ATTI

DELLA

SOCIETÀ TOSCANA

DI

SCIENZE NATURALI

RESIDENTE IN PISA

MEMORIE - SERIE B

VOL. LXXXVI - ANNO 1979

ARTI GRAFICHE PACINI MARIOTTI - PISA - 1979

INDICE

Atti Soc. Tosc. Sci. Nat., Mem., Serie B, **86** (1979)
pagg. 355-362, ff. 3, tabb. 3.

V. MARCHIONNI (*) and A. ROLANDO (*)

INFLUENCE OF BONELLIN ON THE TIME OF SEX INVERSION AND ON FERTILITY IN *OPHRYOTROCHA PUERILIS*

Riassunto — *Influenza della bonellina sul momento della inversione del sesso e sulla fertilità in* Ophryotrocha puerilis. Individui giovani di *Ophryotrocha puerilis* in fase maschile sono stati trattati con bonellina, il pigmento delle femmine mature di *Bonellia viridis* responsabile, secondo alcuni Autori, del viraggio della larva di *Bonellia* verso la mascolinità.

La bonellina ha rivelato in *Ophryotrocha* effetto femminilizzante determinando un significativo anticipo della inversione alla fase femminile ed un incremento del tasso riproduttivo.

Summary — Young individuals of *Ophryotrocha puerilis* in male phase were treated with bonellin, the pigment of *Bonellia viridis* ripe females. The treatment induced a significant anticipation of the female phase and an increase of the reproductive rate.

Key words — Ophryotrocha puerilis - bonellin.

In *Ophryotrocha puerilis*, a proterandric hermaphrodite Polychaete worm, mutual influences between partners in female phase (« Pair culture effect ») have been extensively investigated (HARTMANN and HUTH, 1936; BACCI, 1952; MÜLLER, 1962; PFANNENSTIEL, 1975). Such effects lead to assume, in the mechanism of sex determination of this species, a regulating activity performed by the production of one or several pheromones.

In the course of experiments carried out in order to identify such substances, some assays have also been made by treating *O. puerilis* with bonellin, a pigment which, according to some Authors (PELTER et al., 1976, 1978), should be « responsible for masculinisation in the marine echiuroid *Bonellia viridis* ». This assumption, however, has never been demonstrated, because BALTZER and coll.

(*) Istituto di Zoologia dell'Università di Torino, Via Accademia Albertina, 17 - 10123 Torino.

(1914-1937) and LEUTERT (1975) carried out their own experiments with total extracts, of which bonellin is only one component. The present attempt was suggested by the generally accepted idea that Echiurids are philogenetically related to Polychaetes and hence that there might be a similarity between sexually active substances in *Bonellia* and in *Ophryotrocha*. Moreover the multifactorial type of sex determination demonstrated in *O. puerilis* (BACCI, 1955) provides an interpretation for the existence of multiple sex phenotypes also in *Bonellia* (BACCI, 1965; LEUTERT, 1975).

The experiments on *Ophryotrocha* have been carried out on the offspring of a single couple of animals collected at Mergellina (Bay of Naples, november 1977) and kept in standard conditions (at 18°C, with natural light and with spinach as food). The bonellin employed for treatment has been dissolved in seawater at the concentration of 0.1 γ/ml., since higher concentrations proved to be toxic. The medium has been partially changed twice a week in the course of the experiments.

Anticipation of the female phase. The animals were kept in stock cultures until they reached a lenght of 8-12 chaetigerous segments and were then isolated and subjected to treatment with bonellin, simultaneously control individuals of the same length were isolated. The evaluation of the moment of change from male to female phase and therefore of the degree of maleness or femaleness of each individual was made — following Bacci's method (1955) — by determining the number of chaetigerous segments shown at the time of the first appearance of free oocytes in the coelomic cavities.

The results of the experiments show that bonellin induces an anticipation of the female phase of 0.62 segments in average, a difference which is highly significant ($F = 8.11^{+++}$) (Table 1 and

TABLE 1 - The anticipation of the female phase by treatment with bonellin of isolated individuals.

specimens	n	mean number of segments at sex change	average anticipation	F
treated with bonellin	113	15.34 ± 0.16	0.62	8.11^{+++}
controls	113	15.96 ± 0.15		

Fig. 1). The daily individual growth was also considered and, as the animals were isolated at five different lengths ranging from 8 to 12 chaetigerous segments, the data were assembled in the five corresponding classes. The mean values show (Table 2) that the reduction of the growth rate with age is not different both in the treated and in control specimens. This result leads to exclude that,

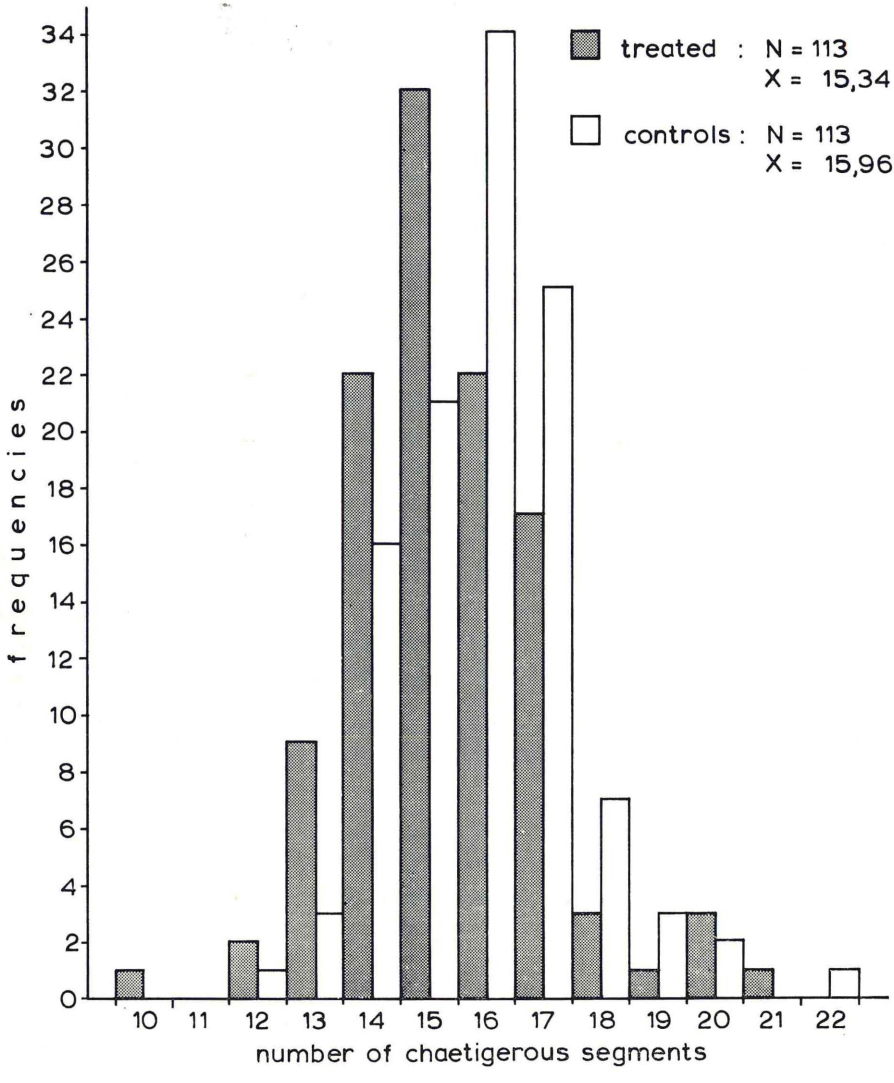

Fig. 1 - The specimens of *O. puerilis* treated with bonellin anticipate the change to the female phase.

TABLE 2 - Average daily growths beginning from different size class.

specimens	n	mean daily growth from different size class measuring in number of segments					daily average growth
		8	9	10	11	12	
treated with bonellin	113	0.94	0.77	0.70	0.68	0.66	0.75
controls	113	0.93	0.80	0.76	0.67	0.68	0.77
total	226						

in the present instance, the bonellin may be regarded as a factor of growth.

Increase of the reproductive rate. 1) 24 animals, 12 specimens treated with bonellin and 12 controls, which inverted at the same length and in the same day, were kept isolated in order to control the ripening of oocytes and the production of egg masses. The treatment with bonellin, carried on for about two months, has determined a remarkable acceleration of the maturation of oocytes and the production of a greater number of egg masses as compared with controls (Table 3, Fig. 2 and Fig. 3). Only one among the specimens treated with bonellin did not lay egg masses and showed resorption of the oocytes after a first phase of oocytes development

TABLE 3 - The increase of the reproductive rate by treatment with bonellin in female phase individuals kept isolated.

specimens	n	number of egg masses	number of egg masses/individual
treated with bonellin	12	22	1.83
controls	12	12	1.00
total	24	34	

but as much as one third of the controls was unable to lay eggs.

2) 12 animals, 6 specimens treated with bonellin and 6 controls, which changed sex at the same number of segments and in the same day, were each mated with an individual in male phase (measuring 10 chaetigerous segments) and kept under observation for a period of 40 days; the egg masses and the eggs number for egg mass of each pair were recorded. As shown in Table 4, the 6 pairs treated

TABLE 4 - The increase of the reproductive rate by treatment with bonellin in pairs.

pairs	n	number of egg masses	number of eggs	eggs/pair/day
treated with bonellin	6	25	7298	32.87
controls	6	19	4976	22.41
total	12	44	12274	

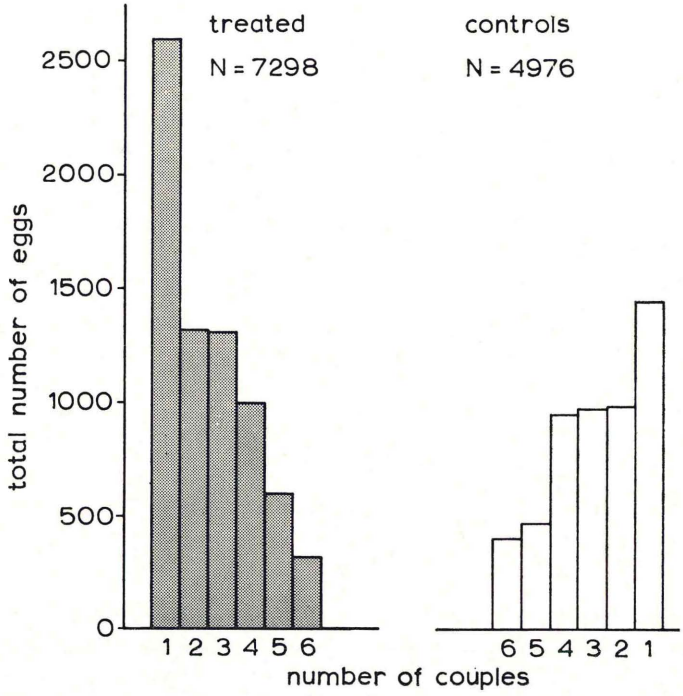

Fig. 2 - Reproductive rate: total number of eggs spawned by each couple treated with bonellin and by each couple of control.

with bonellin laid a greater number of egg masses, a greater total number of eggs and hence showed a greater reproductive rate as expressed by the ratio number of eggs/day (Fig. 2 and Fig. 3).

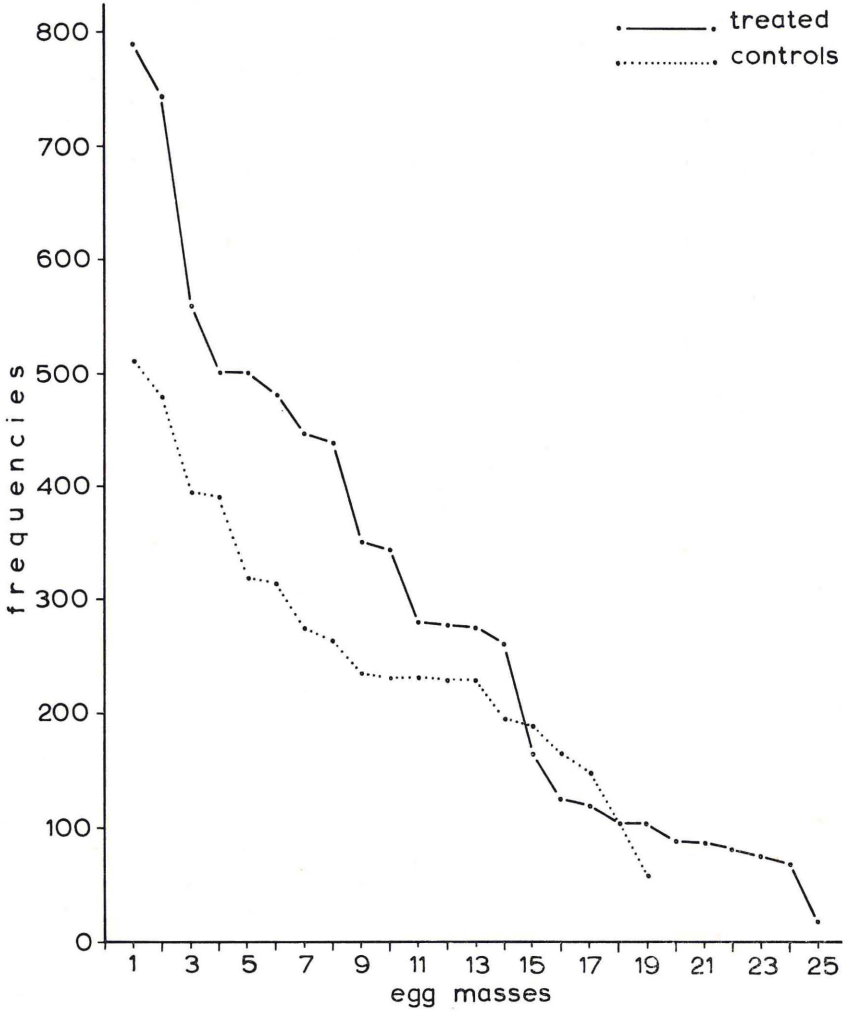

Fig. 3 - Number of eggs/egg masses in couples treated with bonellin and in couples of control.

Parallel experiments were carried on with neobonellin (CARIELLO et al., 1978), another component of the proboscis extracts of *Bonellia,* but no significant results were obtained.

The results of this work do not permit, at the present stage of the research, to put forward any assumption on the mechanism and on the specificity of the feminizing effect of bonellin on *Ophryotrocha*. On the other hand, as mentioned above, experiments demonstrating a specific masculinising action of bonellin on *Bonellia* larvae are completely lacking and we suggest that some features of bonellin point to it as unlikely candidate to the role of sex determining substance.

Especially: 1) bonellin is present in the integument of the whole body, not only of the proboscis, where the larvae adhere preferably, 2) bonellin seems to be the pigment responsible for the negative photobehavior of this Echiurid (LALLIER, 1955), 3) bonellin seems to have, as most integumental zoochromes, a chemical defence role owing to its high toxicity towards other marine animals (MICHEL, 1931; LALLIER, 1955), 4) finally pheromones, in general, are not coloured.

The authors would like to thank G. Prota, L. Cariello, M. De Nicola Giudici and L. Zanetti (Istituto di Chimica Organica dell'Università and Stazione Zoologica, Naples, Italy) for providing them with bonellin and neobonellin.

REFERENCES

BACCI G. (1952) - Diverso comportamento sessuale delle *Ophryotrocha puerilis* di Napoli e Plymouth. *Boll. Soc. Biol. Sper.*, **28**, 4, 1-4.

BACCI G. (1955) - La variabilità dei genotipi sessuali negli animali ermafroditi. *Pubbl. Staz. Zool. Napoli*, **26**, 110-137.

BACCI G. (1965) - Sex determination and sex balance of *Ophryotrocha puerilis*. *Nature*, **207**, 1208-1209.

BALTZER F. (1937) - Entwicklungsmechanische Untersuchungen an *Bonellia viridis*. III. Ueber die Entwicklung und Bestimmung des Geschlechts und die Anwendbarkeit des Goldschmidtschen Zeitgesetzes der Intersexualität bei *Bonellia viridis*. *Pubbl. Staz. Zool. Napoli*, **16**, 89-159.

CARIELLO L., DE NICOLA GIUDICI M., ZANETTI L., PROTA G. (1978) - Neobonellin a new biologically active pigment from *Bonellia viridis*. *Experientia*, **34**, 1427-1429.

HARTMANN M., HUTH W. (1936) - Untersuchungen ü. Geschlechtsbestimmung ü. Geschlechtsumwandlung von *Ophryotrocha puerilis*. *Zool. Jahrb.*, **56**, 389-439.

LALLIER R. (1955) - Recherches sur la toxicité des extraits du Ver marin *Bonellia viridis*. *C.R. Acad. Sci., Paris*, **240**, 1489-1491.

LEUTERT R. (1975) - Sex determination in *Bonellia*. In, Intersexuality in the Animal Kingdom. Reinboth (ed.). Springer Verlag, Berlin.

MICHEL F. (1931) - Ueber den chemischen Schutz der *Bonellia viridis* gegen Frass. *Pubbl. Staz. Zool. Napoli*, **11**, 22-29.

MÜLLER H. (1962) - Ueber die sexualität des Polychaeten *Ophryotrocha puerilis*, ihre Determination und ihren Einfluss auf Drüsentätigkeit Kauapparatentwicklung. *Z. Morph. Okol. Tiere*, **52**, 1-32.

PELTER A., BALLANTINE J. A., FERRITI V., JACCARINI V., PSAILA A. F., SCHEMBRI P. J. (1976) - Bonellin, a most unusual Chlorin. *J. Chem. Soc. Chem. Commun.*, 999.

PELTER A., BALLANTINE J. A., MURRAY-RUST P., FERRITO V., PSAILA A. F. (1978) - The structures of anhydrobonellin and bonellin, the physiologically active pigment from the marine echiuroid *Bonellia viridis*. *Tetrahedron Letters*, **21**, 1881-1884.

PFANNENSTIEL H. D. (1975) - Mutual influence on the Sexual Differentiation in the Protandric Polychaete *Ophryotrocha puerilis*. In, Intersexuality in the Animal Kingdom. Reinboth ed. Springer Verlag, Berlin.

(*ms. pres. il 18 dicembre 1979; ult. bozze il 18 giugno 1980*).