying the number of worms in the enclosures (Schleicherová et al., 2010). These worms have kept plasticity in sex allocation although cultured in dense laboratory populations for generations (Schleicherová et al., 2013). In this experiment, we used worms of the lab culture from the California population.

2.3. *O. adherens*

O. adherens worms were collected in Hawaii, Cyprus, the Canary Islands (Paavo et al., 2000), and Sicily (Simonini et al., 2010). They live in nutrient-rich waters and are found in mussel clusters living in harbors (Paavo et al., 2000). In pairs, each hermaphrodite lays a large egg cocoon (in relation to body size) of about 70 eggs every second day (N200 eggs per week, Paavo et al., 2000). We do not know whether these worms have a plastic sex allocation. In the experiment we used worms of the lab culture from the Canary Islands population.

2.4. *O. gracilis*

O. gracilis worms were collected in the North Sea, where few worms were found after several surveys (Reichert and Buchholz, 2006). Populations of these worms live in coarse sand and thereforemay be exposed to dispersion caused by wave action more heavily than populations of the *O. diadema* or *O. adherens*. Each paired hermaphrodite lays a small egg cocoon of 4–7 eggs per week. In pairs, these worms regularly alternate sexual roles in successive reproductive bouts (Sella et al., 1997). We do not know whether these worms have a plastic sex allocation. In the experiment we used worms of the lab culture from the Helgoland population.

3. Experimental set-up

Worms of all the three species came from laboratory populations that were started approximately 25 years ago. Same age, virgin, non-sibling and ovigerouswormswere tested for their allocation to eggs and sperm as soon as they reached sexual maturity. For *O. diadema* and *O. adherens*, the experimentwas carried out on 45 focalworms per species, for *O. gracilis* on 30 worms. These worms (hereafter, focal worms) were randomly extracted fromthe progeny of asmany pairs of parents, i.e.,worms in the experimentwere not siblings. As soon as they hadmature male and female functions, they were randomly assigned to one of the following three levels of mating opportunities for three weeks: 1) low mating opportunity, where each focal worm had only one mate; 2) intermediate mating opportunity, where each focal worm had 3 potential mates; and 3) large mating opportunity, where each focal worm had 11 potential mates. The whole experiment was performed in 2007 and was concluded in three months.
In *O. diadema*, we identified the focal hermaphrodite in every pair or group by means of a neutral marker which determines either a yellow or a white coloration of ovigerous worms and their eggs (Sella and Marzona, 1983). The focal worms were yellow-egg worms and their mates were white-egg worms. In *O. adherens* and *O. gracilis*, we identified focal worms by their body size. In hermaphroditic *Ophryotrocha* worms, body size does not affect sex allocation (Lorenzi et al., 2005). Therefore, in both species we chose as focal worms individuals that were 4-chaetigerous-segments longer than their partners. We set up 15 replicates per species and per level of mating opportunity. Because it was difficult to track a focal *O. gracilis* worm in a large group of conspecific worms, we only collected data from low and intermediate levels of mating opportunities for this species.
We carried out thewhole experiment in 10-ml glass bowls placed in a temperature-controlled chamber at 20 °C. The worms of the three species were reared in filtered, natural sea-water with salinity 35, were fed spinach ad libitum and kept in the dark. We changed water in the bowls once a week.

3.1. Cost of female function

Following Schärer and Ladurner (2003),we estimated the cost of the female function as the ratio of the area of eggs laid to the area of the body of the laying worm, assuming that these measures are estimates of resource investment. For O. gracilis, we calculated this ratio from data published by Sella and Ramella (1999). For *O. diadema* and *O. adherens* these data were not available from the literature. Therefore, we took digital pictures of the egg cocoons and the body of laying worms (n = 38 for *O. diadema* and n = 22 for *O. adherens*) at magnifications of 125× with a Stereo Microscope with Integrated LED Illumination and HD Camera (Leica EZ4 HD). Both measures of egg area (mean value of 3 randomly chosen eggs in each cocoon) and body area (area of the body of the laying worm) were taken in pixel using Paint.NET.

3.2. Measure of phenotypic plasticity in female function

For each worm, we measured the female function by measuring the following variables: 1) resource investment in eggs (total number of eggs laid by each focal worm ∗ (ratio between the mean egg area and the mean body area)), 2) total number of egg cocoons, and 3) mean time interval between egg layings. Therefore, for each worm, we had a value (raw value, see below) for each variable associated with the female function. To this aim, we counted the number of egg cocoons and eggs laid by focal worms twice a week for three weeks. After counting, we removed the egg cocoons from the bowls to avoid mate competition by juveniles in protandrous phase.We assigned egg maternity as follows. The egg cocoons spawned by *O. diadema* focal worms were easily distinguished from those laid by their partners because of their yellow egg-color. The egg cocoons spawned by *O. adherens* and *O. gracilis* focal worms were distinguished from those laid by their partners by noting the physiological status of focal worms at each check (i.e., whether they had mature eggs in their coeloms).When we had any uncertainty in assigning the maternity of eggs either to focals or to their mates, we excluded the case from data analysis.

3.3. Measure of phenotypic plasticity in male function

At the end of the experiment, we measured the investment in the male function (i.e. the number of sperm) across the three levels of mating opportunities. This measure requires a heavy manipulation of worms that cannot be done during experiments, as we squeeze worms gently under the microscope until sperm ooze out of their body. Since it takes *O. diadema* worm three days to replenish sperm reserves (Sella, 1990), we kept focals isolated for three days before measuring their sperm (thus allowing sperm replenishment in case they had fertilized eggs just before). Two blind observers counted the number of sperm under a phase-contrast microscope at 400× magnification. Spermcounts by the two observers were highly significantly correlated (Pearson correlation, r = 0.923, P b 0.0001) and we used their mean

values in the statistical analysis. Therefore, for each worm, we had a value (raw value, see below) for the only variable associated with the male function. Because counting sperm in focal worms took four days, we entered the day of measurement as a covariate in the analysis to control for the potential effects of the isolation period on sperm number.

3.4. Measure of body growth

At the first and last check,we measured the body size of focalworms as the number of chaetigerous segments, to control for resource investment in body growth (as opposed to investment in reproduction) during the experiment. There is no size-dependent sex allocation in *O. diadema* (Lorenzi et al., 2005). However, the body growth of the worms during the experiment was used to further control for potential associations between level of mating opportunities and body size.

4. Statistical analysis

The three studied species differed in the number of eggs laid. Different scales create problems in data analyses aswe expect larger variance in traits with larger mean values. If plasticity is measured as the difference between absolute trait values (e.g. number of eggs), it might appear that phenotypic plasticity is larger in species which produce larger mean number of eggs. Standardization of data equalizes variances. Therefore, we standardized all individually-measured-trait values (raw data) by the speciesmean of the given trait, in order to provide a biologically more meaningful species comparison.We standardized (Z scores) the raw values of the four variables associated with the female and male functions, namely, resources invested in eggs, number of egg cocoons, interval between egg-layings and sperm numbers (raw value minus the species-specific mean-value divided by the speciesspecific standard deviation), after checking for normality. We entered these standardized values as dependent variables and species and mating opportunity as fixed factors in four General Linear Models (GLMs). GLMs are implementations of Univariate Analyses of Variance and are based on Ordinary Least Squares.We followed a model simplification procedure, removing non-significant interactions and then nonsignificant terms until the model contained nothing but significant terms (Crawley, 1993; Wilson and Hardy, 2002). The factor focal worm-marker (either an egg-color or a body-size marker), introduced in a first GLM, was removed after testing it had no significant effect on female investment ($F_{1,110} = 0.335$, $P = 0.495$). We entered body size (as measured at the end of the experiment) as a covariate to control for potential effects of mating opportunities on body size.
We compared body growth among species and among mating opportunities using a Generalized Linear Mixed Model (GLMM) for repeated measures and count data (Poisson distribution, link function: log).
Statistical analyses were performed using IBM SPSS version 20.

5. Results

5.1. Costs of female function

O. diadema worms produced eggs with a mean diameter of 310.46 ± 9.79 μm (data from n = 38 egg cocoons of 38 worms). *O. adherens* worms produced eggs with a mean diameter of 137.59 ± 8.22 μm (data from n = 22 egg cocoons of 22 worms). An egg size of 200 μm was reported for *O. gracilis* (Sella and Ramella, 1999).
The resource investment in one egg (as measured by the ratio between the mean egg area and the mean body area) was 0.0112 in *O diadema*, 0.0164 in *O adherens* and 0.0210 in *O gracilis*. However, these values must be related to the size of egg cocoons,which differs between species. In our experimental conditions, *O. diadema* worms produced between 5 and 34.3 eggs per egg cocoon. Therefore, the resource investment in one egg cocoon varied between 0.056 and 0.384 in *O. diadema*. *O. adherens* worms produced between 10 and 52.5 eggs per egg cocoon and the resource investment in one egg cocoon varied between 0.164 and 0.861. *O. gracilis* worms produced between 3 and 8 eggs per egg cocoon and the resource investment in one egg cocoon varied between 0.063 and 0.168.

5.2. Measures of phenotypic plasticity in female function

The worms of the three species were differently plastic in the three variables that measured their female function.

5.3. Resource investment in eggs

O. diadema and *O. adherens* worms decreased their resource investment in eggs with increasing mating opportunities, whereas *O. gracilis* worms did not change it (Fig. 1A, Table 1). This is shown statistically by the significant interaction term species * mating opportunity ($F_{3,106} = 6.241$, $P = 0.001$) and graphically by the non-parallel responses to the opportunities for mating by worms from the three species (Fig. 1A) (the covariate body size was removed from the model because it was not significant). In within-species comparisons, *O. diadema* and *O. adherens* worms significantly adjusted their resource investment in eggs to variation in mating opportunities, whereas *O. gracilis* worms did not (*O. diadema*: $F_{2,41} = 63.202$, P b 0.0001; *O. adherens*: $F_{2,40} = 8.863$, $P = 0.001$; *O. gracilis* $F_{1,25} = 0.415$, $P = 0.526$).

5.4. Number of egg cocoons

The worms of the three species differed in how they adjusted the number of egg cocoons to mating opportunities (interaction species * mating opportunity: $F_{3,105} = 3.721$, $P = 0.014$; covariate body size: $F_{1,105} = 0.768$, $P = 0.383$)

(Fig. 1B, Table 1). In *O. gracilis* worms this trait was not plastic. In within-species comparisons, *O. diadema* and *O. adherens* worms significantly decreased the number of egg cocoons to increasing mating opportunities, whereas O. gracilis worms did not (*O. diadema*: factor mating opportunity: F2,40 = 54.329, P b 0.0001, covariate body size: F1,40 = 1.409, P= 0.242; *O. adherens*: mating opportunity F2,39 = 3.264, P = 0.049, body size: F1,39= 3.866, P= 0.056; *O. gracilis*: mating opportunity F1,24 = 0.408, P = 0.529, body size: F1,24= 0.089, P= 0.768) (Fig. 1B, Table 1).

5.5. Time interval between egg-layings

Finally,mating opportunities significantly influenced the time intervals between egg-layings (interaction species * mating opportunity F3,105 = 2.306, P = 0.081; mating opportunity: F2,105 = 20.815, P b 0.0001; covariate body size: F1,105 = 3.005, P = 0.086) (Fig. 1C, Table 1). Inwithin-species comparisons, *O. diadema* worms significantly increased these intervals asmating opportunities increased,whereas *O. adherens* and *O. gracilis* worms did not (*O. diadema*: factor mating opportunity: F2,40 = 38.374, P b 0.0001, covariate body size: F1,40 = 0.025, P = 0.874; *O. adherens*: mating opportunity F2,39 = 2.518, P = 0.094, body size: F1,39= 3.910, P= 0.055; *O. gracilis*: mating opportunity F1,24 = 0.314, P = 0.580, body size: F1,24 = 0.716, P = 0.406) (Fig. 1C, Table 1).

5.6. Magnitudes of plasticity in resource investment in eggs

The magnitudes of plasticity in resource investment in eggs, number of egg cocoons and time intervals between egg-layings (as calculated by the difference between themean value of the variable at intermediate mating opportunities and that at low mating opportunities) were all larger in *O. diadema* and *O. adherens* than in *O. gracilis* (see Fig. 1 A,B,C).

5.7. Measure of phenotypic plasticity in male function

Worms did not vary their number of sperm in response to mating opportunities and this result was consistent among species (interaction species * mating opportunity F3,98 = 1.819, P = 0.149; species: F2,98 = 0.441, P = 0.645; mating opportunity: F2,98 = 1.071, P = 0.347; covariate body size: F1,98 = 1.059, P = 0.306; covariate day of measurement: F1,98= 0.864, P=0.355).

5.8. Body growth

Hermaphrodites grew during the experiment, as shown by the significant differences between their body sizes at the beginning and at the end of the experiment (GLMM, F1,105 = 7.481, P = 0.007). However, body growth was not significantly different by species (F2,105 = 1.818, P = 0.167) or by mating opportunities (F2,105= 0.434, P= 0.649).

6. Discussion

Our results show that the three species differed in their sexallocation responses to mating opportunities. Both *O. diadema* and *O. adherens* worms plastically changed their female allocation in relation to mating opportunities and these changes were consistent whatever variable (resource investment in eggs, number of egg cocoons and time interval between egg-layings) we used in the comparison. In contrast, *O. gracilis* worms did not exhibit any phenotypic plasticity in these traits in our experimental conditions. The three species did not show any significantly plastic response to mating opportunities in their investment in spermnumber. This finding confirms previous results in *O. diadema* (Lorenzi et al., 2005), in which hermaphrodites adjust their behavior to mating opportunities and compete more aggressively asmating opportunities – and rivals on the male role – increase, while the number of sperm does not increase (Lorenzi et al., 2006). Similarly, some fish adjust their behavior – and not their sperm numbers – to mate competition (Scaggiante et al., 2005). In *O. adherens* and *O. gracilis* worms, resources might trade-off as well between egg production and behavioral mate competition, rather than between egg and sperm production. Indeed, pre-mating sexual selection might be stronger than post-mating sexual selection (Lorenzi and Sella, 2008). In support of this hypothesis, it is worth recalling that the *Ophryotrocha* species produce very few aflagellate sperm and have to deposit them on their partner's eggs, inside the jelly cocoon (Oug, 1990; Premoli and Sella, 1995). It is likely that worms get more egg fertilized if they compete to get closer to their egg-releasing partner than if they produce large number of immotile sperm.

If sperm production is cheaper than egg production (Di Bona et al., 2010; Ramirez Llodra, 2002), our results may suggest that the selection for sex allocation plasticity might be stronger on the traitwith the largest costs. This hypothesis is supported by data from other hermaphroditic species. For example, the male function is largely plastic in barnacles where it is likely to involve relatively high costs. Barnacles fully develop their penises before every reproductive season varying their shapes adaptively according to environmental conditions (population density and wave exposure, Hoch and Levinton, 2012). In some hermaphrodites phenotypic plasticity in sex allocation is expressed only under starvation (e.g., Locher and Baur, 2002; Schärer et al., 2005). Resource budgets become stringent under starvation and penalties for sex allocation/mating opportunities mismatches cannot be paid.

O. diadema worms strongly decreased the amount of resources they invested in the female function when the number of rivals increased from low to intermediate mating opportunities. These results confirm that these worms make accurate estimates of their social environment as they respond to changes from one to three adult conspecifics (Schleicherová et al., 2006, 2010). Instead, *O. adherens* worms processed variations between low and intermediatemating opportunities, but did so less accurately than *O. diadema* worms did. Perceptual and response abilities vary among species, as they are

shaped by the ecology of the species (Shettleworth, 2010) and empirical data show that polychaetes exhibit some level of numerical acuity in evaluating social group size (Minetti et al., 2013; Schleicherová et al., 2006, 2010).

In *Ophryotrocha* worms, the magnitude of phenotypic plasticity in the female function may not depend only on the species-specific costs of the female function. The phenotypic plasticity in female allocation may also be associated with the fact that worms live in intertidal communities in which density often fluctuates (Prevedelli et al., 2005; Reichert and Buchholz, 2006 in *Ophryotrocha* worms) and mating opportunities may vary accordingly. Measuring to what extent the densities of the natural populations of the three species are stable – using density as a proxy for mating opportunities – would help us understand whether the magnitude of plasticity in sex allocation is a response to fluctuations in mating opportunities, and would possibly help explain why *O. diadema* worms might be more plastic than *O. adherens* in their female function,whereas *O. gracilis* was not plastic at all, in our experimental conditions. Such measures of population densities are not available, to our knowledge, possibly because the hermaphroditic *Ophryotrocha* populations are very rare (e.g. Prevedelli et al., 2005; Simonini et al., 2010). Population densities and their fluctuations are likely to contribute to shape the mating systems in these worms. *O. diadema* worms conditionally reciprocate sexual roles when they are in isolated pairs; they swap eggs and retaliate cheating (Sella, 1985). This mating system is a good example of howselection promotes cooperation (Crowley et al., 1996; Dugatkin, 2002), suggesting that these worms have been selected for optimizing their reproductive behavior under very low population densities. Nevertheless, they opportunistically adjust their sex allocation as mating opportunities increase (Lorenzi et al., 2005, this paper). Little is known about the mating system of *O. gracilis*. Although thesewormsmake egg swapping as *O. diadema* worms, they do it less regularly than *O. diadema* (Premoli and Sella, 1995). Finally, focused studies on the mating system of O. adherens worms are lacking. These hermaphrodites do not exhibit preferences for reciprocating partners (Paavo et al., 2000), suggesting that they are not specialized in egg swapping. Additionally, pairs of worms stay stuck to each other by means of specialized secretions — which might contribute to reduce mate competition in crowded populations. Therefore, *O. adherens* worms might have been selected less strongly than *O. diadema* worms for perceiving small variations in mating opportunities and adjusting their sex allocation. In summary, both the relative costs ofmale and female functions and species-specific characteristics of the mating system may have influenced the magnitude of phenotypic plasticity in sex allocation.

Acknowledgments

We are particularly grateful to Prof. Bertil Åkesson from the University of Göteborg who kindly provided O. adherens and O. gracilis worms. We thank Carla Debernardi and Liliana Ramella for their help with the experiment. We also thank J. Auld and two anonymous referees for constructive comments on earlier versions of the manuscript.

This work was funded by the ItalianMinistry of University and Scientific Research (MIUR) (PRIN 2008 YS7AP3 to G.S.) and the University of Turin (ex 60% to M.C.L. and G.S.). [RH]

References

Åkesson, B., 1976. Morphology and life cycle of Ophryotrocha diadema, a new polychaete species from California. Ophelia 15, 23–35.

Auld, J.R., Agrawal, A.A., Relyea, R.A., 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proc. R. Soc. Lond. B 277, 503–511.

Baeza, J.A., 2007. Male mating opportunities affect sex allocation in a protrandricsimultaneous hermaphroditic shrimp. Behav. Ecol. Sociobiol. 61, 365–370.

Barbosa, F., 2012. Males responding to sperm competition cues have higher fertilization success in a soldier fly. Behav. Ecol. 23, 815–819.

Brauer, V.S., Schärer, L., Michiels, N., 2007. Phenotypically flexible sex allocation in a simultaneous hermaphrodite. Evolution 61, 216–222.

Candolin, U., Reynolds, J.D., 2002. Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (Rhodeus sericeus). Proc. R. Soc. Lond. B 269, 1549–1553.

Charnov, E.L., 1982. The Theory of Sex Allocation. Princeton University Press, Princeton NJ.

Crawley, M.J., 1993. GLIM for Ecologists. Blackwell Scientific, Oxford.

Crowley, P.H., Provencher, L., Sloane, S., Dugatkin, L.A., Spohn, B., Rogers, L., Alfieri, M., 1996. Evolving cooperation: the role of individual recognition. Biosystems 37, 49–66.

Dahlgren, T.G., Åkesson, B., Schander, C., Halanych, K.M., Sundberg, P., 2001. Molecular phylogeny of the model annelid Ophryotrocha. Biol. Bull. 201, 193–203.

Delph, L.F.,Wolf, D.E., 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. New Phytol. 166, 119–128.

Di Bona, V., Lorenzi, M.C., Sella, G., 2010. Functional males in pair-mating outcrossing hermaphrodites. Biol. J. Linn. Soc. 100, 451–456.

Dugatkin, L.A., 2002. Animal cooperation among unrelated individuals. Naturwissenschaften 9, 533–541.

Gage, M.J.G., 1995. Continuous variation in reproductive strategy as an adaptive response to population density on the moth Plodia interpunctella. Proc. R. Soc. Lond. B 261, 331–337.

Hart, M.K., Svoboda, A., Mancilla-Cortez, D., 2011. Phenotypic plasticity in sex allocation for a simultaneously hermaphroditic coral reef fish. Coral Reefs 30, 543–548.

Hoch, J.M., Levinton, J.S., 2012. Experimental tests of sex allocation theory with two species of simultaneously hermaphroditic acorn barnacles. Evolution 5, 1332–1343.
Hughes, R.N.,Manríquez, P.H., Bishop, J.D.D., 2002. Female investment is retarded pending reception of allosperm in a hermaphroditic colonial invertebrate. Proc. Natl. Acad. Sci. U. S. A. 99, 14884–14886.
Locher, R., Baur, B., 2002.Nutritional stress changes sex-specific reproductive allocation in the simultaneously hermaphroditic land snail Arianta arbustorum. Funct. Ecol. 16, 623–632.
Lorenzi, M.C., Sella, G., 2008. A measure of sexual selection in hermaphroditic animals: parentage skew and the opportunity for selection. J. Evol. Biol. 21, 827–833.
Lorenzi, M.C., Sella, G., 2013. In between breeding systems: neither dioecy nor androdioecy explains sexual polymorphism in functionally dioecious worms. Integr. Comp. Biol. 53, 689–700.
Lorenzi, M.C., Sella, G., Schleicherová, D., Ramella, L., 2005. Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. J. Evol. Biol. 18, 1341–1347.
Lorenzi, M.C., Schleicherová, D., Sella, G., 2006. Life history and sex allocation in the simultaneously hermaphroditic polychaetewormOphryotrocha diadema: the role of sperm competition. Integr. Comp. Biol. 46, 381–389.
Lorenzi, M.C., Schleicherová, D., Sella, G., 2008. Sex adjustments are not functionally costly in simultaneous hermaphrodites. Mar. Biol. 153, 599–604.
Lorenzi, M.C., Sella, G., Schleicherova, D., 2014. Demographic costs of sex allocation: hermaphrodites perform better in small populations. Ital. J. Zool. (in press).
Lorenzi, M.C., Schleicherova, D., Sella, G., 2014. Multiple paternity and mate competition in non-selfing, monogamous, egg-trading hermaphrodites. Acta Ethol. http:// dx.doi.org/10.1007/s10211-013-0169-x (in press).
Minetti, C., Sella, G., Lorenzi, M.C., 2013. Population size, not density, serves as cue for sex ratio adjustments in polychaete worms. Ital. J. Zool 80, 547–551.
Morrow, E.H., 2004. How the sperm lost its tail: the evolution of aflagellate sperm. Biol. Rev. 79, 795–814.
Oug, E., 1990. Morphology, reproduction, and development of a new species of Ophryotrocha (Polychaeta: Dorvilleidae) with strong sexual dimorphism. Sarsia 75, 191–201.
Paavo, B., Bailey-Brock, J.H., Åkesson, B., 2000.Morphology and life history of Ophryotrocha adherens sp. nov. (Polychaeta, Dorvilleidae). Sarsia 85, 251–264.
Premoli, M.C., Sella, G., 1995. Sex economy in benthic polychaetes. Ethol. Ecol. Evol. 7, 27–48.
Prevedelli, D., Massamba N'Siala, G., Simonini, R., 2005. The seasonal dynamics of six species of Dorvilleidae (Polychaeta) in the harbour of La Spezia (Italy). Mar. Ecol. 26, 286–293.
Ramirez Llodra, E., 2002. Fecundity and life-history strategies in marine invertebrates. Adv. Mar. Biol. 43, 87–170. Reichert, K., Buchholz, F., 2006. Changes in the macrozoobenthos of the intertidal zone at Helgoland (German Bight, North Sea): a survey of 1984 repeated in 2002. Helgol. Mar. Res. 60, 213–223.
Richardson, D.S., Burke, T., 2001. Extrapair paternity and variance in reproductive success related to breeding density in Bullock's orioles. Anim. Behav. 62, 519–525.
Scaggiante, M., Rasotto, M.B., Romualdi, C., Pilastro, A., 2005. Territorial male gobies respond aggressively to sneakers but do not adjust their sperm expenditure. Behav. Ecol. 16, 1001–1007.
Schärer, L., 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. Evolution 63, 1377–1405. Schärer, L., Ladurner, P., 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proc. R. Soc. Lond. B 270, 935–941.
Schärer, L., Sandner, P., Michiels, N.K., 2005. Trade-off betweenmale and female allocation in the simultaneously hermaphroditic flatworm Macrostomum sp. J. Evol. Biol. 18, 396–404.
Schleicherová, D., Lorenzi, M.C., Sella, G., 2006. How outcrossing hermaphrodites sense the presence of conspecifics and suppress female allocation. Behav. Ecol. 17, 1–5.
Schleicherová, D., Lorenzi, M.C., Sella, G., Michiels, N.K., 2010. Gender expression and group size: a test in a hermaphroditic and a gonochoric congeneric species of Ophryotrocha (Polychaeta). J. Exp. Biol. 213, 1586–1590.
Schleicherová, D., Lorenzi, M.C., Sella, G., 2013. Do stable environments select against phenotypic plasticity in hermaphroditic sex allocation? Ital. J. Zool. 80, 358–363.
Sella, G., 1985. Reciprocal egg trading and brood care in a hermaphroditic polychaete worm. Anim. Behav. 33, 938–944. Sella, G., 1990. Sex allocation in the simultaneous hermaphroditic polychaete worm Ophryotrocha diadema. Ecology 71, 27–32.
Sella, G., Lorenzi, M.C., 2000. Partner fidelity and egg reciprocation in the simultaneously hermaphroditic polychaete worm Ophryotrocha diadema. Behav. Ecol. 11, 260–264.
Sella, G., Lorenzi, M.C., 2003. Increased sperm allocation delays body growth in a protandrous simultaneous hermaphrodite. Biol. J. Linn. Soc. 78, 149–154.
Sella, G., Marzona, M., 1983. Inheritance, maternal influence and biochemical analysis of an egg color polymorphism in Ophryotrocha diadema. Experientia 39, 97–98.
Sella, G., Ramella, L., 1999. Sexual conflict and mating system in the dorvilleid genus Ophryotrocha and the dinophilid genus Dinophilus. Hydrobiologia 402, 203–213.
Sella, G., Premoli, M.C., Turri, F., 1997. Egg trading in the simultaneously hermaphroditic polychaete worm Ophryotrocha gracilis (Huth). Behav. Ecol. 8, 83–86.
Shettleworth, S.J., 2010. Cognition, Evolution and Behavior. Oxford University Press.

Shine, R., Langkilde, T., Mason, R.T., 2003. The opportunistic serpent: male garter snakes adjust courtship tactics to mating opportunities. Behaviour 140, 1509–1526.

Shuker, D.M., Pen, I.,West, S.A., 2006. Sex ratios under asymmetrical local mate competition in the parasitoid wasp Nasonia vitripennis. Behav. Ecol. 17, 345–352.

Simonini, R., Massamba-N'Siala, G., Prandi, V., Prevedelli, D., 2009. Distribution of the genus Ophryotrocha (Polychaeta) in Italy: new records and comments on the biogeography of Mediterranean species. Vie Milieu 59, 79–88.

Simonini, R., Prandi, V., Massamba-N'Siala, G., Martino, M.P., Castelli, A., Prevedelli, D., 2010. Diversity, habitat affinities and diet of Ophryotrocha species (Polychaeta, Dorvilleidae) living in Mediterranean harbour habitats. Vie Milieu 60, 27–38.

Tan, G.N., Govedich, F.R., Burd, M., 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, Helobdella papillornata (Euhirudinea: Glossiphoniidae). J. Evol. Biol. 17, 574–580.

Thornhill, D.J., Dahlgren, T.G., Halanych, K.M., 2009. Evolution and ecology of Ophryotrocha (Dorvilleidae, Eunicida). In: Shain, D.H. (Ed.), Annelids inModern Biology. JohnWiley & Sons, Hoboken, NJ, pp. 241–256.

Wedell, N., Gage, M.J.G., Parker, G.A., 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17, 313–320.

Westheide, W., 1984. The concept of reproduction in polychaetes with small body size: adaptation in interstitial species. Fortschr. Zool. 29, 265–287.

Wilson, K., Hardy, I.C.W., 2002. Statistical analysis of sex ratios: an introduction. In: Hardy, I.C.W. (Ed.), Sex Ratios: Concepts and Research Methods. Cambridge Univ. Press, Cambridge, UK, pp. 48–92.

Figures and Tables

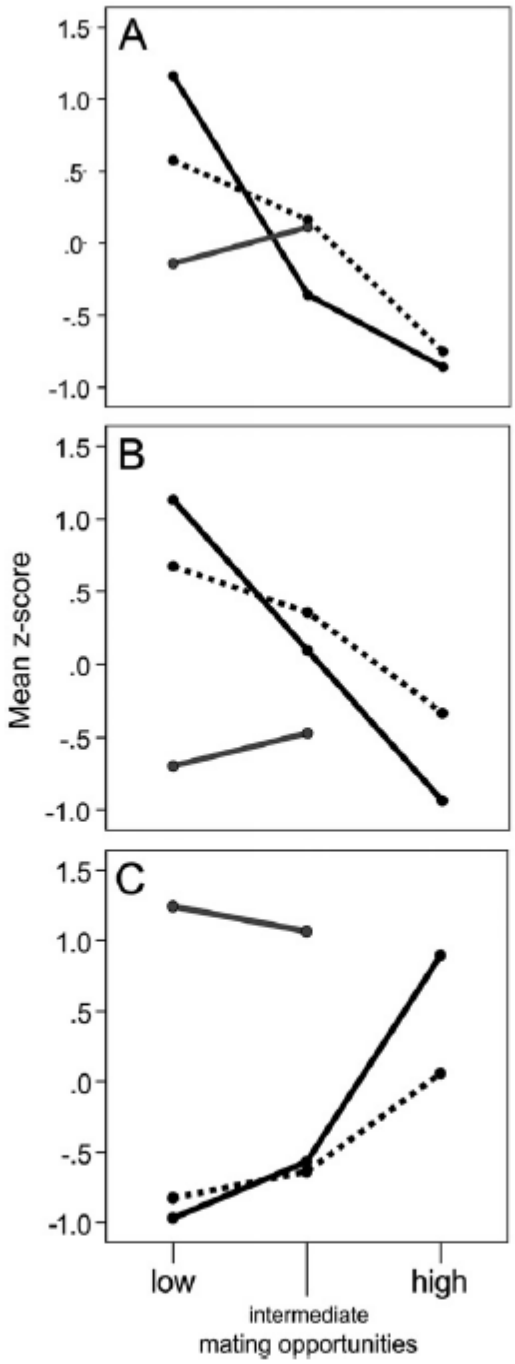

Fig. 1. The phenotypic plasticity in the female function in the three species *O. diadema* (black line), *O. adherens* (dashed line) and *O. gracilis* (gray line) as a response to the levels of mating opportunities (low, intermediate and high mating opportunities for *O. diadema* and *O. adherens*, low and intermediate mating opportunities for *O. gracilis*). The graphs show the estimated marginal means of the Z-scores of the three variables that describe the female function: A = resource investment in eggs, B = number of egg cocoons, C = intervals between egg-layings.

Table 1
Mean values (± s.e.) of the traits that describe female function and body size in *O. diadema*, *O. adherens* and *O. gracilis* in low, intermediate and high mating opportunities.

Species	Mating opportunities	Resource investment in eggs	Number of egg cocoons	Time interval between egg-layings (days)	Total number of eggs	Body size (chaetigerous segments)
O. diadema	Low	1.06 ± 0.09	4.40 ± 0.21	4.95 ± 0.26	94.33 ± 7.64	18.67 ± 0.29
	Intermediate	0.39 ± 0.04	2.80 ± 0.18	7.93 ± 0.51	34.27 ± 3.47	17.33 ± 0.23
	High	0.16 ± 0.03	1.29 ± 0.19	15.75 ± 1.58	14.43 ± 3.06	17.36 ± 0.23
O. adherens	Low	1.53 ± 0.26	2.64 ± 0.37	9.34 ± 1.55	93.50 ± 16.03	14.79 ± 0.28
	Intermediate	1.19 ± 0.17	2.27 ± 0.27	11.25 ± 1.40	72.60 ± 10.14	15.20 ± 0.33
	High	0.43 ± 0.10	1.36 ± 0.27	14.64 ± 1.88	26.21 ± 6.08	14.21 ± 0.24
O. gracilis[*]	Low	0.06 ± 0.03	0.50 ± 0.20	18.90 ± 2.10	2.92 ± 1.20	34.67 ± 1.12
	Intermediate	0.09 ± 0.04	0.80 ± 0.30	15.75 ± 2.56	4.40 ± 1.82	36.93 ± 0.69

[*] Due to our inability to track a focal *O. gracilis* worm in a large group of conspecific worms, we only collected data from low and intermediate levels of mating opportunities for this species.