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Morphological and cytological aspects of sex inversion in a protogynous hermaphrodite, *Epinephelus microdon* (*Teleostei, Serranidae*)

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Abstract — *The sexuality of the grouper **Epinephelus microdon**, caught by diving in the area of Tahiti Island, from January 1986 to March 1987, is investigated. Small specimens are females, males occur among the largest fishes, revealing the protogyny of **Epinephelus microdon** as in other Epinephelinae (Smith, 1959). Nineteen fishes among 233 samples studied are in sex inversion. The change of sex (from female to male) occurs during the post-spawning time and extends throughout the sexual rest period (from June to September) in fishes which are about 1 kg in weight. Using light and electron microscopic criteria, the sex inversion of **Epinephelus microdon** is revealed, in one hand by the identification in the ovary of some seminiferous cysts including spermatocytes or spermatids, scattered in the parietal part of the ovarian lamellae, and, on the other hand by a massive spermatogonial proliferation which develops throughout the gonad according to a centripetal orientation and which is followed by an early spermatogenic activity. These findings are hypothesized to be two consecutive events. Before and during the spermatogonial proliferation, ultrastructural investigations have allowed us to detect the presence of numerous primordial germ cells (PGCs), showing a mitotic activity. PGCs, characterized by irregular outlines and a high electron density, are both undifferentiated (high nucleus to cell ratio, abundant ribosomes, scarce membrane organelles) and bipotential cells (similar ultrastructural features in females and in males). These early germ cells are involved, as well as spermatogonia (which arise from them), in the testicular ontogenesis which takes place, in the ovary, during the sex inversion.*

RESULTS AND DISCUSSION

Hermaphroditism and gonadal sex-inversion in Teleosts have been reviewed by Atz (1964) and Reinboth (1970, 1983). In the family of Serranids, two patterns of hermaphroditism have been identified

(Smith, 1959) : synchronous hermaphroditism and protogynous hermaphroditism, which seem especially common in *Epinephelinae*, as noted by Smith (1959).

The present study deals with the sexuality of the grouper *Epinephelus microdon*, caught by diving in the area around the Tuamotus (French Polynesia), from February 1986 to February 1987. Sexual maturity was variable according to the individuals and occured from March to September. The spawning time in 1986 was from the end of April to the end of May, whereas in 1987 it began in February. The females have oval-shaped ovaries, hollow organs in which ovarian lamellae are perpendicular to the major axis. The testes of the males are hollow organs too, with lamellae in which spermatogenesis can be discerned. Such a pattern of the testes is unusual in Teleosts, but is classically described in groupers (Mc Erlean and Smith, 1964; Smith, 1964; Liem, 1968; Reinboth, 1968, 1970; Bruslé, 1982) and suggests a protogyny in *E. microdon.* Furthermore, as small specimens are females and the larger specimens are males, the protogynous hermaphroditism of *E. microdon* seems to be corroborated.

Among 232 groupers examined, 13 fish undergoing sex-inversion were identified. Sex-change, from female to male, occurs during the post spawning time and continues throughout the sexual rest period, from June to September, in fish which are about 1 kg in weight. In February-March, it is possible to find some sex-inverting groupers with gonads displaying male germ cells close to oocytes in the early stages of vitellogenesis.

Using light and electron microscopic data the first cytological indication of sex-inversion is the occurence of seminiferous cysts scattered in the parietal part of the ovarian lamellae. Spermatocytes or spermatids are found in cysts, this being the first event of spermatogenic activity in the ovary. The gonads of some sex-inverting *E. microdon* show another pattern in the shape of massive spermatogonial proliferation (spermatogonia being clustered in nests) which develops throughout the ovary according to a centripetal orientation and which is shortly followed by spermatogenic activity. These findings are assumed to be two consecutive events. Seminiferous cysts have been described in previous studies (Smith, 1959, 1964; Reinboth, 1967; Bruslé and Bruslé, 1975; De Moussac, 1986; Abu-Hakima, 1987), but spermatogonial proliferation has not been detected, either because it escaped the attention of the researchers or because *E. Microdon* shows a different pattern.

Before and during spermatogonial proliferation, ultrastructural investigations enabled the presence of numerous primordial germ cells (PGCs) to be detected. These early germ cells, previously identified in Mugilids (Bruslé, 1980, 1989), Serranids (Bruslé, 1983) and Labrids (Bruslé, 1987), have the same general ultrastructural features in *E. microdon* as those described in these families. PGCs are characterized by their irregular outlines and high electron density, and are conspicuously different from spermatogonia (regular outlines, low electron density, endowed with more membrane organelles). PGCs have the following features : undifferentiated cells (high nucleus to cell ratio, abundant ribosomes, few membrane organelles); bipotential cells (similar ultrastructural features in males and females) which differentiate into oogonia or spermatogonia, as revealed

by intermediate steps. The presence of PGCs, established in all fish at each stage of the sexual cycle, shows that these early germ cells constitute a permanent germ stock in the gonad. In sex-inverting fish, PGCs are easily identified because they are more numerous than in males or females (especially during spermatogonial proliferation) and because they divide. These early germ cells are therefore involved, like spermatogonia which arise from them, in the testicular ontogenesis which takes place in the ovary during sex-inversion. The participation of PGCs as bipotential cells, being at the origin of the male germ line, has been previously detected in the protogynous hermaphrodite, *Coris julis* (Bruslé, 1987) during the sex change. Several authors have put forward the hypothesis of undifferentiated cells being involved during sex-inversion, but they failed to detect them (undifferentiated cells : Duchac, 1981; Bentivegna and Rasotto, 1983; undifferentiated gonia : Reinboth, 1962, 1967, 1970; Mc Pherson, 1977; bipotential gonocytes : Yeung and Chan, 1987).

Since Smith (1959), some investigators have believed that the identification of degenerative processes inside a gonadal tissue reveals sex-inversion (Moe, 1969; Lissia Frau et *al.*, 1977; Shapiro, 1977; De Mousac, 1986; Garrat, 1986; Abu-Hakima, 1987). However, the evolution and intensity of this degeneration are often too difficult to evaluate to be really significant. In sex-inverting *E. microdon*, it is possible to detect in addition to some atretic oocytes, a large number of eosinophilic granulocytes. Quite often in phagocytotic acitivity, these immune cells characterized by their homogenous, dense granules and endowed with enzymes suggest a true degeneration of the female tissue.

In conclusion, the protogynous hermaphroditism of *E. microdon* is established from our morphological and cytological investigations. The cytological modalities of sex-inversion are characterized by the occurence of seminiferous cysts scattered in the ovarian wall probably followed by massive spermatogonial proliferation in which primordial germ cells, undifferentiated and bipotential cells, are involved.

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