Self-Fertilisation in *Ophryotrocha labronica*

Ophryotrocha labronica (Bacci and La Greca[1]) is a newly described species which has a haploid chromosome complement of three metacentric chromosomes[2]. It is protandrous hermaphroditic and it has been discovered in the course of new researches on sex determination of *Ophryotrocha*[3].

The first appearence of oocytes in this species takes place at a medium length of 13 chaetigerous segments, which thus differs considerably from the means observed in the geographic subspecies of *Ophryotrocha puerilis*, namely *O. puerilis puerilis* of the Mediterranean and *O. puerilis siberti* from the Atlantic. In the former, oocytes first appear at a mean length of 18,21 segments, in the latter at 20,36 chaetigerous segments[4].

Cytological research has demonstrated the presence of male pronuclei in oocytes which were freshly laid by isolated individuals (Fig. 1), and this observation rules out the possibility of parthenogenetic development in such instances. The anaphase of the first maturation was still progressing in the inseminated eggs.

A study of the sequence of sex phases explains the origin of self-fertilisation in *O. labronica*: young individuals of 7–8 chaetigerous segments sometimes show an intense primary spermatogenesis. Spermatogonia and spermatocytes adhere to the dissepiments and to the intestinal walls, and sperms and spermatids fill out the coelome cavities. A few nutrient cells are present in the gonads and oocytes begin to grow in individuals of 9–10 segments. Germinal male elements are gradually eliminated and they are totally absent when oocytes attain medium sizes.

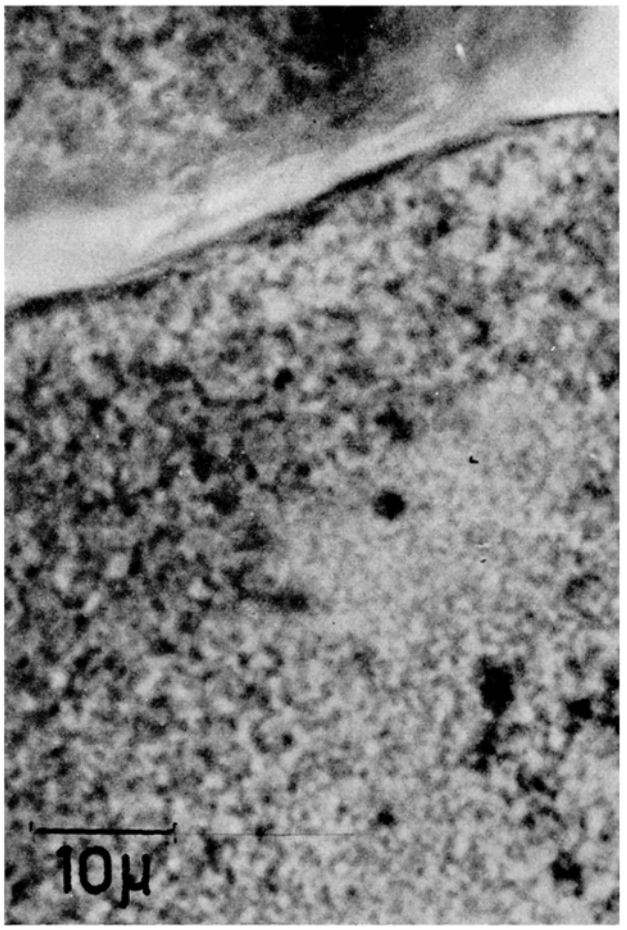

Fig. 1. Male pronucleus visible in a clear zone of an egg laid by an isolated individual (Feulgen, light green).

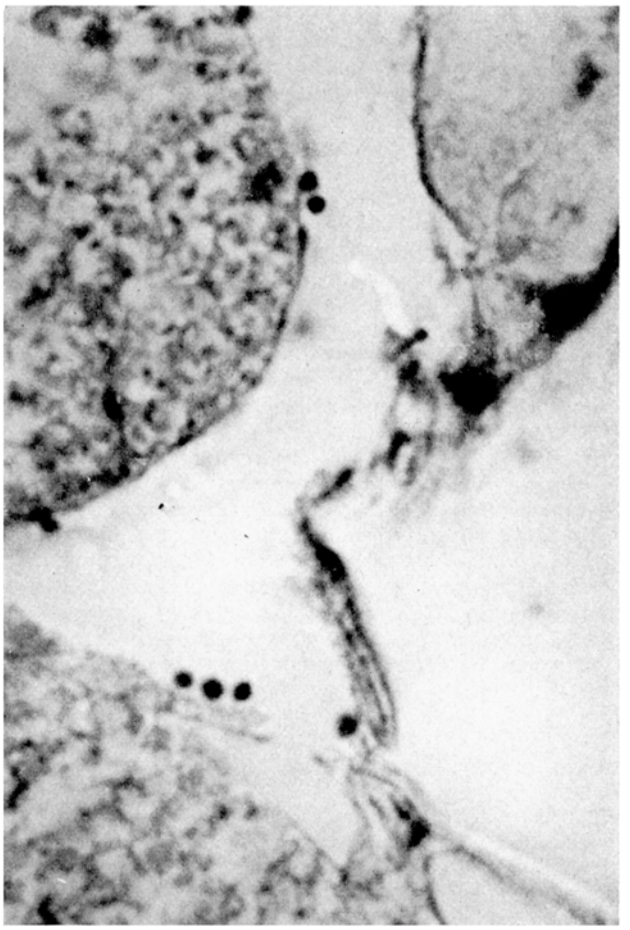

Fig. 2. Groups of sperms in ripe female phase individuals (Iron haematoxilin).

The culture methods used for *O. puerilis*[5] have been employed also for this new species and strains from individuals collected at the Leghorn Aquarium have been studied[6].

The following results were obtained: isolated individuals, kept in Boveri dishes with filtered sea-water, laid eggs that developed normally and gave rise to normal larvae after 8–10 days of development. This result suggested the possibility of parthenogenetic development which has already been observed in *Grubea pusilla*, another Polychaete[7].

[1] G. Bacci and M. La Greca, Boll. Zool. *27*, in print (1960).

[2] U. Parenti, R. C. Accad. Lincei *27*, in print (1960).

[3] G. Bacci *et al.*, Exper. *7*, 222 (1951); R. C. Accad. Lincei *28*, 92 (1960).

[4] G. Bacci and M. La Greca, Nature *171*, 1115 (1953).

[5] G. Bacci, Pubbl. Staz. zool. Napoli *26*, 110 (1955).

[6] The hearthiest thanks are due to Prof. Razzauti and to the staff of the Leghorn Aquarium.

[7] C. Hauenschild, Naturwissenschaften *42*, 611 (1955).

[8] G. Cognetti, Boll. Zool. *23*, 275 (1956); R. C. Accad. Lincei *24*, 325 (1958).

Older female phase individuals with ripe oocytes show again a few spermatocytes and sperms among oocytes which are ready to be laid. Sperms are always very scarce and they form small groups which are often adjacent to the oocytes (Fig. 2). Spermatocytes are generally found in clusters along the intestinal wall. The rapid secondary spermatogenesis is thus responsible for the subsequent self-fertilisation in *O. labronica*.

Similar processes were demonstrated by COGNETTI in the Asteroid species *Asterina pancerii*[8].

Researches are now being carried on in order to ascertain whether primary and secondary spermatogenesis takes place in all individuals of *O. labronica*.

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Istituto di Zoologia, Università di Modena, and Acquario di Livorno (Italy), June 16, 1960.

Riassunto

Ophryotrocha labronica (BACCI e LA GRECA)[1] è ermafrodita proterandrica e si dimostra che molti individui presentano una fugace spermatogenesi secondaria che rende possibile l'autofecondazione.

Isolierung von Lysergsäure-Alkaloiden aus der mexikanischen Zauberdroge Ololiuqui (*Rivea corymbosa* (L.) Hall. f.)

Die Samen von *Rivea corymbosa* (L.) Hall. f., der alten aztekischen Zauberdroge «Ololiuqui»[1] und von *Ipomoea tricolor Cav.* (*Convolvulaceae*) werden auch heute noch von manchen Indianerstämmen Südmexikos für magische Zwecke verwendet.

R. G. WASSON verdanken wir Muster dieser beiden Drogen, die in Oaxaca (Mexiko) gesammelt worden waren. Durch schonende Extraktion konnte aus beiden Samen Alkaloidfraktionen gewonnen werden, die im Dünnschicht-Chromatogramm mindestens 5 übereinstimmende Indolverbindungen erkennen liessen. Es gelang, die 3 Hauptkomponenten zu kristallisieren und chemisch zu identifizieren.

Substanz A: Aus Methanol kristallösungsmittelfreie Prismen, Smp. 232–240° (korr.) Zers., $[\alpha]_D^{20} = +105° (\pm 7°)$ ($c = 0{,}1$ in Chloroform/Methanol 1 : 1). Gefunden: C 71,8; H 6,6; O 6,2; N 15,5%. Berechnet für $C_{16}H_{17}ON_3$ (267,3): C 71,9; H 6,4; O 6,0; N 15,7%. UV-Spektrum: Maxima bei 240 mμ ($\log \varepsilon_{max} = 4{,}3$) und bei 311 mμ ($\log \varepsilon_{max} = 3{,}9$). Dieses Spektrum ist mit demjenigen der Lysergsäure und Isolysergsäure praktisch identisch[2]. Diese physikalisch-chemischen und analytischen Daten entsprechen den Eigenschaften von d-Lysergsäure-amid. Die vollkommene Übereinstimmung der IR-Spektren von Substanz A und von d-Lysergsäure-amid (Iso-ergin)[3] bestätigt die Identität dieser beiden Verbindungen.

Substanz B: Aus Methanol kristallösungsmittelhaltige Prismen vom Smp. 136°(korr.), $[\alpha]_D^{20} = +383° (\pm 6°)$ ($c = 0{,}1$ in Chloroform/Methanol 1 : 1). Die im HV bei 120° getrocknete Verbindung lieferte folgende Analysenwerte: C 71,6; H 6,6; O 6,2; N 15,4%. Berechnet für $C_{16}H_{17}ON_3$ (267,3): C 71,9; H 6,4; O 6,0; N 15,7%. UV-Spektrum: identisch mit demjenigen von Substanz A. Diese Daten stimmten auf d-Isolysergsäure-amid (Ergin)[4]. Die Identität von Substanz B mit Ergin wurde durch die übereinstimmenden IR-Spektren bestätigt.

Substanz C: Aus Aceton massive kristallösungsfreie Polyeder vom Smp. 213–215° (korr.) Zers., $[\alpha]_D^{20} = -195°$ ($c = 0{,}2$ in Pyridin). Gefunden: C 74,6; H 7,9; O 6,5; N 11,0%. Berechnet für $C_{16}H_{20}ON_2$ (256,3): C 74,9; H 7,9; O 6,2; N 10,9%. UV-Spektrum: Maxima bei 222 mμ ($\log \varepsilon_{max} = 4{,}6$), bei 282 mμ ($\log \varepsilon_{max} = 3{,}9$) und bei 291 mμ ($\log \varepsilon_{max} = 3{,}8$). Diese Daten stimmten mit den Eigenschaften von Chanoclavin[5] überein. Die Identität von Substanz C mit diesem erstmals aus saprophytischen Kulturen des Mutterkornpilzes von *Pennisetum typhoideum* Rich. isolierten Alkaloid wurde durch den Vergleich der IR-Spektren, die vollkommene Übereinstimmung zeigten, bestätigt.

Das Vorkommen von Lysergsäure-Alkaloiden, die bisher nur in niederen Pilzen der Gattung *Claviceps* gefunden wurden, in der Pflanzenfamilie der *Convolvulaceae* ist ein unerwarteter, phytochemisch interessanter Befund.

Die Analysen wurden im mikroanalytischen Laboratorium SANDOZ (Leitung Dr. W. SCHÖNIGER) ausgeführt, die Spektren in der spektral-analytischen Abteilung (Leitung Dr. H. G. LEEMANN) aufgenommen. Für wertvolle Diskussion der Spektren danken wir Herrn Dr. H. G. LEEMANN bestens.

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Pharmazeutisch-chemisches Laboratorium SANDOZ, Basel, 28. Juli 1960.

Summary

The psychotomimetic active principles of the ancient aztec drug «Ololiuqui» (*Rivea corymbosa* (L.) Hall. f.) have been found to be alkaloids of the ergot type. Three of the components could be crystallized and identified, i.e. ergine (isolysergic acid amide), isoergine (lysergic acid amide) and chanoclavin. The same alkaloids were found in the seeds of the related convolvulaceous vine *Ipomoea tricolor* Cav.

[1] Vgl. R. E. SCHULTES: *A contribution to our knowledge of Rivea corymbosa.* The narcotic ololiuqui of the Aztecs. Botanical Museum of Harvard University, Cambridge (Massachusetts) 1941.

[2] W. A. JACOBS, L. C. CRAIG und A. ROTHEN, Science *83*, 166 (1936).

[3] S. SMITH and G. M. TIMMIS, J. Chem. Soc. *1936*, 1440.

[4] S. SMITH and G. M. TIMMIS, J. Chem. Soc. *1938*, 764.

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Further Studies on Corticotropin Releasing Factor (CRF); Corticotropin Releasing Activity of Synthetic Peptides

In a preceeding communication[1] we have reported that it was possible to obtain, from hog posterior pituitary powder, a fraction free of vasopressin and yet having a strong CRF activity. By using the method of assay that we have described[1,2], we have now obtained peptide fractions of which 0.5–1.0 μg is as active as 4 μg of a standard preparation called CRF 91. These fractions have been obtained through the following operations: (1) Extraction of the posterior pituitary powder with dilute acetic acid; (2) Fractionated precipitation with acetone; (3) Countercurrent distribution according to the classical method of Craig; (4) Zone electrophoresis according to the method

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