are extremely sensitive to hydrogen bonding and reflect the skeletal conformations of the molecule. The peaks seen in GA′ in these regions—the singlet at 1665 cm^{-1} (see Fig. 2a) and the activity at 1231, 1245, and 1285 cm^{-1}—are similar to the Raman spectra (*11, 12*) of model polypeptides with antiparallel β hydrogen bonding (no Raman studies have been reported on model compounds with parallel β hydrogen bonding). The activity on the high-frequency sides of the 1665- and 1231-cm^{-1} peaks may also indicate the presence of other types of carbonyl groups (for example, nonhydrogen bonding carbonyl groups).

Figure 2, b to d, shows the region 1600 to 1700 cm^{-1} for GA′ dissolved in $CHCl_3$, dioxane, and Me_2SO, respectively. The first two solution spectra closely match the powder spectrum of Fig. 2a. GA′ tends to aggregate due to the low solubility of these solvents; however, a similar spectrum appears for nonsaturated solutions of GA′-methanol. The spectrum of GA′ in Me_2SO differs considerably. The amide I peak is shifted from 1665 to 1685 cm^{-1} and a right asymmetry appears. In the amide III region the peak at 1285 cm^{-1} disappears and the peak at 1231 cm^{-1} is reduced in intensity.

An amide I vibration as high as 1685 cm^{-1} is unusual for an ordered conformation. A similar peak is seen in random coil glucagon (*11*), where it is assigned to completely unsolvated amide carbonyl groups. The powder peaks at 1231 and 1285 cm^{-1} become, in Me_2SO, broad regions of activity centered about 1240 and 1265 cm^{-1}. Previous studies in deuterated Me_2SO using other techniques have been interpreted as suggesting that GA′ exists in an ordered conformation that is not α helical (*8*). Whether the conformation of GA′ in Me_2SO is random coil or ordered is not clear, but it is certainly different from any conformation we find in powder, $CHCl_3$, dioxane, or methanol.

Note added in proof: Our powder data are also consistent with a very recently proposed antiparallel double-helical dimer structure (*13*).

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Chromosomes of the Chocolate Gourami: A Cytogenetic Anomaly

Abstract. *The chocolate gourami,* Sphaerichthys osphromonoides, *has the lowest chromosome number reported for fishes, with* $2n = 16$ *and* $n = 8$. *Other members of the family Belontiidae have somatic numbers of 42 to 48. Chromosome instability is demonstrated by the presence of somatic abnormalities and meiotic multivalents.*

Sphaerichthys osphromonoides is a tropical fish belonging to the largest family of anabantoids, the Belontiidae. This species is native to Malaya and Sumatra and is thought to have originated from a line that came from an anabantid ancestor when an explosive phase of adaptive radiation occurred during the Upper Cretaceous and Lower Tertiary (*1*). This gourami is one of the most specialized belontiids and is classified in the subfamily Trichogasterinae. It is considered by aquarists to be a delicate species that is hypersensitive to temperature change and other external stimuli. The chocolate gourami is not a prolific breeder. Despite intensive studies, its breeding habits are not completely understood (*2*). Until now, *Sphaerichthys* has not been studied cytogenetically.

Specimens used in this study consisted of 21 females and 10 males which were obtained from various tropical fish dealers. Both mitotic and meiotic chromosomes were enumerated ac-

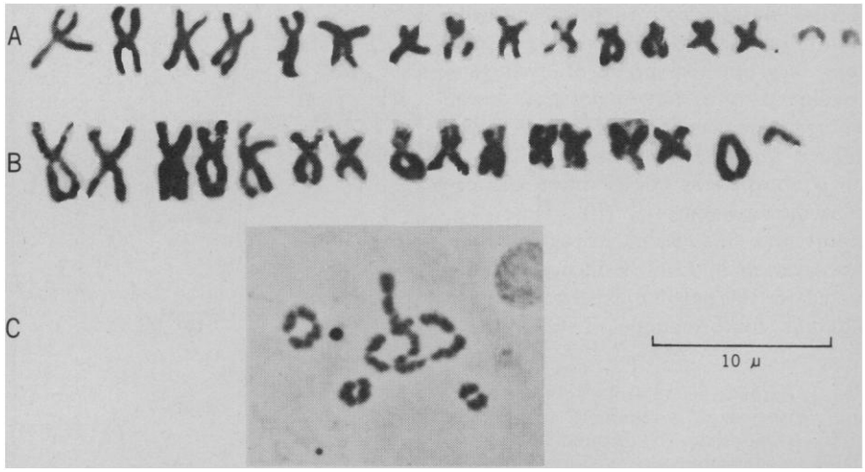

Fig. 1. Mitotic and meiotic chromosomes of the chocolate gourami. (A) Normal somatic complement showing a diploid number of 16. (B) Abnormal somatic complement showing a heteromorphic acrocentric pair. (C) Meiotic spread from testis showing a ring hexavalent, a rod quadrivalent, and three bivalents for a haploid number of 8.

cording to methods reported elsewhere (*3*). In all, 78 mitotic and 15 meiotic spreads were examined. The low meiotic spread count reflects the measure of difficulty in obtaining mature testicular material from male specimens. Ruptured somatic and meiotic spreads were not included in this study.

The modal diploid count of *Sphaerichthys* is 16 (Fig. 1A), of which 14 are metacentric and 2 are acrocentric. Heteromorphic chromosomes did not consistently appear for any given pair in the complement of either the male or female. Thus, sex determination appears to be genic rather than chromosomal. Of the 78 karyotypes analyzed, 38 (49 percent) contained chromosome pairs that did not match. These unequal matchings were evident in pairs 6, 7, and 8, with the greatest number occurring in the last two acrocentrics (Fig. 1B). These mismatched chromosomes differed according to size and chromosome type. Eight of the aberrant karyotypes contained a submetacentric paired with an acrocentric. The remaining abnormal karyotypes contained two acrocentric chromosomes that were unequal in size. These designated abnormal karyotypes were judged to be real rather than artifact primarily because the metaphase spreads were well isolated from each other, thus reducing the probability that chromosomes could be exchanged in as many as 49 percent of the figures scored as abnormal. Other possibilities which could produce artifacts, such as preparation of the photokaryotype, differential chromosome coiling, and induced metaphase damage during slide preparation, were all considered improbable owing to the structural arrangement of the heteromorphic pairs believed to be aberrant. Also, there were no chromosome fragments, chromatid breaks, or gaps within the metaphases studied.

Aberrant chromosomes were not limited to any one specific tissue, since chromosome preparations from gill, spleen, and fin tissue of individual specimens all contained abnormalities. Furthermore, unequal matchings were not limited to either sex. Both females and males displayed aberrations with equal frequency.

The haploid chromosome number from testicular preparations is 8. These eight chromosome pairs are synapsed into five structures (Fig. 1C). Six are normal bivalents, four are arranged end to end, forming a single rod quadrivalent, and six are synapsed as a large ring hexavalent. The relationship of these unusual meiotic configurations to specific somatic chromosomes could not be determined with certainty. In 12 of the 15 meiotic spreads from three different males, similar ring configurations were evident. Such ring structures are indicative of translocations.

Table 1. Chromosome numbers of some fishes belonging to the family Belontiidae.

Subfamilies of Belontiidae	Chromosome number		Fundamental number	Reference
	$2n$	n		
Macropodinae				
Betta splendens	42	21	48	(*7*)
Macropodus opercularis		21		(*8*)
Trichogasterinae				
Sphaerichthys osphromonoides	16	8	30	This study
Colisa fasciatus	48	24	48	(*9*)
Trichogaster sumatranus	48	24	48	This study
Trichogaster trichopterus		24		(*8*)

Chromosomes of representatives of Belontiidae are given in Table 1. Except for *Sphaerichthys*, all these representatives have $2n$ numbers between 42 and 48 and a fundamental arm number of 48. With a $2n$ of 16 and a fundamental number of 30, an apparent case of chromosome reduction is represented in the chocolate gourami. Other examples of chromosome reduction in fishes are lacking. From extensive studies Ohno (*4*) believes the primitive teleost ancestor had approximately 48 acrocentric chromosomes. More specialized teleosts arose from this ancestor through gene duplication. In some instances this did not involve a change in number or structure. In others, the mechanisms of pericentric inversions, translocations (especially centric fusions), deletions, and duplications (including polyploidy) were responsible for an increase in chromosome number (dysploid increase). In like manner, the same mechanisms could account for decreases in chromosome number (dysploid decrease).

The most spectacular example of dysploid decrease in another vertebrate is the Indian muntjac deer, *Muntiacus muntjak* (*5*), in which a $2n$ of 7 for the male and 6 for the female apparently stemmed from an ancestor with a diploid number of 46. One of the proposed mechanisms for this chromosome reduction was the rearrangement of acrocentric chromosomes from an ancestor through centric fusions.

Brown (*6*) proposes that the primary mechanism for dysploid decrease is the translocation of active genes between nonhomologous chromosomes, resulting in one chromosome that is genetically active and one that is eventually eliminated owing to gene loss. Should the newly formed chromosome become homologous, the result is the elimination of a chromosome pair.

It is proposed that all these well-known mechanisms occurred in producing the 16 chromosomes of the chocolate gourami from a larger ancestral complement of 42 to 48, with translocations accounting for the major reductions. Such occurrences of dysploid decrease would gradually reduce the ability of the species to undergo adaptive change, since the probability of vital gene loss would be great. Also, the presence of multivalents in such a reduced complement indicates difficulty during synapsis and segregation. Further chromosomal instability is reflected in that 49 percent of the somatic complements analyzed contain nonhomologous groupings. These cytological findings, plus the knowledge that it is a highly specialized and delicate species, suggest that at this point in time the chocolate gourami is bordering on genetic extinction.

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