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# Origin of Germ Cells, Sex Determination, and Sex Inversion in Medusae of the Genus *Clytia* (Hydrozoa, Leptomedusae): The Influence of Temperature

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*ABSTRACT* In Cnidaria, a separation between soma and germline remains unclear. In this work, we studied the origin of germinal cells and determination of the sexual phenotype in *Clytia hemisphaerica* and *Clytia* sp. Colonies of *C. hemisphaerica* were cultivated and the medusae liberated by each colony raised until maturity. Two hermaphrodite colonies were obtained, liberating male and female medusae. These two colonies and their medusae were raised at 15°C, 21°C, or 24°C. The medusae budded and cultured at 24°C were mainly female (80%). In contrast, if the medusae were released at 15°C, at whatever temperature they were raised later, they were mainly male (85%). The same occurred if, after release at 24°C but before the formation of the gametes, they were kept at 15°C for at least 24 hr. We suggest that there are two subpopulations of germ cells. The female line will be dominant at 24°C but temperature sensitive, with inhibition of this line by a temperature drop to 15°C, this inverting the population sex-ratio. The irreversible action of a temperature drop to 15°C supports the view that the germ cells are isolated very early. In *C. hemisphaerica*, hermaphrodite medusae were never observed. On the contrary, in *Clytia* sp., probably a new species, we have found male, female, but also hermaphrodite specimens. This is the second definite example of hermaphroditism described in any hydromedusan. The transformation of female into hermaphrodite then into male specimens occurs at 13°C. These results demonstrate the unstable character of genetic sex determination in cnidarians, at least in certain species. *J. Exp. Zool. 287:233–242, 2000.* 

The theory of the continuity of the germline was developed by Weissmann (1892) from the study of the development of 35 cnidarian species. Since then, curiously enough, the Cnidaria are one of the rare groups where a separation between soma and germline is still discussed or even denied (Nieuwkoop and Sutasurya, ’81; Tardent, ’85; Thomas and Edwards, ’91).

In Cnidaria, besides the two epithelial cell lines that form and regenerate the ectoderm and endoderm, there are interstitial cells (stem cells) that, in hydra, make up some 20–30% of the cell population. It was long supposed that these cells were totipotent and able to differentiate either into somatic cells (giving rise in particular to cnidocytes and to neurons) or into germ cells (Brien, ’66; Nieuwkoop and Sutasurya, ’81).

This concept of totipotent interstitial cells has been discussed for a dozen years, particularly in the work on *Hydra* by Littlefield (for a review, see Littlefield, ’94), Sugiyama and Sugimoto (’85), and Nishimiya et al. (’93). These authors have used clones of “epithelial” hydra (Marcum and Campbell, ’78) without interstitial cells. By making grafts or reaggregations with normal hydra, they have shown that there probably are three distinct subpopulations of interstitial cells that are unipotent stem cells committed to somatic cells, spermatogenesis, and ovogenesis.

The studies on *Hydra* equally approach the problem of sex determination and the sexual phenotype. In most metazoans, sex determination is genetic, and sex is irreversibly defined at fertilization. But there may be in some groups of animals (nematodes, insects, fish, reptiles, etc.)

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environmental (nongenetic) sex determination, most often by temperature. The best-documented example is that of reptiles where, besides the crocodilians, in which sex is entirely determined by temperature during a precocious developmental phase, in lizards and tortoises there are examples where sex is determined genetically and others where it is determined by the environment (Johnston et al., '95).

Among hydras, there are species where the sexes are separate and others that are hermaphrodite. Based on a 1:1 sex ratio in the populations obtained following sexual reproduction, it seems that in the separate sex species, sex determination is genetic (Bosch and David, '86; Littlefield, '94) without excluding a possible environmental effect on the sexual phenotype (Littlefield, '86; Thomas and Edwards, '91).

In all triploblasts, sex determination consists of a cascade of determinations initially somatic, with the differentiation of the gonads and sexual characters, then germinal, with the determination of direction of the gonocytes toward the male and female lines. The sex of the germ cells corresponds to that of the gonad that accommodates them. Transplanted into an embryo of the opposite sex, they differentiate according to the sex of the gonad and not according to their chromosomal sex (McCarrey and Abbot, '79). It has also been shown that in *Xenopus*, the first germ cells transplanted in ectopic regions of young embryos may differentiate into somatic cells (Wylie et al., '85)

In hydras and apparently in all cnidarians, there is no double sex determination, first somatic and then germinal, but uniquely a germinal determination. In these animals, there are no primary or secondary sexual characters, male and female are identical and there are no somatic gonad tissues. What is incorrectly termed the gonad is simply an accumulation of germ cells between the ectoderm and endoderm.

In the absence of somatic sex determination, the sexuality of the gametes in hydras is the result of interactions between the precursors of the male and female germ cells. This has been shown in the cases of spontaneous or experimental sex changes in dioecious species (Campbell, '85). Thus, if interstitial cells from a male hydra are grafted onto a female hydra, sex change is induced, probably linked with the arrest of oogenesis by a factor secreted or induced by the male gonocytes of the graft (Littlefield, '94). A monoclonal antibody, AC2+, recognizes spermatozoids and their stages of differentiation specifically (Littlefield et al., '85; Littlefield, '86). It is possible to demonstrate the constant presence of AC2+ cells in male hydras, their absence in female hydras, and that sex changes result either from the acquisition of AC2+ cells (masculinization) or from their loss (feminization). All experiments have established that the presence of cells of the male line inhibits the development of the female line. Furthermore, it has been shown that in male specimens of dioecious *Hydra oligactis*, the male sexual phenotype is not expressed except at temperatures below 12°C. At 22°C, genetically male specimens may become female (Littlefield, '86).

All recent work has been on various species of hydras. Hydra is, in many respects, an atypical cnidarian: it is a freshwater animal, whereas the great majority (11,000 species) are marine; it lacks alternation of generations, whereas the theoretical cnidarian cycle consists of a free sexual stage (= medusa) and a fixed asexual stage (= polyp); and the form in which in some species there are sex changes, whereas in the medusa (single sex) sex seems irreversibly determined. In this bibliographic context, it seemed interesting to approach the problems of the origin of the germ cells, the determination of sex genetic and/or environmental, and the establishment of the sexual phenotype in another cnidarian to see if the results obtained on a very unusual cnidarian may be typical of the group as an whole. The genus *Clytia* was chosen, which has a wide geographical distribution. *Clytia* is an hydrozoan (Hydrozoa, Leptomedusae) with an asexual benthic stage (Fig. 1B,C) that buds medusae in its gonotheca. In the medusae, four gonads differentiate, either male or female, lying on the four radial canals (Fig. 1A,M). The greater part of this study was made on *Clytia hemisphaerica*, but some observations on another *Clytia species* (undetermined and probably new) (Fig. 2) are also added. Hermaphrodite specimens of this second species were found in

Fig. 1. *Clytia hemisphaerica.* **A:** Female gonad. **B:** Hydranth. **C:** Details of the hydrotheca. **D:** Adult medusa raised for 3 months in the laboratory. Additional canals and gonads can be seen. **E:** Young medusa at the time of its release from the gonotheca. Note the absence of visible gonads. **F–L:** Medusae raised at 21°C and aged 1, 2, 3, 5, 8, 14, and 21 days, respectively. **M:** Medusa obtained by breeding; this stage is still immature but the sex is determinable with differential interference contrast (DIC) microscopy. **N:** Immature ovary with young oogonia (arrowheads) (DIC). **O:** Immature testis with spermatogonia (arrowhead) (DIC). Scale bars: A, 250 μm; B, 1 mm; C, 200 μm; D, 2 mm; E, 100 μm; F–L, 1 mm; M, 2 mm; N, O, 0, 50 μm.

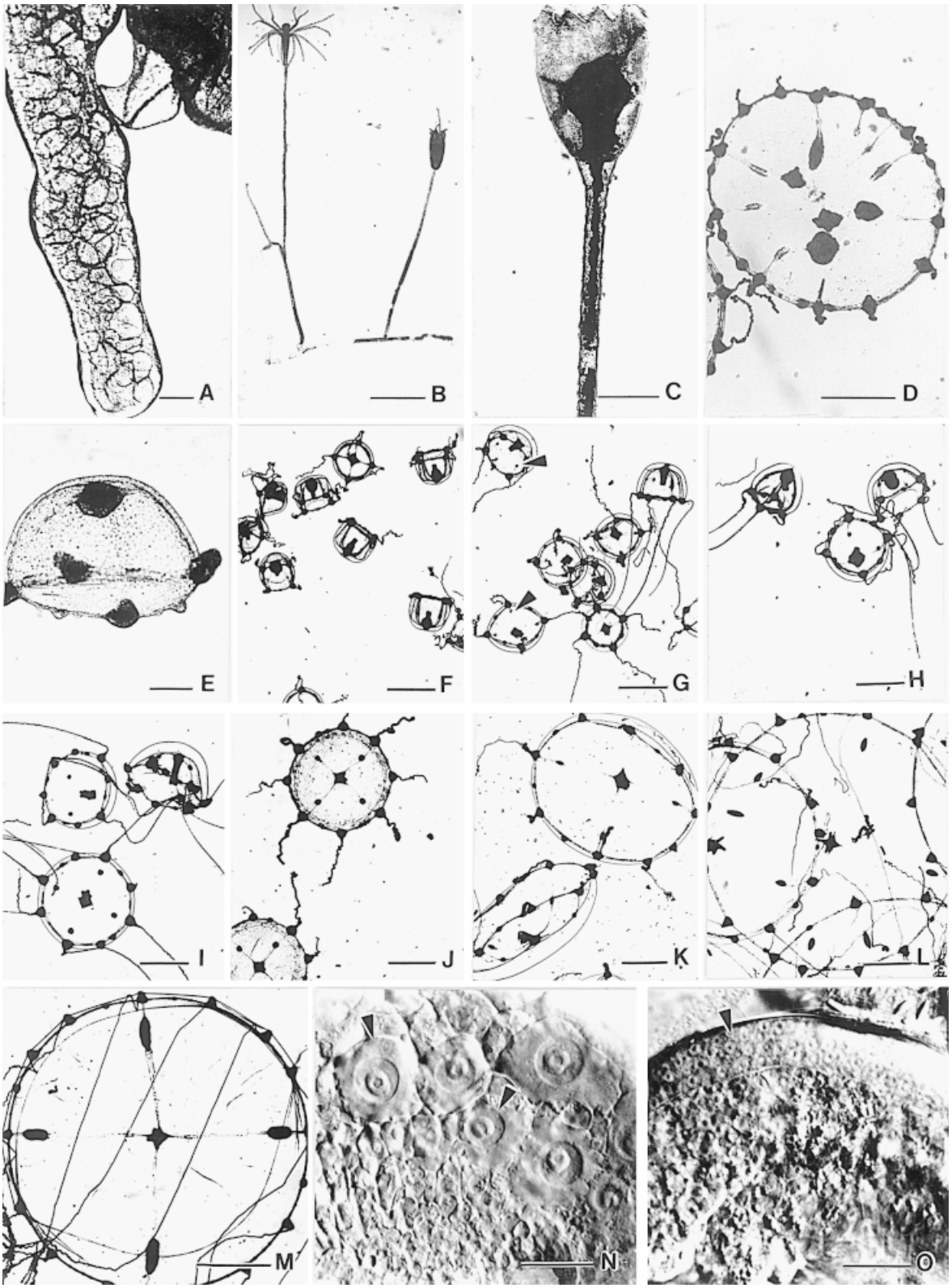


Figure 1.

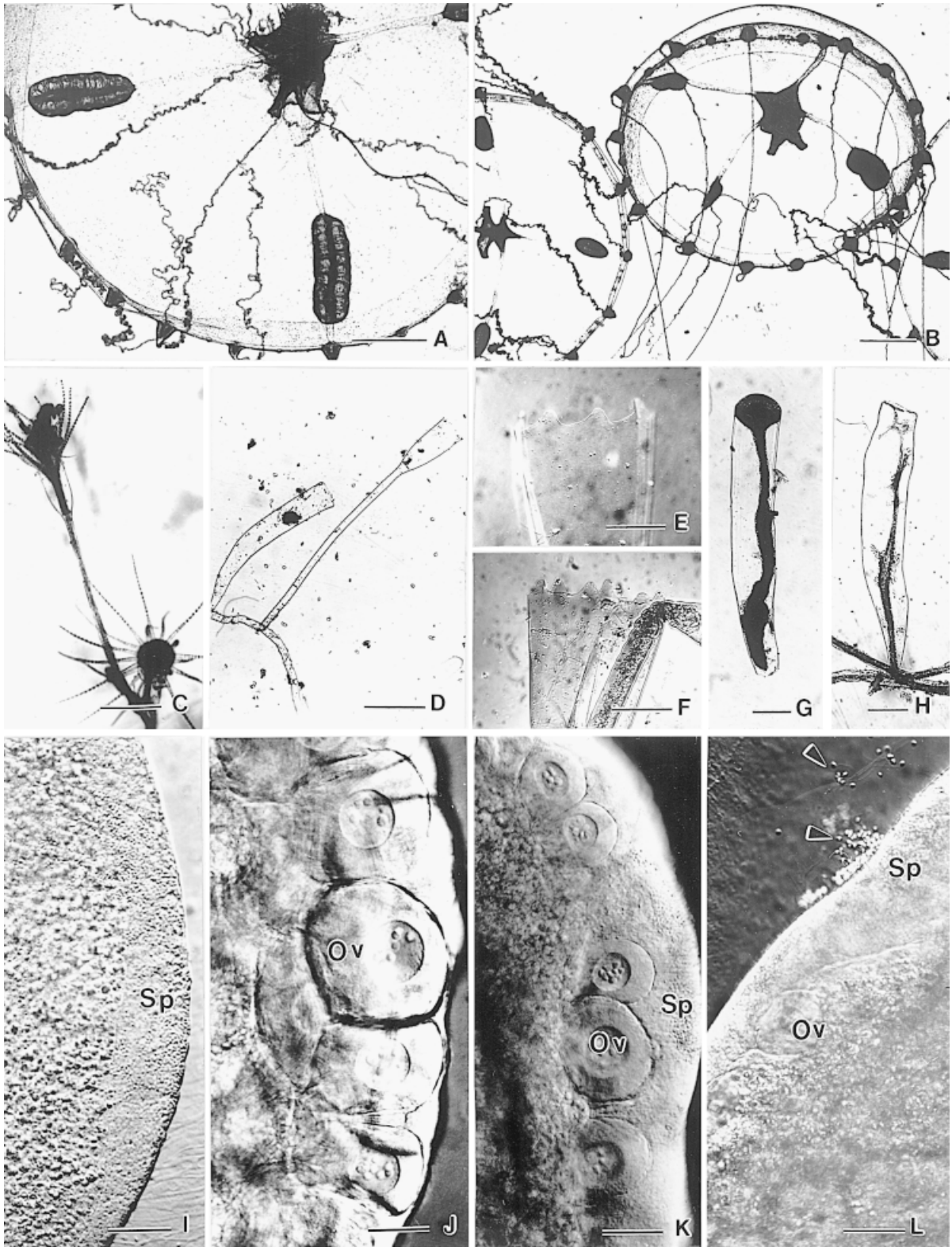


Fig. 2. *Clytia species.* **A:** Female medusa. **B:** Two male medusae. **C:** Hydranth obtained by breeding. **D:** Gonotheca and hydrotheca. **E** and **F:** Details of the hydrotheca aperture. **G** and **H:** Gonothecae. **I:** Gonad of a male medusa differential interference contrast (DIC) microscopy. **J:** Gonad of a female medusa (DIC). **K:** Gonad of a hermaphrodite medusa (DIC). **L:** Gonad of a hermaphrodite medusa releasing spermatozoids (arrowheads) (DIC). Scale bars = A, B, 1 mm; C, D, 0, 5 mm; E, F, 100 μm; G, H, 250 μm; I–L, 50 μm. Sp, spermatozoids; Ov, ovocyte.

situ or raised in the laboratory. So far as we are aware, this is the second definite example of hermaphroditism described in any hydromedusan.

## MATERIALS AND METHODS

### *Obtaining budding colonies of* C. hemisphaerica

Medusae of *C. hemisphaerica* (Linnæus, 1767) were collected in spring from the surface waters (0–50 m) of the bay of Villefranche-sur-Mer (Mediterranean, nr. Nice, France) with townets of 680 μm mesh. The specimens were kept in the dark for 24 hr, and gamete emission was induced by transferring them into the light (Honeger et al., '80). After fertilization, the eggs were isolated and kept in crystallizing dishes in a thermostatically controlled chamber at 24°C, until the metamorphosis of the planula larvae into fixed polyps. A single polyp was retained in each dish. Fed daily with *Artemia* nauplii, each polyp gave rise to a colony liberating medusae, which were raised until their sex could be determined in vivo using interference-contrast microscopy by the presence of oocytes or spermatozoids. Nine colonies were studied: three released only male medusae and four released only female medusae, but two colonies released both male and female medusae. These two colonies were used in our experiments.

### *Effect of temperature*

The two hermaphrodite colonies and the medusae that they had budded were raised either at 15°C (temperature close to the winter minimum in the northwest Mediterranean) or at 21°C or at 24°C. Following each temperature change, we waited a week before using the medusae released. At the time they were set free from the gonothecae, the medusae were some 0.5 mm in diameter and had no gonadal pouches (Fig. 1E).

In each experiment, the medusae were fed daily for 5 min on *Artemia* nauplii. At the end of this time all had usually captured nauplii. A longer feeding period led to overeating, which increased mortality in the cultures. The medusae were raised up to gamete differentiation to determine the sex ratio of the population (Fig. 1F–M). The number of medusae present is given for each experiment in the corresponding table.

### *Observation and culturing of* Clytia *sp.*

We have found intermittently, from September to March, specimens belonging to the genus *Clytia* but that we have not been able to identify as a known species (Fig. 2). These specimens were male, female, or hermaphrodite.

This species was raised in the laboratory (a single culture at 13°C) and studied by light and electron microscopy (after fixation by the method of Eisenman and Albert ['82] and embedding in Spurr resin).

## RESULTS

### *Sex ratio of a population of medusae budded and raised at 15°C*

Two successive cultures were made, resulting in 38 and 28 medusae at the end of the experiments. The medusae achieved sexual maturity 4 to 5 weeks after being released. Their sexual phenotype was checked with interference contrast microscopy (Fig. 1N,O). Table 1 gives the results obtained. They show that at 15°C, around 85% of the medusae were male.

### *Sex ratio of a population of medusae budded and raised at 24°C*

More medusae were released at 24°C than at 15°C. Two successive cultures resulted in 39 and 42 medusae at the end of the experiments that had achieved gonad maturity 3 to 4 weeks after their release (Table 2).

In this case, the sex ratio is the opposite of that obtained at 15°C, with a great preponderance of female medusae (around 80%).

These results show that in our experiments using medusae released by hermaphrodite colonies, the sexual phenotype of medusae of *C. hemisphaerica* varies according to temperature. To decide at what time temperature can influence sex determination, we varied the temperature at the time that the medusae are released by the benthic colony.

### *Sex ratio of a population of medusae budded at 15°C and raised at 24°C*

After their release at 15°C, 2 groups of medusae were transferred and maintained at 24°C un-

*TABLE 1. Sex ratio in populations of* C. hemisphaerica *medusae liberated and raised at 15°C*

| No. of specimens at the end of the experiment[1] | Females | Males |
|---|---|---|
| 38 (a) | 6 | 32 |
| 28 (b) | 4 | 24 |
| Total: 66 | 10 | 56 |
| Sex ratio | 0.15 | 0.85 |

[1](a) indicates 50 specimens at the beginning of the experiment; (b), 38.

*TABLE 2. Sex ratio in populations of* C. hemisphaerica *medusae liberated and raised at 24°C*

| No. of specimens at the end of the experiment[1] | Females | Males |
|---|---|---|
| 39 (a) | 31 | 8 |
| 42 (b) | 33 | 9 |
| Total: 81 | 64 | 17 |
| Sex ratio | 0.79 | 0.21 |

[1](a) indicates 50 specimens at the beginning of the experiment; (b), 55.

*TABLE 4. Sex ratio in populations of* C. hemisphaerica *medusae liberated at 24°C and raised at 15°C*

| No. of specimens at the end of the experiment[1] | Females | Males |
|---|---|---|
| 29 (a) | 5 | 24 |
| 26 (b) | 4 | 22 |
| Total: 55 | 9 | 46 |
| Sex ratio | 0.16 | 0.84 |

[1](a) indicates 45 specimens at the beginning of the experiment; (b), 40.

til their gametes matured (Table 3). The sex ratio is not significantly different from that of medusae that were kept at 15°C after their release at 15°C.

### *Sex ratio of a population of medusae budded at 24°C and raised at 15°C.*

In the two cultures under these conditions, the mortality of the medusae was much greater than in the previous experiments (Table 4). The sex ratio showed a strong dominance of males, and the results were comparable both to those with medusae released and raised at 15°C and to those obtained with medusae budded at 24°C then transferred to 15°C.

This first series of results indicates that for medusae formed and released at 15°C, raising external temperature does not modify their sexual phenotype and that the sex ratio is always in the neighborhood of 80% male to 20% female specimens. In contrast, when they were formed at 24°C, they became predominantly female medusae (around 80%) if kept at this temperature, but their sexual phenotype could be modified if they were cultured at 15°C, the temperature for which an inversion of the population sex ratio had been found.

### *Action of lowered temperature for different times on young medusae budded at 24°C*

After their release, three lots of 50 medusae were first cultured at 24°C until they were 3–4 mm across. At this stage, the gonad pouches were visible on the four radial canals, but oogonia and spermatogonia could not be identified under interference contrast.

The remaining specimens were then divided into six lots of 20 medusae. Five lots were placed at 15°C for different lengths of time (12 hr, 24 hr, 36 hr, 4 days, 7 days). The sixth lot was kept at 24°C.

It seems (Table 5) that a relatively short (24 hr) reduction in temperature from 24°C to 15°C suffices to invert the population sex ratio. Note, however, that inversion of the sexual phenotype does not seem to be increased by longer treatments (36 hr, 48 hr, 4 days, 7 days) but that, in contrast, a transfer for only 12 hr changes the sex ratio, though less evidently (44% of females).

### *Sexual phenotype of medusae budded and raised at 21°C (Fig. 1E–M)*

A single culture was made under these conditions. After 4 weeks, the sex of the 52 specimens remaining could be determined: 30 were female (58%) and 22 were male (42%).

### *Action of temperature on adult specimens*

Unlike dioecious hydras, where, when a single clone is cultured for numerous generations, sex changes may occur spontaneously, the sexual phenotype of a medusa, once determined, has up to now been considered irreversible. We have cultured adult medusae of *C. hemisphaerica* for 6 months. Some regularly underwent temperature changes, but whatever the length and magnitude

*TABLE 3. Sex ratio in populations of* C. hemisphaerica *medusae liberated at 15°C and raised at 24°C*

| No. of specimens at the end of the experiment[1] | Females | Males |
|---|---|---|
| 42 (a) | 6 | 36 |
| 27 (b) | 5 | 22 |
| Total: 69 | 11 | 58 |
| Sex ratio | 0.16 | 0.84 |

[1](a) indicates 54 specimens at the beginning of the experiment; (b), 40.

*TABLE 5. Action of temporary drop to 15°C on the sex ratio in populations of* C. hemisphaerica *medusae*

| Duration of the period at 15°C | No. of mature specimens | Females | Males |
|---|---|---|---|
| 0 hr | 17 | 13 | 4 |
| 12 hr | 16 | 7 | 9 |
| 24 hr | 17 | 2 | 15 |
| 36 hr | 16 | 3 | 13 |
| 4 d | 15 | 2 | 13 |
| 7 d | 14 | 2 | 12 |

of these (between 13°C and 24°C), there were no sex changes. In some, supernumerary gonads of the same sex (Fig. 1D) developed secondarily along supernumerary canals. Thus, temperature can only modify the sexual phenotype during the precocious development of the medusae of *C. hemisphaerica* budded by hermaphrodite colonies.

### *Observations on* Clytia *sp.*

The medusa of this species measures at maturity 5–10 mm across. It is relatively rare, occurring in an irregular fashion between September and March. Apart from the hermaphroditism of some specimens, the medusae are characterized by the color of the manubrium and tentacular bulbs (green under epifluorescence and yellow under transmitted light). The color is not related to a particular diet, for it is found in young medusae born in the laboratory and fed on *Artemia*.

We have not been able to identify our specimens with any described *Clytia* species. Figure 2C–H shows details of colonies obtained in the laboratory and is given for information. The specimens found in situ were of both sexes: 90 males (Fig. 2B,I; Fig. 3E,F) and 61 females (Figs. 2A,J and 3A) were isolated from September 1994 to March 1996, together with 10 hermaphrodite specimens. The latter could show in the same gonad both mature male and female gametes (Fig. 2K,L). These in vivo observations were confirmed with semithin sections and by electron microscopy (Fig. 3B–D).

The small number of specimens collected and the difficulties that we encountered in inducing the metamorphosis of the planulas into fixed polyps have not permitted a systematic experimental approach to any possible effect of temperature on the sexual phenotype. However, 40 medusae that, according to observation in vivo, were either male or female but not hermaphrodite and were kept for a week at 13°C (the temperature of the sea when they were collected in March). At the end of this time, three hermaphrodite specimens were found among them. The 21 male and the 16 female medusae remaining were separated and kept for a further week at 13°C. None of the male changed sex. In contrast, four of the 16 female specimens had become hermaphrodites. In these hermaphrodites, the gonads were either hermaphrodite, having at once oocytes and spermatozoides, or they were all male or all female. After several days, all the gonads had become solely male. Thus, sex inversion had been followed in vivo from female to male via a hermaphrodite stage.

## DISCUSSION

The influence of temperature on sex determination has been shown in the dioecious *Hydra oligactis* (Littlefield, '86). Male specimens raised at 18°C reproduce solely by asexual budding. At this temperature, spermatogonial multiplication (detectable by the monoclonal antibody AC2+) is followed by precocious degeneration. At temperatures below 12°C, gametogenesis results in the liberation of spermatozoids. In contrast, if the temperature is raised to 22°C, some specimens change sex with the disappearance of AC2+ cells, precursors of the male line, and the differentiation of oogonia that develop to maturity.

In these dioecious *Hydra* species, it is accepted (for review, see Littlefield, '94) that the male specimens have both male and female gonocytes. The male gonocytes inhibit the differentiation of the female gonocytes present by the diffusion of an egg-suppressing molecule. In contrast, female specimens do not possess or differentiate other than female gonocytes. In hermaphrodite species, it is supposed that although two populations of male and female germ cells exist, the male gonocytes do not induce the secretion of an egg-suppressing molecule (Littlefield, '94).

In our experiments on *C. hemisphaerica,* the medusae budded and cultured at 24°C were mainly female (around 80%). In contrast, if the medusae were released at 15°C, whatever the temperature at which they were raised later, the medusae were mainly male (80%). The same occurred if after release but before the formation of the oogonia and spermatogonia they underwent a fall in temperature to 15°C for not less than 24 hr. Finally, if polyps and medusae were cultured permanently at 21°C, a sex ratio of about 1:1 resulted (58% female, 42% male).

By analogy, with the results obtained from hydra, it may be suggested that in the colonies and young medusae of *C. hemisphaerica* of our experiments, there are precursors of male and female lines. The female line will be dominant at 24°C, the sexual phenotype then being female in 80% of the specimens but temperature sensitive, with inhibition or destruction of this line by a temporary temperature drop (24 hr) to 15°C, this inverting the population sex ratio. The irreversible action (in 80% of the specimens) of a temperature drop to 15°C even before the release of the medusae supports the view that the germ cells are isolated very early.

In *C. hemisphaerica* we have followed changes in the sex ratio in populations of young medusae

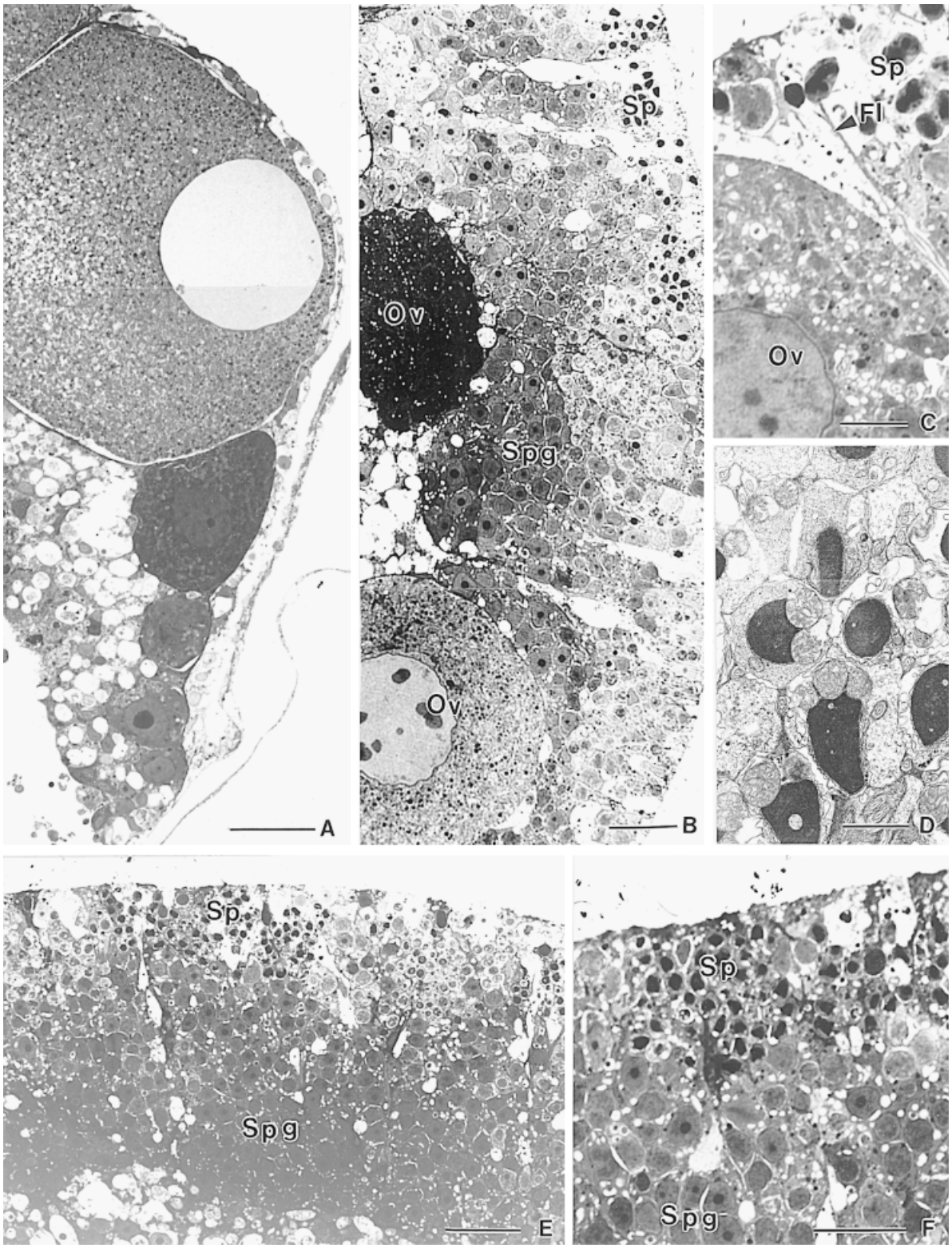


Fig. 3. *Clytia species.* **A:** Semithin section of a female medusa with oocytes at various stages of development. **B:** Gonad of a hermaphrodite medusa; oocytes and spermatozoids are both present. **C:** Detail of a hermaphrodite gonad; the spermatozoids are near an oocyte. **D:** Ultrathin section in a hermaphrodite gonad confirming the presence of spermatozoids. **E:** Gonad of a male medusa. **F:** Detail of a male gonad with spermatogonia and spermatozoids. Scale bars = A, 40 μm; B, 20 μm; C, 10 μm; D, 2 μm; E, 50 μm; F, 20 μm. Fl indicates flagellum; Spg, spermatogonia; Sp, spermatozoids; Ov, oocyte.

as a function of temperature, but we have never observed phenotypically hermaphrodite specimens. In contrast, in *Clytia* sp., a closely related species, we have found hermaphrodite specimens in situ, and we have followed in vivo the transformation of phenotypically female specimens into hermaphrodite specimens, simultaneously liberating eggs and spermatozoids (with self-fertilization possible), then into male specimens only liberating spermatozoids. These sex changes were seen when female medusae were cultured at 13°C. Male specimens raised in the same conditions never changed sex. Furthermore, whereas male and female specimens of *Clytia* sp. were found occasionally from September 1994 to March 1995, then from November 1995 to March 1996, the hermaphrodite specimens found in situ (10 in all) were all collected in March when the sea water column temperature was about 13 ± 0.5°C. It may be suggested that in this species (as in *C. hemisphaerica*), a temperature drop favors the male phenotype.

If it is admitted that in *Clytia* sp., as in the hydras, sexuality depends at bottom on interactions between the precursors of the male and female germ cells, the cascades of interactions between these precursors must be much more complex than in hydras. In fact, in *Clytia* sp., the same specimen may be successively female, hermaphrodite with differentiation of two germ lines, then male. Similarly, in *C. hemisphaerica*, where sex determination mechanisms have been studied not at the individual but at the population level, it seems that whatever the experimental conditions, some individuals seem to escape environmental control of sex determination for 100% of individuals of the same sex are never obtained.

Hydrozoan colonies (3,000 species) are dioecious, with the exception of the siphonophores (Naumov, '69). Nevertheless, several rare examples of hermaphrodite species have been found, but only in the three genera *Eleutheria*, *Cladonema,* and *Amphogona*, medusae are released. *Eleutheria dichotoma* (Hartlaub, 1886) is, so far as we are aware, the only hydromedusa whose hermaphrodite character is accepted. In the other species at first described as being hermaphrodite, *Cladonema radiatum* and *Amphogona apsteini*, the simultaneous or successive presence of male and female gonads in the same specimen has been contested (Mayer, '10).

In general, genetic sex determination involving sex chromosomes is contrasted with environmental sex determination. At the same time, the existence in closely related species (turtles, for example) of the two types of determination suggests that the two mechanisms cannot be radically different (Johnston et al., '95).

We have seen that the sexual phenotype of the medusae of *Clytia hemisphaerica* can be changed by changing the temperature, suggesting that sex is not determined genetically. But apart from the colonies on which we experimented, we also obtained colonies that budded only male medusae or only female medusae, whatever the cultural conditions (in seven of the nine colonies cultured).

In hydras and hydrozoans generally, the 1:1 sex ratio following sexual reproduction has led to the conclusion that sex determination is genetic (Tardent, '85; Littlefield, '94). This conclusion was confirmed by Buhrer ('81), who dissociated and then mixed cells of male and female polyps of *Podocoryne carnea* (Anthomedusae). The aggregates obtained regenerated mosaic colonies that released hermaphrodite medusae.

Nevertheless, in hydras that have been studied systematically in consequence of the maintenance of clones from a single egg kept for many generations, it has been observed that genetically female hydras, characterized from one generation to the next by the absence of precursor cells for the male line, can abruptly become male with the acquisition of AC2+ cells. Conversely, sex change induced by raising the temperature in male specimens of *Hydra oligactis* is linked to the loss of AC2+ cells hitherto present. Such observations demonstrate the labile character of genetic sex determination in these organisms, and there is, moreover, a high degree of plasticity. Examples of transdifferentiation have been described, going so far as the transformation of striated muscle cells into functional gametes (Schmid and Adler, '86).

Our own observations on siphonophores (colonial hydrozoans) also illustrate the possible influence of the environment on the sexual phenotype. For example, *Chelophyes appendiculata* (a calycophoran siphonophore) specimens found in situ are always hermaphrodite, carrying at the same time on the same stolon both male and female gonads. In culture, all specimens become either male or female, none remain hermaphrodite.

It is supposed that sex chromosomes have been secondarily acquired in the course of evolution and that sex determination was at first solely controlled environmentally. It is thus scarcely surprising that in such primitive metazoans as are the cnidarians both types of sex determination may (at least in certain species) coexist and cooperate.

## ACKNOWLEGMENTS

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