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CYTOGENETIC STUDIES OF SIAMESE FIGHTING FISH (*BETTA SPLENDENS* REGAN)

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Summary

Domesticated Siamese fighting fishes are characterized into short fin and long fin types. The short fin fishes are much more aggressive than the long fin type. The purpose of this study was to determine whether they belong to the same species based on karyotype and hybridization evidence.

Metaphase karyotypes of the short and long fin types are similar. Diploid number and arm number are 42 and 56 respectively. Chromosomes are graded into 3 groups, the first sixteen pairs are large size, the 17th and 18th pairs are medium size, and the last three pairs are small size. The centromeric type consist of seven pairs of submetacentric and fourteen pairs of acrocentric. In this investigation, heteromorphic sex chromosome was not detected. However, polymorphism of chromosome no. 3 was observed in both types of fish.

Sixteen pairs of reciprocal crosses and twenty backcrosses were performed. The rates of hatching and survival of F_1 offspring from the reciprocal crosses of the short fin and the long fin types show no statistically significant differences. Backcrosses of ten male and ten female F_1 offspring were completely fertile. Therefore, both types of Siamese fighting fish should be regarded as the same species, **Betta splendens** *(Regan).*

Introduction

The karyotype is defined as the basic chromosome set of a species, and is further characterized as to form and size of chromosome as well as the diploid number. It is generally recognized as a definite species character since the morphology of the chomosome among individuals of the same species is reasonably constant[1]. Karyotype analysis can be useful both in elucidating the phylogenetic relationships of species belonging to the same group or closely related groups. In fact, relationships within natural groups of species can scarcely be considered complete in an evolutionary sense without good karyotypic data to reinforce conclusion based on morphological criteria.

Mayr[2] has described that group of actually or potentially and interbreeding natural populations, which are reproductively isolated from other such groups, are recognized as species. Species isolating mechanism has also been classified as premating mechanisms that prevent interspecific crosses; postmating mechanism tend to reduce the success of interspecific crosses. The former category includes seasonal and habitat isolation, ethological isolation and mechanical isolation that prevent sperm transfer. Postmating mechanisms include gametic mortality, zygote mortality, inviability, total sterility or partial sterility of hybrids.

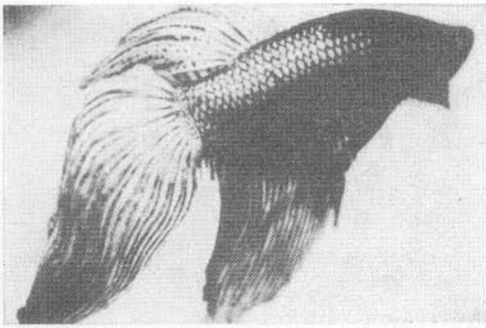
The domesticated Siamese fighting fishes are systematically named *Betta splendens* (Regan)[3]. Nevertheless, two distinct types of morphology are recognized i.e. a short fin and a long fin (Fig. 1). The short fin type shows itself to be much more aggressive in fighting behavior than the long fin type. Special attention has been paid to the question of whether cytotaxonomic classification is in agreement with one based on morphological characters. This report presents a comparative study on metaphase karyotypes of these two morphologically distinct groups of Siamese fighting fish and data on the hybridization experiments between the two groups in order to justify their species status.

Materials and Methods

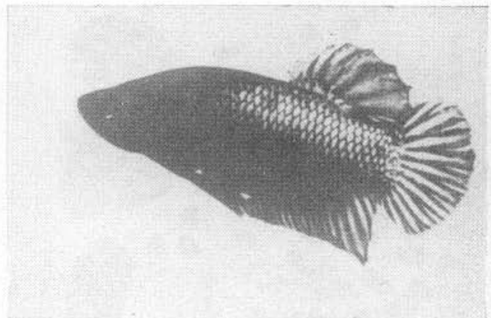
Specimens used in this study were obtained from two different collections. The short fin fish (Fig. 1b, 1d) was from Chachaungsau province and the long fin fish (Fig. 1a, 1c) from Bang Bua, Bangkok.

The metaphase chromosomes were prepared according to the method described by Davission[4]. After overnight staining in Giemsa solution, the air dried chromosome slides were permanently mounted in Permount. Karyotyes were made from photographs of a 5500 times enlargement of well-spread and unbroken metaphase cells. The karyotype of each group was determined on the basis of twenty cells. The karyotype of each group was determined on the basis of twenty cells which were obtained from mature animals of eleven males and ten females of short fin fishes; twelve males and eleven females of long fin fishes.

For each chromosome, long arm (Ll), short arm (Ls) and total length (LT) were measured. The calculations were made for relative length (R.L. $= \frac{LT \times 1000}{\Sigma LT}$) and arm ratio (C.I. $= \frac{Ll}{LT}$)[5]. Centromeric chromosomes were classified by means of the position of centromere. The chromosome was considered to be the metacentric (M), submetacentric (Sm) or acrocentric (A) if arm ratio fall within the range of 0.50–0.69,



a



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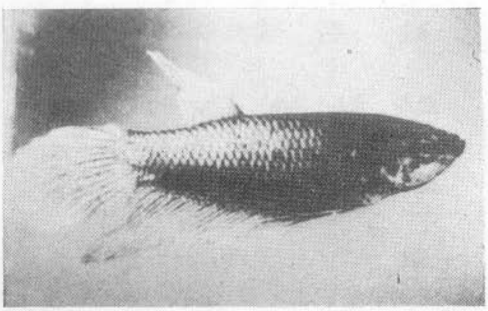

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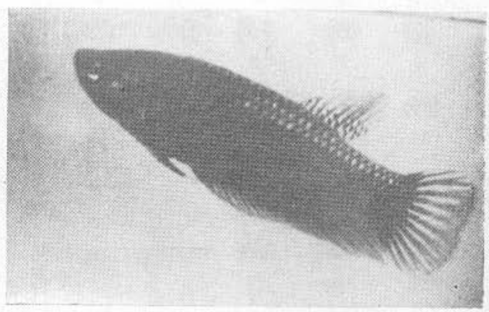

d

Fig. 1: Siamese fighting fish, a) long fin male b) short fin male c) long fin female and d) short fin female

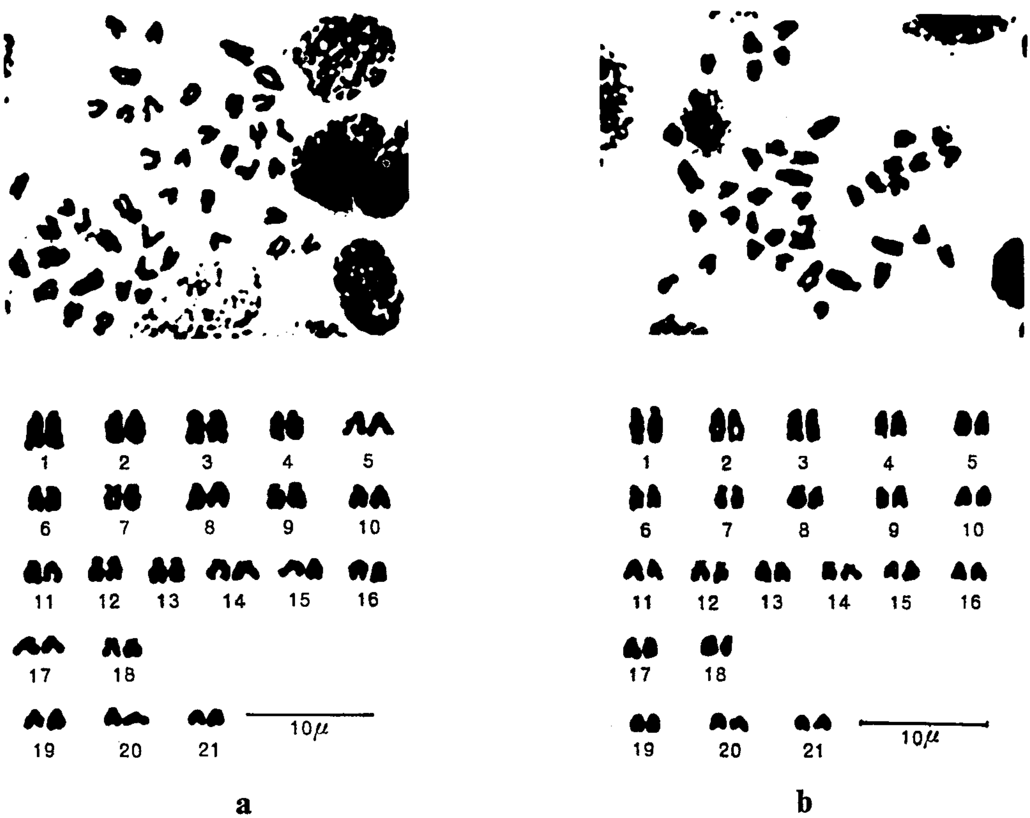

Fig. 2: Mitotic metaphase (upper) and karyotype (lower) of Siamese fighting fish, a) long fin fish and b) short fin fish

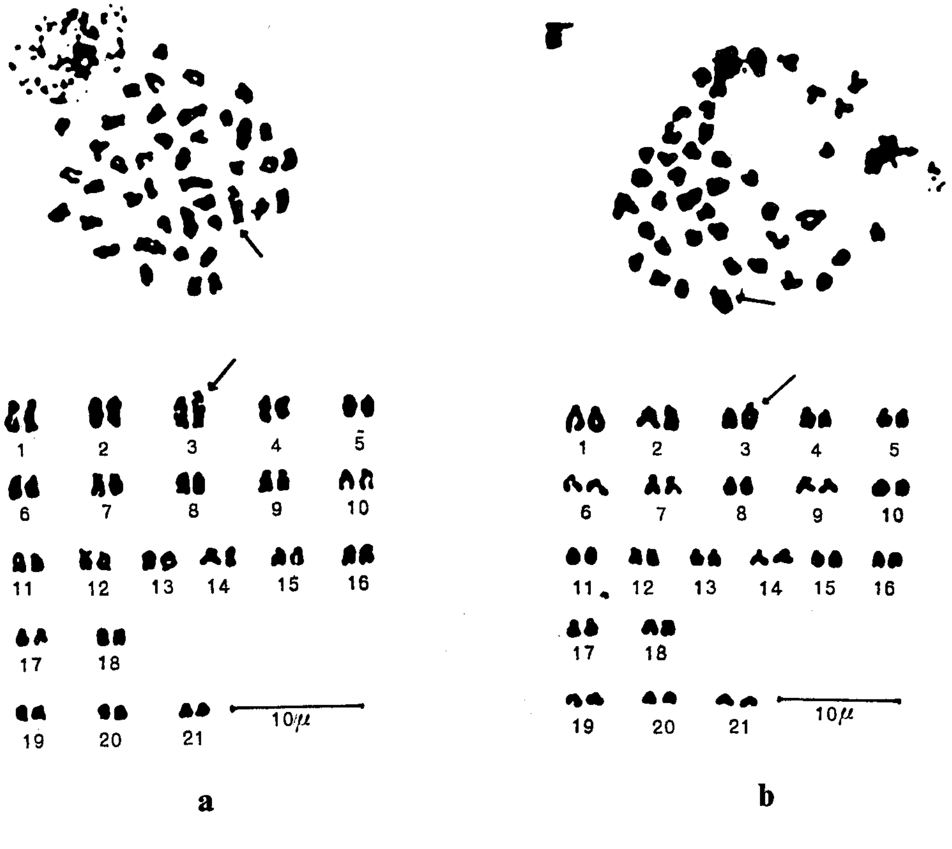

Fig. 3: Chromosomal polymorphism on the third chromosome (arrow) of a) long fin fish and b) short fin fish

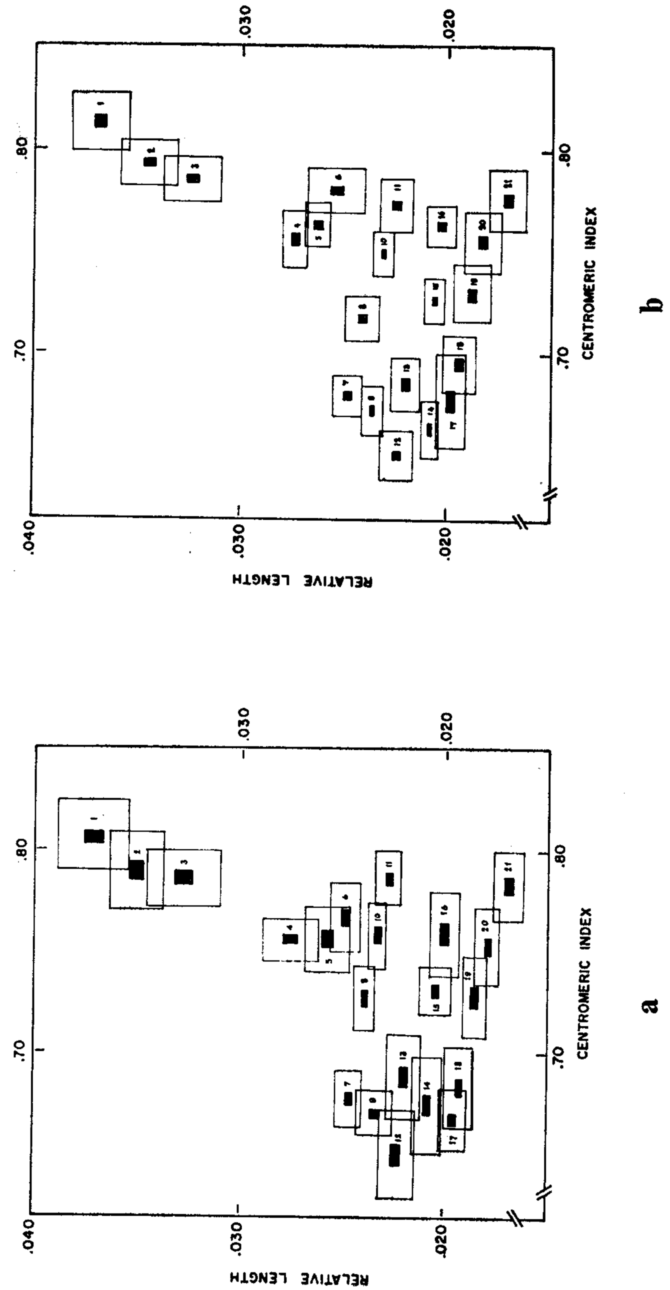

Fig. 4: Two-dimentional karyograms of a) long fin and b) short fin Siamese fighting fish.

0.70–0.75 and 0.76–1.0 respectively. Acrocentric chromosomes were further divided into short-short arm (SSA) and long-short arm (LSA) types[6]. Chromosomes were grouped into large, medium and small sizes. Arm numbers were also studied[7]. Arm ratio and relative length served for the construction of the two-dimensional karyogram[8].

In hybridization experiments, adult males and females about six months old were taken from breeding stocks maintained in the laboratory. Each pair of fish were bred in aquaria of about 30 l capacity, with water temperature about 23.5–29.0°C and pH 6.8–7.2

At the egg laying time, the numbers of eggs were counted while they were slowly sinking to the bottom of the aquaria. 48 hours after fertilization, the numbers hatching were recorded. Numbers of survival were determined on the sixth week. Percentages of hatching and survival were calculated as follows:

$$\text{Percentage of hatching} = \frac{\text{total number of hatching}}{\text{total number of eggs}} \times 100$$

$$\text{Percentage of survival} = \frac{\text{total number of survival}}{\text{total number of hatching}} \times 100$$

The number of fish used is shown in Table I.

TABLE I: FERTILITY OF HYBRIDS OF SHORT AND LONG FIN SIAMESE FIGHTING FISHES.

crossing[a]		No. of pairs	Fertility[b]	% of hatching[c]	% survival[c]
Inbreeding	S♀ × S♂	8	F	97.33 ± 0.17	9.00 ± 0.20
	L♀ × L♂	8	F	98.05 ± 0.09	10.97 ± 0.14
Reciprocal-crosses	S♀ × L♂	8	F	98.29 ± 0.07	11.25 ± 0.16
	L♀ × S♂	8	F	97.95 ± 0.15	11.19 ± 0.14
Backcrosses	Hsl♀ × S♂	3	F		
	Hsl♀ × L♂	2	F		
	Hls♀ × S♂	3	F		
	Hls♀ × L♂	2	F		
	Hsl♂ × S♂	3	F		
	Hsl♂ × L♂	2	F		
	Hls♂ × S♂	2	F		
	Hls♂ × L♂	3	F		

Critical Value of F = 4.99, $P < .05$, df = 7,7

[a]S = short fin; L = long fin; Hsl = short fin ♀ × long fin ♂; Hls = long fin ♀ × short fin ♂

[b]F = fertile [c]Mean ± S.E.

Results and Discussion

Two dimensional karyograms of short fin (Fig. 4b) and long fin (Fig. 4a) are quite similar. The karyotypes (Fig. 2) of these two types of fish investigated are:

Diploid number	= 42
Arm number	= 56
Chromosome size:	
large	= 16 pairs (1–16)
medium	= 2 pairs (17–18)
small	= 3 pairs (19–21)
Centromeric type:	
submetacentric	= 7 pairs (7, 9, 12–14, 17, 18)
acrocentric	= 14 pairs (1–6, 8, 10, 11, 15, 16, 19–21)
Polymorphic chromosome: 3rd pairs (Fig. 3a, 3b)	
Heteromorphic sex chromosome: not present	
Percentage of SSA:	
short fin type	= 71.38
long fin type	= 78.75

Results from reciprocal crosses in each group show that percentages of hatching and survival of the F_1 offspring between the short fin and long fin types are not significantly different. Further, backcrosses of ten females of F_1 offspring were completely fertile (Table I).

The karyotype was early recognized as a definite species character. The metaphase chromosome configurations among individuals of the same species are generally constant. However, departure in morphology of chromosomes from the species pattern is largely attributed by recognizable aberrations especially paracentric inversion, centric fusion and the different amount of heterochromatin[9]. In general, a particular karyotype can be designated as a representative of the species and in some instances even of the genus.

Since chromosomes spontaneously undergo breakage and recombination to form inversions and/or translocation, it is logical that karyological differences between related species have come about by a gradual series of changes that have altered the visible appearance of the chromosomes. Gradual changes in gene sequence are *prima facie* an important evolutionary feature.

Chromosome number ($2n = 42$) in both types of Siamese fighting fish as revealed in this investigation is in agreement with previous reports[10, 11]. The karyotype of these two types of fish have shown to be very similar. However, there is only a slight difference in the percentage of SSA. In addition, these two types of Siamese fighting fish showed similar polymorphism of the third chromosome. F_1 hybridization tests between short fin and long fin fishes were quite successful, producing prolific F_1 offspring which were fully fertile. The clearly suggests that these two types of fishes which are morphologically quite different belong to the same taxon. Such morphological differences could be due to genetic variation followed by the long separation in breeding and raising. In addition, F_1 hybrids showed the same karyotype as those of both parents.

Sex reversal and sex determination have long been problems in *Betta splendens*. From the investigation thus far, little can be said about the sex chromosome in *Betta splendens*. Bennington[10] has reported heteromorphic sex chromosomes in the primary spermatocytes of Siamese fighting fish. His finding was based on a slower movement of a certain chromosome than the others and he found that this chromosome retained a heteropycnotic character throughout late leptotene. However, there is no evidence of photographs to support his interpretation. Švardson and Wickbom[11] disagreed with Bennington's results.

With regard to sex reversal in *Betta splendens*, there were some reports that sex reversal occurred in the long fin females spontaneously[12] or after the removal of ovaries[13]. These ovarectomized fish developed the external morphology characteristic of males but spawned as normal females producing a large number of offspring. Subsequent autopsy showed evidence of active spermatogenesis histologically. The young of these mating were of both sexes[13, 14]. These results showed that male characteristics were not controlled by heteromorphic chromosome.

In the present investigation, heteromorphic sex chromosomes were not observed. Thus, it is likely that sex determination of *Betta splendens* does not depend on sex chromosomes, but may be due to the combination of genes distributed on a single or several pairs of autosomes. Sex characterisics depend on the balanced conditions of these genes and other factors such as hormones and external environment.

Acknowledgements

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บทคัดย่อ

ปลากัดไทยที่เพาะเลี้ยงกันอยู่ในปัจจุบัน มีลักษณะภายนอกแตกต่างกันพอจะแยกได้ เป็น 2 พวก คือ พวกหางและครีบสั้น นิสัยดุร้าย และพวกหางและครีบยาว นิสัยไม่ดุร้ายเท่าพวกแรก งานวิจัยนี้ศึกษาแคริโอไทพ์และการผสมพันธุ์ระหว่างปลากัดไทย 2 พวกดังกล่าว และศึกษาว่าปลากัดไทยมีโครโมโซมเพศหรือไม่

จากการศึกษาแคริโอไทพ์ของปลากัดไทยทั้ง 2 พวก ปรากฏว่ามีจำนวนโครโมโซมเท่ากับ 21 คู่ จำนวนแขนโครโมโซมเท่ากับ 56 จากขนาดของโครโมโซม แบ่งได้เป็น 3 กลุ่ม คือ คู่ที่ 1–16 เป็นกลุ่มที่มีขนาดใหญ่ คู่ที่ 17–18 เป็นขนาดกลาง และคู่ที่ 19-21 เป็นขนาดเล็ก ชนิดของโครโมโซมมีเพียง 2 ชนิด คือ สับเมตาเซนตริค 7 คู่ และอโครเซนตริค 14 คู่ ไม่ปรากฏว่ามีโครโมโซมเพศ พบลักษณะโพลีมอร์ฟีสที่โครโมโซมคู่ที่ 3 ลักษณะที่พบจากการศึกษาโครโมโซมข้างต้นทั้งหมด พบได้เหมือนกันทั้งในปลากัดไทยพวกที่มีหางและครีบสั้นและพวกที่มีหางและครีบยาว

ในการศึกษาการผสมพันธุ์ระหว่างปลากัดไทยทั้ง 2 พวก ได้ศึกษา reciprocal crosses 16 คู่ และ backcross 20 คู่ ผลการศึกษา reciprocal crosses พบว่าปลากัดไทยทั้ง 2 พวกสามารถผสมพันธุ์กันได้เปอร์เซ็นการฟักเป็นตัวและการอยู่รอดของลูกผสม ไม่มีความแตกต่างกับลูกที่เกิดจากการผสมระหว่างปลากัดไทยพวกเดียวกันโดยทางสถิติการศึกษา backcross พบว่าลูกผสมตัวผู้ 10 ตัวและตัวเมีย 10 ตัว สามารถผสมพันธุ์กับปลากัดไทยพันธุ์แท้ทั้ง 2 พวก และให้ลูกที่มีการเจริญเติบโตเหมือนปกติ

ผลของการศึกษาดังกล่าวนี้ สนับสนุนว่าปลากัดไทยพวกหางและครีบสั้น และพวกหางและครีบยาว จัดอยู่ใน species เดียวกัน (*Betta splendens* Regan)