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SEXUAL CONDITIONS IN A POPULATION OF *OPHRYOTROCHA* *ROBUSTA* (ANNELIDA POLYCHAETA) FROM GENOA

Riassunto — *Condizioni sessuali di una popolazione di Ophryotrocha robusta (Annelida Polychaeta) di Genova.* Sono state studiate le condizioni sessuali di un ceppo gonocorico di *Ophryotrocha robusta* raccolto nel porto di Genova. E' stato possibile individuare alcuni caratteri sessuali secondari della specie e accertare anche la presenza di intersessi maschili. Inoltre, sulla base dei risultati preliminari ottenuti saggiando la stabilità sessuale di questa popolazione, è stata suggerita l'esistenza di fenomeni di inducibilità sessuale nei giovani.

Abstract — The sexual conditions of a gonochoric strain of *Ophryotrocha robusta* collected in Genoa have been studied. It has been suggested that a few parameters studied are to be considered as secondary sexual characters. The preliminary results obtained by testing the sexual stability of this population seem to indicate the existence of sexual inducibility in juveniles. In the end, analogously to that observed in *Ophryotrocha labronica*, the presence of a few male intersexes has been ascertained.

Key words — *Ophryotrocha* / sexual conditions / *Annelida*.

Ophryotrocha robusta is a gonochoric species (ÅKESSON (1975), *nomen nudum*), so far undescribed, on whose reproductive biology, however, some data have already been reported (ÅKESSON, 1975).

A catch made in Genoa allowed us to collect, together with several specimens of *Ophryotrocha labronica* and *O. puerilis*, a few *Ophryotrocha* which were kindly identified by Prof. B. Åkesson (Zoological Institute, University of Göteborg, Sweden) as *O. robusta*.

The present research has been carried out on the sexual conditions of this Ligurian strain.

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MATERIAL AND METHODS

The strain employed in the present study was collected in the harbour of Genoa in October 1981 and was named Genoa 1 (Ge 1). The animals were cultured in sea water (37.5‰ salinity) at a temperature of 18°-19°C; the food consisted of ground frozen spinach, delivered once a week.

The following parameters of the population were studied:

a) sex ratio, b) number of chaetigerous segments at the time when the early oocytes appear in the females, c) time (in days) when the early oocytes appear in the females, d) number of chaetigerous segments at the time when the definitive upper jaw (d.u.j.) appears in males and females, e) time (in days) when the d.u.j. appears in males and females, f) number of dorsal glands at the time when the d.u.j. appears in males and females, g) number of eggs per egg mass. The a) parameter (sex ratio) was obtained by breeding in isolation the progeny of 10 pairs; the progeny of 4 pairs was also employed to obtain the b) to f) parameters. The d) and e) parameters were computed from the time when the hatching of eggs took place (i.e. free swimming early larvae). The g) parameter (number of eggs per egg mass) was computed out of a total of 50 egg masses laid by as many pairs (partners were about two months old) raised in separate dishes.

The conditions of sexual stability of the population were also studied. At first the sexual stability of the adults was checked and in order to do this 150 pairs of females and 150 of males were set up and cultured for 40 days. To check the sexual stability of the juveniles (namely to test whether the sexual differentiation of young specimens is influenced by intraspecific interactions) two series of experiments were undertaken. The first series consisted of pairs of juveniles and of two control juveniles per pair cultured in isolation. All the juveniles were employed with the same number of chaetigerous segments ranging from 5 to 7. In the second series, pairs of an adult female and a juvenile were formed, a control juvenile for each pair being cultured at the same time (in this case too the juveniles had the same number of chaetigerous segments, ranging from 5 to 7).

The results obtained by the first series of tests on sexual stability in the juvenile, were compared to those obtained previously by us by similarly conducted experiments on a strain of *Ophryotrocha labronica* (Naples V strain) (unpublished data).

In the end some specimens of *O. robusta* displaying a typical male phenotype (as shown by their d.u.j. and the high number of dorsal glands) and a few oocytes in the coelomic cavities, were also examined. A few of the latter specimens, together with some males were anaesthetized in a 7% $MgCl_2$ water solution, fixed in a $HgCl_2$ solution (Heidenhain's), embedded in paraffin and cut according to a frontal plane (sections were 4 μm thick). Some preparations were stained by Heidenhain's iron hematoxylin, others by Hemalum-eosin.

RESULTS

Parameters of the population

The results yielded by the study of the parameters are summarized in Tab. 1. The sex ratio (50.6% males) is close to that of the population studied by ÅKESSON in 1975 (53.6% males). The d.u.j. appears earlier and at a lower number of chaetigerous segments in the males than in the females (22.56 days and 13.01 segments versus 23.49 days and 13.79 segments); moreover the

TAB. 1 - Parameters of the population of *Ophryotrocha robusta* from Genoa.
N = number of specimens.

* In the g) parameter N = total number of eggs.

parameters	mean \pm standard error		N	
	♂ ♂	♀ ♀	♂ ♂	♀ ♀
a	50.6%	49.4%	1039	1015
b	—	12.48 \pm 0.04	—	554
c	—	21.16 \pm 0.08	—	554
d	13.01 \pm 0.03	13.79 \pm 0.04	560	554
e	22.56 \pm 0.10	23.49 \pm 0.13	560	554
f	3.19 \pm 0.04	0.66 \pm 0.04	560	554
g*	—	376 \pm 19.02	—	18800

number of dorsal glands is higher in the males than in the females (3.19 versus 0.66). All these differences are statistically significant (F test, P values < 0.001). The females of this strain seem to spawn a larger amount of eggs per egg mass than the females of Åkesson's strain (376 versus 201.2). Nevertheless, the number of eggs per egg mass certainly depends on environmental factors (e.g. feeding

conditions, population density etc.) and therefore it is not a reliable comparative parameter.

Sexual stability

The experiments on adults confirmed the stability of their sexual condition. The 300 pairs raised did not show any sex inversion and this fact seems to indicate that the population is firmly gonochoric.

Nevertheless, the sexual stability of the juveniles does not seem to be so rigorous. In fact 54.5% of the pairs formed between two juveniles (out of a sample of 266 pairs) differentiated into a heterosexual pair, while only 42.1% of the control pairs differentiated in the same way. Similar results were also obtained in *O. labronica*; in this case 46.2% of juvenile pairs (out of a sample of 171) turned out to be heterosexual pairs versus 39.2% of the control pairs.

Moreover in *O. robusta* 62.4% of the juveniles cultured together with an adult female (out of a sample of 186 pairs) differentiated into males while only 48.9% of the control juveniles differentiated in the same way. The data on sex inducibility experiments on juveniles are reported in Tab. 2.

TAB. 2 - Sexual inducibility data in *Ophryotrocha robusta* and in *O. labronica*.
Results of the sexual differentiation in the juveniles.

	juv. x juv. number of pairs				juv. x ♀ number of specimens		
	♂ ♂	♀ ♀	♂ x ♀	Tot.	♂ ♂	♀ ♀	Tot.
Paired	57 (21.4%)	64 (24.1%)	145 (54.5%)	266	116 (62.4%)	70 (37.6%)	186
Controls	75 (28.2%)	79 (29.7%)	112 (42.1%)	266	91 (48.9%)	95 (51.1%)	186

O. robusta

	juv. x juv. number of pairs			
	♂ ♂	♀ ♀	♂ x ♀	Tot.
Paired	26 (15.2%)	66 (38.6%)	79 (46.2%)	171
Controls	36 (21.0%)	68 (39.8%)	67 (39.2%)	171

O. labronica

Specimens with male phenotype

8 specimens with male external characters and a few oocytes in their coelomic cavities were observed. 3 specimens were paired to a female and 2 of these pairs normally gave progeny. Histological preparations of 2 specimens revealed the existence of oocytes (40-60 μm in diameter) and free spermatozoa in the coelomic cavities (Fig. 1, in Fig. 2 a normal male). The other 3 specimens were paired with adult males and cultured for 40 days; no production of egg masses and no growth of the oocytes were observed (furthermore one specimen resorbed its own oocytes).

Such specimens should be considered as male intersexes, in keeping with what is already reported in *O. labronica* (ROLANDO & GIORDA, 1982).

DISCUSSION

Parameters of the population

The study of some parameters of a population collected in Genoa revealed that a few male characters are different at a significant level from female ones. They are: 1) the number of dorsal glands (at the time when the d.u.j. appears) 2) the number of chaetigerous segments at the time when the d.u.j. appears and 3) the time when the d.u.j. appears. In analogy with what claimed by Pfannenstiel in *O. labronica* (1976), it is suggested that these parameters are to be considered as secondary sexual characters.

In particular the number of dorsal glands certainly is a secondary sexual character since preliminary observations revealed that the number of dorsal glands remains higher in the males than in the females also even after the appearance of the d.u.j..

Sexual stability

The experiments on sexual stability confirm on the one hand the gonochoric condition of the species under study and the sexual stability of adults but, on the other hand, they seem to indicate the existence of sex induction phenomena in the juveniles. In fact, in the pairs of *O. robusta* juveniles, sexual differentiation differs significantly from that in the control pairs (in particular, a higher number of heterosexual pairs was found in the pairs of juveniles than in the control pairs) (χ^2 test, $P < 0.001$).

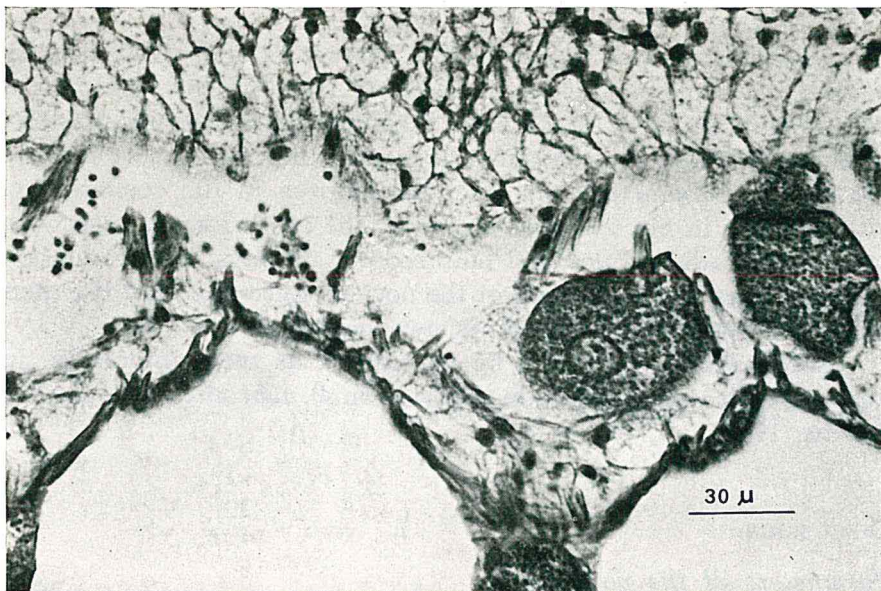


Fig. 1 - Oocytes and free spermatozoa in the coelomic cavities of a male intersex of 16 chaetigerous segments. Hemalum-eosin.

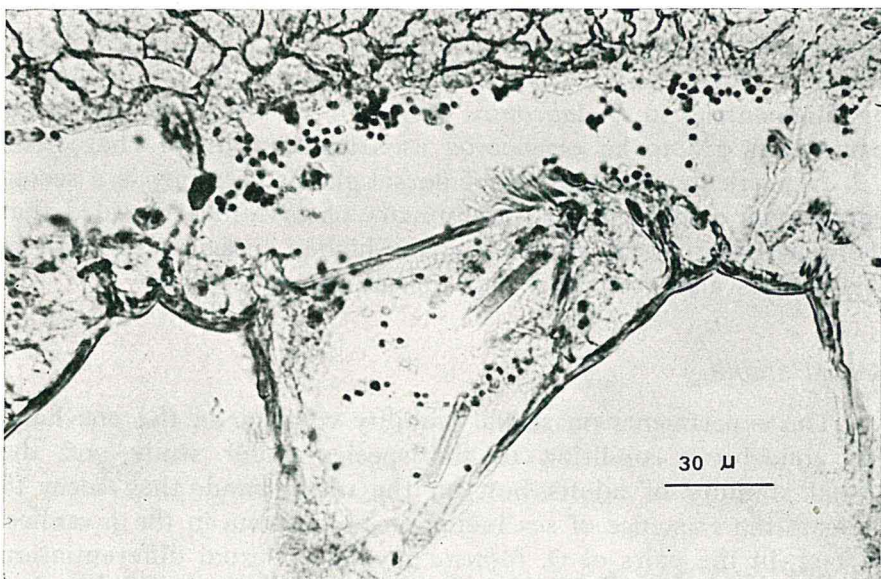


Fig. 2 - Free spermatozoa in the coelomic cavities of a male of 17 chaetigerous segments. Heidenhain's iron hematoxylin.

Similar data were obtained in *O. labronica* in which, however, the sexual differentiation of the paired juveniles is not significantly different from that of control juveniles ($P < 0.10$, not significant).

In *O. robusta* also the juveniles paired to an adult female differentiated into a significantly higher number of males than the control juveniles ($P < 0.001$).

These results suggest that *O. robusta* is a labile gonochoric species.

Intraspecific interactions that control sex expression are well known in the proterandrous hermaphrodite species *O. puerilis* (HARTMANN & HUTH, 1936; MÜLLER, 1962; PFANNENSTIEL, 1975 and 1977; MARCHIONNI & ROLANDO, 1981).

Sexual stability was ascertained in a few populations of the gonochoric species *O. labronica* (ÅKESSON, 1970 and 1972).

Nevertheless, in *O. labronica* inducible hermaphroditism (BACCI et alii, 1979) and the intersexuality phenomena observed (ÅKESSON, 1975; PFANNENSTIEL, 1976 and 1977, ROLANDO & GIORDA, 1982) suggest the existence of a certain sexual lability.

The findings of sex induction recorded in the present investigation in *O. robusta* would be a further indication of sex plasticity in the genus *Ophryotrocha*. The latter results in *O. robusta* require confirmation, and additional tests are needed to clarify whether also adult males are able to induce sexual differentiation in the juveniles.

Male intersexes

The sexual condition of the *O. robusta* specimens with male phenotype is without doubt a display of intersexuality (according to Goldschmidt's 1915 original definition, cf. BACCI, 1965). Hermaphroditism can be ruled out since the specimens studied maintained their ability to fertilize and, paired with adult males, did not spawn their oocytes (which could be also resorbed). Moreover these specimens displayed a quite normal male courtship activity; this, on account of the accurate discrimination between sexes displayed by some gonochoric *Ophryotrocha* species (ROLANDO, 1981 and in preparation), confirms the substantial male condition of these specimens. Therefore they were functionally males and the presence of oocytes should be regarded as a phenomenon of male intersexuality.

Both the sex induction phenomena and the displays of intersexuality seem to point to *Ophryotrocha robusta* as a very suitable species for studies of sexuality in the genus *Ophryotrocha*.

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