Corydoras aeneus : A Diploid - Tetraploid Fish Species Complex

by

Nanette Diffoot

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Zoology

APPROVED:

Dr. Bruce Turner, chairman

Dr. David West

Dr. Richard Burian

May 23, 1986

Blacksburg, Virginia

<u>Corydoras</u> <u>aeneus</u> : A Diploid - Tetraploid Fish Species Complex

by

Nanette Diffoot

Dr. Bruce Turner, chairman

Zoology

(ABSTRACT)

<u>Corydoras</u> <u>aeneus</u> is an armoured catfish found in the upper amazon regions of South America. It is a member of the family Callichthyidae. Studies done with this species of fish showed that <u>C.</u> <u>aeneus</u> is a tetraploid with a chromosome number of 132 (Scheel et al., 1972). Dunham et al. (1980) reported 120 chromosomes. The <u>C.</u> <u>aeneus</u> used in this study were bought from direct importers. We had four samples of fishes supposedly coming from Brazil (Belem), Guyana, Peru and Trinidad. During our initial studies in an attempt to karyotype <u>C.</u> <u>aeneus</u> we came across individuals with a highly reduced chromosome number. A diploid form of <u>C.</u> <u>aeneus</u> was discovered. Only those fishes from our Belem sample were diploid. A comparison of the diploid and tetraploid forms was done. Both forms were karyotyped, the tetraploid form of <u>C.</u> <u>aeneus</u> has 134 chromosomes and the diploid has 56. Physically both forms looked exactly the same. Morphometric as well as meristic data was collected from 131 fishes and analyzed by multivariate, discriminant and contingency chi- square analyses. The results obtained do not suggest any absolute morphological differences between the diploid and the tetraploid forms anymore than between tetraploids.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

POLYPLOIDY

An immediate consequence of the duplication event in a polyploid organism is an increase in nuclear DNA. Cavalier - Smith (1978) argues that autopolyploidy in protozoa, plants and animals may commonly result from selection for large cell size. He argues that selection for optimum nuclear and cell size can explain all the observed variations in polyploidy. An increase in nuclear DNA is usually accompanied by an increase in cell size.

All genes are duplicated in a new autotetraploid; it has four doses of every gene. The four homologues eventually diverge into two independently inherited pairs of chromosomes. Ferris and Whitt (1977) suggest that following gene duplication many of the copies are silenced early in evolution. Those duplicate genes which remain expressed are now available to diverge in structure and acquire new functions. Haldane (1933) suggested that one of the duplicate loci may become non-functional through the accumulation and eventual fixation of deleterious mutations at one locus while the other locus continues to perform the original function. Pseudogenes are a potential example of this phenomenon (Fritsch et al. 1980).

In catostomid fishes, retention of duplicate gene expression has been associated with species which are conservative at the morphological level (Ferris and Whitt, 1977). Phenotypically advanced organisms are expected to lose more genetic information. Hinegardner and Rosen (1972) suggest that highly specialized fish tend to have less DNA per cell than the more generalized or less evolved fishes of the same phyletic grouping. After an initial DNA increase by polyploidy the first stage of evolution will be the loss of the expression of redundant genes. Then over a longer period the DNA which is not expressed is physically eliminated (Ferris and Whitt, 1977). Functional diversification of the four original homologues so that one original linkage group is split into two separate linkage groups is necessary if the acquired state of tetraploidy is going to contribute to the creation of new gene loci. Preferential formation of two separate bivalents instead of one quadrivalent is the prerequisite for diploidization. Structural heterozygosity must be created among the four original homologues (Ohno, 1970).

If disomic inheritance has been established recently or is still incomplete, the duplicated loci will be retained. Given sufficient time for evolution to take place, loss of the duplicate gene expression might occur. If both alleles are expressed in equal frequencies a null allele can be established by protecting itself from selection by having the normal allele at the other locus. Some loci may diverge structurally but their regulation remains the same. Other loci can diverge in their developmental or tissue specific expression (Allendorf and Thorgaard, 1984).

How important is polyploidy in evolution? Stebbins (1977) and White (1978) state that duplicate genes reduce the chances of evolving new genetic types. The replacement of an allele shared by two disomic loci or a single tetrasomic locus is a much longer process than is replacing an allele at a single disomic locus (Allendorf and Thorgaard, 1984).

Ohno (1970) says that gene duplications are absolutely essential for "progressive evolution". He sees evolutionary change as being caused by the addition of genes with new functions. This can only occur following gene duplication so that one gene can diverge and carry out a new function while the other gene maintains it normal product.

Ohno (1970) has hypothesized that one or more rounds of polyploidy have been important in the early formation of the chordates and that this explains in part the great diversity of related isozymes present in vertebrates today.

Polyploidy has been extensively studied in two families of fishes, the catostomids and salmonids. The catostomids seem to have arisen through a polyploid event which occurred fifty million years ago and the salmonids through a much more recent tetraploid event.

Salmonids

Nelson (1976) described three major salmonid taxa. They apparently share a common tetraploid origin. Ohno and his coworkers (Ohno 1968, 1970, 1974) when studying the evolution from fishes to mammals by gene duplication proposed that salmonids were tetraploids. The evidence presented by Ohno and coworkers is the following (Ohno, 1970):

1. Salmonids have 60-90% DNA of that of mammals.

2. They have about twice the DNA per cell as fish species in related families. *Thymallus thymallus* has 60% as much DNA per cell as mammals.

3. Multivalent formation at meiosis has been observed in various species of salmonids.

4. A high incidence of duplicated enzyme loci are observed.

Using two different pairs of duplicated LDH loci produced since the duplication event Lim et al. (1975) found an amount of divergence similar to that between two homologous lactate dehydrogenase genes in species that have been separated an estimated one hundred million years (Allendorf and Thorgaard, 1984).

The tetraploid event seems to have been the result of a single autotetraploidy step in a common ancestor of all salmonid fishes (Allendorf and Thorgaard, 1984). Rediploidization of the chromosomes in salmonids does not appear to be complete, as shown by multivalent formation observed at meiosis (Ohno, 1970). Duplicate pairs of loci are still observed with no evidence of divergence. Some of these loci show tetrasomic inheritance (May et al., 1982).

Around 70% of fish species have chromosome numbers of 2N=44 to 52 (Gold et al.,1980). These are predominantly acrocentric or subtelocentric. The diploid ancestors of salmonids probably had around 48 acrocentric chromosomes, the tetraploid having 96. In fact most salmonids have around 100 chromosome arms and diploid chromosome numbers between 56 and 84. The chromosome arms have been conserved at 100, yet the chromosome number has reduced by centric fusions (Allendorf and Thorgaard, 1984).

Salmonids have been shown to tolerate aneuploidy as well as triploidy (Davisson et al.,1972; Ohno, 1972; Cuellar and Uyeno,1972; Thorgaard et al., 1981). Cuellar and Uyeno (1972) found a triploid individual among eighteen specimens of _Salmo gairdneri_. The diploid had 2N=60 and the triploid 3N=90. They suggest that this was probably due to supression of the second meiotic division. The fact that salmonids can be triploid and be viable suggests that an ancestral salmonid might tolerate a polyploid step (Allendorf and Thorgaard, 1984).

Catostomids

The family Catostomidae (suckers) comprises twelve genera and about sixty species of fishes confined to freshwaters of North America and eastern Asia. Though the catostomids may have originated in Asia, they did not radiate there perhaps because of competition from the diverse array of cypriniformes characteristic of that continent (Ferris, 1982).

Indications are that the group evolved from an ancestor similar to the minnow family Cyprinidae (Uyeno and Smith, 1972) . Uyeno and Smith (1972) showed that species of catostomids have twice the number of chromosomes and cellular DNA content of related families of Cypriniformes. Using fossil evidence they concluded that the family descended from a single tetraploid event about 50 million years ago. During the fifty million year of post-polyploid evolution the catostomids have returned to a functional diploid state at over half the enzyme loci examined (Ferris and Whitt, 1977). Sufficient time has elapsed for many changes to occur at the structural level of the genes (Ferris and Whitt, 1976).

Uyeno and Smith (1972) were the first to describe the karyotypes of suckers. At the chromosome level, all genera have conserved a tetraploid karyotype with 2N=100 compared with 2N=50 for most cypriniformes. In addition, the DNA content of catostomid species is twice that of typical diploid fishes (Ferris and Whitt, 1976). No cyprinids or catostomids have chromosome numbers intermediate between 50 and 100 (Ferris and Whitt, 1972), but three asian cyprinids similar in size and habitats to catostomids possess double the chromosome number and up to two times the DNA of other cyprinids. These are Cyprinus carpio (carp), Carassius auratus (goldfish) and Barbus barbus (Uyeno and Smith, 1972).

The apparent doubling of the chromosome number and DNA content of cells of catostomid fishes and the lack of intermediate numbers suggests that the catostomid karyotype evolved by tetraploidy from a cyprinid-like ancestor with 2N=50 chromosomes (Ferris, 1982).

Since the time of polyploidization the average catostomid has functionally lost approximately 53% of its duplicate genes with a range of 35-65% (Ferris and Whitt, 1976). This moderately rapid loss of the expression of redundant genes (Ferris and Whitt, 1976) appears to be the first stage of evolution. The next step is the physical elimination of this unexpressed DNA.

Species of the primitive subfamilies have retained more functional duplicate genes than those of the "advanced" subfamilies (Ferris and Whitt, 1982). Species conservative at the morphological level are conservative with respect to the retention of duplicate gene expression.

The polyploidy event was apparently the result of allopolyploidization (Ferris, 1982). Hybridization is a common phenomenom among cypriniform fishes (Hubbs, 1955) and is also known in the catostomids. Dauble and Buschbom (1981) present evidence of hybridization between sympatric <u>Catostomus</u> <u>macrocheilus</u> and <u>Catostomus</u> <u>columbianus</u>.

Catostomids seem to have had a rapid change to disomic inheritance (Ferris and Whitt, 1978). Changes in the presumed regulatory genes and charge substitutions in most duplicate isozymes giving a different electrophoretic mobility, suggest that protein divergence has indeed occurred to a considerable extent in catostomids (Ferris, 1982). Changes in relative expression of duplicates both within and among tissues have been shown to occur. Ferris and Whitt (1979) showed that the majority of duplicates are differentially expressed among tissues. They are discriminated by regulatory genes.

The duplicate loci retained will continue to diverge structurally and in their tissue regulation. Eventually, many are expected to become distinct. Those duplications expressed in equal proportions in many tissues should show ever increasing tissue specificity of one of the duplicates. At the DNA level a gradual loss is expected. Fossil data suggest that the Cobitidae are almost as old as the Catostomidae. Asian fossils of cyprinids from the Paleocene and catostomids from the Eocene indicate separate histories spanning at least fifty million years. (Uyeno and Smith, 1972). Several species of Cyprinidae and several cobitid loaches are polyploids (Hitsumachi et. al., 1969). Twenty enzyme loci among four species of loaches have been used to examine the extent of gene duplication at each locus (Ferris and Whitt, 1977). _Botia macrancatha_ and _Botia modesta_ are tetraploids, and their genes have already begun to diverge in their structure and regulation (Ferris and Whitt, 1977). The number of chromosomes in _B. macrancatha_ is 2N=100 as opposed to 2N=50 in the diploid loaches.

Ueno and Ojena (1976) showed that three species of Cobitid fishes, _Cobitis biwae_, _C. taenia taenia_ and _C. taenia striata_ had different chromosomal forms. _C. biwae_ had two types of chromosome number, 2N=48 and 2N=96. _C. taenia taenia_ had 2N=50, 2N=86 and 2N=94. _C. taenia striata_ had 2N=50 and 2N=98 chromosomes. They suggest that a diploid- tetraploid relationship might have existed in these species.

Corydoras

The genus _Corydoras_ is a member of the family Callichthyidae (armoured catfishes). Around 80 species have been described, and new catfishes seem to be making their appearance almost every year. Their systematics is not well understood. _Corydoras_ is restricted to the upper Amazon regions of South America.

Most of the species described by Nijssen (1970) have been based on the pigmentation. _Corydoras_ is known for a wide diversity of color patterns. Strauss (1985) points out that it is difficult to quantify and make objective comparisons. Nijssen (1970) thinks that when you work with fishes with such remarkable color patterns it is valid to assign taxonomic value to these because the color patterns and variation correlate with the geographical distribution of the species. The color pattern is remarkably constant in each species, and the intensity of pigmentation depends on the local nature of the bottom (Nijssen, 1970).

The ground color of _Corydoras_ is tan or greyish. Black and brown pigments are often present on the body, head, fins and barbels. The area between the coracoid processes is white. Small spots may occur on the head, body and fins. These may form vertical bars on the caudal fin. Black or brown pigment may form a stripe near the junctions of the lateral body scutes and near the dorsal fin region (Nijssen,1970). C. aeneus has a ground color of pale yellowish brown. It has a greyish head, paler ventral to the eye level. A dark blotch covers the anterior part of the opercle. The dorsal part of the body is a dark bluish grey from head to the base of the caudal fin and extends to the uppermost parts of the ventrolateral body scutes. The dorsal adipose and caudal fins are reddish brown, the rest of the fins are tan. (Nijssen, 1970).

Electrophoretic analysis shows interspecific variation in Corydoras (Scheel et. al.,1972). In terms of their genetic constitution Corydoras has among the highest karyotypic variability for any genus of fish thus far examined (Gold et al.,1980). Hinegardner and Rosen (1972) as well as Scheel et al. (1972) have suggested that several species of Corydoras are tetraploids. These are Corydoras aeneus and Corydoras julii, with a N=66 and 46 respectively (Scheel et. al., 1972). Dunham (1980) reported N=60 for Corydoras aeneus. The percentage of duplicate genes expressed in the presumptive tetraploid C. aeneus is 37.5% (Dunham et. al., 1980).

The haploid chromosome number in Corydoras ranges from 24 to 60 (Dunham et al., 1980). This is consistent with the presence of diploid as well as tetraploid species in the genus. C. aeneus and C. julii are both tetraploid. Hinegardner and Rosen (1972) state that all the close relatives of Corydoras have a lower DNA content than this genus. They propose that Corydoras evolved from a recent ancestor in which the DNA content was suddenly increased.

An increased cellular DNA content correlates with an increased percentage of duplicate genes in Corydoras (Dunham et al., 1980). Corydoras species have haploid DNA ranging from 2.3 to 4.4 pg per cell (Dunham et al., 1980). There does not seem to be a relationship between cellular DNA content and the generalized versus specialized condition. This family of catfishes is an exception to the general trend that the more generalized species within a group have a higher cellular DNA content than more specialized members of that group. Dunham et al.(1980) also point out that variation in cellular DNA content among species of Corydoras is not accompanied by corresponding morphological variation. Dunham et al. (1980) suggest that this lack of morphological differentiation is consistent with the idea that the genus has undergone a rapid evolutionary radiation. The fact that C. aeneus and C. julii have a high DNA level with an approximate doubling of chromosome number suggest a recent tetraploidization event. (Dunham et al., 1980).

In an attempt to karyotype C. aeneus we came across specimens with a sharply reduced chromosome number, these were presumptive diploids previously unreported. This study we attempt to assess the relationship relationship between the diploid and tetraploid forms of C. aeneus.

METHODS

The fishes used in this study were purchased from direct importers, so we do not know their precise localities in South America. The samples came from Belem (Brazil), Guyana, Peru and Trinidad. Of the 134 fishes used in the study, 45 were from Belem, 41 from Guyana, 12 from Peru and 36 from Trinidad.

CHROMOSOME PREPARATION

Chromosome spreads were prepared by the procedure of Kligerman and Bloom (1977). The chromosome spreads were photographed by a Nikon FA camera mounted on a Nikon microscope at 1000X. The best spread from each locality was used to construct its respective karyotype.

MORPHOMETRIC ANALYSIS

Morphological criteria are commonly used to delineate species in *Corydoras*. Nijssen and Isubrucker (1970) did an extensive morphometric analysis of 5211 specimens of the genus *Corydoras*, reviewing over 100 species and describing 7 new species. Using this review as a guide, 131 specimens of *Corydoras aeneus* were analyzed morphometrically. Four populations were represented and 16 variables were studied, including 11 measurements of anatomical features and 5 direct counts.

The measurements taken were the following : least depth of the caudal peduncle (DCP), length of the bony orbit (LBO), head length (HL), standard length (SL), snout length (SN), distance between the snout and articulation point of the dorsal spine (SND), distance between the snout and articulation point of the pectoral spine (SNP), length of the least width of the interorbital (WI). (See figure 1 for a schematic presentation of these measurements .) All the measurements were taken in millimeters using a dial caliper.

The first 23 specimens analyzed had 5 replicates of each measurement, due to the lack of previous experience in carrying out this task. The rest of the specimens had 2 replicates. All the measurements were taken in millimeters using a dial caliper.

Since more than one observation was taken for each measurement a mean was calculated for each variable. To minimize any potential size effect the data were transformed by dividing the measurements bd, hl, lds, snd, sop into the standard length (sl) and dcp, lbo, sn and wi into the head length (hl).

The counts done on each specimen of _C. aeneus_ were: the number of dorsal body scutes (DBS), the number of ventral body scutes (VBS), number of pre-adipose scutes (PAS), number of caudal fin rays (C) and the number of dorsal fin rays (D). (See figure 2 for a schematic representation of these counts).

For statistical analysis the measurements and counts were treated differently. A contingency chi square test was used for the counts while various tests were used to analyze the measurements. These were the following:

Test for Normality

Normality was tested at the univariate level. Conclusions were made on multivariate normality from these tests, since univariate normality should imply multivariate normality.

Since our sample size is less than 50 the Shapiro Wilk Statistic (SAS Institute, 1985), was used to test univariate normality of the measurements. Our null hypothesis is that our data are normally distributed. If our alpha value is less than .10 we reject the null hypothesis and accept that our data are not normally distributed. This test was performed on the 11 variables of the four localities. In support of the test these plot options were done.

1. Stem and Leaf

    This plot constructs a histogram using the values of the variables. By looking at the plot we can see if the data follow a bell shaped curve characteristic of a normally distributed population or if they are skewed.

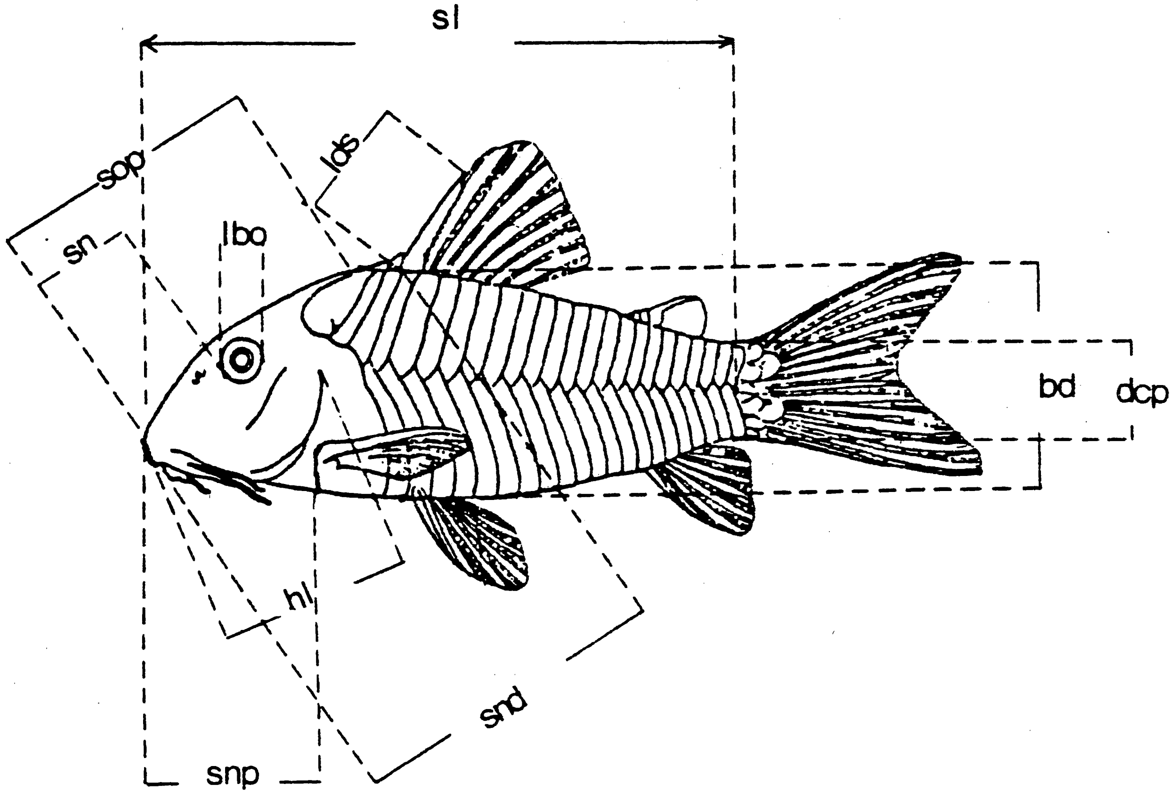

Figure 1. Variables Measured on Corydoras aeneus: The measurements taken on each fish of C. aeneus were the following, bd: body depth, dcp: least depth of the caudal peduncle, hl: head length, lbo: length of the bony orbit, lds: length of the dorsal spine, sl: standard length, sn: length of the snout, snd: distance between the snout tip and the junction of the bases of the predorsal scutes and the dorsal spine, snp: distance between the snout tip and the articulation point of the pectoral spine, sop: distance between the snout tip and the posterior edge of the occipital process, wi: least depth of the interorbital (not shown in the figure). All the measurements were taken in millimeters using a dial caliper.

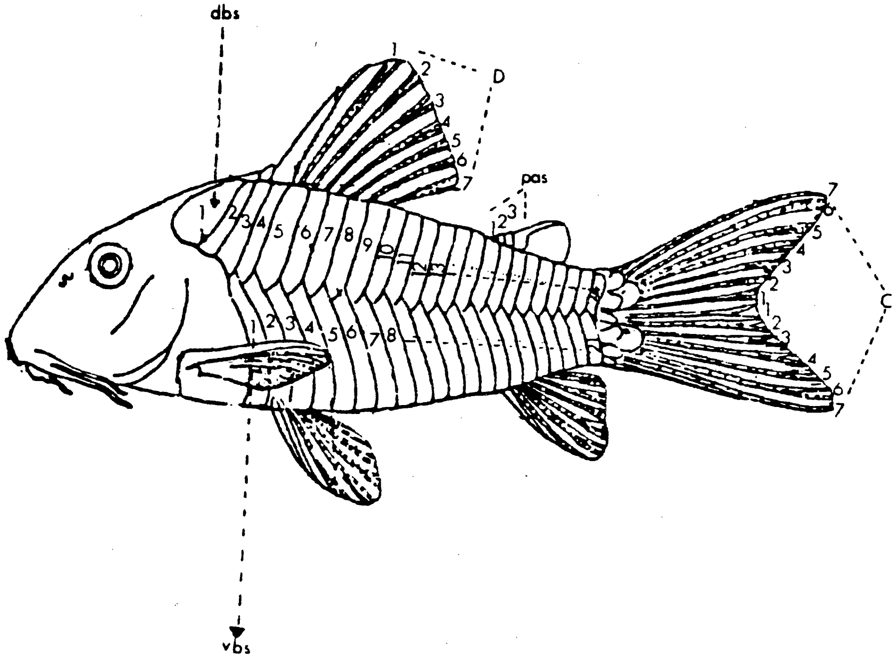

Figure 2. Counts Taken on Corydoras aeneus: Five counts were done on each fish. These were, C: number of caudal fin rays, D: number of dorsal fin rays, dbs: number of dorsal body scutes, pas: number of pre-adipose scutes, and vbs: number of ventral body scutes.

2. Box Plot

The values for each one of the measurements are organized into a box. This box contains 50% of the values. The median is identified by a line running across the box and the mean by a + sign. Leaving both ends of the box we will observe two lines which end with the smallest and largest value of the variable.

3. Normal Probability

This option plots the values of each measurement against the standard deviation for its respective variable. It gives some idea of how the data are distributed around their respective means.

Multivariate analysis

MANOVA Test

A multianalysis of variance was performed for check for heterogeneity among the four localities with respect to each one of the eleven variables measured. Our null hypothesis is that there is no heterogeneity among the four localities. If any test is significant for any one of the variables,we reject this hypothesis for the particular variable. We then say that there is significant heterogeneity among the four localities.

Discriminant Analysis (SAS Institute, 1985)

Assuming multivariate normality, its main objective is to classify the observations into two or more groups on the basis of one or more numeric variables by computing linear or quadratic discriminant functions.

The main purpose of the test is to predict. This test sets up four groups (one for each locality) based on the differences between localities in terms of the measurements taken. Then it predicts to which group each specimen belongs based on its measurements. It will give us an idea of how well morphological differences have been established between groups.

CANDISC Analysis (SAS Institute, 1985)

This discriminant procedure computes linear combinations of the variables which best summarize the differences among the classes and computes scores for each observation in the linear combinations. We can plot different combinations of the CAN values created by the analysis and see how each one of the localities is spaced relative to the others. The plots suggest how well separated the localities are from each other morphologically in terms of the variables measured.

RESULTS

KARYOTYPES

The correct chromosome number of Corydoras aeneus is not known. Scheel et al. (1971) reported 2N=132, while Dunham et al. reported 2N=120 with 4.4 pg of DNA content. Chromosome spreads of the tetraploid form of C. aeneus in our samples from Guyana and Peru have 134 chromosomes (Figure 3 and 4), but due to poor chromosome spreads from Trinidad the chromosome number remains to be determined. The presumptive C. aeneus from Belem had a uniform chromosome number of 56 (Figure 5 and 6). No tetraploids were detected in the Belem sample, and no apparent diploids in any of the others.

MORPHOMETRIC ANALYSIS

The morphometric data and the means and standard deviations are shown in table 1 and 2 respectively.

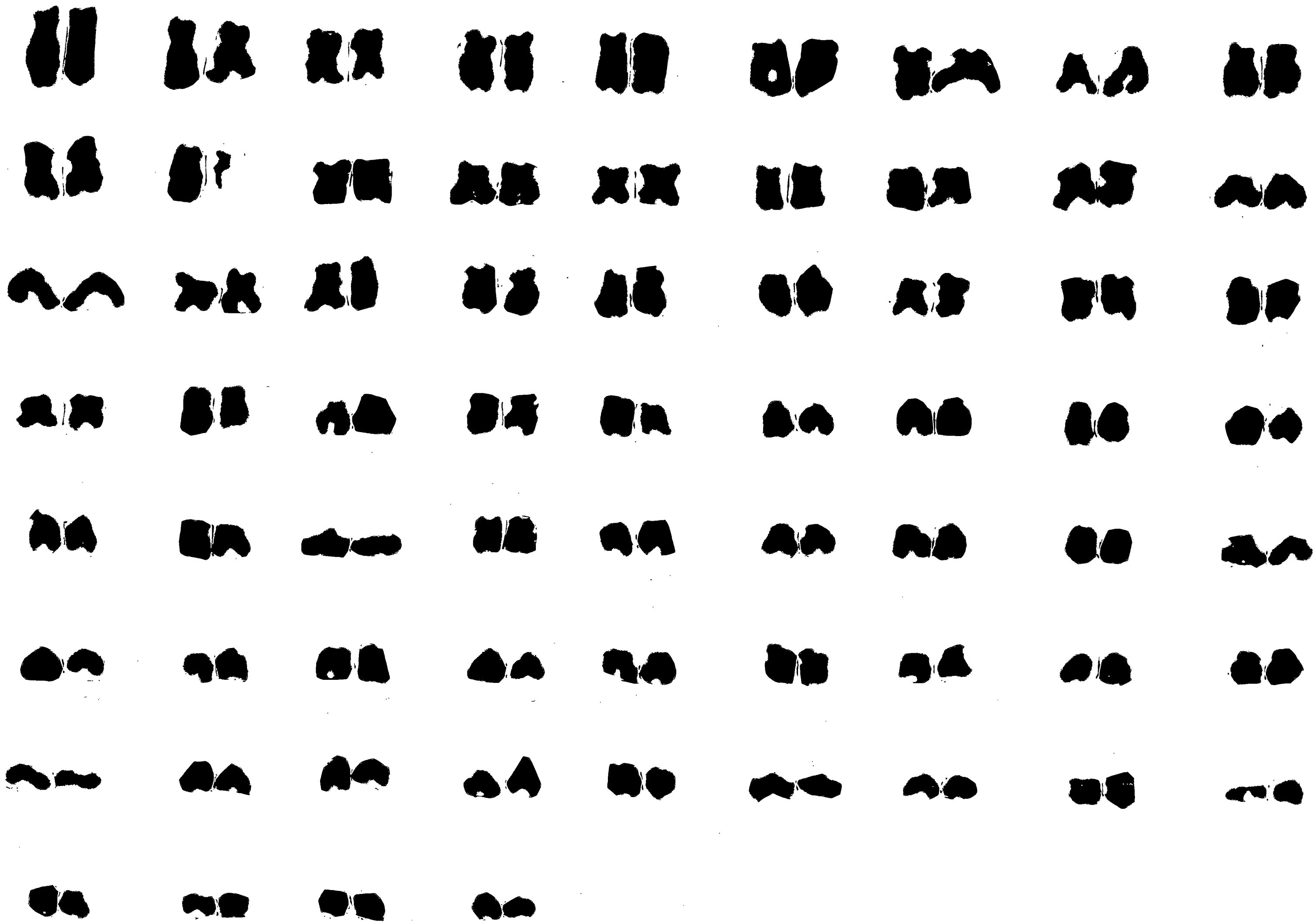

Figure 3. Mounted Karotype of a Female <u>Corydoras</u> <u>aeneus</u> From Peru

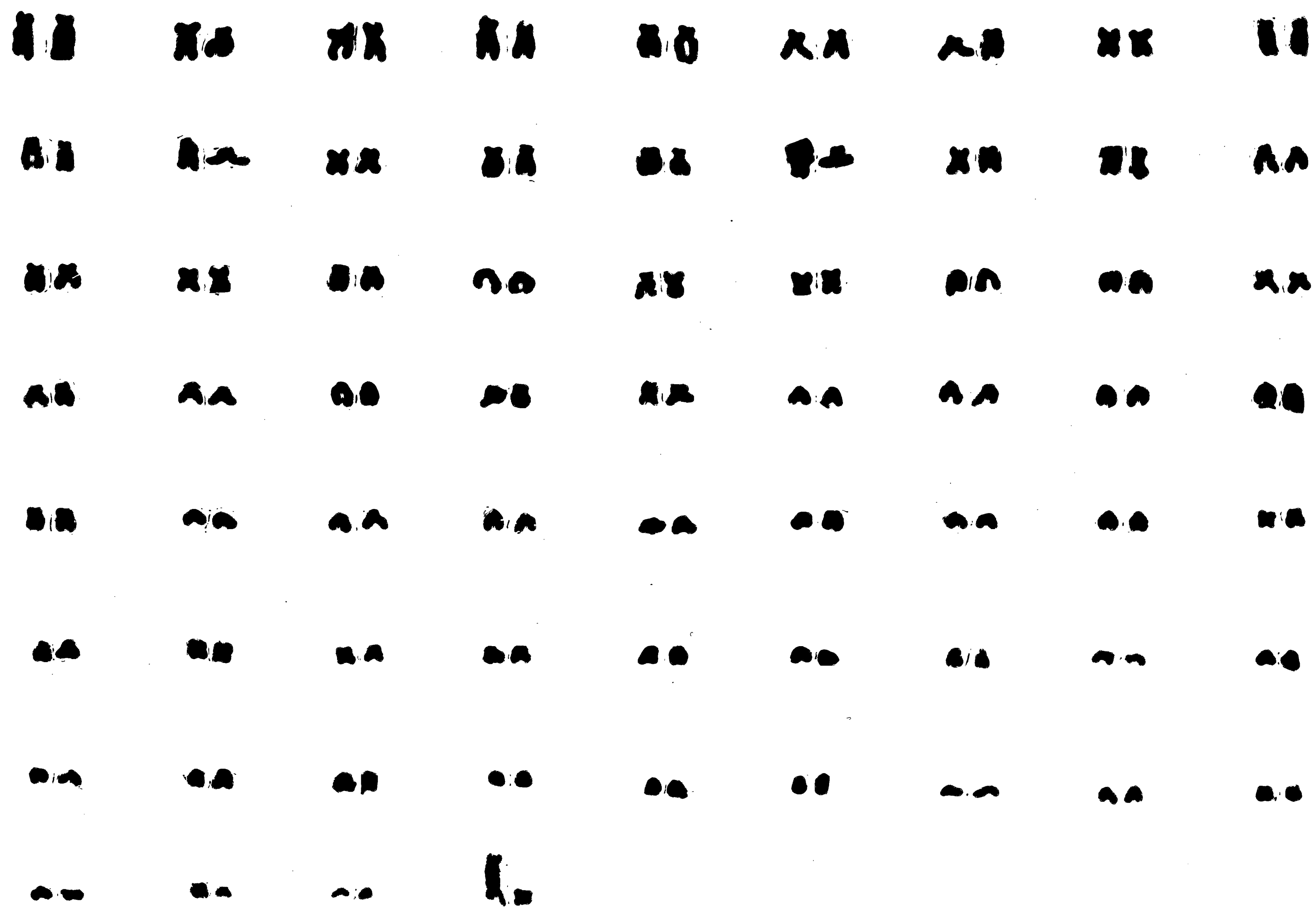

Figure 4. Mounted Karyotype of a Male Corydoras aeneus From Peru

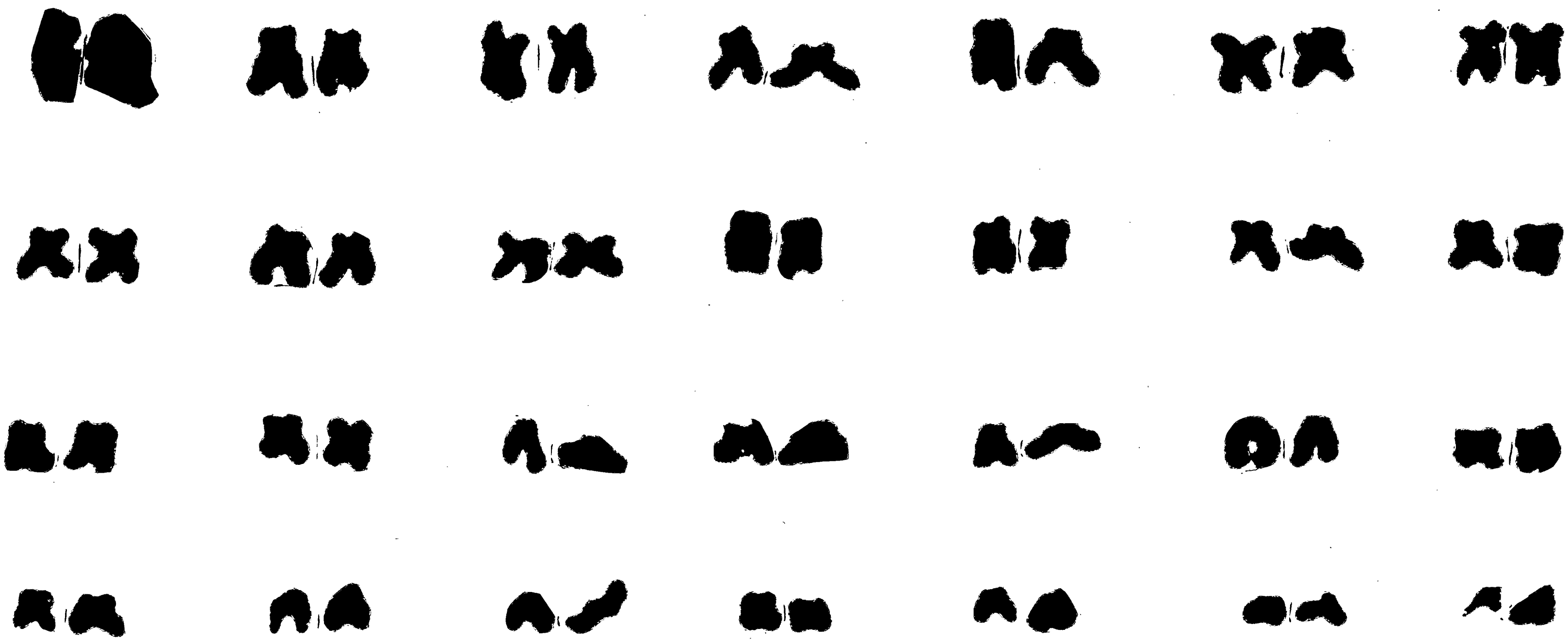

Figure 5. Mounted Karyotype of a Female Corydoras aeneus From Belem

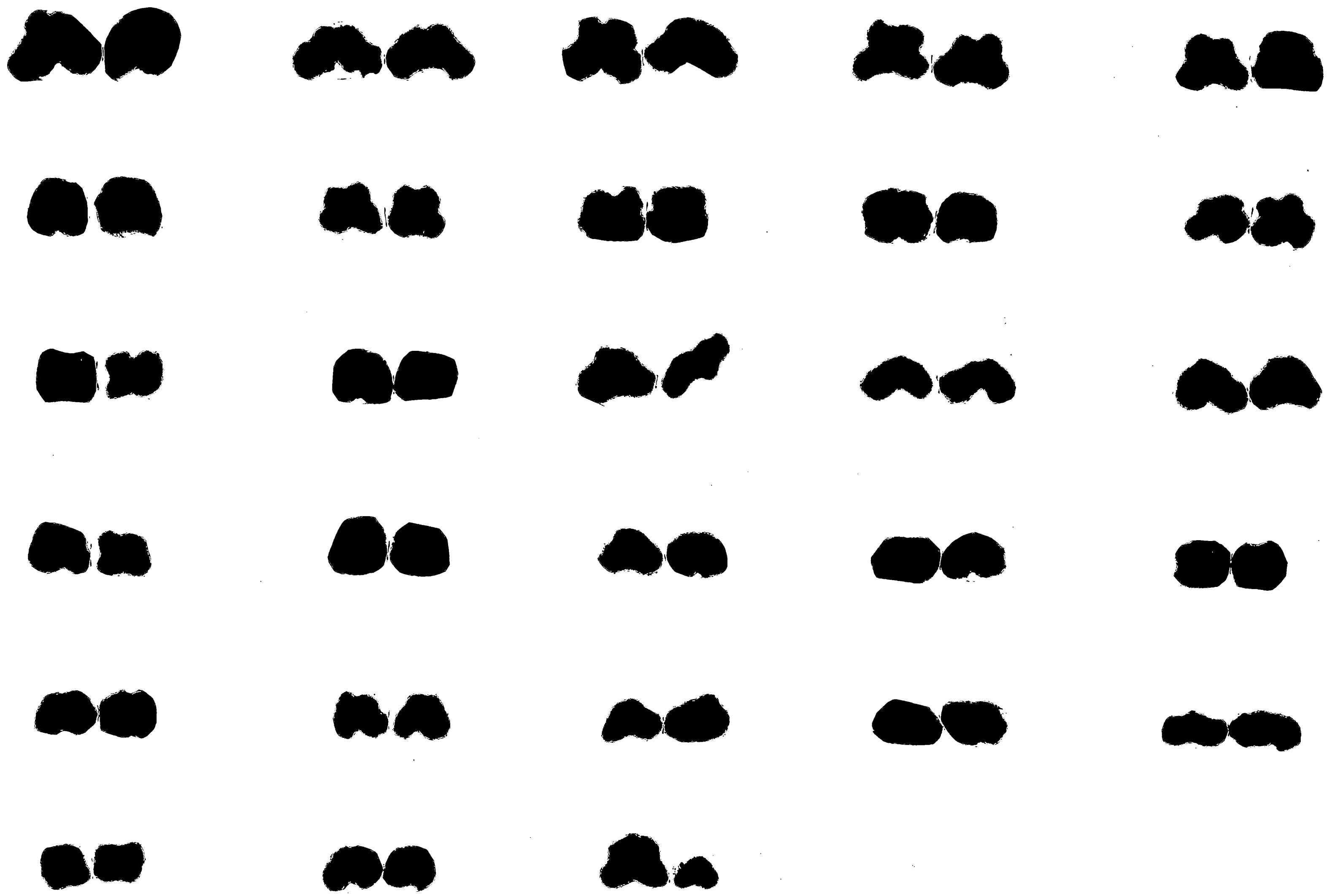

Figure 6. Mounted Karyotype of a Male Corydoras aeneus From Belem

Table 1. Results of the Measurements Taken on <u>C. aeneus</u>

The measurements BD, Hl, LDS, SND, SNP, and SOP were divided into the standard length (SL). DCP, LBO, SN and WI were divided into the head length (HL). For an explanation of the measurements see figure 1.

OBS	BD	HL	LBO	LDS	SL	SN	SND	SNP	SOP	DCP	WI	LOCAL
1	2.80	3.00	3.45	5.35	35.30	1.64	1.88	3.62	2.23	1.99	2.23	BELE
2	2.96	3.03	4.24	5.20	41.94	1.67	1.94	3.84	2.33	2.14	2.33	BELE
3	2.73	2.94	4.48	5.36	40.88	1.62	1.87	3.83	2.19	2.06	2.28	BELE
4	2.95	3.31	3.79	4.92	37.82	1.47	1.93	4.17	2.24	2.08	2.16	BELE
5	2.86	3.12	4.47	5.30	49.27	1.62	1.89	3.72	2.32	2.33	2.31	BELE
6	2.99	3.06	4.19	5.23	39.54	1.64	1.94	3.79	2.30	2.08	2.28	BELE
7	2.72	2.96	4.30	5.04	48.38	1.45	1.79	3.56	2.15	2.15	2.21	BELE
8	2.74	2.94	4.59	5.06	50.00	1.67	1.84	3.62	2.30	2.24	2.30	BELE
9	2.75	2.91	4.02	4.76	38.58	1.72	1.87	3.88	2.29	2.22	2.49	BELE
10	3.00	3.07	3.70	4.51	41.48	1.71	1.98	.	2.35	2.31	2.38	BELE
11	2.91	2.95	3.51	5.08	37.26	1.61	1.88	.	2.24	2.17	2.37	BELE
12	2.74	3.13	4.30	4.59	34.15	1.75	1.83	4.09	2.18	1.93	1.96	BELE
13	3.04	3.03	4.70	5.30	48.38	1.60	1.97	4.03	2.35	2.31	2.50	BELE
14	2.62	2.81	4.57	4.76	46.78	1.58	1.79	3.42	2.18	2.39	2.52	BELE
15	2.92	3.00	3.90	4.95	37.48	1.69	1.85	3.65	2.26	2.23	2.45	BELE
16	2.85	2.84	4.00	4.88	35.25	1.73	1.87	3.42	2.26	2.22	2.44	BELE
17	2.72	2.92	4.17	5.40	37.80	1.55	1.85	3.51	2.23	2.09	2.27	BELE
18	2.63	2.85	4.73	5.17	31.30	1.83	1.77	4.29	2.09	1.90	2.27	BELE
19	2.55	2.85	3.84	5.07	33.45	1.82	1.83	4.19	2.10	2.00	2.35	BELE
20	2.50	2.95	3.95	4.91	32.53	1.85	1.84	4.31	2.11	2.05	2.23	BELE
21	2.58	3.13	3.79	5.34	37.35	1.79	1.90	4.42	2.25	2.03	2.15	BELE
22	2.45	2.94	3.70	5.15	32.30	1.67	1.78	4.75	2.04	1.93	1.93	BELE
23	2.56	3.05	3.76	5.78	40.20	1.85	1.84	4.44	2.20	2.03	2.24	BELE
24	2.70	3.08	4.24	5.53	44.40	1.63	1.95	4.21	2.34	2.06	2.22	BELE
25	2.56	3.11	3.76	5.00	37.15	1.53	1.88	3.79	2.16	1.99	2.11	BELE
26	2.71	3.12	3.74	5.85	37.60	1.52	1.89	4.42	2.31	1.99	2.13	BELE
27	2.48	2.92	4.02	5.26	33.40	1.73	1.88	4.31	2.18	2.06	2.28	BELE
28	2.84	2.87	4.80	5.06	36.18	1.61	1.89	4.11	2.25	2.08	2.69	BELE
29	2.49	2.78	4.80	4.53	32.38	1.73	1.85	4.36	2.25	2.06	2.45	BELE
30	2.58	2.97	3.71	5.13	38.25	1.76	1.88	4.20	2.25	2.13	2.32	BELE
31	2.73	2.95	4.65	5.55	34.95	1.57	1.81	4.36	2.17	2.01	2.18	BELE
32	2.69	3.00	4.12	5.10	33.38	1.71	1.84	4.41	2.15	2.06	2.25	BELE
33	2.75	3.06	4.01	5.82	34.63	1.64	1.86	4.30	2.16	2.10	2.25	BELE
34	2.78	3.13	4.02	5.25	41.75	1.63	1.88	4.21	2.28	2.09	2.26	BELE
35	2.76	3.08	4.19	5.41	41.65	1.57	1.81	4.38	2.24	2.06	2.29	BELE
36	2.85	3.46	3.62	5.57	40.10	1.47	1.93	3.88	2.27	1.93	2.00	BELE
37	3.10	2.77	4.69	5.05	29.28	1.60	1.88	3.61	2.25	2.12	2.78	BELE
38	3.27	3.05	4.08	5.60	36.10	1.60	2.00	3.78	2.39	2.08	2.88	BELE
39	2.88	3.30	3.70	5.01	34.83	1.38	1.88	3.39	2.16	2.06	2.00	BELE
40	2.74	3.12	4.46	5.90	45.90	1.57	1.94	4.03	2.41	2.00	2.22	BELE
41	2.74	3.14	4.16	5.67	36.88	1.65	1.91	3.98	2.33	1.95	2.19	BELE
42	2.82	3.06	4.94	5.30	47.18	1.59	1.95	3.87	2.42	2.13	2.35	BELE
43	2.75	3.06	4.04	4.95	46.00	1.66	1.85	3.67	2.24	2.20	2.32	GUYA
44	2.88	3.07	4.01	5.27	46.16	1.65	1.92	3.61	2.28	2.32	2.19	GUYA

45	2.79	3.23	3.98	4.82	46.79	1.60	1.90	4.31	2.26	2.12	2.14	GUYA
46	2.96	3.19	4.31	5.27	51.10	1.66	1.96	4.14	2.40	2.46	2.30	GUYA
47	2.88	2.99	4.37	4.87	41.80	1.68	1.93	3.97	2.36	2.31	2.22	GUYA
48	2.88	3.03	4.24	4.86	44.92	1.52	1.91	3.60	2.30	2.34	2.34	GUYA
49	2.74	2.98	4.49	4.75	46.10	1.66	1.90	3.85	2.27	2.34	2.44	GUYA
50	2.79	2.97	4.37	4.78	46.10	1.59	1.84	3.74	2.27	2.30	2.38	GUYA
51	2.82	2.90	4.34	4.52	45.00	1.55	1.87	3.61	2.31	2.38	2.38	GUYA
52	2.89	3.03	4.03	4.61	44.23	1.62	1.86	3.84	2.33	2.18	2.24	GUYA
53	2.70	3.04	4.17	4.99	46.03	1.54	1.84	3.87	2.26	2.22	2.26	GUYA
54	2.86	3.28	3.80	5.07	47.67	1.61	2.00	3.89	2.41	2.08	2.25	GUYA
55	2.79	3.01	4.30	4.96	45.20	1.55	1.91	4.24	2.32	2.41	2.37	GUYA
56	2.83	2.94	4.17	5.12	45.98	1.60	1.86	3.51	2.25	2.39	2.34	GUYA
57	2.84	3.12	4.02	5.53	47.63	1.63	1.84	4.05	2.27	2.32	2.33	GUYA
58	2.82	3.05	3.93	4.96	45.23	1.64	1.85	3.82	2.26	2.22	2.28	GUYA
59	2.72	2.99	4.41	4.81	41.18	1.62	1.91	3.86	2.27	2.27	2.34	GUYA
60	2.91	3.08	4.48	5.52	50.00	1.57	1.92	3.59	2.42	2.33	2.34	GUYA
61	2.75	3.14	4.23	5.84	52.83	1.60	1.92	3.94	2.38	2.30	2.19	GUYA
62	2.86	2.99	4.38	5.79	52.25	1.58	1.88	3.37	2.37	2.32	2.46	GUYA
63	2.76	2.86	4.20	4.90	44.48	1.58	1.85	3.75	2.22	2.34	2.40	GUYA
64	2.71	3.00	3.93	4.65	42.83	1.61	1.83	3.97	2.24	2.13	2.26	GUYA
65	2.81	3.02	4.15	5.25	50.55	1.66	1.91	3.79	2.33	2.40	2.47	GUYA
66	2.94	3.07	4.23	4.72	45.10	1.63	1.93	3.77	2.40	2.21	2.39	GUYA
67	2.92	3.12	4.51	5.08	49.93	1.64	1.93	4.07	2.33	2.25	2.22	GUYA
68	2.87	3.12	3.87	5.18	46.23	1.59	1.85	4.24	2.31	2.23	2.11	GUYA
69	2.73	3.00	4.10	5.21	44.30	1.62	1.83	3.67	2.23	2.21	2.27	GUYA
70	2.73	3.04	4.05	4.57	50.23	1.63	1.92	4.09	2.32	2.26	2.19	GUYA
71	2.80	2.84	4.22	4.80	45.45	1.56	1.84	3.68	2.22	2.39	2.39	GUYA
72	2.75	2.80	4.27	5.26	43.10	1.63	1.81	3.67	2.22	2.46	2.33	GUYA
73	2.80	2.98	4.06	5.66	44.70	1.60	1.81	3.47	2.30	2.39	2.29	GUYA
74	2.76	2.88	4.20	4.59	42.93	1.64	1.86	3.94	2.23	2.36	2.40	GUYA
75	2.89	3.21	3.75	4.70	42.45	1.64	1.89	3.78	2.34	2.15	2.33	GUYA
76	2.74	2.81	4.29	5.40	45.20	1.62	1.76	3.74	2.13	2.40	2.31	GUYA
77	2.63	2.81	4.14	5.04	41.58	1.60	1.76	3.52	2.22	2.28	2.31	GUYA
78	2.79	2.98	4.24	4.78	47.60	1.65	1.86	3.82	2.24	2.32	2.33	GUYA
79	2.77	2.96	4.24	4.89	46.10	1.62	1.86	3.76	2.31	2.29	2.33	GUYA
80	2.71	3.01	3.93	5.05	46.18	1.61	1.85	4.01	2.31	2.19	2.30	GUYA
81	2.99	2.99	3.86	4.99	42.75	1.62	1.89	3.81	2.26	2.40	2.36	GUYA
82	2.84	3.11	4.11	5.34	51.10	1.57	1.96	3.83	2.38	2.22	2.32	GUYA
83	2.72	2.87	4.19	5.23	44.83	1.56	1.75	3.63	2.18	2.35	2.35	GUYA
84	2.93	3.50	3.24	4.64	42.09	1.94	1.90	2.96	2.26	1.76	1.91	PERU
85	2.93	3.50	3.23	4.64	42.09	1.94	2.00	4.14	2.37	1.82	1.86	PERU
86	2.81	3.19	3.23	4.32	39.10	1.79	1.91	4.33	2.22	1.97	1.96	PERU
87	2.94	3.36	3.14	4.86	44.00	1.89	1.92	4.14	2.31	1.99	2.10	PERU
88	2.91	3.43	3.47	5.11	44.56	1.67	1.95	3.63	2.38	1.91	2.10	PERU
89	.	3.49	3.37	4.81	39.75	1.53	1.86	4.64	2.20	1.88	1.84	PERU
90	2.88	3.06	3.95	4.94	41.98	1.75	1.98	3.85	2.34	2.19	2.18	PERU
91	3.03	3.03	3.93	5.37	42.83	1.65	1.95	3.68	2.32	2.17	2.17	PERU
92	2.84	2.85	4.22	5.13	40.95	1.64	1.86	4.06	2.17	2.20	2.28	PERU
93	2.87	3.15	3.62	4.93	41.05	1.74	2.02	4.14	.	2.14	2.14	PERU
94	2.85	2.91	4.27	4.91	42.85	1.64	1.86	3.49	2.22	2.23	2.27	PERU
95	2.89	3.19	3.80	2.52	45.15	1.72	1.99	4.64	2.41	2.10	2.08	PERU

96	2.82	2.83	4.00	5.15	37.33	1.62	1.83	3.58	2.15	2.18	2.36	TRIN
97	2.57	2.84	4.00	4.94	35.80	1.62	1.79	3.48	2.17	2.15	2.17	TRIN
98	2.63	2.82	3.81	5.04	33.80	1.63	1.86	3.58	2.25	2.16	2.30	TRIN
99	2.56	2.76	4.11	4.49	38.08	1.70	1.77	3.46	2.15	2.16	2.30	TRIN
100	2.70	2.93	4.40	4.92	39.95	1.65	1.82	3.58	2.22	2.05	2.10	TRIN
101	2.56	2.98	3.91	4.59	39.10	1.51	1.81	3.91	2.22	1.98	2.15	TRIN
102	2.61	2.96	3.97	5.02	41.15	1.70	1.82	3.99	2.19	1.98	2.14	TRIN
103	2.70	2.74	4.37	5.25	37.80	1.62	1.74	3.52	2.14	2.27	2.24	TRIN
104	2.64	2.84	4.14	5.04	38.18	1.67	1.80	3.52	2.21	2.26	2.25	TRIN
105	2.87	2.82	4.19	5.11	37.20	1.71	1.81	3.64	2.13	2.25	2.20	TRIN
106	2.61	2.79	4.03	4.84	33.70	1.69	1.83	3.65	2.16	2.10	2.09	TRIN
107	2.48	2.89	4.11	4.48	38.55	1.65	1.80	3.50	2.11	2.05	2.21	TRIN
108	2.69	2.89	3.84	5.08	34.95	1.81	1.86	3.94	2.18	2.15	2.15	TRIN
109	2.52	2.81	4.11	5.27	37.00	1.56	1.74	3.68	2.14	2.18	2.19	TRIN
110	2.66	2.99	4.17	5.07	39.30	1.63	1.84	3.96	2.23	2.06	2.19	TRIN
111	2.64	3.09	3.97	4.62	37.75	1.67	1.78	3.85	2.20	1.84	2.02	TRIN
112	2.66	2.97	4.33	4.86	42.50	1.71	1.86	3.74	2.30	2.17	2.10	TRIN
113	2.64	2.89	4.12	5.66	39.33	1.62	1.84	3.85	2.22	2.13	2.27	TRIN
114	2.71	2.82	4.12	4.80	36.00	1.64	1.80	3.79	2.18	2.12	2.30	TRIN
115	2.61	2.64	4.19	4.39	34.90	1.59	1.72	3.70	2.03	2.20	2.32	TRIN
116	2.74	2.75	4.14	4.89	34.23	1.64	1.78	3.91	2.13	2.30	2.32	TRIN
117	2.48	2.74	4.42	4.32	36.28	1.65	1.77	3.39	2.13	2.20	2.19	TRIN
118	2.47	2.65	4.24	4.83	37.05	1.61	1.72	3.74	2.04	2.40	2.34	TRIN
119	2.62	2.72	4.10	5.00	35.15	1.62	1.80	3.45	2.12	2.23	2.29	TRIN
120	2.67	3.20	3.61	5.09	38.78	1.48	1.91	.	2.27	2.00	1.96	TRIN
121	2.72	2.84	3.98	5.04	34.55	1.56	1.85	.	2.21	2.06	2.31	TRIN
122	2.65	3.14	4.29	5.14	43.45	1.68	1.86	4.37	2.25	2.01	2.14	TRIN
123	2.59	3.27	3.83	4.76	38.00	1.61	1.80	4.42	2.09	1.78	1.88	TRIN
124	2.64	3.02	3.59	4.92	33.60	1.50	1.76	3.65	2.16	1.98	1.96	TRIN
125	2.71	3.29	3.46	4.36	38.68	1.42	1.86	3.93	2.23	1.90	1.99	TRIN
126	2.69	3.27	4.03	4.26	36.25	1.50	1.80	4.17	2.12	1.85	2.02	TRIN
127	2.56	3.24	3.60	4.63	35.85	1.47	1.77	4.07	2.18	1.79	1.84	TRIN
128	2.66	2.74	4.15	4.51	31.58	1.62	1.82	3.71	2.16	1.84	2.01	TRIN
129	2.71	3.32	3.61	4.85	35.05	1.67	1.94	4.21	2.33	1.72	1.93	TRIN
130	2.84	3.24	3.82	4.83	39.58	1.47	1.89	3.70	2.24	2.01	2.04	TRIN
131	2.75	2.88	3.84	4.59	34.33	1.78	1.91	3.82	2.27	2.16	2.22	TRIN

Test for Normality

The results of the Shapiro-Wilk test are shown in table 3. Of the 44 normality tests 9 were significant at an alpha of .10. 80% of the data is normally distributed.

An example of the plot options done is shown in figure 3. Results correlate with the results of the Shapiro-Wilk test supporting the fact that most of our data give evidence of being normally distributed.

Box plots of the different variables for the locations were placed side by side for comparison (figure 4-14).

The results of these normality tests suggest that we have multivariate normality. We proceeded to do the multianalyses.

Table 2. Mean and Standard Deviation of the Variables Measured

VARIABLES	BRAZIL	GUYANA	PERU	TRINIDAD
BD	2.76(.178)	2.81(.080)	2.90(.060)	2.65(.093)
DCP	2.09(.114)	2.29(.094)	2.03(.162)	2.07(.162)
HL	3.02(.141)	3.01(.113)	3.22(.233)	2.93(.197)
LBO	4.14(.395)	4.16(.190)	3.63(.403)	4.02(.241)
LDS	5.21(.342)	5.04(.329)	4.68(.732)	4.85(.306)
SL	38.65(5.31)	46.09(2.93)	42.20(1.83)	37.08(2.60)
SN	1.64(.107)	1.61(.037)	1.74(.129)	1.62(.087)
SND	1.88(.055)	1.87(.054)	1.93(.057)	1.82(.052)
SNP	4.00(.344)	3.82(.215)	3.98(.485)	3.78(.259)
SOP	2.24(.087)	2.29(.066)	2.29(.081)	2.18(.066)
WI	2.30(.194)	2.31(.081)	2.07(.150)	2.15(.140)

( ) Standard Deviation

Table 3. Probability < W For The Populations of C. aeneus

The results of the normality tests for the variables measured are given below. W is the symbol for the Shapiro-Wilk normality test done. The numbers given are the probabilities obtained from the test for each variable within each locality. Any probability lower than .05 suggests that the data for the respective variable are not normally distributed.

Variable	bd	dcp	hl	lbo	lds	sl	sn	snd	snp	sop	wi
Brazil	474	.046	.095	.123	.537	.039	.690	.495	.199	.820	.028
Guyana	.711	.431	.448	.621	.139	.031	.449	.416	.731	.493	.518
Peru	.655	.345	.319	.245	.010	.896	.444	.366	.610	.540	.404
Trin.	.488	.386	.010	.197	.471	.837	.217	.620	.093	.939	.099

Multivariate Analyses

MANOVA Test

Multianalysis of variance was performed on each variable using all 4 localities. With 3 and 121 degrees of freedom every test was significant at an alpha of .05 (table 4). We reject the null hypothesis that the localities were not different from one another. Morphological differences appear to exist between the fish from the different localities.

```
VARIABLE=BD

               MOMENTS                                    QUANTILES(DEF=4)                           EXTREMES

N                42   SUM WGTS          42   100% MAX      3.27     99%      3.27          LOWEST    HIGHEST
MEAN        2.76286   SUM           116.04    75% Q3      2.865     95%     3.091            2.45       2.99
STD DEV    0.178015   VARIANCE   0.0316892    50% MED      2.74     90%     2.997            2.48         3
SKEWNESS   0.473535   KURTOSIS    0.417404    25% Q1     2.6275     10%     2.515            2.49       3.04
USS         321.901   CSS          1.29926     0% MIN      2.45      5%    2.4815             2.5        3.1
CV          6.44313   STD MEAN   0.0274682                           1%      2.45            2.55       3.27
T:MEAN=0    100.584   PROB>|T|      0.0001   RANGE         0.82
SGN RANK      451.5   PROB>|S|      0.0001   Q3-Q1       0.2375
NUM ^= 0         42                          MODE          2.74
W:NORMAL   0.970636   PROB<W         0.474
```

```
   STEM LEAF                     #      BOXPLOT
     32 7                        1         0
     32
     31
     31 0                        1         |
     30                                    |
     30 04                       2         |
     29 569                      3         |
     29 12                       2         |
     28 5568                     4      +-----+
     28 024                      3      |     |
     27 5568                     4      |  +  |
     27 0122334444              10      *-----*
     26 9                         1      |     |
     26 23                       2      +-----+
     25 56688                    5         |
     25 0                        1         |
     24 589                      3         |
     24
        ----+----+----+----+
   MULTIPLY STEM.LEAF BY 10**-01
```

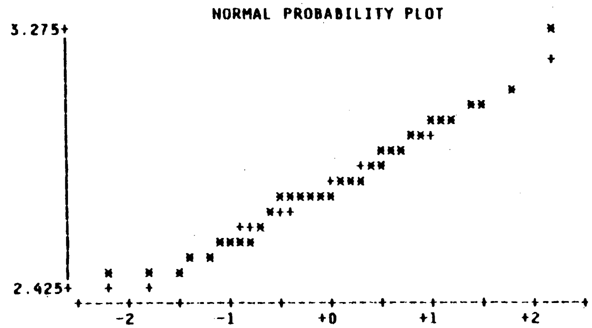

Figure 7. Normality Tests For Variable Bd Of The Fishes From Belem: These tests are an example of the normality tests done on each variable for each population. The first part gives you general information of the data and the results of the Shapiro-Wilk test (represented by W). In the stem and leaf plot a histogram is constructed using the data for the variable. To the right of the plot a column labelled number tells you how many values fall within each one of the intervals set. For an explanation of the box plot see figure 8. The normal probabilty plot plots each one of the values against standard deviation.

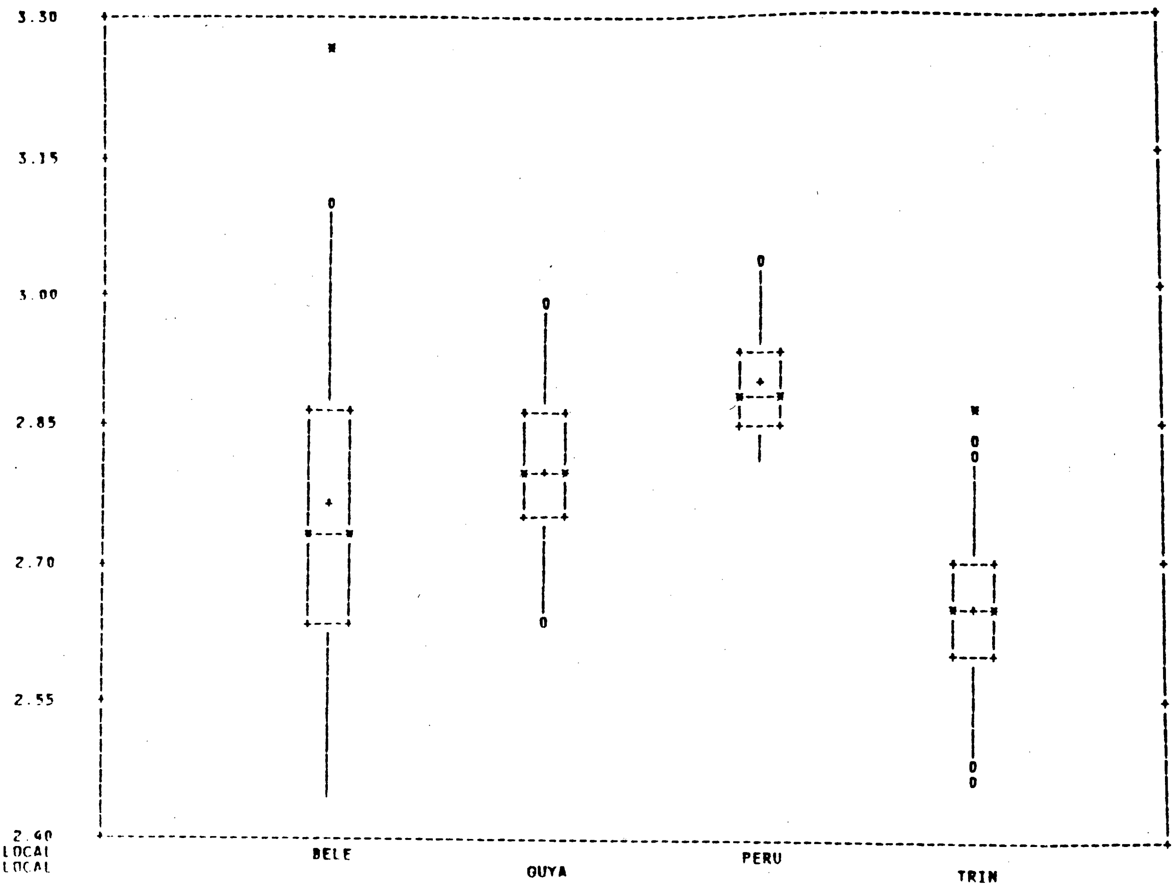

Figure 8. Schematic Plots For Variable BD : Four box plots are arranged side by side for comparison. Each box plot is a schematic representation of how the data is distributed. The box itself contains 50% of the data for the particular variable. The line running across the box plot represents the median. The median divides the box into two smaller boxes. These two smaller boxes do not have to be of similar size. The central vertical lines called "whiskers" extend from the box as far as the data extend. 0 represents a mild outlier and * represents an extreme outlier. For an explanation of the variables see figure 1.

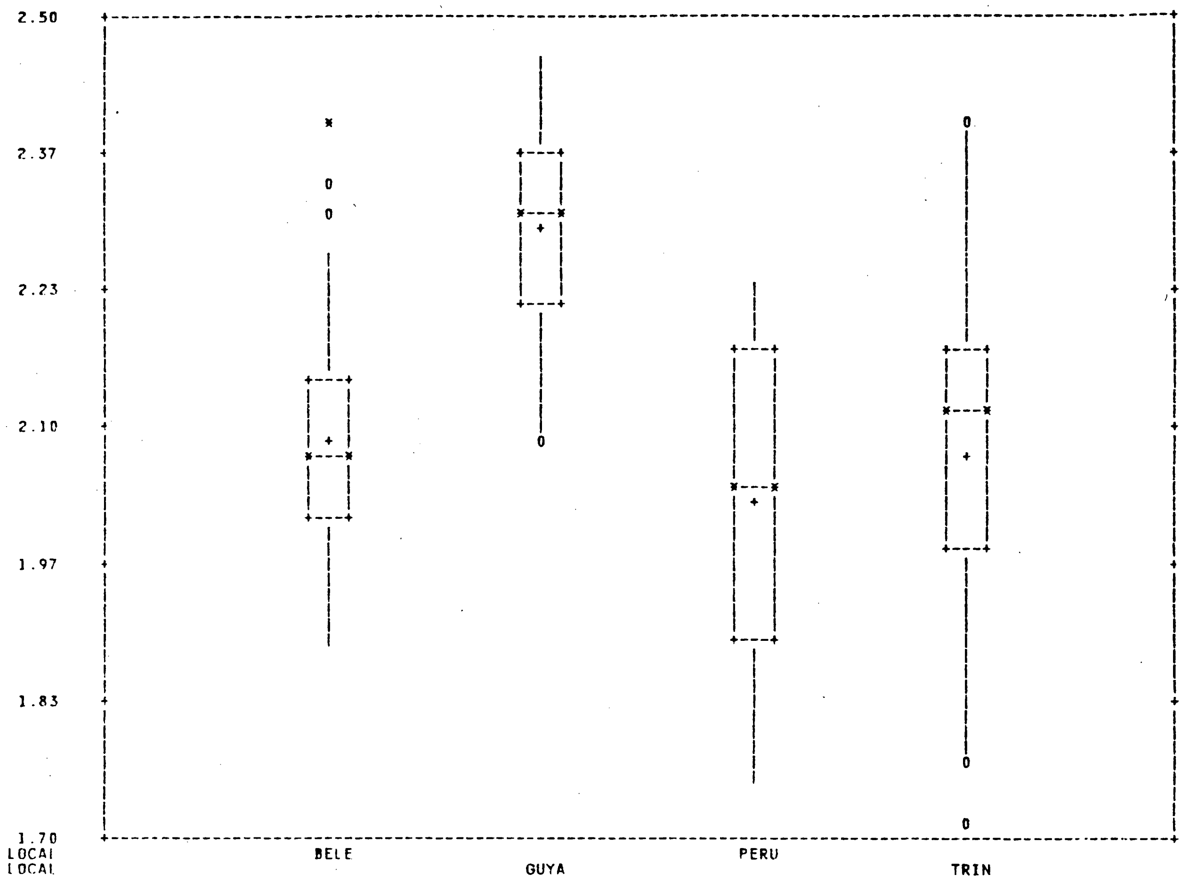

Figure 9. Schematic Plots For Variable DCP: For an explanation see figure 8.

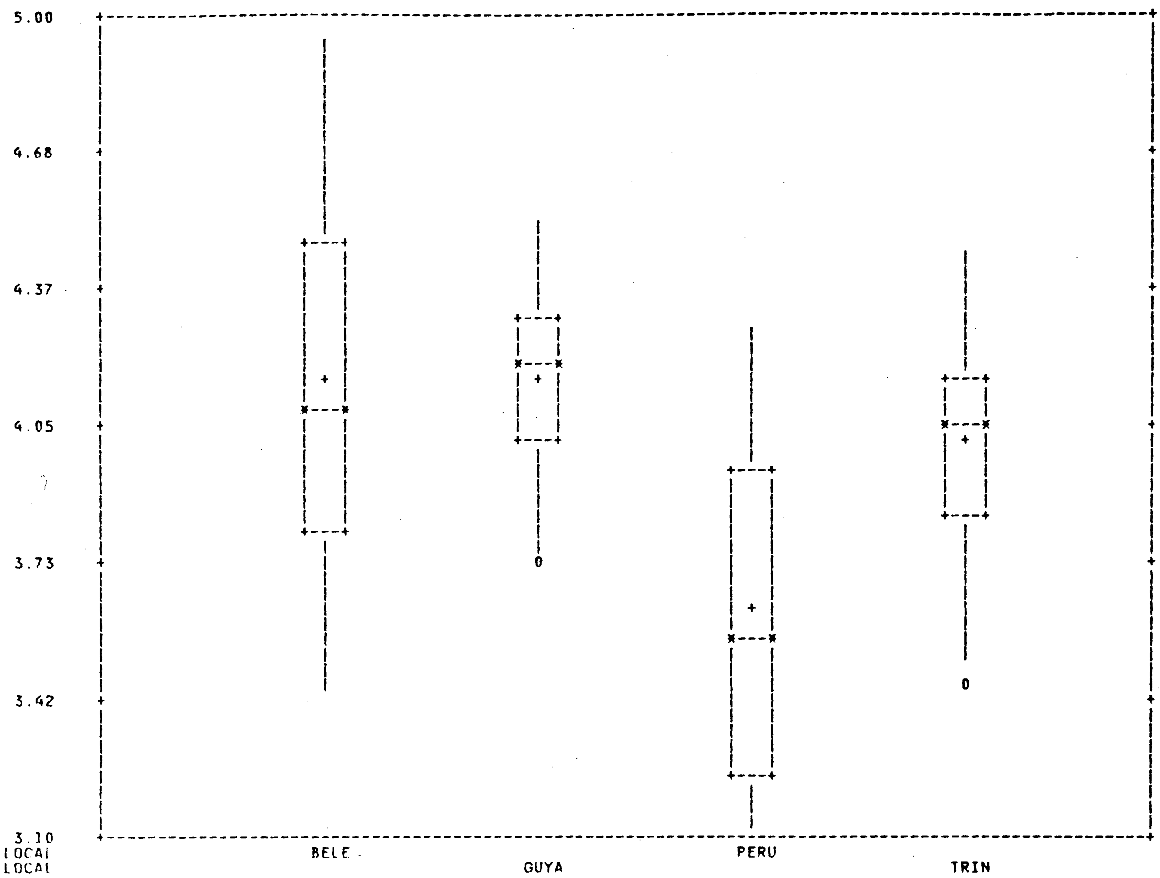

Figure 10. Schematic Plots For Variable LBO: For an explanation see figure 8.

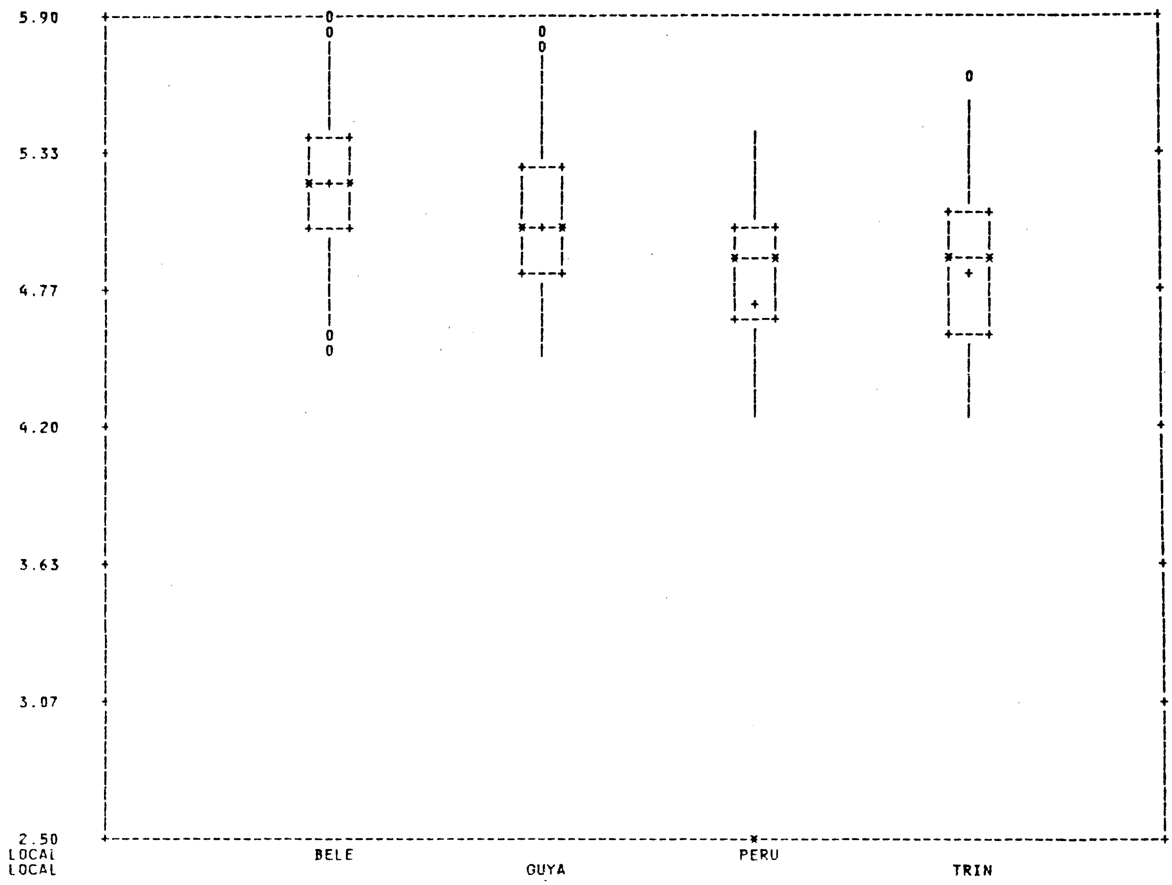

Figure 11. Schematic Plots For Variable LDS: For an explanation see figure 8.

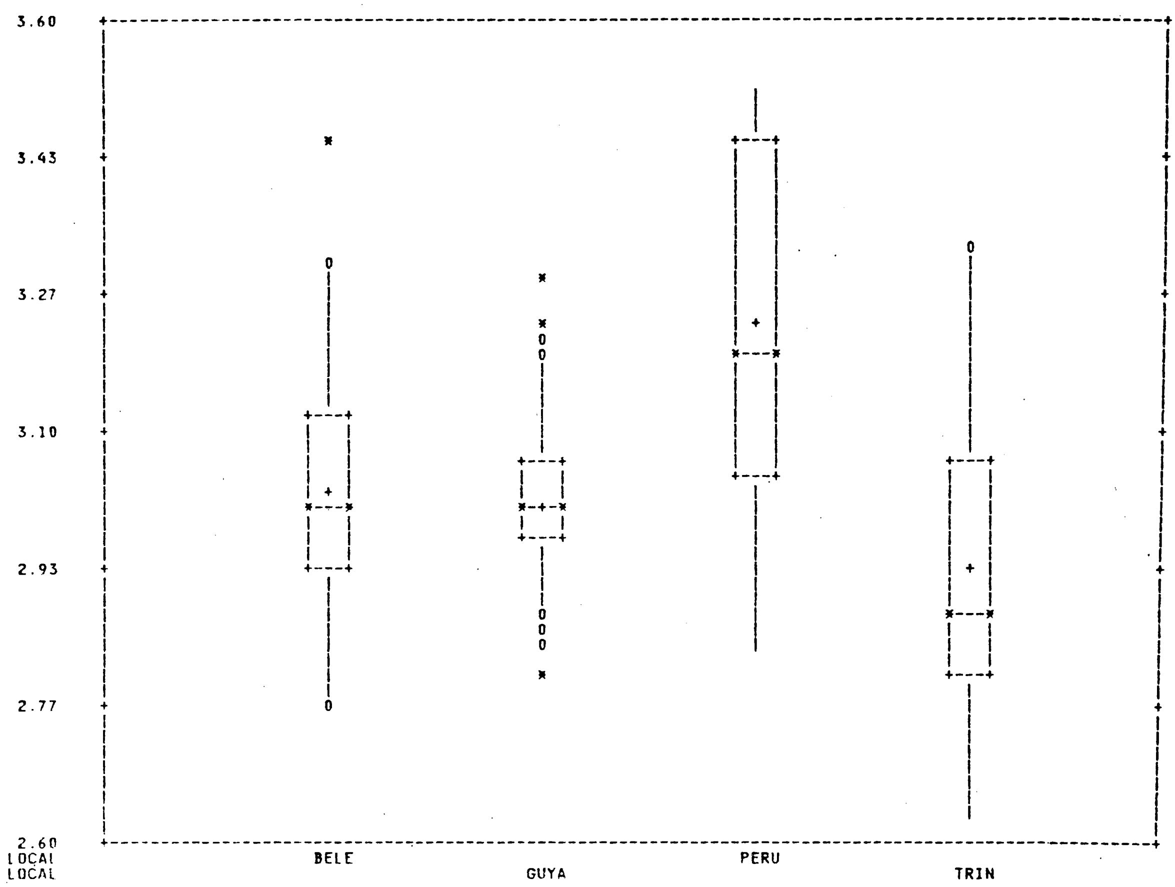

Figure 12. Schematic Plots For Variable HL: For an explanation see figure 8.

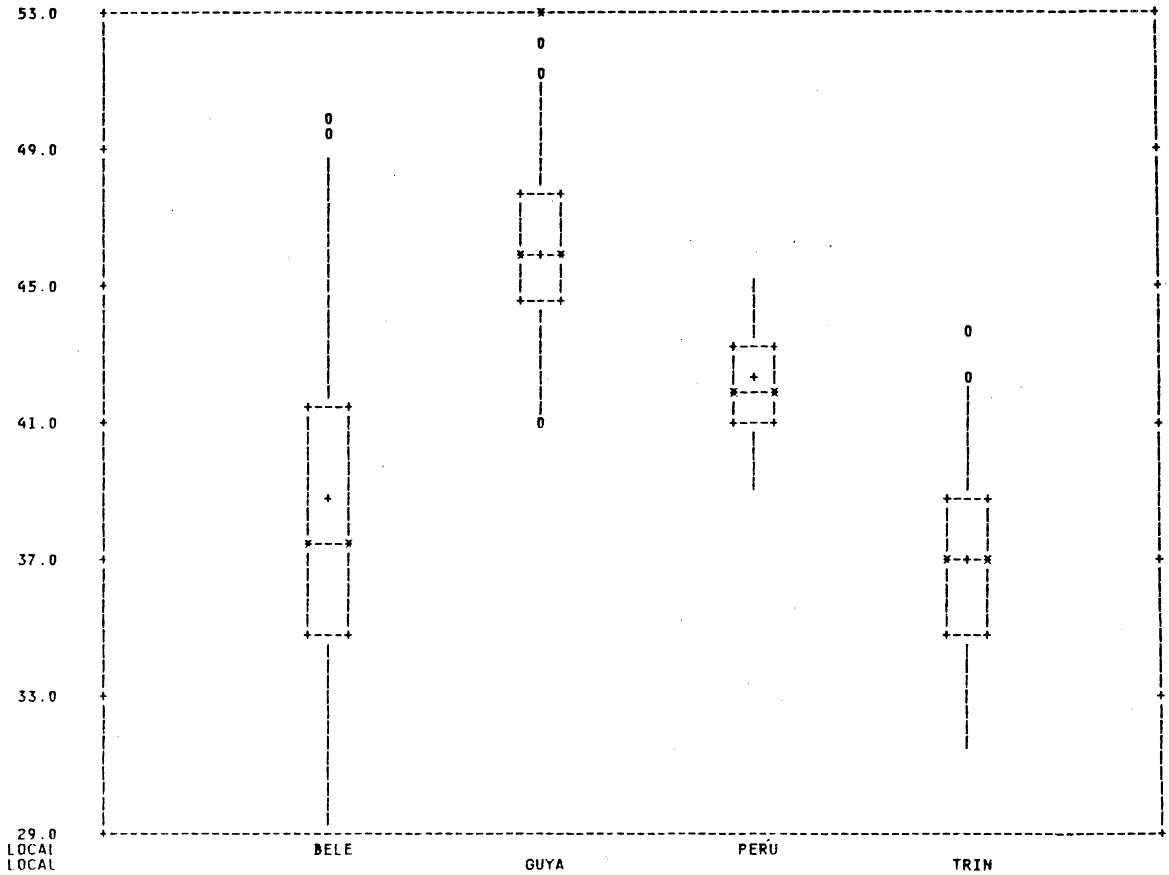

Figure 13. Schematic Plots For Variable SL: For an explanation see figure 8.

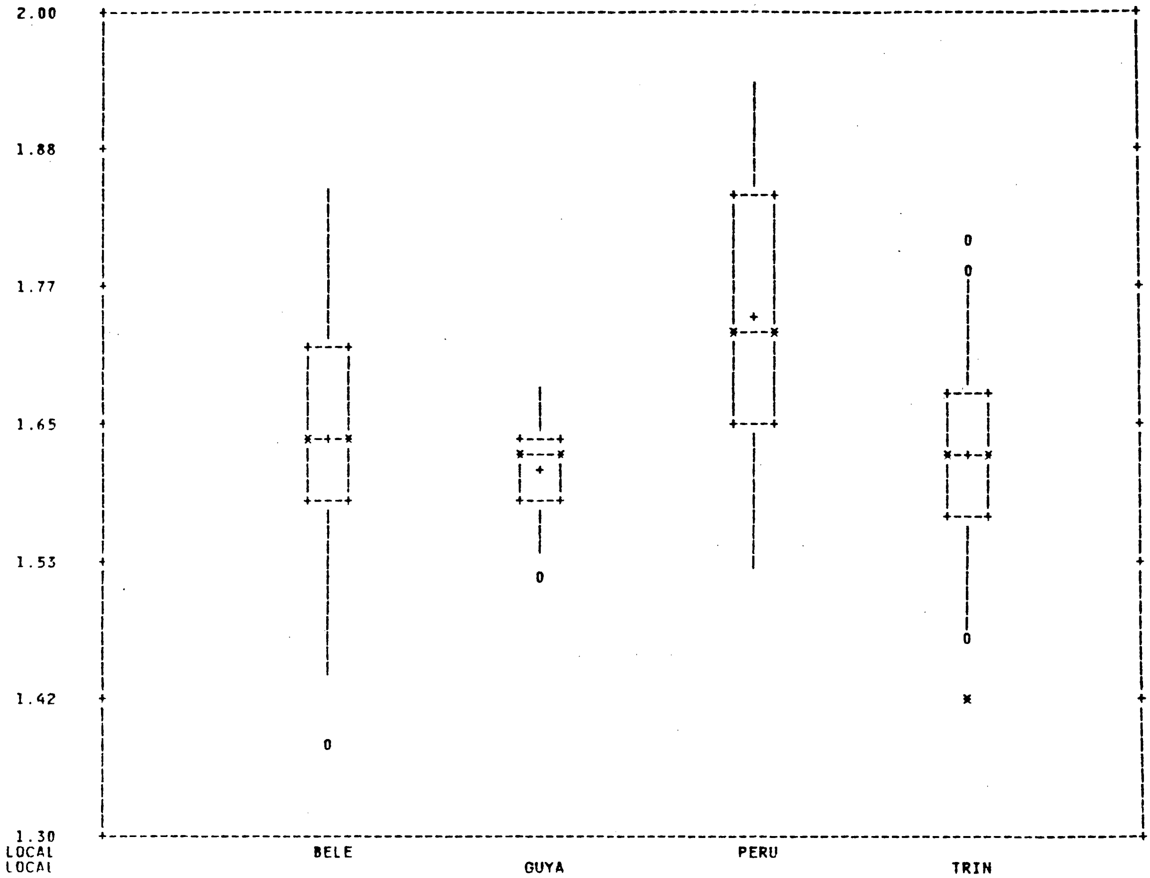

Figure 14. Schematic Plots For Variable SN: For an explanation see figure 8.

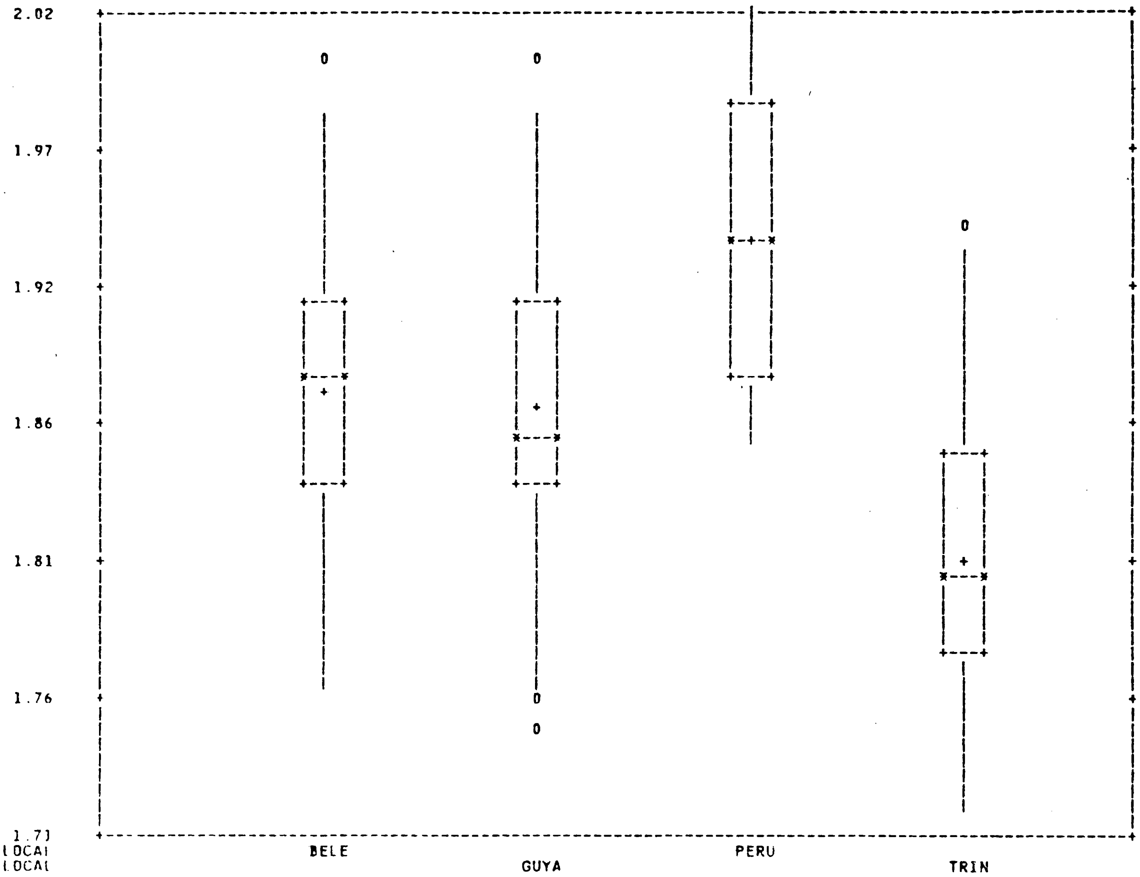

Figure 15. Schematic Plots For Variable SND: For an explanation see figure 8.

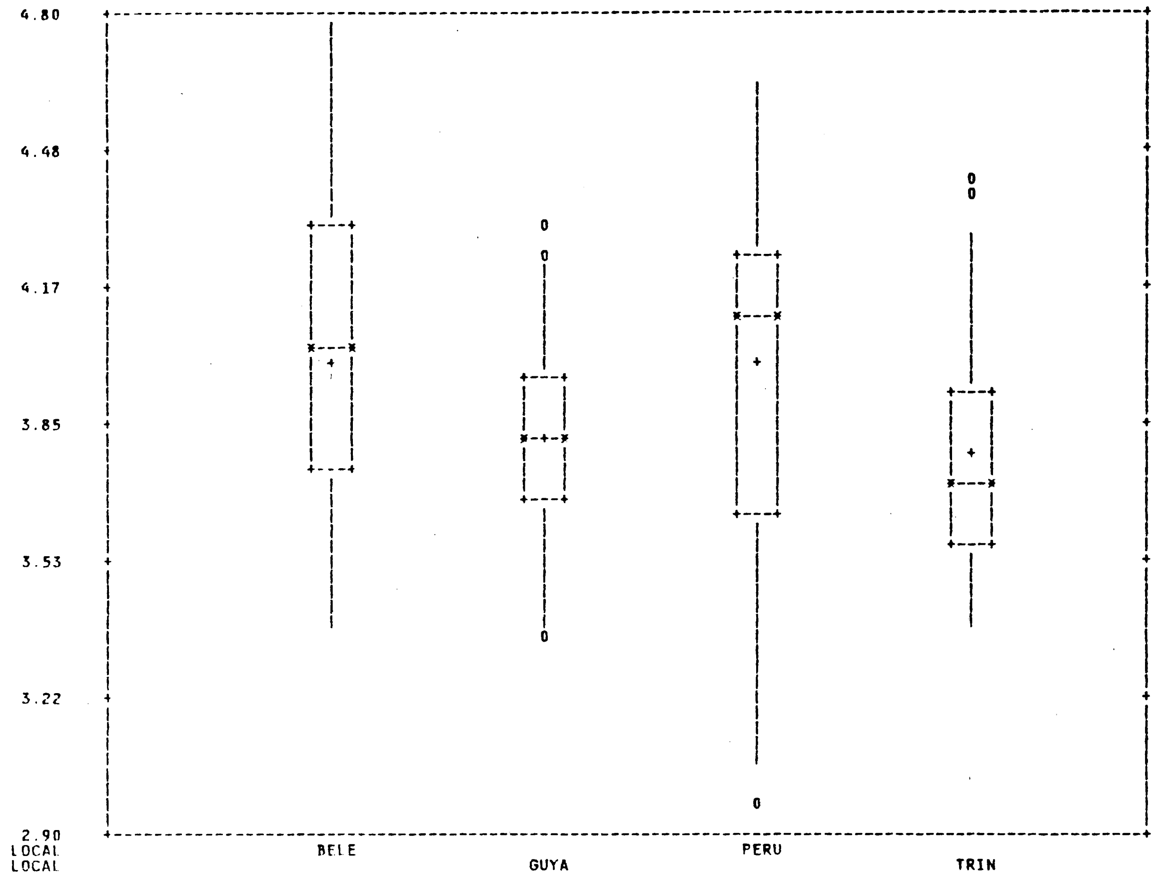

Figure 16. Schematic Plots For Variable SNP: For an explanation see figure 8.

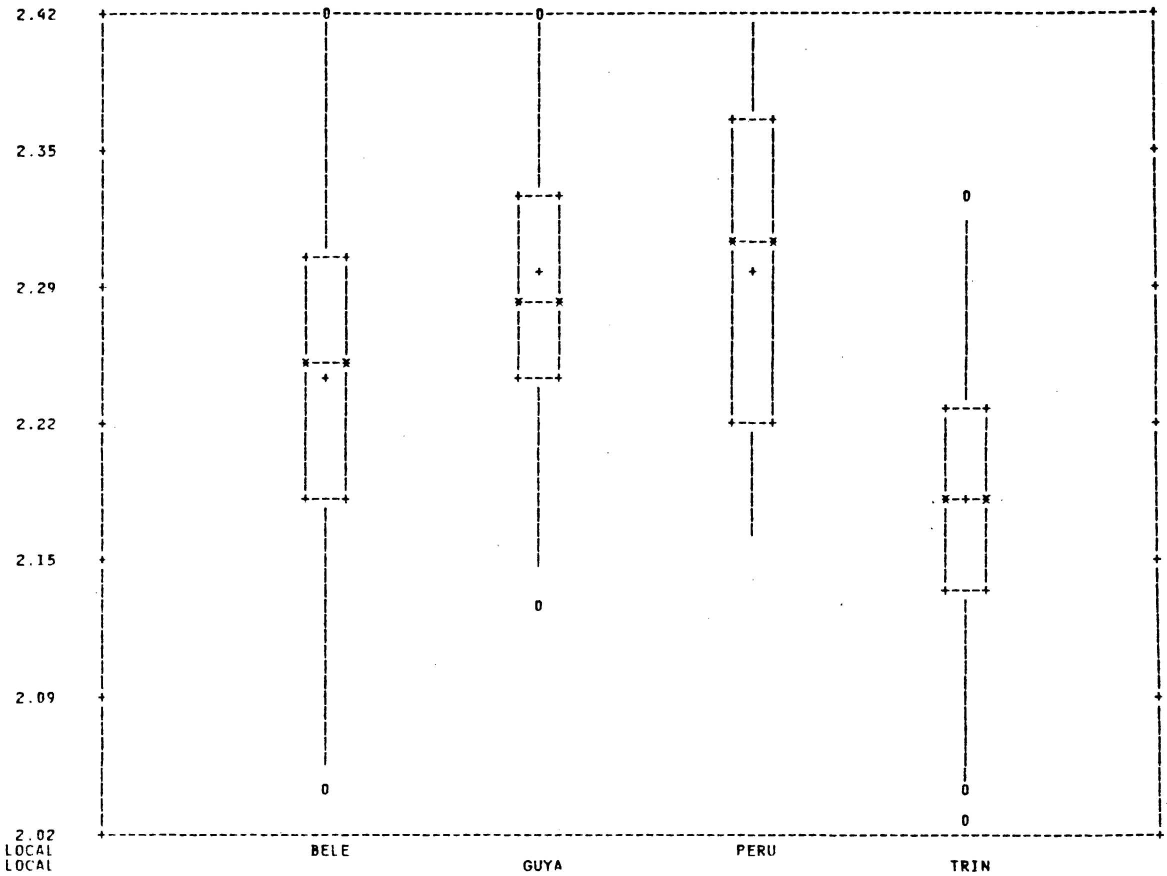

Figure 17. Schematic Plots For Variable SOP: For an explanation see figure 8.

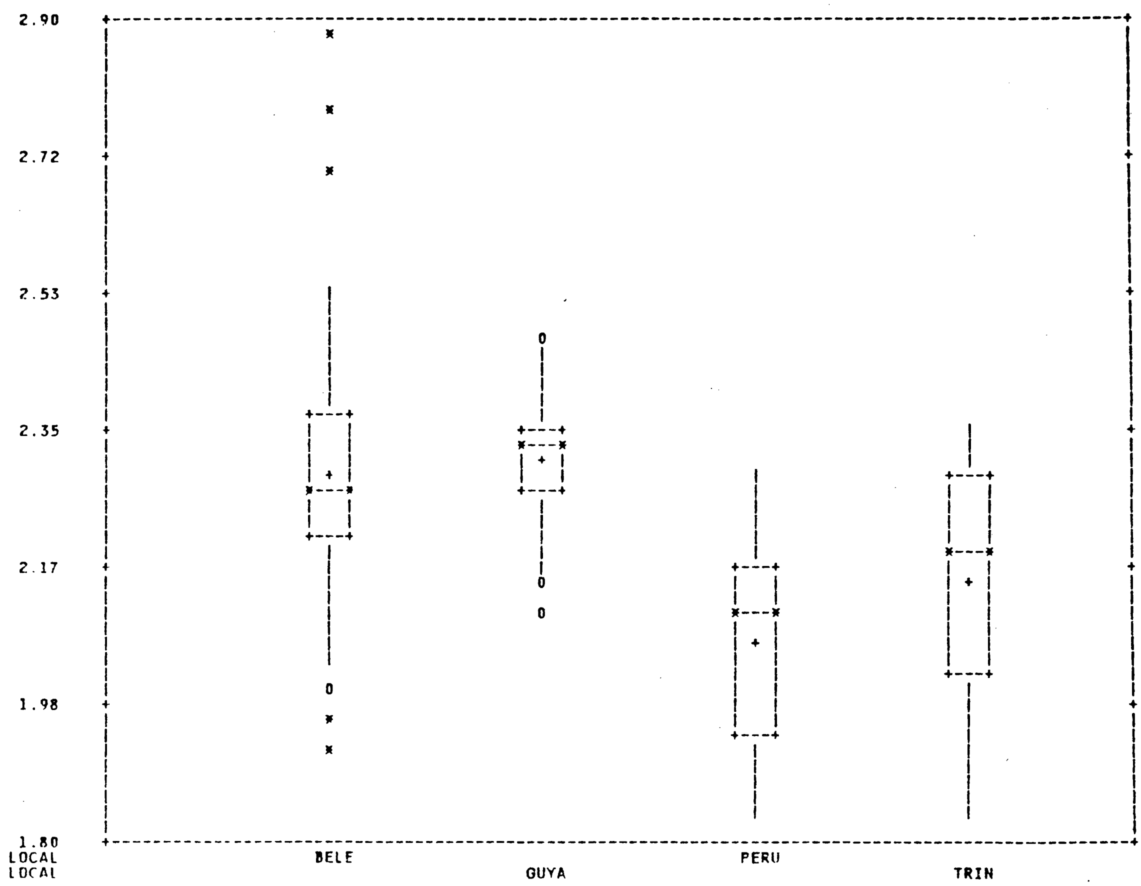

Figure 18. Schematic Plots For Variable WI: For an explanation see figure 8.

Table 4. MANOVA Tests For Continous Variables

The results of the multianalysis of variance are given below. The F value determines if we reject or not the null hypothesis. The null hypothesis is that there is no heterogeneity between the different localities. The probability of obtaining such F (PR>F) values are all .0001 with one exception. We reject the null hypothesis with a probability equal to or less than .05.

VARIABLE	MEAN	F VALUE	PR > F	R. SQUARE
bd	2.754	16.21	.0001	.2867
dcp	2.147	27.02	.0001	.4012
hl	3.007	7.76	.0001	.1614
lbo	4.086	9.40	.0001	.1890
lds	5.013	9.79	.0001	.1953
sl	40.970	42.29	.0001	.5118
sn	1.637	8.75	.0001	.1783
snd	1.862	17.57	.0001	.3034
snp	3.872	4.21	.0073	.0946
sop	2.244	16.44	.0001	.2896
wi	2.245	12.39	.0001	.2350

CANDISC Analysis

Three lines are necessary to separate the four populations of _C. aeneus_; therefore, three canonical values were constructed per variable in the candisc analysis. By combining the canonical values in all possible ways separation of the populations into discrete groups is observed (figure 15-17). When we plot CAN 1 against CAN 2 we observe two groups, one consisting of Guyana and Peru and the other of Belem and Trinidad. Belem is not completely blended in with Trinidad, but is to its right. Peru is shifted to the left closer to Guyana yet not mixed in . A plot of CAN 1 versus CAN 3 tends to move Belem slightly away from Trinidad, separating them even more but a little overlap is still observed. CAN 2 versus CAN 3 does not say much except that it moves Peru away from the other populations to the bottom right hand side. These results suggest that some morphological differences exist between the different groups studied of _C. aeneus_.

Discriminant Analysis

Giving each population equal probability membership to a given locality is well predicted (see figures 5-6). Guyana and Trinidad seem to have the best established criterions for predictability. The small sample size for Peru might be the cause of the 10% error in prediction observed.

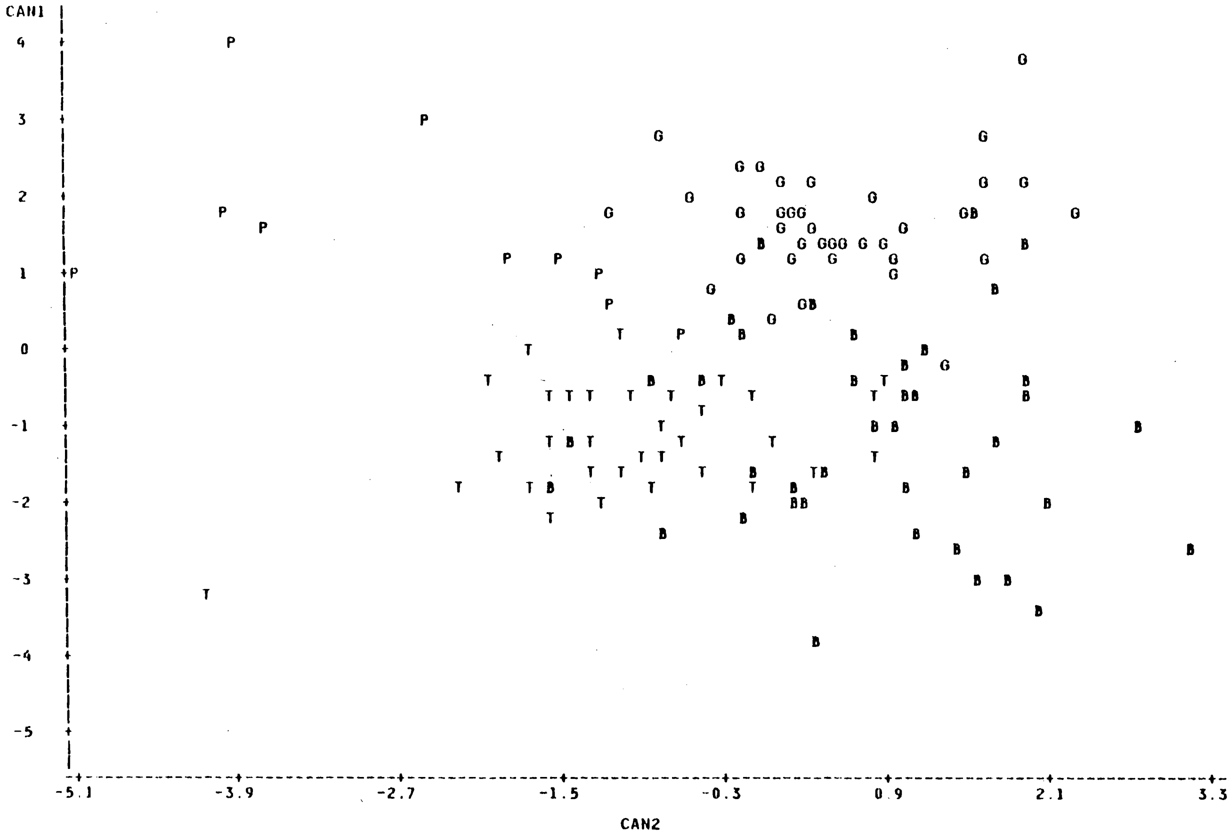

Figure 19. Plot Of CAN 1 * CAN 2: The CAN values are constructed by the Candisc analysis. The analysis constructs a linear function of those variables which best describes the differences between the localities. Since we have four localities we need a maximum of three lines to separate the localities from one another. These are CAN 1, CAN 2 and CAN 3. Different combinations of these CAN values were made to see how well the localities are separated from each other. The letters B,G,P and T stand for Brazil, Guyana, Peru and Trinidad respectively.

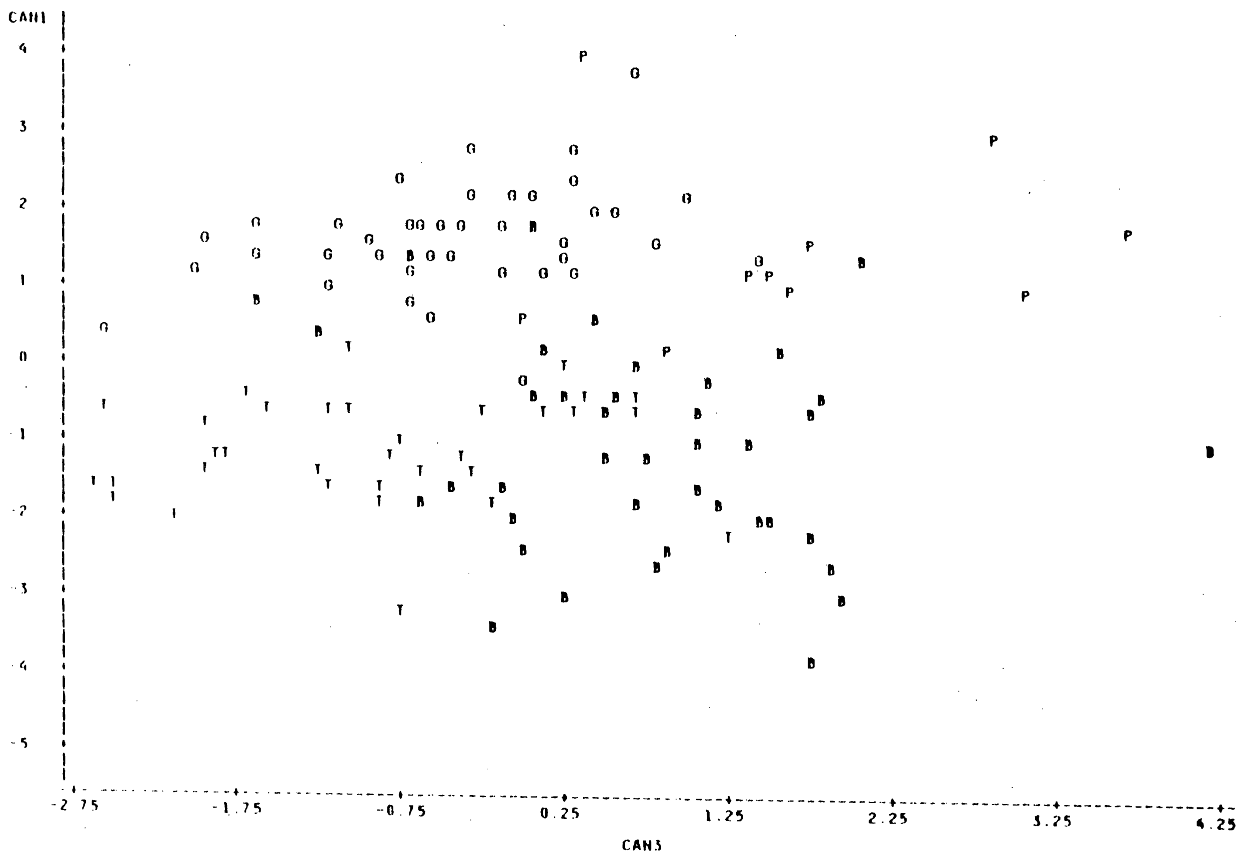

Figure 20. Plot Of CAN 1 * CAN 3: For explanation see figure 19.

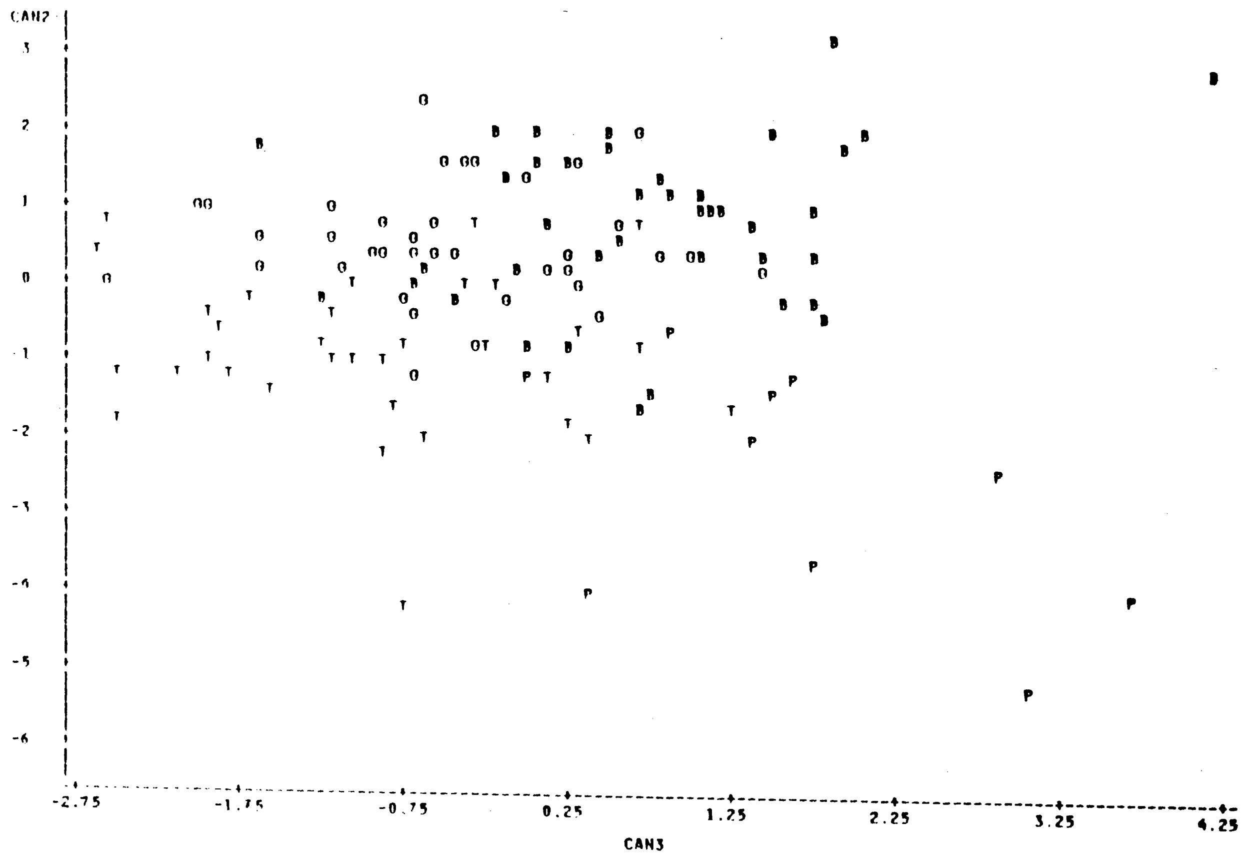

Figure 21. Plot Of CAN 2 * CAN 3: For an explanation see figure 19.

Table 5. Prior Probabilities Assigned To Each Locality

In the discriminant analysis each fish is assigned equal probability of membership to the four localities, eventhough the frequencies vary between localities.

LOCALITY	FREQUENCY	PRIOR PROBABILTY
Brazil	40	0.250
Guyana	41	0.250
Peru	10	0.250
Trinidad	34	0.250

Table 6. Number of Observations and Percents Classified Into Locality

A total of 125 fishes were used in the discriminant analysis. The analysis predicts to which of the four localities each one of these fishes belongs based on the measurements taken (see figure 1). The table below gives the number of fishes predicted into each locality and what percentage this number represents from the total number of fishes we had in each locality.

FROM LOCALITY	BRAZIL	GUYANA	PERU	TRIN	TOTAL
BELEM	38	2	0	0	40
	95.00	5.00	0.00	0.00	100.00
GUYANA	0	41	0	0	41
	0.00	100.00	0.00	0.00	100.00
PERU	0	1	9	0	10
	0.00	10.00	90.00	0.00	100.00
TRINIDAD	0	0	0	34	34
	0.00	0.00	0.00	100.00	100.00
TOTAL	38	44	9	34	125
PERCENT	30.40	35.20	7.20	27.20	100.00

Discrete Variables

The results of the chi-square contingency test performed on these data is shown in table 7. The localities were combined in all possible ways. A significant difference was observed for the number of preadipose scutes. The tests suggest that part of this difference exists between Guyana and Trinidad and to some extent between Belem and Guyana,

All the fishes had 7/7 caudal fin rays and I-7 (one spine and seven rays) dorsal fin rays, yet, of the 15 specimens from Peru 7 had I-6 dorsal fin rays. One specimen from Belem and one from Trinidad had I-6, none from Guyana.

Table 7. Results of the Chi-Square Tests of the Discrete Variables

A contingency chi-square test was done for three variables using different combinations of the localities. The letters B, G, P, and T stand for Brazil, Guyana, Peru and Trinidad respectively. We reject our null hypothesis at a probability equal to or less than .05. For an explanation of the variables see figure 2.

LOCALITIES	VARIABLE	X VALUE	DF	P
B, G, T	dbs	6.245	4	.20>p>.10
B, G	dbs	5.458	2	.10>p>.05
B, T	dbs	1.000	2	.70>p>.60
G, T	dbs	2.580	2	.30>p>.20
B, G, T	vbs	2.050	4	.90>p>.80
B, G, P, T	pas	15.495	6	.025>p>.01
B, G, P	pas	9.93	4	.05>p>.025
B, G, T	pas	14.97	4	.01>p>.005
G, P, T	pas	12.02	4	.025>p>.01
B, G	pas	6.83	2	.10>p>.05
B, P	pas	1.41	2	.50>p>.40
B, T	pas	1.91	2	.40>p>.30
G, P	pas	1.77	2	.50>p>.40
G, T	pas	11.12	2	.005>p>.0025

DISCUSSION

When we compared the diploid and tetraploid forms of _C. aeneus_ the only difference was in their color patterns. The diploid aeneus-like individuals had a darker brownish color across the ventrolateral body scutes, while the tetraploid form had a lighter shade of brown across this same area. This difference in color did not persist in captivity. Nijssen (1970) suggested variation in color pattern in _Corydoras_ within members of the same species coming from different regions. The intensity of pigmentation will depend on the nature of the bottom. If this is in fact what is happening the diploid form lives in waters different from those in which the tetraploids are present. The tetraploid specimens we used were definitely from different locations but nothing is known about the nature of the water from which they came. 89 tetraploid specimens were used in the study, none showing this lighter coloration. Nijssen (1970) found specimens of _C. aeneus_ with more intense pigmentation living in darker bottomed waters. If the diploid form has a darker coloration due to the nature of its habitat then we must assume that the three tetraploid forms all live in waters with a similar bottom. An alternative explanation for this phenomenom is that the difference in color has a genetic basis, and that the difference in color between the diploid coming from Belem and the tetraploids coming from Guyana, Peru and Trinidad is the result of a genetically determined color pattern within each group. The possibility that all three tetraploid populations have similar coloration because they live in waters of similar nature cannot be excluded.

If we examine the karyotypes more closely the tetraploid form of C. aeneus from Peru has N=67, the diploid form from Belem has a N=28. These karyotypes appear to show heterogameity in the male, but we cannot be certain due to the limited number of spreads with which to verify this. Autotetraploidy does not seem to be the polyploid event which produced the tetraploid C. aeneus. Assuming the C. aeneus-like sample used in this study gave rise to the tetraploid form by duplication of its ancestral parental genome the event would require at least three sets of this genome present in the initial tetraploid form. Ferris and Whitt (1977) have suggested that the second step in establishing disomic inheritance in a recently produced polyploid organism is the physical elimination of DNA. The tetraploid individuals presumably from Guyana and Peru have 2.3 times the number of chromosomes present in the diploid form. If we assume that the tetraploid has eliminated some DNA there had to be more than two genomes of this diploid present. This is very unlikely because the gametes produced would be genetically unbalanced. The tetraploid form has retained 35% of its duplicate loci, while 65% of its duplications have been silenced or eliminated (Dunham et al., 1980). Perhaps the diploid form had a higher DNA content when the tetraploid event occurred and that it too has eliminated some DNA since then. Our results concerning the tetraploid chromosome number are relevant to Guyana and Peru only. We were unable to karyotype material from Trinidad due to the lack of good spreads. The possibility that the population on Trinidad is not the result of the same tetraploid is questionable. Is there more than one tetraploid form of C. aeneus as well as more than one diploid population in the fresh waters of South America?

Nearly all the morphological data are normally distributed (See results). Those variables which are not normally distributed had values centered around the same number or they were clustered to one side of the curve with a few variables at the other end. Nevertheless, of the 44 tests performed 36 showed the variables to be normally distributed. 95% of the values obtained fell within two standard deviations of their respective means.

The Manova tests were all highly significant (See table 4). We reject the null hypothesis that the populations are the same although the test does not stress which populations differ the most. Some morphological differences have been fixed in the populations, discriminating between the localities.

The CANDISC analysis tries to separate the populations by computing linear combinations of the variables which best reflect any differences among the populations, and then computing scores for each observation on the linear combination. Since we are working with four populations, three lines were computed in an attempt to separate the populations. If we plot these CANDISC values two at at time (See figures 15-17) we can get some idea of how well these lines separate the populations and better yet, how discriminating are these variables between populations. CAN 1 separates Guyana and Peru from Belem and Trinidad, CAN 2 pulls Peru out of Guyana and CAN 3 separates Trinidad from Belem. In all three CANDISC plots there is a sharp separation between Guyana and Trinidad, no matter which combination of CAN values we used. Most of the heterogeneity among localities implied by the MANOVA analysis might be due to the sharp separation between Guyana and Trinidad.

Discriminant analysis classifies the observations into two or more groups using morphological variables (see table 6). A _C. aeneus_ of unknown locality has equal probability of belonging to each population. Using the functions created by the analysis, predictability was high (See table 6). Of the 40 measured Belem specimens 38 were predicted to belong to Belem two to Guyana. The fishes coming from these two localities were morphometrically similar. All the 41 Guyana specimens were predicted to be from Guyana. The same held for Trinidad, all 34 specimens being predicted correctly. Both Guyana and Trinidad seem to have a defined range of values for these measurements. The CANDISC analysis showed a sharp separation between them. Fishes from these two localities have diverged into two morphologically recognizable populations within their species. The analysis suggests that there is enough difference between these populations that we can predict to which population a fish will belong by taking the eleven measurements described. 90% of the Peruvian fishes were correctly classified, and one fish was classified as coming from Guyana.

The discrete variables were not significantly different between populations, with the exception of the pre-adipose scutes. The test for the difference in number of dorsal body scutes for Belem and Guyana is right on the border of rejection, suggesting again established morphological differences. The tests performed on the number of pre-adipose scutes (pas) was significant among the populations of Belem, Guyana, Peru and Trinidad. A break down of this ultimately showed a highly significant difference between Guyana and Trinidad, in support of our previous suggestion of divergence of Guyana and Trinidad as distinctive morphological groups within the species.

All the tests suggest differences between Guyana and Trinidad. Differences between the other populations are only suggestive. Nothing distinguishes the diploid form from the tetraploid form any more than the tetraploids from each other. Morphologically nothing seems to distinguish the diploid form. Was this diploid form one of the ancestral parental genomes which contributed to the formation of a tetraploid *C. aeneus*? To answer the question would require going directly to South America for collections. Precise locality data are essential. Perhaps more than the diploid form exists in South America. Allotetraploidy seems to be involved . If this is true it would be the first case reported in catfishes. Catostomids have lost approximately 53% of their duplicate since the time of their tetraploid event fifty million years ago. *C. aeneus* has lost 62.5% (Dunham et al., 1980), but the rate of gene loss is unknown.

CONCLUSIONS

1. C. aeneus is apparently diploid-tetraploid species complex. Allotetraploidy may be involved. An electrophoretic study of live material may give some idea of how closely related the diploid form is to the tetraploid C. aeneus. Collections from known localities of South America are essential in this study. Tetraploid and diploids from different sites need to be compared. DNA hybridization could be another powerful technique used. The tetraploid form from Guyana and Peru has 134 chromosomes while the C. aeneus-like diploid ostensibly from Belem, Brazil has 56.

2. If this kind of variation is present in other species of Corydoras morphological studies alone will fail to define the biological species. A survey of the chromosome number in other species of Corydoras would be necessary.

BIBLIOGRAPHY

Allendorf, F.W. and G.H. Thorgaard. 1984.
Tetraploidy and the evolution of salmonid fishes.
Evolutionary Genetics of Fishes, Plenum Press, New York. 1-54.

Cavalier-Smith, T. 1978.
Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox.
Journal of Cell Science 34:247-278.

Cuellar, O. and T. Uyeno. 1972.
Triploidy in rainbow trout.
Cytogenetics 11:508-515.

Dauble, D. and R. Buschbom. 1981.
Estimates of hybridization between two species of catostomids in the Columbia River. Copeia 4:802-810.

Davisson, M.T. ,J.E. Wright and L.M. Atherton. 1972.
Centric fusion and trisomy for the LDH-B locus in brook-trout.
Science 178:992-994.

Dunham, R. , D. Philipp and G.S. Whitt. 1980.
Levels of duplicate gene expression in armoured catfishes.
The Journal of Heredity 71:248-252.

Ferris, S.D. and G.S. Whitt. 1977.
Duplicate gene expression in diploid and tetraploid loaches (Cypriniformes, Cobitidae). Biochemical Genetics 15:1097-1112.

Ferris, S.D. and G.S. Whitt. 1977.
Loss of duplicate gene expression after polyploidization.
Nature 265:258-260.

Ferris, S.D. and G.S. Whitt. 1978.
Phylogeny of tetraploid catostomid fishes based on the loss of duplicate gene expression. Systematic Zoology 27:189-206.

Ferris, S.D. and G.S. Whitt. 1979.
Evolution of the differential regulation of duplicate genes after polyploidization. Journal of Molecular Evolution 12:267-317

Ferris, S.D. 1984.
Tetraploidy and the evolution of the catostomid fishes. 1984.
Evolutionary genetics of Fishes.
Plenum Press, New York. 55-94.

Fritsch; E.F., M.L. Richard and T. Maniatis. 1980.
Molecular cloning and characterization of the human B-like globin gene cluster. Cell 19:959-972.

Futuyma, D.J. 1979.
Evolutionary Biology Sinauer Associates Inc., Sunderland Massachussetts. 565pp.

Gold, J.R., W.J. Karel and M.R. Strand. 1980.
Chromosome formulae of North American fishes.
The Progressive Fish Culturist 42:10-23.

Haldane, J.B.S. 1933.
Two new allelomorphs for heterostylism in primula.
American Naturalist 67:559-560.

Hinegardner, R. 1968.
Evolution of cellular DNA content in Teleost fishes.
The American Naturalist 102:517-523.

Hinegardner,R. 1972.
Cellular DNA content and the evolution of Teleostean fishes.
The American Naturalist 106:621-644.

Hitosumachi,S., M. Sasaki and Y. Ojima. 1969.
A comparitive karyotype study in several species of Japanese loaches (Pisces, Cobitidae). Japanese Journal of Genetics 44:157-160.

Kligerman, A.D. and S.E. Bloom. 1977.
Rapid chromosome preparations from solid tissues of fishes.
Journal Of Fish Res. Board Can. 34:266-269.

Klug, W.S. and M.L. Cummings. 1983.
Concepts of Genetics. Charles E. Merrill Publishing Co., Columbus Ohio. 614pp.

Lim, S., R. Kay and G. Bailey. 1975.
Lactate dehydrogenase isoenzymes for salmonid fish, evidence for unique and rapid functional divergence of duplicated H4 lactate dehydrogenases.
The Journal of Biological Chemistry 250:1790-1800.

May, B., J.E. Wright and K. Johnson. 1982.
Joint segregation of biochemical loci in Salmonidae. III. Linkage associations in Salmonidae including data from rainbow trout (Salmo gairdneri). Biochemical Genetics. 20:29-39.

Nelson, J.S. 1984.
Fishes of the World.
Second Edition; John Wiley and Sons, New York. 523pp.

Nijssen, H. 1970.
Revision of the Surinam catfishes of the genus Corydoras Lacepede, 1803. Beaufortia 18:1-75.

Nijssen, H. and J.H. Isubrucker. 1980.
A review of the genus Corydoras Lacepede, 1803 (Pisces, Siluriformes, Callichthyidae). Bijdragen Tot De Dierkunde 50:190-220.

Ohno, S. 1970.
Evolution by gene duplication. Springer-Verlag, New York. 160pp.

Ohno, S. 1970.
The enormous diversity in genome sizes of fish as a reflection of natures extensive experiments with gene duplication. Transactions of American Fisheries Society 99:120-130.

Ohno, S., U. Wolf and N. Atkin. 1968.
Evolution from fish to mammals by gene duplication. Hereditas 59:169-187.

SAS User's Guide: Basics
1982. SAS Institute Inc., Cary, North Carolina.

SAS User's Guide: Statistics.
1985. SAS Institute Inc., Cary, North Carolina.

Scheel, J., V. Simonsen and A.O. Gyldenholm. 1972.
The karyotypes and some electrophoretic patterns of fourteen species of the genus Corydoras. Sonderdruck aus Z.f. Zool. Systematik u. Evolutionsforschung 10:144-152.

Sparrow, A.H. and A.F. Nauman. 1976.
Evolution of genome size by DNA doublings. Science 192:524-529.

Stebbins, G.L. 1976.
Processes of Organic Evolution
Second Edition; Prentice Hall, Inc., Engelwood Cliffs, New Jersey. 191pp.

Thorgaard, G.H. and G.A. Gall. 1979.
Adult triploids in a rainbow trout family. Genetics 93:961-973.

Thorgaard, G.H. and M.E. Jazwin. 1981.
Polyploidy induced by heat shock in rainbow trout. Transactions of the American Fisheries Society 110:546-550.

Ueno, K. and Y. Ojima. 1976.
Diploid-tetraploid complexes in the genus Cobitis.
Proceedings of the Japan Academy 52:446-449.

Uyeno, T. and G.R. Smith. 1972.
Tetraploid origin of the karyotype of Catostomid fishes.
Science 175:644-646.

White, M.J.D. 1978.
Modes of Speciation.
W.H. Freeman and Company, San Francisco California. 427pp.

The vita has been removed from the scanned document