

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## Labile Sex Expression and the Evolution of Dioecy in Ophryotrocha Polychaete Worms

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/150315> since 2016-06-23T10:54:48Z

*Published version:*

DOI:10.1007/s11692-014-9297-0

*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)



# UNIVERSITÀ DEGLI STUDI DI TORINO

1

2

3 This is an author version of the contribution published  
4 on: Questa è la versione dell'autore  
5 dell'opera: [**Evolutionary Biology DOI: 10.1007/s11692-014-**  
6 **9297-0**]

7 The definitive version is available at:

8 La versione definitiva è disponibile alla URL:

9 [<http://link.springer.com/article/10.1007/s11692-014-9297-0>]

10

11

12 **Labile sex expression and the evolution of dioecy in *Ophryotrocha***

13 **polychaete worms**

14

15

16

17

18 S. Meconcelli<sup>§</sup>, M. C. Lorenzi<sup>§</sup>, G. Sella<sup>§</sup>

19

20

21 <sup>§</sup> Department of Life Sciences and Systems Biology, Università di Torino, Turin, Italy.

22

23

24

25

26

27 *correspondence:* Stefania Meconcelli, Department of Life Sciences and Systems Biology,

28 Università di Torino, Via Accademia Albertina 13, 10123 Turin, Italy. Tel.: +39 011 670

29 4511; e-mail: stefania.meconcelli@unito.it

30

31 Short title: Labile sex expression in *Ophryotrocha* worms

32

33

34

## 35 **Abstract**

36 Labile sex expression is considered to play a key role in the evolution of breeding systems and in the  
37 transition from hermaphroditism to dioecy, according to the evolutionary models proposed for plants. While in  
38 hermaphrodites sex allocation within the individual can be plastically adjusted in response to social environment,  
39 in dioecious species it is predicted to be fixed. However, labile sex expression in the form of gender plasticity  
40 can still be present in dioecious species of animals with environmental sex determination. It is still unclear how  
41 gender plasticity is involved in the evolution of breeding systems and what its role is in the transition from  
42 hermaphroditism to dioecy. We assessed the degree of plasticity in gender expression in three dioecious species  
43 of polychaete worms of the genus *Ophryotrocha*. We found sexual polymorphism and plasticity in sex  
44 expression during the juvenile phase to be a response to social environment. The majority of juveniles reared  
45 with an adult female or male expressed the gender opposite of that of the partner, so as to form heterosexual  
46 pairs. On the basis of these findings we outline a possible evolutionary pathway of the transition from  
47 hermaphroditism to dioecy in the genus *Ophryotrocha*.

48

49

### 50 **Keywords:**

51 Gender plasticity; pseudohermaphrodite; monoecy; evolutionary transition; environmental sex  
52 determination

53

54

55

56

57

## 58 **Introduction**

59 Labile sex expression is widespread among plants and animals (Charnov and Bull 1977; Korpelainen  
60 1990,1998; Delph and Wolf 2005). Natural selection is expected to favor organisms with labile sex expression  
61 when individual fitness as a male or female is strongly influenced by environmental factors and when parents  
62 cannot predict in which environment the offspring will live (Charnov and Bull 1977).

63        Given their lack of mobility, plants are highly exposed to environmental variations and are consequently  
64 more prone to adapt to different environments plastically (Bazzaz 1991). Indeed plants are often characterized by  
65 labile sex expression in response to different environmental conditions (Freeman et al. 1980). As a consequence  
66 of this high lability in sex expression, there is a large variety of breeding systems in plants in addition to dioecy  
67 and hermaphroditism – namely, gynodioecy, androdioecy and subdioecy (or trioecy) (Renner and Ricklefs  
68 1995; Ehlers and Bataillon 2007). These latter breeding systems are considered to represent intermediate stages  
69 in the evolutionary transition between hermaphroditism and dioecy (Charlesworth and Charlesworth 1978;  
70 Freeman 1997; Delph 2005; Barrett 2013). For this reason, labile sex expression is considered to have an  
71 important role in the evolution of breeding systems and in the transition from hermaphroditism to dioecy  
72 (Freeman 1997; Delph and Wolf 2005; Crossman and Charlesworth 2013).

73        In animals, labile sex expression in the form of plasticity in gender expression is generally observed  
74 when the mechanism of sex determination is environmental (Charnov and Bull 1977; Mankiewicz et al. 2013).  
75 Environmental sex determination involving phenotypic plasticity in gender is common in invertebrates (Leonard  
76 2013), while in vertebrates it has been found only in fishes and reptiles (Bull 1983; Godwin et al. 2003; Sarre et  
77 al. 2004). The environmental factors which influence sex expression in invertebrates, fish and reptiles are both  
78 abiotic (e.g., temperature, photoperiod, nutrition, density, pH, UV light, metabolic products, salinity and light)  
79 and biotic (e.g., parasites, exposure to the opposite sex, social cues and host characteristics in parasitoids) (Bull  
80 1983; Korpelainen 1990; Godwin et al. 2003; Sarre et al. 2004).

81        Adaptation of sex expression to the environment is also a common feature of hermaphroditic plants and  
82 animals (Charnov 1977). Both are able to allocate reproductive resources to female and male function in  
83 response to environmental conditions, such as population size or mating opportunities (Pannell 1997; Charnov  
84 1977; Korpelainen 1998; Schärer 2009; Schleicherová et al. 2014).

85        Sex allocation theory mainly focuses on species with fixed sex expression, while several species display  
86 labile sex expression. According to sex allocation theory, dioecious species are only able to change their  
87 offspring sex ratio (Charnov 1982; Schärer 2009). Therefore, within the individual, the expression of gender and  
88 sex allocation are predicted to be fixed, independent of group size variations and uninfluenced by mating  
89 opportunities.

90        In contrast with this prediction, plasticity in gender expression can still be present in dioecious species  
91 that have a hermaphroditic ancestor and environmental sex determination, at least in the developmental stage  
92 (Korpelainen 1998). Little is known about the degree of plasticity in gender expression in dioecious species of

93 animals with environmental sex determination. In some of these species, plasticity in gender expression during  
94 the juvenile phase can be elicited by the gender of a conspecific adult. If that adult represents the only social  
95 environment that the juvenile will experience, as in a low density population, we can expect that the juvenile will  
96 be able to express the gender opposite to that of the adult. There are several examples among invertebrates of  
97 this kind of influence on gender expression: the marine worm *Bonellia viridis* (Echiura) (Bacci 1965; Leutert  
98 1975; Agius 1979; Berec 2005), the siboglinid worms of the genus *Osedax* (Vrijenhoek et al. 2008), the  
99 crustacean parasites *Pachypygus gibber* (Copepoda) (Hipeau-Jacquotte 1978; Becheikh et al. 1998; Michaud et  
100 al. 2004), *Ione thoracica* (Isopoda) and *Stegophryxus hyptius* (Isopoda), some parasitic species of mermithids  
101 (Nematoda) (Parenti 1965) and the dioecious species of the marine polychaete worms of the genus *Ophryotrocha*  
102 (Rolando 1984).

103 In the genus *Ophryotrocha* there are dioecious, simultaneously hermaphroditic and sequentially  
104 hermaphroditic species and all of them show a large extent of labile sex expression in response to social  
105 conditions. Therefore this genus presents us with a target model system for studying the plasticity of gender  
106 expression from an evolutionary perspective. For example, in the sequential hermaphroditic species *O. puerilis*,  
107 when pairs of two females are formed, one of the two worms, usually the youngest one, changes to the male sex,  
108 so as to form a heterosexual pair (Åkesson 1974; Pfannenstiel 1975, 1977; Kegel and Pfannenstiel 1983;  
109 Berglund 1986). In the dioecious species *Ophryotrocha labronica* and other *Ophryotrocha* dioecious species,  
110 sex expression in a juvenile is influenced by the presence of a sexually mature worm so that the juvenile will  
111 develop the sex opposite to that of its partner significantly more often than expected (Bacci et al. 1979; Rolando  
112 1983, 1984). Conversely, abiotic environmental factors have no influence on gender expression (Åkesson 1975;  
113 Prevedelli et al. 1998; Prevedelli and Simonini 2001). Moreover, some *Ophryotrocha* dioecious species cannot  
114 be defined as purely dioecious. The presence of four sexual phenotypes (i.e. pure male, male with a few oocytes,  
115 pure female, and female with a few sperm) has been reported repeatedly (Pfannenstiel 1976; Rolando and Giorda  
116 1982; Rolando 1983; Lorenzi and Sella 2013). Lorenzi and Sella (2013) interpret this sexual polymorphism as a  
117 vestigial trait of an ancestral hermaphroditic state, which was inferred from phylogenetic analyses based on  
118 morphological and molecular markers (Dahlgren et al. 2011; Thornhill et al. 2009).

119 As opposed to plants, in animals it is still unclear how gender plasticity is involved in  
120 the evolution of breeding systems and what its role is in the transition from hermaphroditism  
121 to dioecy. Therefore the study of the variation of plasticity in the expression of the sexual  
122 phenotypes may help to identify a possible evolutionary pathway of the evolution of dioecy

123 from a hermaphroditic ancestor. If plastic sex allocation in response to social group size is one  
124 of the main advantages of hermaphroditism over dioecy (Schärer 2009), we can expect a  
125 reduction or a loss of plasticity in sex allocation in the transitions from hermaphroditism to  
126 dioecy. This reduction of plasticity could be manifested as a decrease in the ability of sensing  
127 and/or responding to environmental stimuli, or as a reduction of the time-window when  
128 plasticity can be expressed. In the present study, we tested for variations in the degree of  
129 gender plasticity of juveniles and adults in three sexually dioecious species of *Ophryotrocha*  
130 worms – *Ophryotrocha labronica*, *Ophryotrocha robusta* and *Ophryotrocha macrovifera*,  
131 according to the social environment they were exposed to – i.e the presence of an adult male  
132 or female. The three species have similar morphology and reproductive biology but they  
133 differ in some genomic aspects (*O. macrovifera* and *O. labronica* have a different number of  
134 chromosomes compared to *O. robusta* (Robotti et al. 1991); and the genome size of *O.*  
135 *macrovifera* is twice that of the other two species (Sella et al. 1993)). The three species  
136 diverge also in their geographical distribution (Simonini 2009; Paxton and Åkesson 2010).

137 In the current study, we found that plasticity in gender expression in the three species  
138 was confined to the juvenile stage, that four sexual phenotypes (pure males, pure females,  
139 males with a few oocytes and females with a few sperm) were expressed in the populations of  
140 the three species and that, in the adult phase, individuals expressed only one of the four sexual  
141 phenotypes. The presence of sexual polymorphism among adults together with plasticity in  
142 the sex expression of juveniles allowed us to outline the transition from ancestral  
143 simultaneous hermaphroditism to dioecy via monoecy (i.e. a situation where the  
144 hermaphroditic organism has distinct female and male gonads) as the most likely evolutionary  
145 pathway (Freeman et al. 1997; Golenberg and West 2013).

146

## 147 **Materials and methods**

**149 Study species and animal rearing**

150 The external morphology and life cycle parameters of *O. labronica*, *O. robusta* and *O. macrovifera* are only  
151 slightly different (Table 1). In the three species mating is achieved by pseudo-copulation, a process of external  
152 fertilization in which partners reach close physical contact before releasing their gametes (Westheide 1984).  
153 Eggs are released in water and are enveloped by a transparent mucous cocoon, through which egg development  
154 can be easily observed. Females grow faster than males and reach sexual maturity at a body size larger than that  
155 of males. Both sperm and oocytes originate from the same clusters of primordial germ cells and then mature  
156 freely floating in the coelom (Pfannenstiel and Grünig 1982; Brubacher and Huebner 2009). Ripe oocytes can be  
157 easily seen from the transparent body walls, while unripe oocytes and sperm can only be observed after intense  
158 manipulations of worms. Sexual dimorphism consists of a wider prostomium and a larger and thicker upper jaw  
159 in males than in females. These traits, together with presence of visible oocytes, make it easy to distinguish  
160 males from females by visual inspection. In addition, males have more rosette glands than females. Rosette  
161 glands are located dorsally one per segment on the posterior segments of the body. The rosette glands have been  
162 described for all the three species (Paxton and Åkesson 2010), but their function has never been investigated.  
163 They can be easily observed under a phase-contrast microscope (250X). Sexual dimorphism in secondary sexual  
164 traits such as prostomium and jaw size and shape allowed us to distinguish only two sexual phenotypes, male  
165 and female, although four sexual phenotypes (pure female, pure male, male with oocytes and female with sperm)  
166 can be identified in these worms by also looking at the types of gametes present in every individual.

167 In *Ophryotrocha* species, the sex determining mechanism and sex ratio control are supposed to be polygenic  
168 (Bacci 1978; Premoli et al. 1996). Polygenic systems are known to be very sensitive to various environmental  
169 effects (Falconer 198; Bull 1983). However in *Ophryotrocha* species, abiotic environmental factors such as  
170 temperature, photoperiod, salinity, artificial or natural marine water and diet do not influence gender expression  
171 (Åkesson 1975; Prevedelli et al. 1998; Prevedelli and Simonini 2001).

172 *Ophryotrocha* species occur interstitially, at relatively low density in shallow, nutrient-rich waters  
173 (Thornhill et al. 2009). *Ophryotrocha labronica* has a cosmopolitan worldwide distribution (Paxton and Åkesson  
174 2010) and inhabits both harbors and brackish water environments (Simonini 2009). *O. macrovifera* is much rarer  
175 than *O. labronica*. It was found in only a few localities along the Mediterranean sea and the North Atlantic  
176 coasts (Paxton and Åkesson 2010; Simonini 2009). *O. robusta* is endemic to the Mediterranean sea, where it  
177 occurs only in a few localities (Paxton & Åkesson, 2010, Simonini, 2009). Because of the low mobility of these



178 worms, different populations are supposed to be quite reproductively isolated (Lanfranco and Rolando 1981;  
179 Sella and Robotti 1986).

180 All experiments were carried out using laboratory populations established several years ago starting from  
181 large samples of worms collected from the wild (*O. macrovifera* from Chioggia, Italy (2006), *O. labronica* from  
182 Alamitos Beach, Long Beach, California, USA (2005) and *O. robusta* from Porto Empedocle, Italy (2010)).  
183 Animals were reared in 30 ml bowls with filtered artificial marine water (33 psu) at a constant temperature of 21  
184 °C and fed with spinach *ad libitum*.

185

### 186 **Experimental design**

187 To test how the presence of an adult male or female influences the expression of the  
188 sexual phenotype in juveniles in the three species, we set up 55 pairs of parents (20 pairs of *O.*  
189 *labronica*, 20 pairs of *O. macrovifera* and 15 pairs of *O. robusta*). From the offspring of these  
190 pairs we selected 330 juveniles (6 per pair) (hereafter “experimental worms”) as soon as they  
191 had a body length of 3 segments with setae. The selected juveniles were assigned to three  
192 treatments (2 experimental worms of each family per treatment) (Figure 1): 1) juvenile paired  
193 with an adult female, 2) juvenile paired with an adult male, and 3) juvenile isolated as a  
194 control. We expected experimental worms to develop the gender opposite to that of their  
195 partner. Therefore, we expected sex ratio in treatment 1) and 2) to differ from the sex ratio in  
196 our control treatment. Adult males and females (hereafter “partners”) used in treatments 1)  
197 and 2) were obtained from the progeny of 108 pairs (36 per species) and were all of the same  
198 age (21 days). When the experimental worms reached a clear sexual differentiation, we sexed  
199 them. They were sexed according to the presence of visible oocytes in females and of a  
200 prostomium and an upper jaw larger in males than in females.

201 To test the effect of the presence of an adult male or female on the expression of the  
202 sexual phenotypes of sexually mature individuals of the three species, we used a subsample of  
203 the sexually mature experimental worms and formed 87 homosexual pairs by pairing each of  
204 them with a partner. If gender plasticity is still present in the adult stage, we can expect

205 worms in homosexual pairs to be stimulated to produce gametes of the sex opposite to that of  
206 their partner's. Ninety heterosexual pairs were set up as controls. To check for the presence of  
207 oocytes in males and sperm in females, we needed to kill worms. Therefore we formed these  
208 pairs relying on external sexual dimorphism only, thus without distinguishing pure females  
209 from females with sperm and pure males from males with oocytes. Pairs were reared for a  
210 time interval that allowed all the heterosexual pairs to lay at least two egg masses. We  
211 guessed that those homosexual pairs in which at least one of the partners had both oocytes and  
212 sperm would have had the opportunity to lay at least one egg mass in that same time interval.

213 All experimental worms were eventually checked for sperm in females or oocytes in  
214 males. To check for the presence of sperm, worms were gently squeezed between two slides,  
215 so that sperm oozed out of the parapodia, and were observed by phase-contrast microscopy  
216 (250X). Oocytes can be easily identified from the transparent body walls of the worms at  
217 250X magnification. Females that had sperm and males that had oocytes were classified as  
218 pseudohermaphrodites, because generally in these worms only one type of gamete is  
219 functional (Baldi et al. 2009; Lorenzi and Sella 2013). In a subsample of worms ( $n = 184$ ; 64  
220 from treatment 1, 57 from treatment 2 and 63 from treatment 3), we measured the  
221 developmental time to sexual differentiation as the number of days from the stage of 3  
222 segments with setae to sexual maturity.

223 In order to check for a correlation between sexual phenotype and number of rosette  
224 glands (Lorenzi and Sella 2013; Paxton and Åkesson 2010), we also measured the number of  
225 rosette glands and the number of segments with setae (as an estimate of body size) in the  
226 same subsample. Measures were taken under phase-contrast microscopy (250X).

## 227 **Statistical analysis**

228 We first focused on sex ratio, i.e., the effect of social environment during the juvenile phase on worm sex  
229 expression. We tested whether the sex ratio (i.e., the frequencies of sexual phenotypes in experimental worms)

230 differed according to treatment in the juvenile phase using a Generalized Linear Mixed Model (GLMM) with  
231 binomial distribution. Sex was assigned based on external morphology, therefore juveniles became either males  
232 (pure males and males with oocytes) or females (pure females and females with sperm). Predictor variables  
233 included species and social environment (i.e. juvenile + male, juvenile + female, isolated juvenile). The sibship  
234 of every experimental worm was added as a random blocking to control for similarities in the proportion of the  
235 different sexual phenotypes within families. Since the sex of worms was not significantly affected by treatment  
236 during the adult phase, in the GLMM we used all the data obtained from the 330 juveniles that entered the  
237 experiment.

238 Then, we focused on how many juveniles matured the gender opposite to their partner's. Using a Generalized  
239 Linear Model (GLM) with Poisson error distribution and a log link function, we analyzed the difference between  
240 the number of experimental worms that matured the gender opposite to their partner's and the number of  
241 experimental worms that matured the same gender as their partner's (heterosexual pairs vs. homosexual pairs). In  
242 this statistical analysis pseudohermaphrodites (males with oocytes and females with sperm) were therefore  
243 excluded. The same statistical analysis was used to compare the number of pseudohermaphrodites among the  
244 three social environments and species.

245         Using a Generalized Linear Mixed Model (GLMM) with Poisson error distribution and a log link  
246 function, we also analyzed the developmental time (i.e., the number of days that passed from the stage of 3  
247 segments with setae to the sexual differentiation stage). Predictor variables included sexual phenotype, species  
248 and social environment. The sibship of every experimental worm was handled as the random factor. Three  
249 different GLMMs, one for every sexual phenotype (males, females and pseudohermaphrodites), were made to  
250 compare the developmental times among the three social environments. As in the previous analysis, predictors  
251 were species and social environment, while sibship was a random factor. We used the results of these statistical  
252 tests only to assess differences in developmental times between social environments within the same sexual  
253 phenotype.

254         For all the analyses, we followed a model selection process based on Aikaike's information criterion  
255 (AIC), which is a measure of model fit. AIC was recorded from models including all possible combinations and  
256 interactions of effects, and we selected the model having the lowest AIC (Quinn and Keough 2002). In the  
257 GLMM and GLM with Poisson error distribution we also checked for overdispersion.

258         We assessed whether the proportion of sexual phenotypes in the adult phase differed between homo-  
259 and hetero-sexual pairs using a  $2 \times 4$  contingency table (Chi-squared test).

260 Finally, we analyzed the number of rosette glands using a Generalized Linear Model with Poisson error  
261 distribution and a log link function. To analyze the number of rosette glands, we used the following factors as  
262 explanatory variables: species, sexual phenotype, social environment and body size. Model selection and  
263 statistical assumptions were checked, as described for the previous analysis.

264 All statistical analyses were performed using the software SPSS 20.

265

## 266 **Results**

267

### 268 **Type and frequency of sexual phenotypes of the experimental worms**

269 In the three species, we found four sexual phenotypes, i.e. 39.3% pure males, 35.6% pure females, 19.1%  
270 females with sperm and 6.0% males with oocytes. The frequencies of males (pure males and male with oocytes)  
271 and females (pure females and females with sperm) were not significantly different among species and were  
272 significantly affected by the gender of the adult to which juveniles were exposed (Table 2 and Figure 2). The  
273 interaction between these two predictors was removed after checking it was non-significant in a preliminary  
274 analysis, which suggested that the social environment had the same impact on the juveniles of the three species.  
275 Statistical comparisons show that the difference in sex ratio among "social environments" is due mainly to the  
276 difference between the environment "juvenile+ female" and the other two social environments (Table 2),  
277 indicating female as the sex able to affect juvenile sexual development.

278 When juveniles reached sexual maturity, they formed true heterosexual pairs with their adult partner  
279 (pure male + pure female) (47.5%) significantly more often than true homosexual pairs (pure male + pure male  
280 or pure female + pure female) (31.1%) (GLM with Poisson error distribution: d.f. = 2,  $\chi^2_{(Wald)} = 19.56$ ,  $P < 0.001$ ;  
281 heterosexual pairs (pure male + pure female) vs homosexual pairs (pure male + pure male or pure female + pure  
282 female),  $B = 0.42$ ,  $\chi^2_{(Wald)} = 6.55$ ,  $P = 0.01$ ). The remaining pairs (21.4%) were composed of at least one male  
283 with oocytes or one female with sperm. In the subsequent analysis, we merged these two intermediate  
284 phenotypes together to form the experimental group of pseudohermaphrodites, since females with sperm and  
285 males with oocytes were relatively rare phenotypes. The number of pseudohermaphrodites depended  
286 significantly on species and social environment (Figure 3) (GLM: species,  $\chi^2_{(Wald)} = 25.74$ , d. f. = 2,  $P < 0.001$ ;  
287 social environment,  $\chi^2_{(Wald)} = 25.74$ , d. f. = 2,  $P < 0.001$ ). The number of pseudohermaphrodites was significantly  
288 higher when juveniles developed in isolation than when they developed together with males ( $B = 0.75$ ,  $\chi^2_{(Wald)} =$   
289  $25.69$ ,  $P < 0.0001$ ) or with females ( $B = 0.27$ ,  $\chi^2_{(Wald)} = 4.41$ ,  $P = 0.036$ ).

290

### 291 **Developmental time to sexual maturity**

292 The developmental time of juveniles was significantly different among species and sexual  
293 phenotypes, but juveniles of the three species adjusted their developmental time to social  
294 conditions in a similar way, although sexual phenotypes responded differently to social  
295 environment (Table 3). The developmental time of juveniles that expressed the same gender  
296 of their adult partner was significantly longer than that of juveniles which expressed the  
297 gender opposite to that of their partner (Table 3 and Figure 4). Overall, juveniles that  
298 developed in isolation had developmental times which were generally intermediate compared  
299 to the developmental times of their conspecifics exposed to adults. The large variations  
300 between species and phenotypes do not allow to identify clear, common effects of isolation on  
301 developmental times (Figure 4).

### 302 **Expression of the sexual phenotypes of sexually mature worms**

303 No differences were observed in the number of sexual phenotypes between worms in  
304 homosexual pairs and worms in heterosexual pairs during the adult phase ( $\chi^2 = 0.43$ , d.f. = 3,  
305  $P = 0.93$ ). Pairing off with a worm of the same sex did not stimulate the production of  
306 gametes of the opposite sex. In those homosexual pairs that were composed of two females,  
307 worms occasionally laid eggs. Egg laying occurred in 4 out of 16 homosexual pairs of females  
308 in *O. robusta*, in 2 out of 39 pairs in *O. macrovifera* and in 5 out of 32 pairs in *O. labronica*.  
309 Therefore in those homosexual pairs at least one of the partners was a female with sperm. We  
310 do not know whether fertilized eggs were the result of a self-fertilization process or whether  
311 the homosexual pairs were functionally heterosexual pairs.

### 312 **Rosette glands**

313 The number of rosette glands was positively associated to body size and varied significantly between species  
314 and sexual phenotypes, but no interaction between the two factors was found (Figure 5). In all the three species  
315 the number of rosette glands was larger in males than in females and pseudohermaphrodites (GLM: species, log-

316 likelihood chi-square ( $G^2$ ) = 19.87, d. f. = 2,  $P < 0.001$ ; sexual phenotype,  $G^2 = 80.20$ , d. f. = 2,  $P < 0.0001$ ;  
317 social environment,  $G^2 = 5.64$ , d. f. = 2,  $P > 0.05$ ; body size,  $G^2 = 170.7$ , d. f. = 1,  $P < 0.0001$ ). The number of  
318 rosette glands was significantly different between males and females ( $B = -0.39$ ,  $\chi^2_{(Wald)} = 1.88$ ,  $P < 0.0001$ ),  
319 males and pseudo-hermaphrodites ( $B = -0.52$ ,  $\chi^2_{(Wald)} = 0.69$ ,  $P < 0.0001$ ), while it was not different between  
320 females and pseudo-hermaphrodites ( $B = -0.13$ ,  $\chi^2_{(Wald)} = 2.64$ ,  $P = 0.10$ ). This means that only two sexual  
321 phenotypes, male and female, can be distinguished according to the number of rosette glands.

322

## 323 **Discussion**

324

325 Our results showed that social environment – i.e. the presence of a sexually mature  
326 partner – influenced the expression of the sexual phenotype in juveniles of the *Ophryotrocha*  
327 dioecious species. The effect was documented 1) by variations of the frequencies of sexual  
328 phenotypes according to the social environment. Indeed juveniles tend to develop so as to  
329 form heterosexual pairs.. Furthermore the absence of a partner stimulated the production of  
330 pseudohermaphroditic sexual phenotypes. Indeed pseudohermaphrodites were significantly  
331 more common among isolated juveniles than among juveniles reared with adults of either sex.  
332 The effect of social environment was also documented 2) by the significantly different  
333 developmental times to the onset of sexual maturity of juveniles. Juveniles which have  
334 matured the same gender of their adult partner needed longer time to reach sexual maturity  
335 than juveniles which had matured the gender opposite to that of their partner's in all three  
336 species.

337 Sex expression was influenced by social conditions only during the juvenile phase for all  
338 the three species. This can be expected in species whose populations have largely fluctuating  
339 densities and live in patchy environments, such as intertidal communities do (Sella and  
340 Ramella 1999; Prevedelli et al. 2005). During the adult phase, frequencies of sexual  
341 phenotypes were no longer influenced by the social environment, as expected in species that

342 underwent selective pressures for sexual specialization towards dioecy. *Ophryotrocha*  
343 dioecious species are therefore another example of labile gender maturation of juveniles in  
344 response to the presence of a sexual mature partner, in addition to those reported by Leutert  
345 (1975), Berec (2005); Bacci (1965), Agius (1979), Hipeau-Jacquotte (1978), Beckeickh et al.  
346 (1998), Michaud et al. (2004), Parenti (1965) and Vrijenhoek et al. (2008).

347         Although the three species differ from each other in their geographical distribution,  
348 genome structure and life cycle, they did not differ in their degree of plasticity in sexual  
349 expression at the end of the juvenile phase. Looking both at the propensity of juveniles to  
350 develop the gender opposite to that of their partner's and to vary in their developmental time  
351 according to their response to social conditions, the three species behaved in a similar way (as  
352 shown from the absence of statistical interactions involving species as a predictor variable).  
353 This interspecific homogeneity can be due either to the phylogenetic proximity (Dahlgren  
354 2001) or to maintenance of plasticity in sex expression during development as an adaptive  
355 response to common selective forces.

356         Not all experimental worms reacted in the same way to the social environment:  
357 31.12% of juveniles matured the same gender of their partner. Nevertheless, they showed a  
358 longer developmental time than that of juveniles which developed the gender opposite to that  
359 of their adult partner's. This result suggests that in *Ophryotrocha* worms the degree of sensing  
360 and/or responding to stimuli from adult partners is also influenced by genetic variations  
361 between individuals. In a similar way social environments influence juveniles sexual  
362 development differently: looking at the external morphology of experimental worms only  
363 adult females are able to influence the sex of juveniles (Figure 2). However when looking at  
364 gametes production we can asses also a influence of adult males on juveniles sexual  
365 development since the number of juveniles developed to pseudohermaphrodites is lower when  
366 juveniles are paired with males compare to isolated juveniles (Figure 3). According to these

367 results,, the most recent theories about phenotypic plasticity (West-Eberhard 2003; Ah-King  
368 and Nylin 2010; Golenberg and West 2013), identify two factors involved in determining the  
369 final sexual phenotype: 1) variations in the sequences of regulatory genes responsible for the  
370 control of alternative developmental pathways and 2) environmental stimuli.

371 The results of our experiment made it possible for us to outline a possible evolutionary  
372 pathway of the evolution of dioecy from a hermaphroditic ancestral state in *Ophryotrocha*. In  
373 plants, the transition from hermaphroditism to dioecy is thought to have evolved through two  
374 main distinct pathways (Ehlers and Bataillon 2007): from hermaphroditism via gynodioecy to  
375 dioecy and from hermaphroditism via monoecy to dioecy. Gynodioecy refers to the  
376 coexistence in a population of two sexual phenotypes, i.e. pure females and individuals  
377 having both sexual functions (within the same flower or in separate flowers), while monoecy  
378 refers to plants having both sexual functions in separate male and female flowers within the  
379 same individual (Ehlers and Bataillon 2007). In animals the distinction between individuals  
380 having both sexual functions either within the same flower or in separate flowers translates  
381 respectively to syngonic (the same gonads producing both male and female gametes) or  
382 digonic (distinct male and female gonads in the same individual) simultaneous  
383 hermaphrodites (Vega-Frutis et al. 2014).

384 The pathway through gynodioecy (Charlesworth and Charlesworth 1978; Delph and  
385 Wolf 2005) is based on two mutational events. Starting from a population of hermaphrodites,  
386 a first mutation is responsible for the production of pure females, so that the remaining  
387 hermaphrodites will be selected to plastically adjust their sex allocation and becoming  
388 strongly male biased. A second mutation will then generate pure males that will spread and  
389 outnumber the strongly male-biased hermaphrodites. This model relies on a genetic  
390 assumption (the first genetic mutation) and does not include gene x environment interactions  
391 (Freeman 1997). In species evolving through this pathway, gender expression should vary



392 only in hermaphrodites as a consequence of the presence of pure females rather than other  
393 environmental conditions. Moreover, the model predicts that when pseudohermaphroditic  
394 phenotypes are present, they belong to the male gender, i. e. the gender which did not undergo  
395 the first genetic mutation determining male-sterility (Ehlers and Bataillon 2007).

396 In contrast, the pathway through monoecy (Renner and Ricklefs 1995) is based on  
397 mechanisms of regulation of gender expression triggered by variations in environmental cues.  
398 A mutation of the regulatory sequence of sex expression would determine the tendency to  
399 express one gender only, setting the evolutionary stage of dioecy or subdioecy. At this stage  
400 the sexual development of the organism is still directly dependent on the perception of  
401 external environmental cues and therefore it will maintain its ability to adapt to environmental  
402 variations plastically. Following this evolutionary model, during the transition,  
403 pseudohermaphroditic phenotypes should be common and extreme phenotypes (pure male  
404 and pure female) rare, since all individuals retain the ability to express both sexual  
405 phenotypes (Freeman 1997).

406 Our results fit well a possible monoecy pathway in which both the influence of social  
407 conditions on sex expression and the presence of pseudohermaphrodites can be explained. It  
408 is difficult to classify the pseudohermaphroditic phenotypes of dioecious species as syngonic  
409 or digonic, since only clusters of germ cells, and no true gonads, are present. They are  
410 hermaphroditic phenotypes with strong male- or female-biased sex allocation, and with rare  
411 gametes of the opposite sex. However, simultaneous hermaphroditic species of this genus also  
412 have spatially separate male and female sections (in the first 2-3 body segments these  
413 hermaphrodites produce only sperm, while in the remaining segments they produce only  
414 oocytes) (Åkesson 1974; Schleicherová et al. 2014). Therefore, they resemble digonic rather  
415 than syngonic simultaneous hermaphrodites.

416 In plants, the main selective force favoring the transition to dioecy via monoecy is  
417 sexual specialization (Freeman 1997 and references therein). In animals, selective pressures  
418 leading to sexual specialization are poorly known (but see Weeks 2012). In the populations of  
419 the hermaphroditic ancestor of the dioecious *Ophryotrocha* species, selection for sexual  
420 specialization would have been responsible for the appearance of pseudohermaphrodites (in  
421 which both types of gametes are present but only one type is functional) and then of pure  
422 males and pure females. One may wonder why pseudohermaphrodites still coexist with pure  
423 males and pure females in the existing populations of *Ophryotrocha*. According to Ehlers and  
424 Bataillon (2007) and Lorenzi and Sella (2013) selection for sexual specialization may become  
425 less strong or ineffective when pseudohermaphrodites are strongly biased towards one of the  
426 two genders. In the *Ophryotrocha* dioecious species, the dichotomy between sexual  
427 dimorphism at the morphological level and sexual polymorphism at the gamete level is  
428 illustrated well by the number of rosette glands. This sex-related trait allowed us to  
429 distinguish only two reproductive morphs (males and females), while at the gamete level four  
430 sexual phenotypes exist (pure male, pure female, male with oocytes and female with sperm).  
431 If we can find out more precisely what the function of rosette glands is, we can more easily  
432 understand what the selective pressures are that act for sexual specialization and hence drive  
433 the evolution of dioecy in this genus.

434

## 435 **Acknowledgments**

436 We thank Liliana Ramella, Chiara Nebiolo and Alessandra Lerda for assistance in the  
437 laboratory. We are grateful to Vincent Marsicano for help with linguistic revision.

438

439 **References**

- 440 Agius, L. (1979). Larval settlement in the echiuran worm *Bonellia viridis*: settlement on both  
441 the adult proboscis and body trunk. *Marine Biology*, 53(2), 125-129.
- 442 Ah-King, M., & Nylin, S. (2010). Sex in an evolutionary perspective: just another reaction  
443 norm. *Evolutionary biology*, 37(4), 234-246.
- 444 Åkesson, B. (1974). Reproduction and Larval Morphology of Five Ophryotrocha Species  
445 (Polychaeta, Dorvilleidae). *Zoologica Scripta*, 2(4), 145-155.
- 446 Åkesson, B. (1975). Reproduction in the genus Ophryotrocha (Polychaeta,  
447 Dorvilleidae).[Conference paper]. *Pubblicazioni della Stazione Zoologica, Napoli*.
- 448 Bacci, G. (1965). Sex determination. Pergamon Press, Oxford.
- 449 Bacci, G. (1978). Genetics of sex determination in Ophryotrocha (Annelida, Polychaeta).  
450 *Marine organisms: genetics, ecology, and evolution. Plenum Press, New York*.
- 451 Bacci, G., Lanfranco, M., Mantello, I., & Tomba, M. (1979). A new pattern of  
452 hermaphroditism (inducible hermaphroditism) in populations of Ophryotrocha  
453 labronica (Annelida Polychaeta). *Experientia*, 35(5), 605-606.
- 454 Baldi, C., Cho, S., & Ellis, R. E. (2009). Mutations in two independent pathways are  
455 sufficient to create hermaphroditic nematodes. *Science*, 326(5955), 1002-1005.
- 456 Barrett, S. C. (2013). The evolution of plant reproductive systems: how often are transitions  
457 irreversible? *Proceedings of the Royal Society B: Biological Sciences*, 280(1765),  
458 20130913.
- 459 Bazzaz, F. (1991). Habitat selection in plants. *American Naturalist*, 137(supplement: hab).

- 460 Becheikh, S., Michaud, M., dé ric Thomas, F., Raibaut, A., & Renaud, F. (1998). Roles of  
461 resource and partner availability in sex determination in a parasitic copepod.  
462 *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1402),  
463 1153-1156.
- 464 Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs.  
465 *Ecology*, 67(4), 836-845.
- 466 Brubacher, J. L., & Huebner, E. (2009). Development of polarized female germline cysts in  
467 the polychaete, *Ophryotrocha labronica*. *Journal of morphology*, 270(4), 413-429.
- 468 Bull, J. J. (1983). *Evolution of sex determining mechanisms*: The Benjamin/Cummings  
469 Publishing Company, Inc.
- 470 Charlesworth, B., & Charlesworth, D. (1978). A model for the evolution of dioecy and  
471 gynodioecy. *American naturalist*, 975-997.
- 472 Charnov, E. L., & Bull, J. (1977). When is sex environmentally determined?
- 473 Crossman, A., & Charlesworth, D. (2013). Breakdown of dioecy: models where males acquire  
474 cosexual functions. *Evolution*.
- 475 Dahlgren, T. G., Åkesson, B., Schander, C., Halanych, K. M., & Sundberg, P. (2001).  
476 Molecular phylogeny of the model annelid *Ophryotrocha*. *The Biological Bulletin*,  
477 201(2), 193-203.
- 478 Delph, L. F., & Wolf, D. E. (2005). Evolutionary consequences of gender plasticity in  
479 genetically dimorphic breeding systems. *New Phytologist*, 166(1), 119-128.
- 480 Ehlers, B. K., & Bataillon, T. (2007). 'Inconstant males' and the maintenance of labile sex  
481 expression in subdioecious plants. *New Phytologist*, 174(1), 194-211.

- 482 Falconer, D. (1981). Introduction to Quantitative Genetics Longman Inc. *New York, NY*.
- 483 Freeman, D., Harper, K., & Charnov, E. (1980). Sex change in plants: old and new  
484 observations and new hypotheses. *Oecologia*, 47(2), 222-232.
- 485 Freeman, D. C., Doust, J. L., El-Keblawy, A., Miglia, K. J., & McArthur, E. D. (1997).  
486 Sexual specialization and inbreeding avoidance in the evolution of dioecy. *The*  
487 *Botanical Review*, 63(1), 65-92.
- 488 Godwin, J., Luckenbach, J. A., & Borski, R. J. (2003). Ecology meets endocrinology:  
489 environmental sex determination in fishes. *Evolution & development*, 5(1), 40-49.
- 490 Golenberg, E. M., & West, N. W. (2013). Hormonal interactions and gene regulation can link  
491 monoecy and environmental plasticity to the evolution of dioecy in plants. *American*  
492 *journal of botany*, 100(6), 1022-1037.
- 493 Hipeau-Jacquotte, R. (1978). Existence de deux formes sexuelles mâles chez le copépode  
494 ascidicole Notodelphyidae Pachypygus gibber (Thorell, 1859). *Comptes Rendus de*  
495 *l'Academie des Sciences, Paris*, 287 D, 253-256.
- 496 Kegel, B., & Pfannenstiel, H.-D. (1983). Evaluation of the pair-culture effect in Ophryotrocha  
497 puerilis (Polychaeta: Dorvilleidae). I. Pair-culture effect and sex ratio. *Helgoländer*  
498 *Meeresuntersuchungen*, 36(2), 205-213.
- 499 Korpelainen, H. (1990). Sex ratios and conditions required for environmental sex  
500 determination in animals. *Biological Reviews*, 65(2), 147-184.
- 501 Korpelainen, H. (1998). Labile sex expression in plants. *Biological Reviews*, 73(2), 157-180.
- 502 La Greca, M., & Bacci, G. (1962). Una nuova specie di Ophryotrocha delle coste tirreniche  
503 (Annelida, Polychaeta). *Italian Journal of Zoology*, 29(1), 7-18.

- 504 Lanfranco, M., & Rolando, A. (1981). Sexual races and reproductive isolation in  
505 Ophryotrocha labronica La Greca and Bacci (Annelida, Polychaeta). *Italian Journal of*  
506 *Zoology*, 48(3-4), 291-294.
- 507 Leonard, J. L. (2013). Williams' Paradox and the role of phenotypic plasticity in sexual  
508 systems. *Integrative and comparative biology*, 53(4), 671-688.
- 509 Leutert, R. (1975). Sex-determination in Bonellia. In *Intersexuality in the animal kingdom*  
510 (pp. 84-90): Springer.
- 511 Lorenzi, M. C., & Sella, G. (2013). In between breeding systems: neither dioecy nor  
512 androdioecy explains sexual polymorphism in functionally dioecious worms.  
513 *Integrative and comparative biology*, 53(4), 689-700.
- 514 Mankiewicz, J. L., Godwin, J., Holler, B. L., Turner, P. M., Murashige, R., Shamey, R., et al.  
515 (2013). Masculinizing effect of background color and cortisol in a flatfish with  
516 environmental sex-determination. *Integrative and comparative biology*, 53(4), 755-  
517 765.
- 518 Michaud, M., de Meeûs, T., & Renaud, F. (2004). Environmental sex determination in a  
519 parasitic copepod: checking heterogeneity and unpredictability of the environment.  
520 *Marine Ecology Progress Series*, 269, 163-171.
- 521 Pannell, J. (1997). Variation in sex ratios and sex allocation in androdioecious *Mercurialis*  
522 *annua*. *Journal of Ecology*, 85(1), 57-69.
- 523 Parenti, U. (1965). Male and female influence of adult individuals on undifferentiated larvae  
524 of the parasitic nematode *Paramermis contorta*. *Nature*, 207, 1105-1106.

- 525 Paxton, H., & Åkesson, B. (2010). The Ophryotrocha labronica group (Annelida,  
526 Dorvilleidae)—with the description of seven new species. *Zootaxa*, 2713, 1-24.
- 527 Pfannenstiel, H.-D. (1975). Mutual influence on the sexual differentiation in the protandric  
528 polychaete Ophryotrocha puerilis. In *Intersexuality in the animal kingdom* (pp. 48-56):  
529 Springer.
- 530 Pfannenstiel, H.-D. (1976). Ist der Polychaet Ophryotrocha labronica ein proterandrischer  
531 hermaphrodit? *Marine Biology*, 38(2), 169-178.
- 532 Pfannenstiel, H.-D. (1977). Experimental analysis of the “Paarkultureffekt” in the protandric  
533 polychaete, *Ophryotrocha puerilis* Clap. Mecz. *Journal of Experimental Marine*  
534 *Biology and Ecology*, 28(1), 31-40.
- 535 Pfannenstiel, H.-D., & Grünig, C. (1982). Yolk formation in an annelid (*Ophryotrocha*  
536 *puerilis*, polychaeta). *Tissue and Cell*, 14(4), 669-680.
- 537 Prevedelli, D., & Simonini, R. (2001). Effects of diet and laboratory rearing on demography  
538 of *Dinophilus gyrotilatus* (Polychaeta: Dinophilidae). *Marine Biology*, 139(5), 929-935.
- 539 Prevedelli, D., & Vandini, R. Z. (1998). Effect of diet on reproductive characteristics of  
540 *Ophryotrocha labronica* (Polychaeta: Dorvilleidae). *Marine Biology*, 132(1), 163-170.
- 541 Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*:  
542 Cambridge University Press.
- 543 Renner, S. S., & Ricklefs, R. E. (1995). Dioecy and its correlates in the flowering plants.  
544 *American Journal of Botany*(5), 596-606.
- 545 Robotti, C., Ramella, L., Cervella, P., & Sella, G. (1991). Chromosome analysis of 9 species  
546 of *Ophryotrocha* (Polychaeta, Dorvilleidae). *Ophelia*, 625-632.

- 547 Rolando, A. (1984). The sex induction hypothesis and reproductive behaviour in four  
548 gonochoristic species of the genus *Ophryotrocha* (Annelida Polychaeta). *Monitore*  
549 *Zoologico Italiano-Italian Journal of Zoology*, 18(4), 287-299.
- 550 Rolando, A., & Giorda, R. (1982). Male intersexes in *Ophryotrocha labronica* La Greca &  
551 Bacci (Annelida Polychaeta). *Monitore Zoologico Italiano-Italian Journal of Zoology*,  
552 16(1), 67-73.
- 553 Sarre, S. D., Georges, A., & Quinn, A. (2004). The ends of a continuum: genetic and  
554 temperature- dependent sex determination in reptiles. *Bioessays*, 26(6), 639-645.
- 555 Schleicherová, D., Sella, G., Meconcelli, S., Simonini, R., Martino, M., Cervella, P., et al.  
556 (2014). Does the cost of a function affect its degree of plasticity? A test on plastic sex  
557 allocation in three closely related species of hermaphrodites. *Journal of Experimental*  
558 *Marine Biology and Ecology*, 453, 148-153.
- 559 Schärer, L. (2009). Tests of sex allocation theory in simultaneously hermaphroditic animals.  
560 *Evolution*, 63(6), 1377-1405.
- 561 Sella, G., & Ramella, L. (1999). Sexual conflict and mating systems in the dorvilleid genus  
562 *Ophryotrocha* and the dinophilid genus *Dinophilus*. In *Reproductive Strategies and*  
563 *Developmental Patterns in Annelids* (pp. 203-213): Springer.
- 564 Sella, G., Redi, C., Ramella, L., Soldi, R., & Premoli, M. (1993). Genome size and karyotype  
565 length in some interstitial polychaete species of the genus *Ophryotrocha*  
566 (Dorvilleidae). *Genome*, 36(4), 652-657.
- 567 Sella, G., & Robotti, C. (1991). Heterozygote deficiency at the phosphoglucose isomerase  
568 locus in a tyrrhenian population of *Ophryotrocha-labronica* (Polychaeta, Dorvillidae).  
569 *Ophelia*, 641-645.



- 570 Simonini, R., Massamba-N'Siala, G., Grandi, V., Prevedelli, D. 2009. Distribution of the  
571 genus *Ophryotrocha* (Polychaeta) in Italy: new reports and comments on the biogeography  
572 of Mediterranean species. *Vie et Milieu*, 59, 79–88.
- 573 Thornhill, D. J., Dahlgren, T. G., & Halanych, K. M. (2009). Evolution and ecology of  
574 *Ophryotrocha* (Dorvilleidae, Eunicida). *Annelids in modern biology*, 242-256.
- 575 Vega- Frutis, R., Macías- Ordóñez, R., Guevara, R., & Fromhage, L. (2014). Sex change in  
576 plants and animals: a unified perspective. *Journal of evolutionary biology*, 27(4), 667-  
577 675.
- 578 Vrijenhoek, R., Johnson, S., & Rouse, G. (2008). Bone- eating *Osedax* females and their  
579 'harems' of dwarf males are recruited from a common larval pool. *Molecular ecology*,  
580 17(20), 4535-4544.
- 581 Weeks, S. C. (2012). The role of androdioecy and gynodioecy in mediating evolutionary  
582 transitions between dioecy and hermaphroditism in the Animalia. *Evolution*, 66(12),  
583 3670-3686.
- 584 West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*: Oxford University  
585 Press.
- 586 Westheide, W. (1984). The concept of reproduction in polychaetes with small body size:  
587 adaptations in interstitial species. *Fortschr. Zool*, 29, 265-287.

588

589

590 **Figure legends**

591

592 **Figure 1** Experimental set up. Juveniles (n = 330) were randomly assigned to one of three  
593 treatments: 1. juvenile paired with an adult female 2. juvenile paired with an adult male 3.  
594 juvenile isolated. When juveniles reached a clear sexual differentiation, a subsample of these  
595 sexually mature worms were screened to verify the presence of sperm (in females) or oocytes  
596 (in males). The remaining worms were used to form homosexual pairs (n = 87) or  
597 heterosexual pairs (n = 90). At the end of the experiment all the worms were checked for  
598 sperm in females or oocytes in males.

599

600 **Figure 2.** Relative frequencies of males (including males and males with oocytes) and females (including females  
601 and females with sperm) in every of the three social environments (juvenile paired with a male, with a female or  
602 isolated). 55.9% of juveniles became males when paired together with females, while only 38.3% developed as  
603 males in pair with an adult male. In a similar way, 61.7% of juveniles developed as females when they  
604 developed together with males, while 44.1% became females in pair with females. Juveniles in isolation  
605 developed 58.8% as females and 41.2% as males.

606

607 **Figure 3.** Frequencies (%) of Ipseudohermaphrodites (female with sperm and male with oocytes) in *O.*  
608 *labronica*, *O. macrovifera* and *O. robusta* depending on the social environment (juveniles paired with a  
609 male, a female or isolated ).

610

611 **Figure 4.** Variations in the developmental time (days) to sexual maturity in *O. labronica*, *O.*  
612 *macrovifera* and *O. robusta* under the effect of the social environment (juveniles paired with a  
613 male, a female or isolated) paneled separately for every sexual phenotype (females, males and  
614 pseudohermaphrodites). The graph shows the means  $\pm$  SE.

615

616 **Figure 5.** Variations in the number of rosette glands relative to body size depending on sexual phenotypes  
617 (female, male, pseudohermaphrodite), paneled separately for *O. labronica*, *O. macrovifera* and *O. robusta*.

618  
 619  
 620  
 621  
 622  
 623  
 624  
 625  
 626  
 627  
 628

**Table 1.** Main differences in the life cycle of the three tested species (mean  $\pm$  SD)

	<i>Ophryotrocha labronica</i>	<i>Ophryotrocha robusta</i>	<i>Ophryotrocha macrovifera</i>
N. Eggs/cocoon	116 $\pm$ 46	134 $\pm$ 51	76 $\pm$ 33
N. segments with setae at hatching	2 $\pm$ 1	0	2 $\pm$ 1
N. segments with setae at $\text{\textcircled{M}}$ definitive upper jaw appearance	15 $\pm$ 2	15 $\pm$ 2	14 $\pm$ 2
N. segments with setae at $\text{\textcircled{F}}$ Oocytes appearance	16 $\pm$ 2	14 $\pm$ 2	14 $\pm$ 2
time from hatching to $\text{\textcircled{M}}$ definitive u.jaw appearance (days)	22 $\pm$ 5	28 $\pm$ 8	21 $\pm$ 7
time from hatching to $\text{\textcircled{F}}$ oocytes appearance (days)	20 $\pm$ 4	26 $\pm$ 6	18 $\pm$ 6

629  
 630  
 631  
 632

633

634

635

636

637

638

639

640

641

642 **Table 2.** Results of the GLMM testing for the effect of species and social environment on the  
643 sex ratio.

644

645

<b>Predictor</b>	<b>Comparisons</b>	<b><i>P</i></b>
species		$F_{2,293} = 0.76$ 0.468
social environment		$F_{2,293} = 4.54$ 0.011
	"J+♂" vs "J+♀"	$t = -2.74$ 0.006
	"J+♂" vs "isolated J"	$t = -0.39$ 0.698
	"J+♀" vs "isolated J"	$t = 2.43$ 0.016
<b>Random effect</b>		<b><i>P</i></b>
	sibship	$z = 1.75$ 0.080

646

647

648

649

650

651

652 **Table 3.** Results of the GLMMs testing a) the effect of species, social environment and  
 653 sexual phenotype on the developmental time to sexual maturity; b) the effect of the social  
 654 environment for each type of sexual phenotype (J = Juvenile).

655

<b>a)</b>		<b>Predictor</b>		<b>P</b>
		species	$F_{2,173} = 11.79$	<0.001
		social environment	$F_{2,173} = 0.46$	0.630
		sexual phenotype	$F_{2,173} = 4.61$	0.011
		social environment X sexual phenotype	$F_{2,173} = 6.35$	<0.001
		<b>Random effect</b>		<b>P</b>
		sibship	$z = 2.63$	0.008
<b>b)</b>		<b>Predictor</b>	<b>Comparisons</b>	<b>P</b>
<b>Females</b>		social environment	$F_{2,43} = 3.75$	0.032
			"J+♂" vs "J+♀"	$t = 2.74$ 0.009
			"J+♂" vs "isolated J"	$t = 1.27$ 0.210
			"J+♀" vs "isolated J"	$t = -1.69$ 0.098
<b>Males</b>		social environment	$F_{2,79} = 9.26$	<0.001
			"J+♂" vs "J+♀"	$t = 3.79$ <0.001
			"J+♂" vs "isolated J"	$t = -3.54$ 0.001
			"J+♀" vs "isolated J"	$t = -0.32$ 0.754
<b>Pseudoherm.</b>		social environment	$F_{2,47} = 1.64$	0.206

656