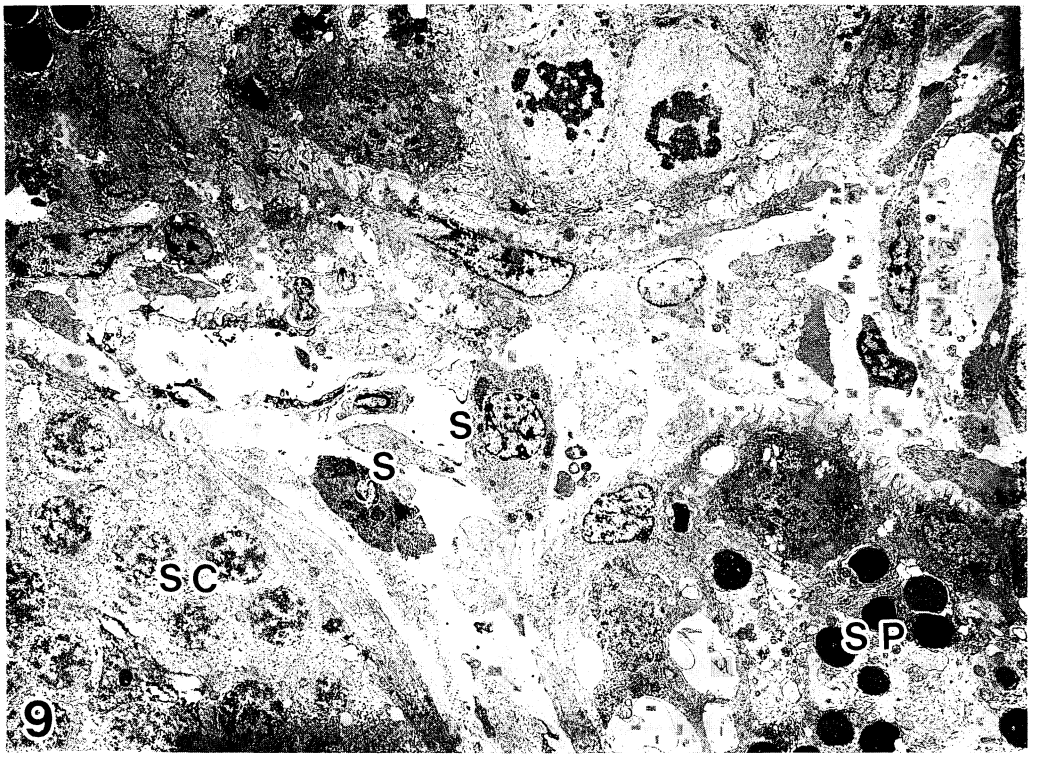


Fig. 9. Electron micrograph of steroid producing cells (*S*) in the testicular tissue of male gonad. Steroid producing cells are distributed in the interstices among lobules. *SC*—spermatocyte; *SP*—sperm. ×2,500.

to produce of T (51.7 pg/mg tissue), 11-KT (101.7 pg/mg tissue) and E_2 (16.3 pg/mg tissue) in response to SGA. Comparing the ability of gonads to produce steroid hormones, male gonads had higher levels of 11-KT production than did female gonads, while female gonads had higher levels of T and E_2 than did male gonads.

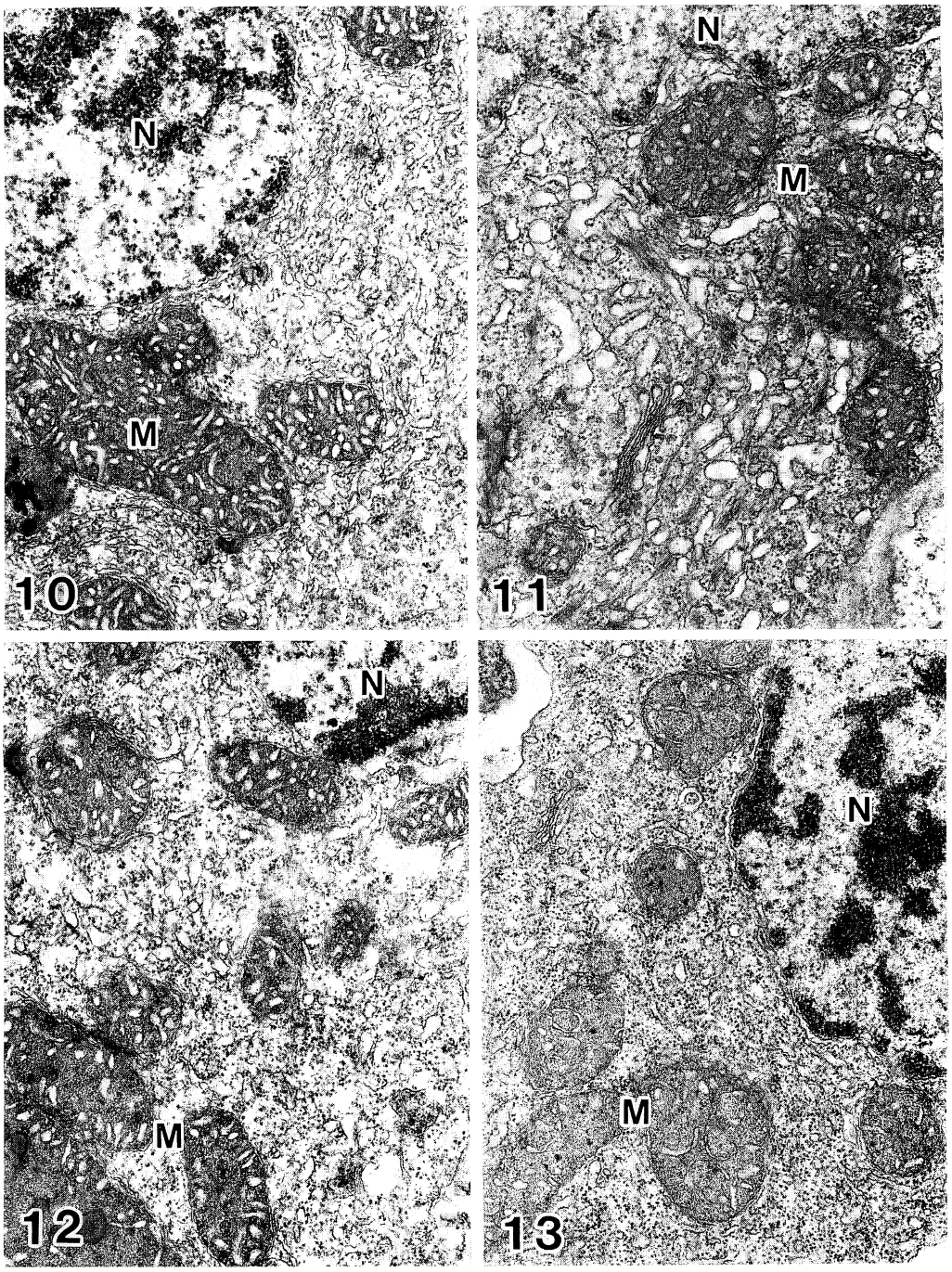
Discussion

The histological observations of the present study are basically in agreement with the reports on gonadal structure in anemonefish by other researchers (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Fricke, 1979; Ochi, 1989; Brusle-Sicard and Reinboth, 1990; Hattori, 1991; Hattori and Yanagisawa, 1991). Namely, males have hermaphroditic gonads with both undeveloped ovarian and testicular tissues, without any boundary between them. Ovaries of females have no testicular tissue.

The studies on distribution and activity of SPC in the gonads of sex-changing fish are very few, though it is essential for understanding relationship between steroid hormones and sex change. Yeung and Chan (1985) have observed the activity level of steroid producing cells in the gonads of protogynous *Monopterus albus* during sex change. The activity of SPC

Figs. 10 and 11. Electron micrographs of steroid producing cells in the testicular tissue (Fig. 10) and in the ovarian tissue (Fig. 11) of the same male gonad which has active spermatogenic tissue. They have well-developed endoplasmic reticulum and mitochondria (*M*) with well-developed tubular cristae. *N*—nucleus. ×25,200.

Figs. 12 and 13. Electron micrographs of steroid producing cells in the testicular tissue (Fig. 12) and in the ovarian tissue (Fig. 13) of the same male gonad which has inactive spermatogenic tissue. They have mitochondria (*M*) with undeveloped cristae and undeveloped endoplasmic reticulum (compare with Figs. 10 and 11). *N*—nucleus. ×25,200.



(Leydig cell) in female phase was immature, and was at the peak of their activity in the mid-intersexual phase. Nakamura et al. (1989) also revealed that Leydig cells increased in size and number in the mid and later stages of sex change in the gonads of protogynous wrasse, *Thalassoma duperrey*. There have been no reports on the activity and distribution of SPC in the gonads of protandrous species. In the

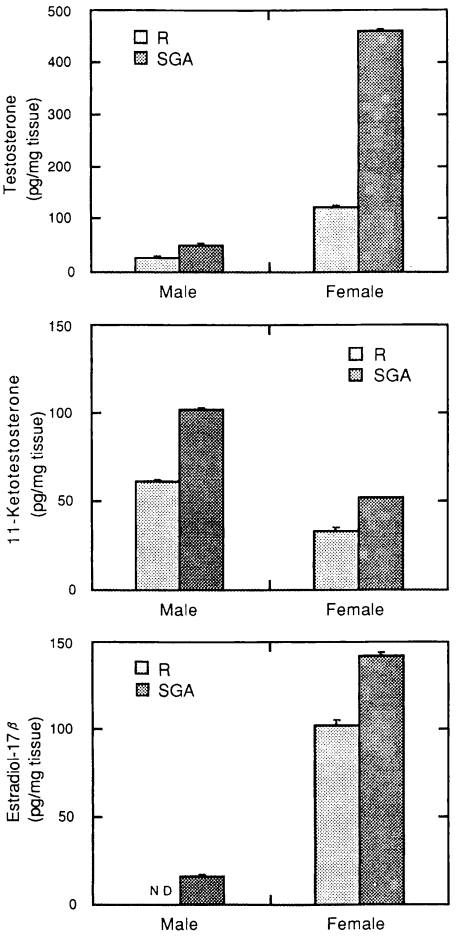


Fig. 14. Effects of salmon gonadotropin (*SGA*) on in vitro secretion of testosterone, 11-ketotestosterone and estradiol-17β by gonadal fragments of *Amphiprion frenatus* male and female. Fragments of the gonads were cultured for 24 h at 25°C in L-15 medium (*R*) or in L-15 medium plus SGA (10 μg/ml).

present study, we confirmed ultrastructurally the distribution of SPCs both in the testicular tissue and in the ovarian tissue of male gonads. Activities of SPCs both in ovarian and testicular tissues are in accord with spermatogenic activity. Namely, SPCs in gonads having active spermatogenic tissue are active in structure, but SPCs in gonads having inactive spermatogenic tissue are inactive. This fact may indicate that SPCs distributed in ovarian tissue also produce the steroid hormones involved in spermatogenesis.

In the ovaries of female anemonefish, SPCs were found in the interstices between oocytes and in the theca layer enclosing the developing oocytes. It would be interesting to find out the origin of these two types of cell. Recently Nakamura et al. (1993) found evidence that steroid producing theca cells enclosing developed oocytes were formed as a result of the enclosure of oocytes by SPCs originating from the area in the vicinity of blood vessels in the ovary of tilapia, *Oreochromis niloticus*. This fact indicates that SPCs move actively in the gonads. Thus, it is highly possible that SPCs distributed in testicular tissue of gonads in male phase move into the interstitial area and theca layer in the process of sex change.

We measured serum steroid hormones by radioimmunoassay, but the levels were not detectable in most samples. This results not only from the small amount of serum but also from the low steroid levels. In contrast, it was possible to measure the steroid hormones in incubation media. This method, in vitro production of steroids by gonadal fragments in the presence of SGA, has been tried in other species of fish (Sakai et al., 1989; Nakamura et al., 1989; Hourigan et al., 1991). Thus, this method seems to be a generally applicable way to determine the steroid profile of gonads in small fish from which blood can not be taken efficiently.

Kime et al. (1991) had demonstrated that E_2 may be involved in the sex inversion of the protandrous sobaity *Sparidentex hasta*, on the basis of the correlation of seasonal changes in serum steroid hormones and the changes of sexual states of the gonads during regression and recrudescence. Thus, it is also important to know the ability of E_2 production in the gonads of anemonefish. In the present study, E_2 production by gonadal fragments of male was low, even though gonadal fragments contained ovarian tissue together with testicular tissue. This fact suggests that the production of E_2 by ovarian tissue in the male gonad is low. Nagahama (1983) demonstrated that testosterone produced in the theca cells is transported to the granulosa cells and aromatized to E_2 in salmonid ovary. In addition Nakamura et al. (1993) revealed that the timing of an increase in serum E_2 levels coincides with the development of granulosa cells in the process of gonadal development in tilapia. These facts indicate that development of granulosa cells is essential for the production of E_2. In this paper we report the observation that granulosa cells enclosing oocytes in the ovarian tissue of male-phase fish are poorly developed. This fact supports the hypothesis that granulosa cells in ovarian tissue of male gonads are capable of support-

ing only a low levels of conversion of T to E_2. This low production of E_2 in the ovarian tissue may bring about the degeneration of oocytes, without inducing further development of oocytes. On the other hand, organella in steroid producing cells and granulosa cells enclosing yolky oocytes in the ovary of female had well-developed, indicating active production of steroids. Moreover, ovarian fragments from fish in female phase produced T and E_2 in response to SGA. T is the precusor of E_2, and E_2 is involved in vitellogenin synthesis in the liver, as widely known in fish (Fostier et al., 1982). Thus, it is interesting to investigate the changes in the levels of E_2 production and development of steroid producing cells and granulosa cells in accompanying with sex change of anemonefish.

Gonadal fragments of males produced T and 11-KT in response to SGA. These may be involved in spermatogenesis. 11-KT was also produced by ovarian fragments of female. High production of 11-KT in females has been reported in gonochoristic tilapia (Eckstein, 1970) and in protogynous wrasse, *Thalassoma duperrey* (Nakamura et al., 1989). 11-KT is thought to be the main androgens in teleost fish (Ozon, 1972). Moreover, this steroid has the highest androgenic potency among natural androgens (Hishida and Kawamoto, 1970). Hourigan et al. (1991) reported that 11-KT is associated with aggressive behaviour in males of *T. duperrey*. However, the role of 11-KT in females is unknown.

It is certain that the development of the testis and the ovary depends on gonadotropins from the pituitary, as in other vertebrates (van Oordt and Peute, 1983). Active spermatogenesis in the male gonad is a strong indicator of active secretion of endogenous gonadotropins. Despite this fact, the reasons for the low production of E_2 and the poor development of ovarian tissue in the male are unknown.

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Literature Cited

Atz, J. W. 1964. Intersexuality in fishes. Pages 145–232 *in* C. N. Armstrong and A. J. Marshall, eds. Intersexuality vertebrates including man. Academic Press, London.

Brusle-Sicard, S. and R. Reinboth. 1990. Protandric hermaphrodite peculiarities in *Amphiprion frenatus* Brevoort (Teleostei, Pomacentridae). J. Fish. Biol., 36: 383–390.

Eckstein, B. 1970. Metabolic pathways of steroid biosynthesis in ovarian tissue of a teleost, *Tilapia aurea*. Gen. Comp. Endocrinol., 14: 303–312.

Fricke, H. W. 1979. Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos*. Z. Tierpsychol., 50: 313–326.

Fricke, H. W. and S. Fricke. 1977. Monogamy and sex change by aggressive dominance in coral reef fish. Nature, 266: 830–832.

Fostier, A., B. Jalabert, R. Billard, B. Breton and Y. Zohar. 1982. The gonadal steroids. Pages 277–372 *in* W. S. Hoar, D. J. Randall and E. D. Donaldson, eds. Fish physiology, Vol. IXA. Academic Press, New York.

Hattori, A. 1991. Socially controlled growth and size-dependent sex change in the anemonefish *Amphiprion frenatus* in Okinawa, Japan. Japan. J. Ichthyol., 38: 165–177.

Hattori, A. and Y. Yanagisawa. 1991. Life-history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii*, in temperate waters of Japan. Env. Biol. Fish., 31: 139–155.

Hishida, T. and N. Kawamoto. 1970. Androgenic and male-inducing effects of 11-ketotestosterone on a teleost, the medaka (*Oryzias latipes*). J. Exp. Zool., 173: 279–284.

Hourigan, T., M. Nakamura,Y. Nagahama, K. Yamauchi and E. G. Grau. 1991. Histology, ultrastructure, and in vitro steroidogenesis of the testes of two male phenotypes of the protogynous fish, *Thalassoma duperrey* (Labridae). Gen. Comp. Endocrinol., 83: 193–217.

Kagawa, H., G. Young, S. Adachi and Y. Nagahama. 1982. Estradiol-17β production in amago salmon (*Oncorhynchus rhodurus*) ovarian follicles: role of the thecal and granulosa cells. Gen. Comp. Endocrinol., 47: 440–448.

Kime, D. E., E. P. Lone and A. Al-Marzouk. 1991. Seasonal changes in serum steroid hormones in a protandrous teleost, the sobaity (*Sparidentex hasta* Valenciennes). J. Fish Biol., 39: 745–753.

Moyer, J. T. and A. Nakazono. 1978. Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. Japan. J. Icthyol., 25: 101–106.

Nagahama, Y. 1983. The functional Morphology of teleost gonads. Pages 223–275 *in* W. S. Hoar, D. J. Randall and E. M. Donaldson, eds. Fish physiology, Vol. IXA. Academic Press, New York.

Nakamura, M., J. Specker and Y. Nagahama. 1993. Ultrastructural analysis of the developing follicle during early vitellogenesis in tilapia, *Oreochromis niloticus*, with special reference to the steroid-producing cells. Cell Tissue Res., 272: 33–39.

Nakamura, M., T. Hourigan, K. Yamauchi, Y. Nagahama

and E. G. Grau. 1989. Histological and ultrastructural evidence for the role of gonadal steroid hormones in the sex change in the protogynous wrasse *Thalassoma duperrey*. Env. Biol. Fish., 24: 117–136.

Nakazono, A. 1991. Functional hermaphroditism. Pages 327–361 *in* Y. Itazawa and I. Hannyu, eds. Fish physiology. Kouseisha-Kouseikaku Co Ltd. Tokyo. (In Japanese.)

Ochi, H. 1989. Mating behaviour and sex change of the anemonefish, *Amphiprion clarkii*, in the temperate waters of southern Japan. Env. Biol. Fish, 26: 257–275.

Ozon, R. 1972. Androgens in fishes, amphibians, reptiles and bird. Pages 329–385 *in* D. R. Idler, ed. Steroids in nonmammalian vertebrates. Academic Press, New York.

Sakai, N., H. Ueda, N. Suzuki and Y. Nagahama. 1989. Steroid production by amago salmon (*Oncorhynchus rhodurus*) testes at different developmental stages. Gen. Comp. Endocrinol., 75: 231–240.

Ueda, H., A. Kambegawa and Y. Nagahama. 1985. Involvement of gonadotropin and steroid hormones in spermiation in the amago salmon, *Oncorhynchus rhodurus*, and goldfish *Carassius auratus*. Gen. Comp. Endocrinol., 59: 24–30.

van Oordt, P. G. W. J. and J. Peute. 1983. The cellular origin of pituitary gonadotropins in teleosts. Pages 137–186 *in* W. S. Hoar, D. J. Randall and E. M. Donaldson, eds. Fish physiology, Vol. IXA. Academic Press, New York.

Yeung, W. S. B. and S. T. H. Chan. 1985. The in vitro metabolism of radioactive progesterone and testosterone by the gonads of protandrous *Rhadosargus sarba* at various sexual phases. Gen. Comp. Endocrinol., 59: 171–183.

Yeung, W. S. B., H. Chen and S. T. H. Chan. 1993a. The in vitro metabolism of radioactive androstenedione and testosterone by the gonads of the protogynous *Monopterus albus* at different sexual phases: a time-couse and seasonal study. Gen. Comp. Endocrinol., 89: 313–322.

Yeung, W. S. B., H. Chen and S. T. H. Chan. 1993b. Effects of LH and LHRH-analog on godnadal development and in vitro steroidogenesis in the protogynous *Monopterus albus*. Gen. Comp. Endocrinol., 89: 323–332.

Yeung, W. S. B., H. Chen and S. T. H. Chan. 1993c. In vitro effects of oLH and LHRH-analog on sex reversal and plasma sex steroid profiles in the female *Monopterus albus*. Gen. Comp. Endocrinol., 90: 23–30.

Yogo, Y. 1987. Hermaphroditism and the evolutionary aspects of its occurrences in fishes. Pages 1–47 *in* A. Nakazono and T. Kuwamura, eds. Sex change in fishes. Tokai University Press, Tokyo. (In Japanese.)

ハマクマノミの生殖腺とステロイドホルモンの産生能

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魚類の性転換と性ホルモンに関する基礎的問題を解決するため，雄性先熟のハマクマノミ (*Amphiprion frenatus*) を用い，生殖腺の微細構造の観察，とりわけステロイドホルモン産生細胞 (SPC) の分布および活性について調べた．さらに生殖腺を生体外でサケ生殖腺刺激ホルモンの存在下で培養し，主な性ホルモンの産生について測定した．ハマクマノミの生殖腺は，今まで知られていたと同じく，雌は発達した卵を持つ卵巣組織のみからなっていた．雄では，精巣組織と未発達な卵巣組織を同時に持つ両性生殖腺であった．両者を隔てる組織は認められないが，互いに分離して分布していた．雄の生殖腺の卵巣組織は，主に周辺仁期の卵母細胞からなっていたが，その多くは退行していた．精巣組織内の精子形成は，活発な個体とそうでない個体とが見られた．雄の生殖腺の両組織中には，多くの SPC が認められ，卵巣組織と精巣組織に分布する SPC の活性は，一致していた．しかも，精子形成が活発なほど高い活性を示した．生体外における生殖腺の性ホルモンの産生は，雄の生殖腺では，雄性ホルモンの 11-ケトテストステロン量が高く，雌の生殖腺では雌性ホルモンのエストラジオールとその前駆体のテストステロン量が高かった．

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