Rudimentary Hermaphroditism and Automictic Parthenogenesis in *Limnadia lenticularis* (Phyllopoda, Conchostraca)

Limnadia lenticularis is a conchostracan phyllopod in which no males occur[1–3]. Only females being known of this species, it has been supposed to propagate exclusively parthenogenetically, but no caryological studies have been made. My observations have been carried out on individuals of *Limnadia lenticularis* from a temporary pool near the Lake Maggiore (Italy)[4], and on specimens reared in the laboratory at room temperature (22 °C about) from the eggs released by those.

The reproductive system of *L. lenticularis* consists of 2 central tubular cavities lying on either side of the digestive tract, with many short sac-like dilations, at the tips of which female germinal regions are placed. From each female germinal region arise clusters of 4 cells (1 oocyte and 3 nurse cells), which, as they increase in size, project forward into the haemocoel bounded by a layer of small follicle cells.

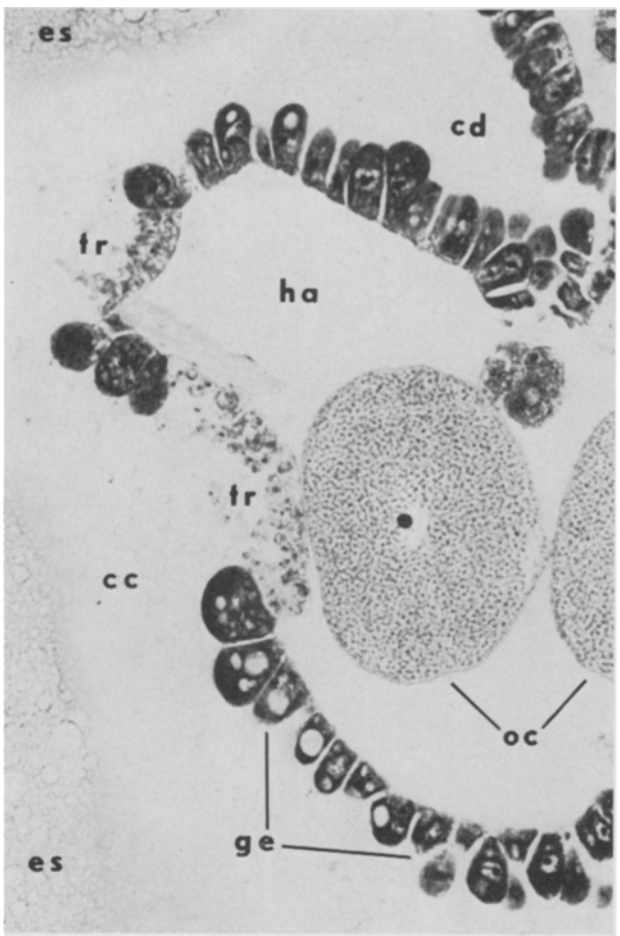

Fig. 1. Part of the hermaphrodite reproductive system of *L. lenticularis* in longitudinal section. *cc*, central cavity; *cd*, sac-like dilation of central cavity; *es*, egg-shell substance; *ge*, lining glandular epithelium of central cavity; *ha*, haemocoel; *oc*, oocytes; *tr*, testicular region. Carnoy. Haematoxylin-eosin. × 370.

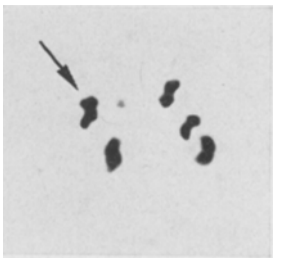

Fig. 2

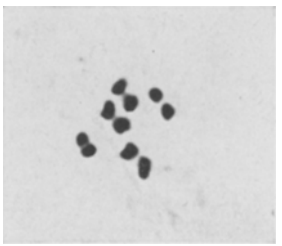

Fig. 3

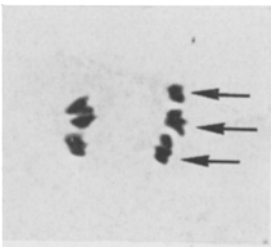

Fig. 4

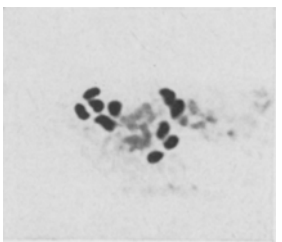

Fig. 6

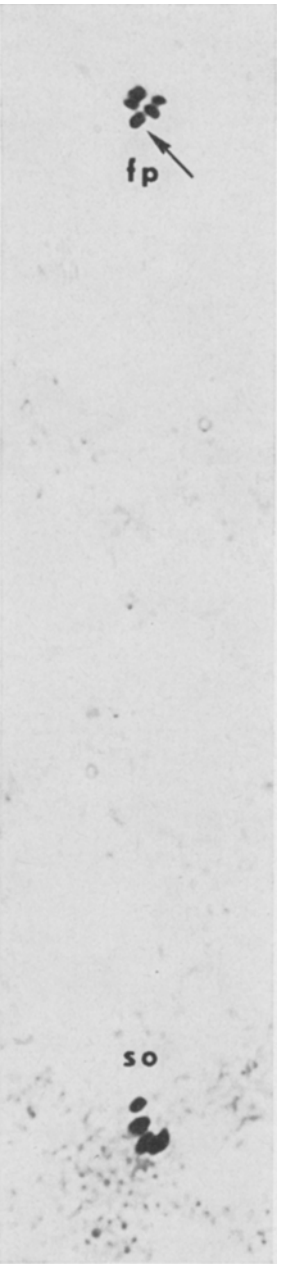

Fig. 5

Fig. 2. Metaphase I with 5 bivalents showing a quadripartite structure (arrow). × 2000.

Fig. 3. Early anaphase I; interzonal connections between disjoining dyads are visible. × 2000.

Fig. 4. Late anaphase I; each dyad is composed of 2 chromatids (arrows). × 2000.

Fig. 5. The 2 haploid groups of 5 dyads derived from the first meiotic division. Arrow indicates the split between the 2 chromatids of a dyad. *fp*, first polar chromosome group; *so*, secondary oocyte chromosome group. × 2000.

Fig. 6. Gathering of the 5 dyads of the first polar nucleus with those of the secondary oocyte. × 2000.

[1] G. O. Sars, Fauna Norvegiae *1*, 1 (Christiania 1896).
[2] T. Gislén, Acta Univ. lund. *32*, 1 (1937).
[3] P. Mathias, Actual. scient. ind. *447*, 1 (1937).
[4] H. M. Fox, Mem. Ist. Ital. Idrobiol. *6*, 205 (1951).

The 2 central cavities and their dilations are lined with a layer of large gland cells, which secrete the egg-shell substance. The glandular epithelium of the central cavity – not the one of their dilations – is here and there interrupted by masses of male germ cells, which are sometimes situated in lobes protruding into the haemocoel (Figure 1).

In these testicular regions, male germ cells appear to be arranged irregularly, and only a few stages of their maturation are evidently visible. The spermatogenesis closely resembles the one of Notostraca[5], but it seems abortive, because sperms are not formed, or are produced in very small number.

Therefore the histological examination of the reproductive system has shown that all the observed individuals, with female somatic features, really were hermaphrodites. The hermaphroditism in Phyllopoda was known only in Notostraca[5,6]; now the author has found it also in Conchostraca[7].

When the oocytes, at the end of vitellogenesis, have fallen into the central cavity, they are covered with their shell, and all at the stage of metaphase of the first meiotic divison are found with 5 bivalents. The oocytes continue and accomplish their maturation after they have been put on the back of their mother.

The study of oocyte maturation with aceto-orcein squash method[8] has shown that 10–15 min after the oocytes have been put on the back of the mother, anaphase I with disjunction of the 5 bivalents takes place (Figures 2, 3 and 4). Typical telophase I is omitted. After anaphase I, 2 haploid groups of 5 dyads are formed, corresponding to the first polar nucleus and to the secondary oocyte 1 respectively (Figure 5). The 2 haploid chromosome groups remain well separate at the periphery of the ooplasm for 70–80 min, then they gather in 1 diploid metaphase plate (Figures 6 and 7). The second maturation division follows with the separation of the 2 chromatids of each dyad, and the formation of 1 diploid polar body, which is probably extruded, and of the mature ovum nucleus, which enters a resting stage. The interphase pronucleus remains at the periphery of the ooplasm until 3 h after oviposition, then it migrates into the middle of the ovum. 4 h and 10 min after the eggs have been put on the back of the mother, the first cleavage mitosis begins with a diploid set of 10 chromosomes (Figure 8).

Conclusions. On the basis of these observations it appears that the population of *L. lenticularis* examined consists of rudimentary hermaphrodites, which reproduce by automictic parthenogenesis. 2 meiotic divisions occur, but only diploid polar body is formed. The restoration of the diploid set of chromosomes takes place through the fusion of the first polar nucleus with the secondary oocyte one[9,10].

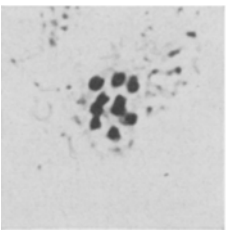

Fig. 7

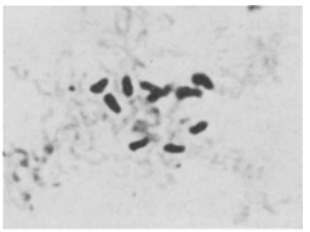

Fig. 8

Fig. 7. Metaphase II with 10 dyads. × 2000.

Fig. 8. Mitotic metaphase plate of late cleavage stage showing 10 chromosomes. × 2000.

Riassunto. È stata studiata una popolazione di *Limnadia lenticularis* costituita da individui con caratteristiche esterne femminili, ma con un apparato riproduttore ermafrodita.

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Istituto di Zoologia dell'Università di Bologna (Italy), 2 December 1968.

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[8] R. STEFANI, Riv. Biol. *56*, 309 (1963).
[9] This investigation was supported by the Consiglio Nazionale delle Ricerche (C.N.R.) of Italy.
[10] The author wishes to thank Prof. R. STEFANI, Director of the Zoological Institute of Cagliari, for the useful suggestions in the aceto-orcein squash method.

Single Giant Larvae of *Ascidia malaca* from Double Eggs

Experiments of fusion of two eggs in invertebrates (sea urchin, nematodes, nemertines) as well in vertebrates (Urodeles) have permitted the analysis of many problems of embryonic development, in particular those concerning the nucleo-cytoplasmic relations.

FAUTREZ[1] has obtained the fusion of 2 unfertilized ascidian eggs and the development of the resulting giant eggs up to the larva stage. According to this author, the giant larvae are not single but, at least for some organs, more or less double. From this result FAUTREZ has deduced that in the unfertilized ascidian egg there are preformed structures. This conclusion contrasts with the conclusion drawn by REVERBERI[2] and REVERBERI and ORTOLANI[3] from the results of the development of the egg fragments: according to these authors the unfertilized ascidian egg is totipotent.

In the course of some experiments we noticed that demembranated eggs which remained in sea water or

[1] J. FAUTREZ, Bull. Acad. r. Belg. *26*, 144 (1940).
[2] G. REVERBERI, Pubbl. Staz. zool. Napoli *11*, 168 (1931).
[3] G. REVERBERI and G. ORTOLANI, Devl. Biol. *5*, 84 (1962).