honis[2, 3]; it is highly probable that each of these populations represents a good biological species. Thus, further detailed morphological comparisons among these populations are also required in order to revise the taxonomy of the Taiwanese *Japalura*.

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The karyotype and genome structure of the pirate perch *Aphredoderus sayanus* (Aphredoderidae: Teleostei)

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Summary. The standard karyotype, genome size (DNA content), and genomic DNA base composition and distribution of the relict paracanthopterygian fish, *Aphredoderus sayanus*, were investigated. Several features of the *A. sayanus* genome appear to be derived rather than primitive conditions. These include a large number (at least 10 pairs) of bi-armed chromosomes, a low genome size, and high DNA asymmetry. This may indicate that *A. sayanus* is not a typical paracanthopterygian fish in terms of its genome structure.
Key words. Karyotype; genome size; DNA base composition; relict fish.

The pirate perch, *Aphredoderus sayanus*, is the only living member of the family Aphredoderidae, and is one of the few freshwater forms in the teleost superorder Paracanthopterygii[1]. The species is endemic to North America and is found primarily in the lowlands of the Atlantic and Gulf slopes and in the Mississippi Valley[2]. Relatively little is known about its biology, although there are published data on pirate perch habitats, food, growth, and reproduction[2, 3]. Evolutionarily, *A. sayanus* is a living relict and presumably represents one of the remnants of an ancient ichthyofauna that occupied the Mississippi Valley prior to the ancestors of most modern day North American fishes[4]. Fossil genera related to *Aphredoderus* are known in North America from as early as the Oligocene[5].

In this note, the standard karyotype, genome size (DNA content), and genomic DNA base composition and distribution of *A. sayanus* are reported. The purposes of the study were to obtain basic genetic information on a poorly known species, and to examine the chromosomal and genomic structure of a paracanthopterygian fish. The latter are not well known genetically since most paracanthopterygian species are marine and difficult to obtain alive. Paracanthopterygians are of systematic interest since they are the putative sister group to the Acanthopterygii, the largest and most diverse of all presumably monophyletic teleost groups[6, 7].

The *A. sayanus* specimens examined in the study were collected by seine from an unnamed tributary of the Navasota River near College Station, Texas. The specimens were returned live to our laboratory and maintained in aerated aquaria until sacrificed. The methods used to prepare, stain, and photograph metaphase chromosomes followed Gold[8]. Genome sizes of two individuals were determined by flow cytometry of erythrocyte nuclei and sperm following the methods of Bickham et al.[9] and using chicken erythrocyte nuclei as the internal standard. Genomic DNA base composition and differential melting rate profiles were generated via thermal denaturation of visceral tissue DNA isolated from two individuals following the methods of Mandel and Marmur[10].

The standard karyotype of *A. sayanus* (fig. 1) contains $2n = 48$ chromosomes as determined from over 150 metaphases taken from four specimens. Fundamental arm number (NF) estimates ranged from 68 to 72 as measured from six different karyotypes. One pair, the largest in the complement (cf fig. 1), was clearly heteromorphic in two of the specimens and may indicate the existence of morphologically differentiated sex chromosomes. These two specimens were male; the other two specimens karyotyped were juveniles and could not be identified as to sex. This means that the heteromorphism detected could well be autosomal. The occurrence of bi-armed chromosomes in the *A. sayanus* karyotype is probably a derived rather than primitive condition. Over 1800 teleost species have been karyotyped, including 15 species from the superorder Paracanthopterygii and over 900 species from the superorder Acanthopterygii[11]. A diploid karyotype of 48 acrocentric chromosomes (i.e., $2n = 48$, $NF = 48$) has been found in most of the major teleost groups, and is the predominant karyotype in the most advanced group, the Acanthopterygii. On this basis, Ohno[12] and others have suggested that the $2n = 48$, $NF = 48$ condition is primitive for teleosts. *A. sayanus* possesses a diploid number of 48 chromosomes, but at least 10 pairs of chromosomes are bi-armed (meta- or submetacentric) and one of these may possibly represent a sex chromosome heteromor-

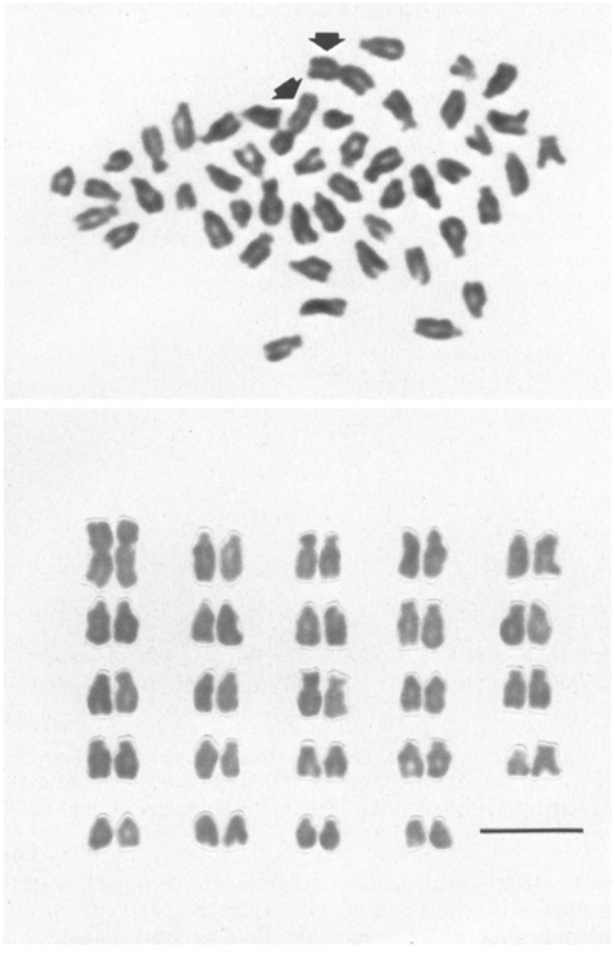

Figure 1. Metaphase and standard karyotype of *Aphredoderus sayanus*. Arrows indicate the heteromorphic chromosome pair (cf text). Bar equals 5 µm.

phism. The latter, if verified, would represent a derived state given the relative infrequency of morphologically differentiated sex chromosomes in teleost fishes [13].

The genome sizes (DNA contents) of the two individuals examined were 1.14 and 1.24 pg of DNA per diploid erythrocyte nucleus. This gives a genome size estimate for *A. sayanus* of 1.19 pg of DNA. The difference in genome size between the two specimens is within the range of DNA values found between individuals within populations of other teleost fishes [14, 15]. The genome size of *A. sayanus* also appears derived in that the estimated value of 1.19 pg of DNA is considerably lower than that found in most other teleost fishes including both paracanthopterygians (12 species assayed, range = 1.4–6.0 pg) and acanthopterygians (ca. 200 species assayed with 95% falling in the range 1.2–2.8 pg) [14–16]. The fact that *A. sayanus* has a low genome size and possesses a unique morphological feature [17] fits well with Hinegardner's [18] hypothesis that, in fishes, genome size reduction accompanies morphological specialization.

The estimated GC base composition (guanine–cytosine base pairs) of *A. sayanus* genomic DNA was 41.1 ± 0.1; the estimated compositional heterogeneity value was 13.1 ± 0.5. The former reflects the proportion of GC (and hence AT or adenine–thymine) base pairs in the DNA; whereas the latter reflects the degree to which the GC and AT base pairs are interspersed within the genome and essentially defines the transition width of the melting curve [10]. The melting rate

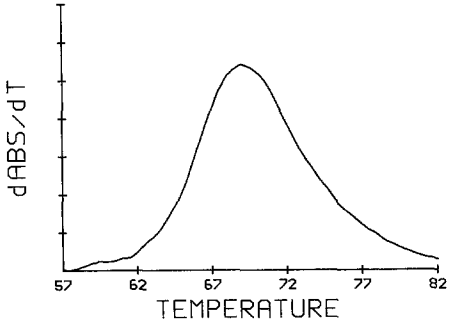

Figure 2. Differential melting rate profile of *Aphredoderus sayanus* genomic DNA. Abscissa – denaturation temperature; ordinate – increase in absorbence for each step increase (0.2 °C) in temperature.

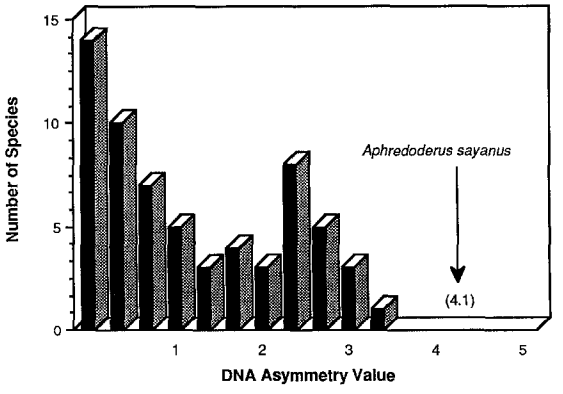

Figure 3. Frequency distribution of DNA base composition asymmetry values for 64 species of teleost fish. Asymmetry values are in absolute units. Data are from Hudson et al. [19] and Karel and Gold [20].

profile of *A. sayanus* DNA is shown in figure 2. The asymmetry value, which is the difference between the mean and modal percent GC values of the melting curve, was 4.1.

The base composition of *A. sayanus* DNA (41.1% GC) appears typical for a teleost fish since of the approximately 110 teleost species examined [19, 20], roughly 90% have percent GC values in the range 39–43. The compositional heterogeneity value and asymmetry value of *A. sayanus* DNA, however, are considerably higher than comparable values observed in the other fishes examined to date (fig. 3). These high values indicate that a considerable fraction of the *A. sayanus* genome has a base composition which differs substantially from the bulk of *A. sayanus* DNA. Regardless of the cause, the structure of the *A. sayanus* genome appears to differ from those of the other fishes examined to date.

Based on the foregoing, several features of the *A. sayanus* genome appear to be derived conditions. These include the *A. sayanus* karyotype, genome size, and DNA base composition asymmetry. This may indicate that *A. sayanus* is not a typical paracanthopterygian fish in terms of its genome structure, and that the Aphredoderidae will not be especially useful in evaluating phylogenetic trends in genome evolution among teleosts. Alternatively, it will be interesting to examine these parameters in the putative sister group to the Aphredoderidae, the cavefishes of the family Amblyopsidae [5].

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Isolation of plant growth regulators from *Pseudomonas amygdali*

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Summary. Trans-zeatin and indole-3-acetic acid were isolated, as the main components of the cytokinin and indole mixtures respectively, from culture filtrates of *Pseudomonas amygdali,* the causal agent of hyperplastic bacterial canker of almond.
Key words. Pseudomonas amygdali; hyperplasia; almond; plant growth regulators; cytokinins; auxins; *trans*-zeatin; indole-3-acetic acid.

Several plant diseases are characterized by growth abnormalities of the infected tissues. Such effects are in many cases determined by an alteration of the physiological hormone balance in the plant[1]. Among phytopathogenic bacteria *Pseudomonas syringae* pv. *savastanoi* (Smith) Young, Dye & Wilkie, *Agrobacterium tumefaciens* (Smith and Towsend) and *Corynebacterium fascians* (Tilford) Dowson produce indole-3-acetic acid (IAA)[2,3] and/or cytokinins[4-7], which have been shown to have an important role in plant pathogenesis[8-10].

Pseudomonas amygdali Psallidas & Panagopoulos is the cause of hyperplastic bacterial canker of almond (*Prunus communis* Arc.). The most characteristic symptoms of the disease, which is present in Greece[11], Turkey[12] and Afghanistan[13], is the formation of perennial cankers on trunks, branches, twigs and shoots. The cankers, which are slow in their development, begin as swellings of the bark, which crack open and become surrounded by swollen cortical tissue.

The development of the symptoms and the final appearance of the cankers suggest the possible involvement of phytohormones in the disease process of *P. amygdali.*

The present study has been undertaken to investigate the ability of *P. amygdali* to produce plant growth substances in vitro. Here we report on the isolation and characterization of 6-(4-hydroxy-3-methylbut-2-enylamino)purine (*trans*-zeatin) and indole-3-acetic acid from cultures of *P. amygdali. P. amygdali* strain NCPPB 2610 was grown at 20 °C in Woolley's[14] medium supplemented with 1.5% peptone (Difco). After 5 days of incubation in shake culture the cells were removed by centrifugation (5000 g, 10 min) and filtration (Millipore, 0.45 µm). The culture filtrate (6.5 l) was lyophilized and the residue redissolved in one tenth of the original volume of distilled water. The pH of the solution was adjusted to 2.5 using HCl 1 N and extracted four times with an equal volume of ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure; the final product was an oily residue. The crude extract analyzed by TLC (silica gel Merck, Kieselgel F_{254}, 0.25 mm, chloroform-methanol, 6:4; chloroform-ethyl acetate-methanol, 2:2:1, v:v:v) showed several bands which reacted positively to indole and indole-3-substituted derivative staining reagent[15,16]. The most intensely stained zone correspond to IAA.

To purify the free IAA the residue was fractioned on a Sephadex LH-20 (Pharmacia, 25-100 µm) column, using chloroform-methanol (6:4, v:v) as an eluent system. The fractions containing IAA (**1**) were pooled and evaporated under reduced pressure, then further purified by TLC (silica gel, chloroform-ethyl acetate-methanol, 2:2:1, v:v:v). The R_f region from the chromatogram corresponding to IAA was scraped off, eluted with methanol and evaporated to dryness under reduced pressure. The purified compound **1** obtained as an oily residue (54 mg corresponding to 8.3 mg/l), when analyzed by TLC (silica gel) and High Performance Thin Layer Chromatography (HPTLC) (silica gel Merck, Kieselgel F_{254} 0.20 mm) using chloroform-methanol (6:4, v:v); chloroform-ethyl acetate-methanol (2:2:1, v:v:v) or *n*-butanol-acetic acid-water (60:15:25, v:v:v) as solvent systems, co-chromatographed with authentic IAA (Fluka A.G.).

The ^{1}H-NMR (table) and UV (MeOH) spectra of **1** were also identical to those of IAA used as a reference compound and consistent with the data reported for indole-3-substituted derivatives[17,18].

The chemical nature of **1** was confirmed by its conversion into the corresponding methyl ester (**2**), by treatment of **1** with ethereal diazomethane. The derivative **2** when analyzed by TLC (silica gel, chloroform-*iso*-propanol, 95:5, v:v), exhibited the same R_f value also by co-chromatography of the methyl ester prepared performing the same reaction on an