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].

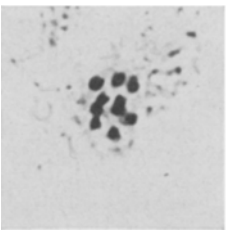

Fig. 7

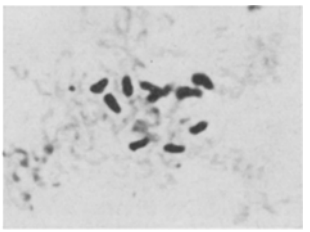

Fig. 8

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.

F. ZAFFAGNINI

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

[5] A. R. LONGHURST, Proc. zool. Soc. London *125*, 671 (1955).
[6] H. M. BERNARD, *The Apodidae* (MacMillan, London 1892).
[7] F. ZAFFAGNINI, Mem. Ist. Ital. Idrobiol. *23*, 129 (1968).
[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).

24 h sometimes stuck and fused. This observation led us to follow the destiny of these giant eggs after their fertilization. In the present communication we report the results which we have obtained.

At fertilization the giant eggs modify their spheric shape: this modification, which is probably determined or accompanied by the redistribution of the plasms, occurs also in the normal eggs. It is followed by other modifications at the extrusion of the polar globules; 4 polar globules are extruded. It seems that only one spermatozoon enters the egg. In fact, thanks to the transparency of the egg, 3 nuclear vesicles have been observed moving in the cytoplasm and fusing. Some fused eggs cleave normally and develop, but only a very few arrive at the larval stage. The eggs which develop normally behave in every detail as a single egg: the 16-cell stage is characterized by the 'micromeres', also bilateral symmetry is evident. The behaviour of the eggs as a unity is ultimately attested by the blastula, gastrula and neurula stages which do not differ, except in size, from the controls. Moreover, the giant larva is completely normal: without any signs of duplication, it has 3 palps like a normal larva, 2 pigmented sensorial spots (again, as in an ordinary larva) and only 1 row of notochordal cells (Figure 1). The study of the sections confirms the unity of the giant larvae.

As remarked above, the double eggs showed, after fertilization, 3 nuclear vesicles: they should then be triploids. The size of the nuclei, measured in different cells, supports this view. In the Figures 2 and 3 the sizes of nuclei in ectodermic and entodermic cells of giant and normal larvae are reported.

The number of cells in the giant larvae was determined on the notochordal cells, which in normal larvae are a fixed number (38). Counts show that the giant larvae also possesses 38 notochordal cells; however, as shown above, they are larger. The counting of chromosomes proved difficult.

Since the double giant eggs behave, in segmentation and development, as a unity and give rise to larvae which are single in all their systems, it appears that also the second condition required to characterize a 'harmonic equipotential system' is fulfilled in the ascidian egg. The first condition, i.e. the capacity of the egg fragments to give rise to normal dwarf larvae, was already shown to be present.

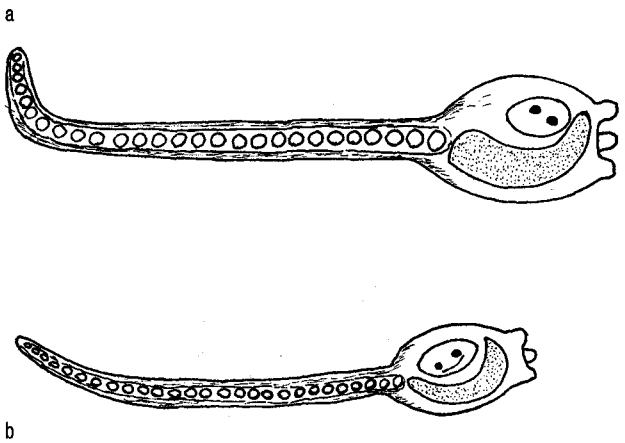

Fig. 1. (a) Giant larvae; (b) normal larvae. × 2500.

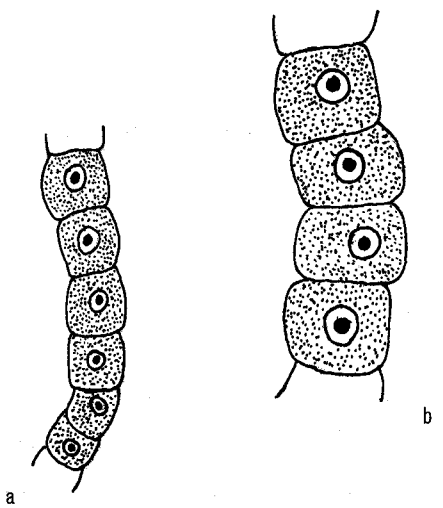

Fig. 2. Ectodermic cells (a) in a diploid and (b) in a giant larva. × 750.

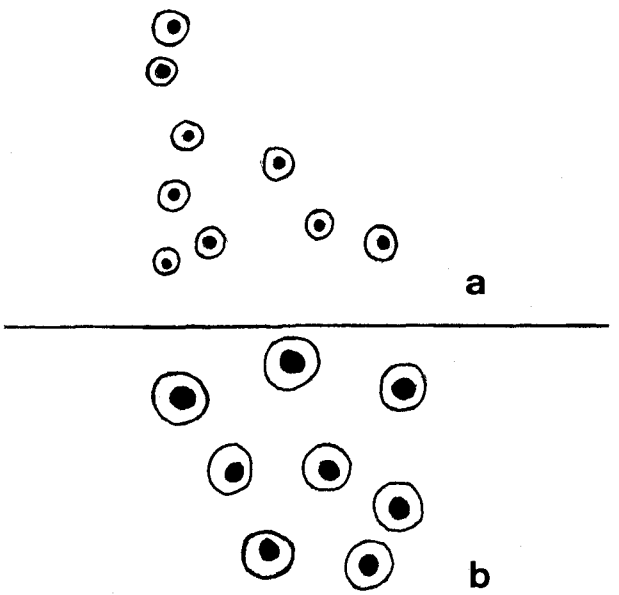

Fig. 3. Nuclei of entodermic cells (a) in a diploid and (b) in a giant larva. × 750.

Riassunto. È stata operata la fusione due a due di uova vergini di *Ascidia malaca*. Le uova giganti così ottenute, sono state successivamente fecondate e il loro sviluppo è stato seguito. Da esse sono state ottenute in numerosi casi delle larve giganti che mostravano unicità in tutti i loro organi. L'osservazione dell'uovo gigante, dopo fecondazione, permette di asserire che le larve giganti sono triploidi.

N. Farinella-Ferruzza
and G. Reverberi

Istituto di Zoologia dell'Università di Palermo (Italy), 23 December 1968.