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Protandrous hermaphroditism in the clown fish *Amphiprion percula* from Andaman and Nicobar islands

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ABSTRACT

Gonadal sex differetiation through histological analysis and social structure were investigated in anemone fish *Amphiprion percula* occuring in Andaman and Nicobar islands. Field observation in different locations of Bay island showed that this species is always seen in association with their host sea anemones *Heteractis magnifica H. crispa* and *Stichodactyla gigantea* as small social groups that include an adult pair and one to three juveniles (sub adults) and the largest fish is usually the female and the next largest one is the functional male. Considerable size difference consistently appeared between the sexually active female and male, and noticed that a hierarchy exists in which the female is the dominant individual in a social group. Histological examination of gonads indicated that all juveniles start their life as male and subsequently changes into females as they reach larger sizes and mature. Based on the histological observation, the gonad was categorized into seven phases: immature phase, pre-ripe male phase, ripe male phase, transitional phase, pre-ripe female phase, ripe female phase-I and ripe female phase-II. All the juveniles had ambosexual gonads with testicular and ovarian tissues. Fishes in ripe female phase-II had ovaries with many fully grown yolky oocytes and vitellogenic oocytes, which were absent in ripe female phase-I, and characterized by the presence of many perivitellogenic oocytes and an ovarian cavity but have no testicular tissue. The study confirmed the sex reversal in A. *percula* from male to female (protandrous hermaphroditism). The field study supported that social structure plays an important role in its sex change.

Introduction

The sea anemone fishes belonging to the family Pomacentridae, sub family Amphiprioninae comprising of genera *Amphiprion* and *Premnas* commonly known as clown fishes and are unique among damselfishes having peculiar behavioural pattern viz. proclivity to live in symbiotic association with species specific sea anemone (Mariscal, 1970 a&b and Fautin, 1986); formation of social groups and permanent pair bonds that sometimes last for years (Fricke, 1974; Allen, 1975a; Bell 1976; Fricke and Fricke, 1977, Fricke 1979; Moyer and Nakazono, 1978; Fricke 1983) in their life stages. In the life history of anemone fishes, the phenomenon of sex reversal is an intriguing component and the details of which have been discovered only in the past three decades. Sex change (Atz, 1964; Yogo, 1987) and hermaphroditism is known in over 100 species of 15 families of reef fishes. Many reef fishes belonging to the families Serranidae, Labridae, Scaridae and Sparidae are protogynous hermaphrodites (Warner *et al*., 1975; Robertson and Warner, 1978; Warner and Robertson, 1978; Hoffman, 1983; Brusle-Sicard *et al*., 1992) in which fishes begin their life as females and later assume the more colourful male phase. First investigation on protandrous sex inversion in clown fishes were reported in *A. bicinctus* from the Gulf of Aqaba, Red Sea and *A. akallopisos* from Aldabra in the Indian Ocean (Fricke and Fricke, 1977). Later, the sex changing mechanism in *A. melanopus* from Guam (Ross, 1978 a and b); *A. frenatus, A. clarkii, A. polymnus, A. perideraion, A. sandaracinos*, and *A. ocellaris* from Japanese waters (Moyer and Nakazono, 1978), *A. clarkii* from Japan (Ochi, 1989a; Hattori and Yanagisawa 1991a&b), *A. frenatus* (Brusle-Sicard and Reinboth, 1990; Brusle-Sicard *et al*., 1991, 1994, Nakamura *et al*., 1994) and *A. melanopus* (Godwin, 1991) were reported. So far no specific study has been made on sex change in the candidate species. Hence in the present study, an attempt has been made to generate information on social structure from field observations, and determination of gonadal phases and sex change through histological observation in *A. percula* from Andaman and Nicobar islands.

Materials and methods

Field observations on social structures and behavior of *A.percula* were studied by snorkeling in the waters of North Bay, Marine Hill, Chidiyattappu, Mini Bay, Wandoor, Havelock, Car Nicobar and Great Nicobar in Andaman and Nicobar islands. For data collection, fishes residing in one sea anemone were considered as one social group or a clutch at each location, and the size of the adults and juveniles (sub adults) recorded. For histological studies, 90 specimens of *A. percula* having standard length between 18 to 64 mm were collected from 20 social groups using hand nets, and brought to the laboratory. The testis and ovaries of different size groups were dissected out from the freshly sacrificed specimen and fixed in 10% neutral buffered formalin. After 24 hours of fixation, they were washed under running tap water over night and stored in 70% ethyl alcohol until further processing. The stored tissues were later dehydrated following the standard procedure in different grades of alcohol series. The tissue were then cleared in chloroform and isopropyl alcohol and impregnated in boiling paraffin and embedded in paraffin wax (melting point 58-60^{0}C). The paraffin wax blocks were cataloged and stored in labeled polyethylene bags. Longitudinal and transverse sections were cut out at 3-5 µm thickness using Fuji Optec rotary microtome. Mayors egg albumin (Gray, 1973) was used as the ahesive for fixing the paraffin ribbon with section on the clean dry slides. The section was deparaffinised, dehydrated and stained with Harris hematoxylin stain and counter stained with eosin (Preece, 1972; Patki *et al*., 1983). The stained slides were observed under Trinocular microscope Olympus CH-40 and photographed for different gonadal phases. Based on the histological studies, gonads of *A. percula* were classified into seven stages as per the classification suggested by Selman and Wallace (1986), Hattori and Yanagisawa (1991 b) and Hattori (1994).

Results

Social structure

Population of *A. percula* is sparsely available in social groupings in coral reef and intertidal zone in the surveyed locations. Field studies indicated that social groups usually consisted of two sexually active adults (female and male) and 1 to 3 juveniles or sub adults (Table 1) with all its social behavior centered on the host sea anemone *H.magnifica*, *H. crispa* and *S.gigantea*. Among these, maximum association of fishes were noticed in *H.magnifica*. Significant size difference consistently appeared among the adult fishes, of which, the female was larger than the male. The second largest fish (male) spend considerable time chasing the next largest individual of the sub adults, which in turn vents its aggression on the smaller fish residing in the same anemone and also the intruders. Once the pair bond is formed, the pairs displayed amicable relationship and typical *Amphiprion* courtship behavior and also showed non-harmful aggressiveness.

Gonadal phases

Histological observation on the gonads of *A. percula* revealed that it changed sex as they mature and normally, all the juveniles start as males and subsequently change into females when they reach larger size in the social group in wild. Based on the histological studies and structural developments (Table 2), the gonad was categorized into following seven phases.

TABLE 1: *Standard length (SL) and sex of* A. percula *examined histologically and number of fishes present in each sea anemone (Social group) from the wild.*

	Rankding of fishes									
	Ist		IInd		IIIrd		IVth		Vth	
Social group	SL (mm)	sex	SL (mm)	sex	SL (mm)	sex	SL (mm)	sex	SL (mm)	sex
1	55	F	41	AM	39	M/F	32	M/F	*	-
2	53	F	33	AM	30	M/F	25	M/F	*	-
3	51	F	41	AM	3.2	M/F	2.5	M/F	*	-
4	60	AF	40	AM	30	M/F	25	M/F	20	M/F
5	56	F	43	AM	33	M/F	25	M/F	20	M/F
6	49	F	40	AM	30	M/F	20	M/F	*	-
7	62	F	53	AM	43	M/F	30	M/F	26	M/F
8	58	F	39	AM	25	M/F	20	M/F	*	M/F
9	64	F	58	AM	46	M/F	35	M/F	24	M/F
10	60	F	51	AM	42	M/F	25	M/F	20	M/F
11	55	F	43	M/F	20	M/F	*	-	*	-
12	54	F	45	AM	*	-	*	-	*	-
13	65	F	50	AM	40	M/F	27	M/F	21	M/F
14	59	F	43	AM	35	M/F	30	M/F	22	M/F
15	60	F	51	AM	32	M/F	23	M/F	21	M/F
16	63	F	50	AM	40	M/F	25	M/F	20	M/F
17	58	F	48	AM	32	M/F	24	M/F	18	M/F
18	61	F	50	AM	36	M/F	25	M/F	20	M/F
19	59	F	47	AM	34	M/F	24	M/F	18	M/F
20	63	F	53	AM	42	M/F	25	M/F	20	M/F

*No fish; F-Female ; AM-Active male and also ambosexual; M/F-ambosexual

TABLE 2: *Gonadal features of* A. percula *in different maturity stages*

Stages of maturity	Gonad features or structures										
	fyo	dyo	ov	oa	ol	oc	op	sc	ss	dt	gv
Immature	-	-	-	-	-	-	+	-	-	-	-
Pre-ripe male	-	-	-	-	-	-	+	+/-	+	-	-
Ripe male	-	-	-	-	-	-	+	+	+	-	-
Transitional phase	-	-	-	-	-	-	+	+	+	-	-
Pre-ripe female	-	-	-	-	+	+	+	-	-	+	-
Ripe female phase-I	-	+	-	+	+	+	+	-	-	+/-	-
Ripe female phase-II	+	+	+	+	+	+	+	-	-	-	+

fyo=Fully grown yolky oocyte; dyo = Developing yolky oocyte;
ov = Oocytes in the vitellogenesis stage; oa=Oocytes in the cortical alveolus stage
ol = Ovigerous lamellae; oc=Ovarian cavity; op=Oocytes in the perinucleolus stage;
sc = Spermatocyte cysts; ss=Spermatids and / or sperm
dt (Degenerated Testicular Tissues) = Degenerated spermatocyte cysts (dsc) & Degenerated spermatids / sperms (ds)
gv = Germinal vesicle
+= Present;- = Absent; +/- = Few or absent

Immature phase **:** In this stage, the gonad had both testicular and ovarian tissues in very immature stage. Gonadal structure was characterized by the presence of perinucleolus oocytes but did not have an ovarian cavity and ovigerous lamellae. Though the gonad is in male stage , it did not have any spermatocytes (Fig. 1A).

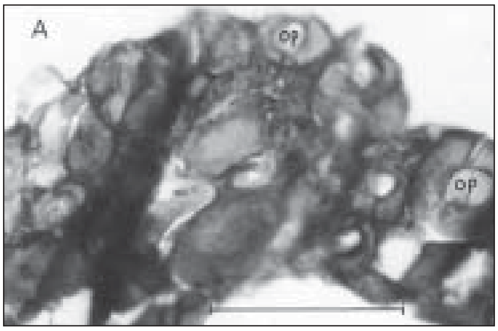

Fig.1. A: Immature gonad showing oocytes in perinucleous stage (op);

Pre-ripe male phase : Gonads had both testicular and ovarian tissues little advanced than the previous. Testicular tissue was constituted by perinucleolus oocytes only. No ovarian cavity and ovigerous lamellae were noticed. The gonad had a complex structure consisting

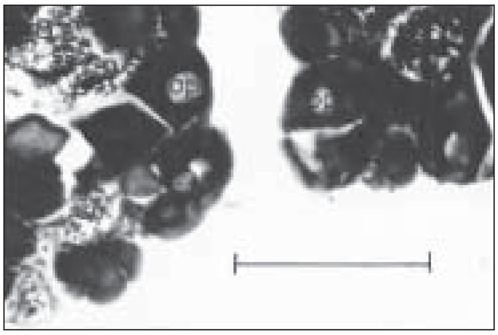

Fig.1. B: Pre-ripe male phase showing spermatocyte cysts (sc), Spermatids/sperm (ss), oocytes in perinucleous stage (op);

of many spermatocyte cysts (Fig. 1 B).

Ripe male phase : The testicular zone was well developed with prominent complex structure consisting of many spermatocyte cysts at various stages of spermatogenesis, spermatids and sperms. Gonads had both testicular and ovarian tissues without any boundary tissues. The ovarian tissue was represented only by perinucleolus oocytes, but did not have an ovarian cavity and ovigerous lamellae (Fig.1 C1 & C2).

Transitional phase : The testicular and ovarian tissue had a complex struc-

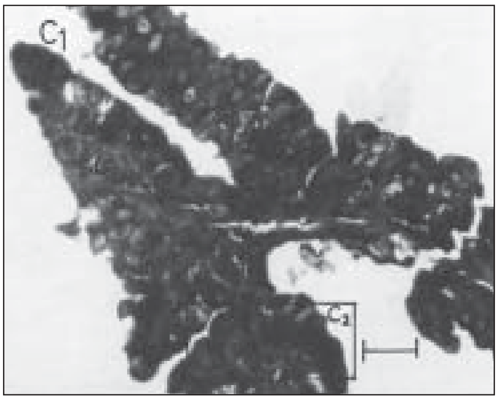

Fig.1. C1 : Ripe male phase showing spermatocyte cysts (sc), Spermatids/sperm (ss), oocytes in perinucleous stage (op);

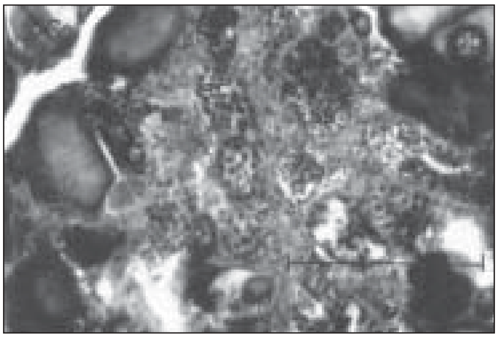

Fig.1. C2 : Part of Ripe male phase gonad in inset of C1 enlarged to show spermatocyte cysts (sc), Spermatids/sperm (ss), oocytes in perinucleous stage (op);

ture. The gonad had oocytes in perinucleolus stage. The testicular tissue was represented by spermatids and sperm occasionally together with a few spermatocyte cysts. Degenerating spermatids and spermatocyte cysts were present but did not have an ovarian cavity and ovigerous lamellae (Fig.1D).

Pre-ripe female phase: This stage was characterized by the presence of ovarian cavity and ovigerous lamellae but all the oocytes were in perinucleolus stage only. Gonad did not have any spermatocyte cysts, spermatids and sperm. Degenerating tissues of spermatocyte

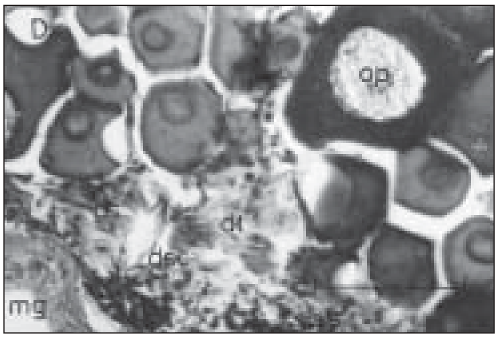

Fig.1. D : Transitional phase showing Spermatids / sperm (ss), oocytes in perinucleous stage (op), Degenerating testicular tissues (dt), Degenerating spermatocyte cysts (dsc), Degenerated sperm (ds), Mesogonium (mg);

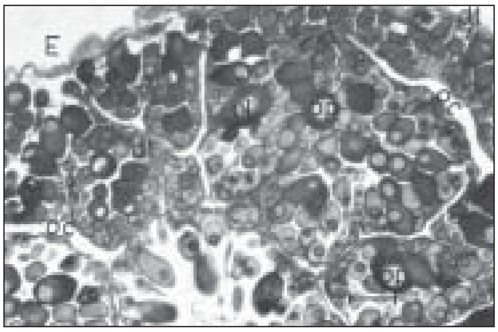

Fig.1. E : Pre-ripe female showing ovarian cavity (oc), oocytes in perinucleous stage (op), Degenerating testicular tissues (dt);

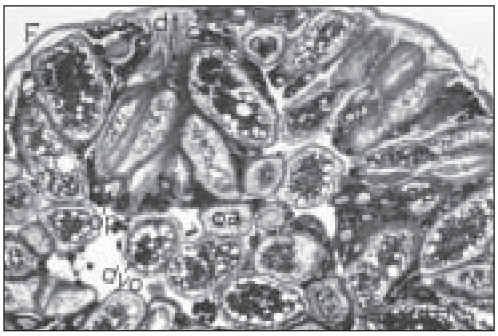

Fig.1. F : Ripe female phase-I showing ovarian cavity (oc), oocytes in perinucleous stage (op), Degenerating testicular tissues (dt); oocytes in cortical alvelolus stage (oa); Developing yolky oocytes (dyo);

cysts or spermatids or sperm. Remnants of degenerating tissues of spermatocyte cysts or spermatids or sperms are present (Fig. 1F).

Ripe female phase-II : This stage is characterized by the presence of more number of fully-grown yolky oocytes and vitellogenic oocytes. In the fully-grown oocytes, the germinal vesicles have almost disappeared and its breakdown can be ascertained by the presence of conspicuous space. Such oocytes are characterized by the appearance of cortical alveoli in the periphery of ooplasm close to the distinct vitelline membrane indicating the induction of final maturation process leading towards ovaluation. The gully-grown oocytes have well-developed and differentiated follicular wall. The ovarian tissue is well developed and the gonad had an ovarian cavity and ovigerous lamellae with various developmental stages of oocytes. No testicular tissue spermatocytes, spermatids or sperm was found in this stage (Fig. 1G).

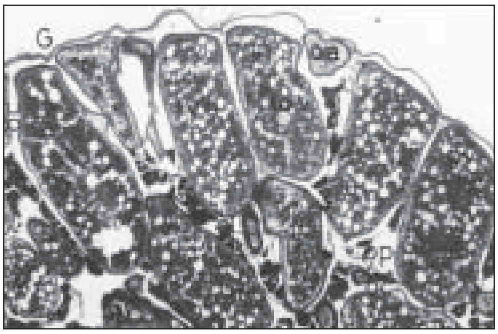

Fig.1. G. Ripe female phase-II showing ovarian cavity (oc), oocytes in perinucleous stage (op), oocytes in cortical alveolus stage (oa); Developing yolky oocytes (dyo); oocytes in vitellogenesis stage (ov), Germinal vesicle (gv), Fully grown yolky oocytes (fyo).

Discussion

The protandrous hermaphroditism in *A. percula* observed in the present study can be considered as a natural adaptation to overcome adverse field situation wherein movement between host sea anemones becomes extremely difficult due to their widely seperated distribution, low population density, and high predation pressures outside their hosts. This adaptation is also reported in various clown fishes (Moyer and Sawyers, 1973; Allen, 1975 a&b; Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Fricke, 1979; Keenleyside, 1979; Moyer, 1980; Thresher, 1984; Warner, 1984; Ochi and Yanagisawa, 1987; Ochi 1986 and 1989 a&b) in tropical waters and these facts strongly influences their social and mating systems (Moyer and Sawyers, 1973; Allen, 1975b; Keenleyside, 1979; Moyer, 1980; Thresher, 1984; Ochi and Yanagisawa, 1987; Ochi 1986 and 1989 a&b). Under such conditions, *A. percula* forms social units wherein two largest specimens of the colony live together as pairs in which the female dominates the male and 1 to 3 juveniles or sub adults are dominated both by the female and the male. The same features were also reported in clown fishes from South East Asian waters (Allen, 1975 a&b; Fricke and Fricke, 1977; Fricke, 1979 and 1983; Moyer and Nakazono, 1978; Ross, 1978b; Ochi and Yanagisawa, 1987; Ochi, 1989a; Hattori and Yanagisawa, 1991a). However no social unit typical of the tropical anemone fishes was noticed in *A. clarkii* at Murote Beach where dense populations of host sea anemones present (Moyer and Sawyers, 1973; Moyer and Nakazono, 1978; Moyer, 1980; Ochi, 1986 and 1989a&b; Yanagisawa and Ochi, 1986; Ochi and Yanagisawa, 1987) and this may be due to its efficient swimming ability than other anemone fishes, and their less dependency on host sea anemone (Allen, 1975a). In Andaman waters *A. percula* were never seen without a host sea anemone.

The largest individuals in each social group had functional ovaries and the second largest fish in the same group is the active male with gonads not only having functional testis in various developmental stages but also possess non-functioning ovarian cells and oocytes which were in young stages with the most developed one in perinucleolus stage. In the case of all smaller fishes, ranking from third to the smallest (sub adults and juveniles) also had non-functioning male and female germ cells within the same gonads (ambosexual gonads) as also been reported in *A. bicinctus* and *A. akallopisos* (Fricke and Fricke, 1977), *A. frenatus, A. clarkii, A. polymnus, A. perideraion, A. sandaracinos, A. ocellaris* (Moyer and Nakazono, 1978), *A. clarkii* (Ochi, 1989a; Hattori and Yanagisawa 1991a,b); *A. melanopus* (Ross, 1978a,b; Godwin, 1991), *A. fernatus* (Brusle-Sicard and Reinboth, 1990; Brusle-Sicard *et al*. 1991, 1994, Nakamura *et al*., 1994) in *A. clarkii* (Hattori and Yanagisawa, 1991 b; Hattori, 1994).

In *A. percula* the ovetestis of all juvenile fishes ranging in size from 18 to 30 mm looked like immature ovaries during early gonadogenesis (Hattori and Yanagisawa, 1991b) with very low level of spermatogenesis whereas the available small area mostly occupied with undeveloped ovarian tissues (Stahlschmidt-Allner amd Reinboth, 1991) with very few oocytes in perinucleolus stage. The ovotestis in pre-ripe male phase also had heterosexual germinal elements with few spermatocyte cysts, spermatids and sperm (testicular tissue) whereas the female part is constituted by some oogonia and perinucleolus oocytes without any ovarian cavity as also recognized in *A. clarkii* (Hattori and Yanagisawa, 1991b). Though juveniles had ambosexual gonads with varying degrees of male and female germ cells, all juvenile fishes were identified as males as ovarian tissues were in inactive conditions in this life stage. Brusle- Sicard and Reinboth (1990) also reported that in anemone fishes, the gonad is also an ovotestis and as the connective tissue is lacking, the heterosexual germinal elements are in direct contact with each other.

In ripe male phase, active spermatogenesis could be clearly evinced by the presence of many spermatocyte cysts at various stages of spermatogenesis, spermatids, sperms and mostly located interiorly in the testicular zone (TZ) whereas the ovarian tissues represented by young oocytes and perinucleolus oocytes are localized greatly on the periphery of gonad and very few were noticed intermingled with the testicular zone without any boundary tissues as also observed in *A.clarkii* (Hattori and Yanagisawa, 1991b) and *A. frenatus* (Nakamura *et al*., 1994 and Moyer and Nakazono, 1978). Gonads in the transitional stage had undergone a reduction in testicular parts and at this stage the number of oocytes showed an increase than the previous stage indicating that this stage is an intermediate stage between ripe male phase and the pre-ripe female. Hattori and Yanagisawa (1991 b) also reported that in *A. clarkii* spermatocyte cysts are lacking in the transitional gonad whereas in *A. melanopus* Godwin (1991) noted a differentiation into mature spermatids before their replacement by oocytes.

In the pre-ripe female phase, the ovarian cavity was well recognizable and all the ovarian tissue were localized on its periphery and the remnants of degenerating tissues of male germ cells were occupied at the outer periphery of ovarian tissues. The ovarian tissues in ripe female phase-I are devoid of vitellogenic oocytes whereas it is present in ripe female phase-II along with more number of fully-grown yolky oocytes. The major distinguishing difference between ripe female phase-I and II is that in former, no vitellogenic oocytes are present. Many brown bodies were also noticed in the ovary indicating the degenerated testicular tissues. Thus the present study confirmed the sex reversal in clown fish *A. percula* from male to female (protandrous hermaphro-ditism) in which the largest and socially dominant fish in a particular anemone is generally the female and had functional ovaries. The second largest fish in the same social group is the active male and had gonads that are functioning testis but also possess non-functioning or latent ovarian cells. If the dominant female dies, the gonads of males ceases to function as testis and the egg producing cells become active, and male not only changes sex, but also grows at an accelerated rate. Simultaneously, the largest of the sub adults becomes the functional male and also may grow even faster, filling the size gap. This adaptation allows continuous reproduction and social structure plays in important role on its sex changing mechanism and gonadal phases.

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