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# ARK - Arizona Rivulin Keepers

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## The Scheel Letters, No. 53; Part 2

### Crossings Part 2: Rivulinae Myers in Cyprinodontidae

#### (16) CAL/MAC 1964

MAC is an Epiplatys. The type locality for this species is Chiloango, N of the Congo mouth. My single MAC female was not willing to spawn in crossings. She normally kept her eggs too long and (tuberculosis?) when at last she spawned the eggs often were not viable. With CAL male she spawned several times and many eggs were harvested. Most of these eggs however apparently were not viable or not fertilized, but some eggs probably were fertilized. I was not able however to trace any blastula, so this cross has to be done once more when both forms are kept in my tanks.

#### (17) CAL/ROL 1964

CAL belongs to the "eastern group of Aphyosemion", whereas ROL belongs to the "western group" or Callopanchax. I was very interested in this cross because of the "Callopanchax character" that developed on the throat of spawning CAL females. See AUS/CAL.

ROL was described from the Freetown area in Sierra Leone. This species belongs to the most difficult (taxonomic matters) group of "ROL-like Aphyosemion" from Sierra Leone and Liberia. The female used for this cross however was not a pure ROL female. The female was coded SL4/ROL (see this cross). However, it was able -at least- to produce some viable eggs.

I had six fertile eggs from three spawnings. The first phases of the embryonal development were normal. The blastula developed and after this the gastrulation produced the deep groove in the yolk when passing the equator. Shortly after the ending of the gastrulation a certain "body" developed at the animale pole and the eggs burst (all six) at that phase of development. Probably this cross will not give away any viable embryo.

#### (17) CAL/SEN 1964

Also for this cross I used a "hybrid female", the SEN-VO/NI female, see SEN/SEN. The "hybrids" produced between individuals from different populations of SEN however do not differ in fertility or viability from individuals of the parent strains.

I had seven eggs and six eggs developed. The blastula was big but apparently it was not abnormal. A short corda developed after the gastrulation. At the same time a big "blister" developed (... cannot read

last line) ...the blister developed such size that the egg became deformed and burst. In these eggs -before the bursting- the blister reached the size (almost) of the yolk ball. In other eggs the blister was smaller and the eggs did not burst. Some eggs did not develop any blister at all. Embryos were very thin and reached 180 degrees on yolk in these eggs. In one or two eggs a working blood system developed and a few elements were seen circulating in veins. After a few days however the circulation stopped. No thrombus was discovered. No further development was noticed and soon the embryos died.

#### **(19) CHA/DAG 1964**

I used a female of the common aquarium strain of DAG (the Monrovia strain). CHA: details on this species will be given in connection with the information on the CHA/SHE crossings. For this particular cross I used a CHA of the Angona-Abra strain. I had many eggs in this crossing. The eggs were kept in the nylon without inspection until the sixteenth day. Then eggs were taken out from the nylon for inspection. In six eggs the embryo was dead or about to die. In seventeen eggs the embryo was still alive. The embryos were rather big and many black pigments were visible. Most embryos were suffering from severe thrombus and their circulation of blood has stopped or was about to stop. In most embryos the heart was still working without being able to circulate any blood. As I consider all embryos as not viable, all were preserved in Bouin. See also DAG/CHA cross.

This result indeed surprised me as I had some idea of a certain relationship between these two species which both are found in SW Ghana. Both species develop a short "sword" at the lower corner of the caudal fin and this character is very rare in *Epiplatys*.

#### **(20) CHA/SHE 1962-64**

Recently Daget & Arnould concluded (I have seen the unpublished manuscript only, 1964) that SHE and SPM are synonyms for CHA. If measurements and counts for these three forms are compared no difference is seen. Also it is evident that these forms form a natural group of relatives. Stenholt Clausen and I have prepared a manuscript for the Aquarium Journal on these three forms and I think that this article will appear rather soon in that magazine. We have seen the types for CHA (Sauvage's individuals), those for SPM (Arnould's species) and also individuals belonging to the lot which Poll had for his description of SHE. We have also had five natural strains of CHA and SHE and a fourth form from Kumasi in Ghana. These individuals were caught by Stenholt Clausen (1962) and Ulf Hannerz (1963). Stenholt Clausen's collection of live individuals from four localities apparently contained two different closely related species. One form or phenotype came from Ayenasi and Tikawbo in SW Ghana and corresponded in most details to SHE, whereas two strains (Angona and Abra) corresponded to CHA (the type individuals). The Kumasi form collected by Hannerz did not correspond to these two forms but was found to be closest to CHA in most details.

On the next page you will find counts for the types of CHA and SPM and the material (SHE) from the Congo Museum and also for the aquarium kept strains of CHA (Angona-Abra) and SHE (Ayenasi-Tikawbo) and of the Kumasi form (KUM) together with counts for various hybrids produced from these live strains.

D= 09 10 11 12 Dm  
CHA 0 3 1 1 10.6

SHE 1 2 1 0 10.0  
SPM 0 8 7 2 10.6  
CHA 0 7 21 11 11.1  
SHE 1 11 41 5 10.9  
KUM 0 3 10 3 11.0  
KUM/SHE 0 0 1 2  
CHA.SHE/CHA 0 0 4 0  
CHA.SHE/KUM 0 4 3 0

A= 14 15 16 17 18 19 Am  
CHA 1? 0 3 0 0 0 16.0?  
SHE 0 0 4 0 0 0 16.0  
SPM 4 6 7 0 0 0 15.2  
CHA 0 4 23 11 0 1 16.2  
SHE 0 17 34 6 0 0 16.0  
KUM 0 8 8 0 0 0 15.5  
KUM/SHE 0 0 1 2 0 0  
CHA.SHE/CHA 0 0 0 2 2 0  
CHA.SHE/KUM 0 0 0 7 0 0

D= 25 26 27 28 29 30 Sqm  
CHA 0 0 0 3 0 0 28.0?  
SHE 0 0 0 4 2 2 28.7  
SPM 0 5 18 7 4 1 28.4  
CHA 0 8 28 19 5 0 27.6  
SHE 0 10 87 18 2 0 27.2  
KUM 1 3 9 9 2 0 27.4

The three "phenotypes" also were breeding true to their characters. The hybrids mentioned above were viable and fertile and no abnormalities were noticed in the reproduction of the F1 and F2 individuals. This means that apparently there are no post-mating isolating mechanisms that hinder an exchange of genes between these three forms. We have also seen various collections of preserved individuals from the British Museum that belong to the CHA-SHE complex. And preserved individuals from Ghana collected by T. Roberts and A. Schioetz. Although many of these individuals have lost their colors more or less, most samples could be placed in one of the three phenotypes mentioned above (with some hesitation sometimes). This means that for our study we had individuals from sixteen different localities in Ghana and from three localities in Ivory Coast (types and paratypes etc.). We have tried to discover the distribution of these three forms in connection to river drainages, rainfall and type of soil. No absolutely clear distribution according to one of these factors could however be found.

SHE in Ghana is restricted to the most humid parts of the rainforest, that is the extreme SW Ghana. This

form lives on a soil type named oxysol and in areas of high rainfall. The SHE phenotype however also occurs in the driest part of the forest, in the north and in the derived savannah. This probably is the SPM phenotype that however does not differ from the SHE phenotype in colors and color patterns. Apparently in Ivory Coast there is an unbroken "chain" of populations from Abidjan (type locality for SHE) to Bouake (type locality for SPM). Apparently it is not so in Ghana.

CHA also occurs in SW Ghana, but in areas that have less rainfall and on a soil named the oxysol-ochrosol intergrades. The water of this area is less acidic and contains more salts. The CHA form or forms which cannot be placed in CHA or KUM but which are "integrades" occur all the way from SW Ghana to the Togo Hills in the east.

The KUM form occurs near Kumasi and at least 50 miles to the east and the west of that city. This form lives on the ochrosols that receive less rainfall and in this area the seasons are more marked ... (can not read last line)

As the CHA and the SHE phenotypes differ markedly in their color patterns and as both forms breed true to these characters and as they apparently do not occur together in nature and do not mix we may consider these two forms as allopatric subspecies of CHA, whereas the KUM form for the present is placed in CHA. The two Ghana forms, here identified as CHA and SHE, develop these characters that can be used to separate live and well preserved individuals (males):

**CHA:** four black crossbars situated between the anterior-most anal fin ray and the root of the caudal fin. In front of these bars there are no such bars above the root of the ventrals (occur frequently in KUM), nor behind the pectorals (occur very rarely in KUM). Much yellow and orange (or red) color, rather evenly distributed in fins and more or less on the body sides. No big red dots on anal fin, just below the root. Brilliance from guanine, usually low (very low in KUM).

**SHE:** black bars as in CHA plus a very marked black bar just behind the pectorals, rarely above the root of the ventrals (occur in Poll's material). No yellow or orange fin color evenly distributed in fins. A conspicuous row of big red dots in anal fin just below the root of that fin. Guanin brilliance very strong, steel blue. Hybrids are intermediate to all these characters.

### **(21) CHE/BIF 1964**

CHE is found in the Congo drainage near Leopoldville and probably this species is restricted to the savannah biotope that occurs in this part of the huge Congo drainage. As SEN is represented in this part of the Congo drainage it would be natural to look after BIF as these two forms occur sympatrically all over the West African savanna (except in Sierra Leone) and in the Nile drainage. In general CHE resembles BIF in shape, the color patterns and in behavior. Probably no other Epiplatys described so far resembles BIF as much as CHE. For this reason the CHE/BIF cross had to be prepared. These two forms develop different hemoglobine patterns however. BIF has his own complicated pattern, whereas CHE develops similar patterns as do SEX, LON, CHA, SHE and DUB. A six-line pattern. This crossing was difficult to arrange as both species are shy and as the individuals were not interested in spawning. I kept two "pairs" in separate tanks. Through about two months the pair CHE/BIF-VO did not spawn a single egg, whereas during that length of time the CHE/BIF-NI.VO produced seven eggs only. Only two eggs

were fertile. One egg developed a normal blastula, but after this effort the egg died. The second egg developed a normal blastula also and an embryo was produced. This embryo did hatch but was not able to swim and died after some days. As hemoglobine pattern, spawning behavior and embryo development indicate, BIF probably is not the close relative of CHE (which I supposed).

#### **(22) CHE/MAC 1964**

Both species occur in the Congo drainage and both are nominal Epiplatys. They differ in hemoglobine patterns (see cross no. 21) as MAC develops the common "Aphyosemion pattern" = four lines. Also this cross was difficult to produce and after several weeks I had six eggs only. One egg developed a normal blastual, but the next day the egg died.

#### **(23) CHE/SEX 1965**

As these two species develop similar hemoglobine patterns I was interested in the results of such crosses as previous crossings between "six line species" have produced rather promising results which indicated close relationship (see SEX/LON and SEX/SHE). At that time I did not know that also DUG and BLO developed this particular pattern. The various SEX females (three) that I used for this cross to my biggest CHE male were not interested in spawning with that male. Also the male was not very interested and when the females did not react on his flirt just after the bringing together of the individuals he gave up and did not pay further attention to the females. No eggs could be harvested when the individuals were kept together for a long time in one tank. I had to store the three females alone in separate tanks and the male in his own tank. Every two or three days I placed a female in the male's tank and hoped that at least some eggs would be spawned. After two months I had fourteen eggs (the largest spawning contained three eggs). Three eggs developed a normal blastula. These eggs (three spawnings) finished their gastrulation and after this one egg died. The second egg developed an "embryo" covering some 180\_ on the yolk. No distinct corda was produced nor was I able to trace distinct organs. Black pigment cells however developed on the yolk. This egg lived for fourteen days. The third egg developed a small embryo. The corda, the head, the eyes and the gill araches were visible, although the embryo measured about 90 degrees only on the yolk. A large flat "blister" developed and the usual thin vein between the throat of the embryo and the inner yolk membrane was visible. No blood elements developed. As the embryo was not considered as viable I preserved the egg after one week.

When I gave up this crossing I spawned my three SEX females one by one with SEX males. They spawned at once and one female gave more than a hundred eggs from that spawning. These results indicate that CHE and SEX are not at all close relatives and that the six line hemoglobine pattern -in this particular case- is not an indication of close relationship. Micro photos of the "best" CHE/SEX embryo in egg have been prepared showing the embryo, the blister and the vein through blister.

#### **(24) CHR/CIN 1961**

Both species are nominal Aphyosemion. CIN was described as a member of the subgenus Fundulopanchax. Indeed CIN has the characteristics of a Fundulopanchax. CIN however differs in some important details from all other members of this subgenus. The male does not develop any red pattern on the throat as all other members of the unit. Instead the fighting or spawning male develops a deep black color on the lower part of the head. This character is found in all known members of the Callopanchax unit and very often among Epiplatys. In Aphyosemion only AUS develops this pattern. The CIN male

does not develop any red markings on the body sides. Red pigments are found (diffused) on the gill covers and behind the pectoral base (the "wound"). CIN males do not make "scissor movements" (folding and unfolding of unpaired fins) as do all other males in nominal *Aphyosemion* and many *Epiplatys*. In this CIN corresponds to species in *Nothobranchius*. Also further details of CIN are rather "Nothobranchius-like". Probably CIN is not a "missing link" between these two nominal genera. See *Aquarium Journal* 1964, Scheel: *A. cinnamomeum*, draft in Killie letters).

Several eggs were spawned, but two eggs only developed. The tail of the embryo "curled up" in one end of the egg. Twelve days after the spawning the embryo has not yet a working blood system. The body of the embryo has many black pigments. The head and the end of the tail of the embryo are distinct, whereas the central part of the embryo is indistinct, shapeless, diffuse masses of cells. A few days later one embryo died. After seventeen days the second embryo has not changed. The whole embryo is still situated in one end of the egg. The egg membrane is deformed, elliptic. A slow circulation of blood elements is seen. The yolk ball is still large, but has become more transparent, water filled? After twenty days the embryo is still alive. The pulse is not regular and no blood elements are seen in the veins. Two days later blood elements circulate again, the pulse is not regular. 24 days after spawning the situation improves. The pulse is regular, the circulation of blood elements is normal and the pectorals move. During the next seven days the development of the embryo apparently has stopped. the circulation of blood decreased and came to a complete stop. 37 days after spawning the embryo died in the egg.

#### **(25) CHR/COG 1959**

See COG/CHR. Fifteen eggs were spawned and six hybrids were raised.

#### **(26) CHR/DAG 1959**

CHR is the genotype for *Aphyosemion*. DAG is a nominal *Epiplatys*. Two CHR males were spawned in separate tanks to two females of the common aquarium strain (Monrovia strain). Fifteen eggs were harvested. Fourteen eggs were fertile. Six days after spawning these eggs contained embryos. After nine days the development of the embryos was rather promising. The development of veins on the yolk probably is too weak. After twelve days some embryos "curl up" in one end of the egg. The development of most embryos still was promising. After nineteen days many embryos were suffering from thrombus. The blood elements formed red clouds here and there on the yolk and in the body of the embryos. Thrombus frequently occurs at the entrance to the heart. Only a few embryos were still circulating their blood. Heart move and so do the pectorals of most embryos that are rather large. After 21 days some dead or dying embryos hatch out of the eggs. Some are still alive. After 24 days all embryos are dead or dying. Preserved.

#### **(27) CHR/LAB 1959**

Both forms belong to the Rivulin fauna of the Lower Congo area and probably these two forms are close relatives. CHR probably does not occur below the falls near Leopoldville, whereas LAB probably does not occur above the falls.

Only two eggs were spawned. After six days these eggs had a pigmented embryo. After nine days the embryo was large, but the development of veins on yolk appears to be insufficient for that size of the embryo. In the second egg the embryo is much smaller, measuring less than 180 degrees on the yolk and

this embryo has no working blood system. After nineteen days the situation has improved much. The largest embryo has already consumed most of the yolk, whereas the small embryo is still far behind in development. Next day the largest embryo hatched out of the egg. Too soon: big yolk sack. The hybrid is not able to swim and stays in egg glass. This embryo died one week later. The second hybrid now consumed the yolk and hatched out.

### **(28) CHR/NIG 1959**

NIG was described as a member of the subgenus *Fundulopanchax* and has the characters of that unit in *Aphyosemion*. NIG however may be considered as a "link" between the subgenera *Fundulopanchax* and *Aphyosemion*. For this reason the CHR/NIG cross is important. A female of the Akure (AK) strain of NIG was used.

Seventeen eggs were spawned and sixteen of these developed. After three days all eggs contained an embryo. After five days the embryo measured 120 degrees on the yolk. No blood, no pigments. After twelve days embryos have reached equal development in eggs. No abnormalities were discovered during the development of the hybrids in eggs. The first hybrid hatched out after 21 days. Fourteen viable hybrids were hatched. One hybrid died during hatching. During their first few weeks the development of the hybrids was normal. A severe crisis developed when the hybrids started maturing and I lost all hybrids but two. Also these two hybrids suffered much from disease (tuberculosis? + *Oodinium*). After maturing these two hybrids recovered. A male and a female developed. The male was very colorful. The blue shine was stronger than in CHR males and equal to fine specimens of NIG or even stronger. Generally this hybrid male was closer to CHR than to NIG. Oddly enough this hybrid developed more red dots on body sides than did the NIG/COG hybrids (COG has many more red dots on the body sides than has CHR and in particular my strain of CHR used for this cross). Fifty eggs were harvested. No egg was fertile. Six months old the female resembled a female of CHR, but from now on the phenotype changed into that of a male. The female did not spawn and probably was an intersex individual. Nine months old the male was a real beauty in colors and in shape of body and fins. It reached the size of a full grown old male of CHR. The yellow fin color was very strong. This male probably is in the Amsterdam Museum.

### **(29) CHR/PET 1958**

PET is a member of *Callopanchax* and this cross like the CHR/NIG cross represents a cross between members of two subgenera in *Aphyosemion*. The detailed report (on loose leaves) for this cross has been lost. The abstract of this report says: 13 fertile eggs developed. Some hybrids were hatched, but they died soon after. As my strain of PET became extinct in 1959 I have not had the possibility to control these results by a new crossing. It is indeed quite extraordinary that the CHR/PET cross produced hybrids that could be hatched.

### **(30) CHR/COG 1959-60**

CHR and COG resemble each other in most traits. Certain strains of CHR are so close to the COG phenotype that even trained killifish hobbyists consider these strains as belonging to the COG species. Within CHR various strains develop variable numbers of red dots on males' body sides. For this cross I used a strain of CHR that develops minimum number of red spots (the SCH phenotype) whereas my strain of COG developed maximum number of red spots for this species. I have also kept a strain of CHR

that developed a similar number of red dots as does the COG phenotype. This strain in CHR probably represents the original CHR phenotype. See Lambert in Ann. Mus. Roy. Afr. Cent. ser. 8 Sci. Zool. 93/1961. I distinguish between individuals of CHR and COG first of all from the shape of the caudal fin. In COG the upper and the lower edges are almost parallel, whereas in CHR the edges produce an angle. Also the produced rays at the corners of this fin are somewhat longer in CHR males. I distinguish between females by the brown reticulated pattern that develops on the sides of COG females and not on females of the two strains of CHR that I kept. Perhaps these "differences" will not keep if more material is studied in the future.

CHR is found all over most parts of the Congo drainage above the falls at Leopoldville, whereas the COG form probably is restricted to waters near that city. I had twelve eggs and only one of these did not give a viable hybrid. No abnormalities were discovered during the development of these hybrids. All eleven hybrids developed into females. Two were lost. When young these females were similar to those from COG. With age they lost however their brown reticulation and they then were just like females of CHR. These females all spawned normal eggs and they were backcrossed to both parent species.

COG/COG.CHR backcross: many eggs were spawned, but only nine eggs developed. Seven days after spawning the development of the embryos was not promising. In one egg the circulation of blood has come to a complete stop. This embryo is about to die. In sex eggs (two had already died) the embryo is still alive. These embryos differed markedly in size and development of the blood system. After thirteen days all these embryos were dead or dying. Some suffered from severe thrombus.

CHR/COG.CHR backcross: for this backcross two males of CHR and three females COG.CHR were used. 28 eggs were spawned. All eggs developed. After four days the development of the embryos differed markedly. 13 days after spawning the first fry hatched out, much too soon for the size of the embryo and the yolk ball. After 18 days I had four viable fry hatched out. Also the fry that hatched much too soon absorbed the yolk and started swimming.

At that time I had 17 eggs left unhatched. In three eggs a large embryo with a good blood system was seen. One egg contained a much smaller embryo that however apparently had developed a normal blood system (at that time). Five eggs contained dying embryos that were almost ripe for hatching. These embryos were suffering from severe thrombus and no blood elements were circulating in the veins. Eight eggs contained small embryos, dead or about to die from unknown reasons.

The report contains no information on the five or six apparently viable embryos in eggs after eighteen days. The total number of viable fry however will not exceed nine out of 28 or about 33%. These results: COG/COG.CHR and CHR/COG.CHR backcrossings indicate that an exchange of genes between these two sympatric species (my form of CHR used for this cross probably originated from Leopoldville) is very difficult and that the two forms are sufficiently genetically separated to be considered as two good species.

F2-Fx generations of the CHR/COG.CHR strain: the few viable fry of the CHR/COG.CHR backcross developed normally and matured as males and females. However these individuals were just like individuals of CHR. Males developed the very low number of red dots on body sides that characterized



the CHR strain. For this reason the pure CHR strain was preserved and only the backcross individuals were kept for further study.

I was not aware of the possibilities or further abnormalities to be expected to occur in F3-Fx generations from this "mixed strain" as I spawned the individuals in planted tanks and found sufficient offspring for my use. Egg development was not controlled. During 1962 however I was aware that the production of fry from this sort of breeding was not normal and two pairs of F3 and/or F4 generations were separated for spawning on nylon under control. I was much surprised to realize that more than half of the number of eggs spawned and fertilized in these embryos gave embryos that died in their eggs as did most hybrids in the F2 generation. Many developing embryos died from thrombus, other embryos from unknown reasons. Individuals that hatched out viable were raised under control. I found that some of these individuals did not grow. These individuals were sorted out and I tried to raise these "dwarfs" on my best food. They did not grow on this. A few were preserved and inspected under the microscope. These individuals apparently belonged to the COG phenotype as their number of red dots (very small indeed, on 10 mm individuals) by far exceeded those found on CHR individuals. After the discovery of these abnormalities I preserved the whole lot of F3-F4 individuals from this strain. "Unbalanced genes" I suppose.

I have no written report on the fate of the six CHR/COG individuals. Probably these individuals were handed over to an aquarist who forgot to report on development, sex ratio and fertility.

My collections contain a few individuals from CHR, COG and their hybrids. On this material I prepared these counts:

D= 08 09 10 11  
COG 0 0 0 2  
COG/CHR 0 3 6 0  
"CHR" 0 0 2 0

A= 14 15 16  
COG 0 1 1  
COG/CHR 1 3 5  
"CHR" 1 0 1

Sq-long= 28 29 30  
COG 0 3 1  
COG/CHR 1 14 3  
"CHR" 1 3 0

"CHR" represents individuals of the CHR phenotype, some of these (most or all) are from the backcross CHR/COG.CHR generations.

### **(31) COG/NIG 1958**

The NIG female was from the Akure (AK) strain. Six eggs were spawned and four eggs developed.

These eggs developed without any visible abnormality and four viable hybrids were hatched. They measured 4.7 mm at that age. By accident these hybrids were mixed up with fry from other species and for this reason they are not considered here. See NIG/COG cross.

### **(32) DAG/CHA 1963**

I used a DAG male of the Bruce Turner strain and a CHA female of the Kumasi strain. See also DAG/DAG and CHA/SHE for further information on these strains. As the Bruce Turner strain in DAG is very close to the DAG from Monrovia and as the Kumasi strain of CHA is close (?) to the Angona-Abra strain of this species this cross corresponds more or less to the cross 19: CHA/DAG.

Seventeen eggs developed. No abnormalities were discovered during the development of the hybrids in eggs. The fry apparently was normal and viable. Fifteen fry were divided into two lots and were raised in two tanks of their own. During the first days the fry (or some of these) were growing normally, but after one week or so it was evident that they were dying one by one from unknown reasons. Not attack of Oodinium was seen. Later on I found hybrids swimming "belly up" and apparently also suffering from spasms. Seven weeks after the spawning I preserved the last live hybrid that at that time also had turned the belly towards the surface of the water, but in other ways behaved as normal. This hybrid measured 10.2 mm (including the caudal fin). D 10, A 16 or 17, Sq-1 about 26. There are four black bars above and behind the anal fin. No such bar is seen in front of that fin. There are however traces of such a bar just behind the pectorals. The throat pattern contains a thin red line along the line of lateral line pit organs just behind the lower lip. Two dark longitudinal lines (as in DAG, not present in CHA) and some diffuse dark pigments (as in CHA, not in DAG) are present on the throat of this minute individual. The relationship between DAG and CHA probably is not as close as I suggested previously.

### **(33) DAG/COG 1958**

See also CHR/DAG. I used the common aquarium strain in DAG. Six eggs were spawned and five eggs developed. After five days the development of the embryo was promising and all embryos already had a working blood system. After ten days the development of the embryos differed markedly. Some embryos apparently do not develop at all. After fourteen days all five embryos are still alive. Heads appear as deformed (see also NIG/COG) and the size of the embryos differs much. After sixteen days I preserved all eggs as one embryo had already died and the remaining four were about to die.

### **(34) DAG/DAG 1963-64**

Recently Daget & Arnoult divided *Epiplatys dageti* Poll in two subspecies. I have seen the manuscript for this paper only. For the aquarium fish, known as "E. chaperi" since 1908, they proposed the name *E. dageti monroviae*, thus indicating that this form originated from Monrovia. The *E. dageti dageti* subspecies originated from Port Bouet near Abidjan in Ivory Coast. Stenholt Clausen and I recently mailed a manuscript on this species to the Aquarium Journal in San Francisco and I think that this article will appear this year in that magazine. I have kept four (or three) strains of DAG and have used three of these in my crossings. These strains are coded: AQ: *E. dageti monroviae* Daget & Arnoult (probably from Monrovia)

BR: a rather similar strain that Bruce Turner from New York sent me in 1963 as "wild *E. chaperi* from

Nigeria"

AW: a strain of *E. dageti dageti*, caught by Stenholt Clausen at Awiebo, SW Ghana in 1962

MO: a strain of *E. dageti monroviae*, caught by Stenholt Clausen near Monrovia in Liberia, summer 1965. Not used in any crossings so far. Does not differ from the "AQ" strain.

The BR strain differs from the common aquarium strain (AQ) only by the development of the black pattern on the body sides. In the AW strain (since 1908) there are four black bars situated in between the anterior-most anal fin ray and the root of the caudal fin. Also there is a similar bar just behind the pectorals. This bar system is extremely constant in this strain. No similar bar is ever seen above the root of the ventrals. In the BR strain additional black bars often occur in between the "normal" bars. These additional bars are seen in females and they develop high on the back and usually they are more like oblong black spots than like real bars. Also in this strain some individuals develop a black bar above the root of the ventrals.

In the AW strain additional bars are even more common and they do not differ from the normal bars in their shape and size. As in other species in *Epiplatys* that develop such additional black bars, these bars are not permanent (some may be permanent) but come and go according to the mood of the fish. In this strain many individuals develop V bars (black bars above ventrals) and the P bar (pectoral bar) is present in most individuals.

In 1965 Stenholt Clausen sent me twelve nature caught individuals of the MO strain. All these individuals corresponded exactly to the AQ strain in their black patterns. No individual developed even traces of any V bar.

In the AQ, BR and MO strains the males develop a conspicuous orange red marking in the center of the throat. This marking does not occur in the AW males (a weak pinkish color might be visible in some live males). If AW males are preserved in alcohol a very conspicuous lemon color develops on the throat. This lemon color extends over just the area that is orange red in the other males. This development of a lemon throat color that is restricted (very marked) to certain parts of the throat is not unique for the AW males as this strange development of color occurs in males of *Aphyosemion*, *Epiplatys* and *Aplocheilus*. So far I have not been able to discover any coherence between this "character" and the usual taxonomy for these fishes. The lemon color disappears after a few days or weeks in alcohol. In my opinion the orange red color seen on the throat of males of *DAG monroviae* is perhaps not identical with the base for the lemon color but at least related to this. The red marking on these males is not a normal pigmentation.

The two subspecies also differ in the development of the guanine shine. This is green blue in the western subspecies and brown to bronze in the eastern subspecies. Males of the AQ strain differ markedly in size when compared with males of the AW strain. BR males are intermediate. The new MO strain apparently does not at all develop the large size, known for the AQ individuals. They correspond to the AW individuals. Probably the AQ strain developed a marked increase in size during the almost sixty years of inbreeding.

AW/BR cross: I produced 21 "hybrids". No abnormalities were discovered during the development of these individuals. They were viable when young, but with age several individuals developed tumors in the gill region and died. Eight individuals were raised to adult size, the remaining fourteen were preserved at various phases of their development after hatching.

The AW/BR males resembled the pure BR males. However, they did not develop any orange red color on the throat. Also females corresponded more to the BR phenotype than to the AW form. The AW/BR males and females were fertile, but they reproduced "freely" in their tanks and the egg development was not kept under control. Females developed more red pigments (concentrated just behind the lower lip) than do females of the pure strains. The offspring of the free breeding individuals, the F2 or (AW/BR)<sup>2</sup> generation, did not develop any orange red color in the few males produced. Red pigments on females' throat differed much.

After this I prepared a BR/AW.BR backcross. Eggs were kept under control during their development. The egg type did not differ from that of the two parent strains (similar, 1.0-1.1 mm). Nine eggs were fertile and these eggs developed without any abnormality. The fry were viable. Only females developed in this cross.

### **BR/AQ and AQ/BR crossings**

Mr. Ib from Dragor near Copenhagen prepared these two types of crossings for me. He found no abnormalities in the development of these "hybrids". From his BR/AQ cross he had a single individual only, a male. From his AQ/BR cross he had twenty individuals. These individuals were fertile and twelve individuals were raised from their spawning.

These BR/AQ, AQ/BR and (AQ/BR)<sup>2</sup> individuals had these markings:

BR/AQ: 1 had 2P, 2V, 8A

AQ/BR: 16 had 2P, 0V, 8A; 4 had 2P, 1V, 8A

(AQ/BR)<sup>2</sup> 11 had 2P, 0V, 8A; 1 had 2P, 2V, 8A

2P = two black bars behind pectorals, one on each side.

1V = one black bar above ventrals. Missing on one side of the body

Counts: DAG-PB = E. dageti dageti from Port Bouet (type locality) from the collections of the Congo Museum

D= 08 09 10 11 Dm

DAG-PB 0 2 0 0 9.0

DAG-AW 0 16 29 4 9.8

DAG-BR 0 9 1 0 9.1

DAG-MO 0 4 1 1 9.5

AW/BR 0 3 4 0 9.6

(AW/BR)<sup>2</sup> 0 2 1 0 9.3

BR/AW.BR 0 2 1 0 9.3

BR/AQ 0 1 0 0 9.0

AQ/BR 0 17 3 0 9.2

(AQ/BR)2 1 2 9 0 9.7

A= 14 15 16 17 Am

DAG-PB 1 2 0 0 14.7

DAG-AW 6 24 16 3 15.9

DAG-BR 2 6 2 0 15.0

DAG-MO 0 4 2 0 15.3

AW/BR 0 0 7 0 16.0

(AW/BR)2 2 0 1 0 14.7

BR/AW.BR 0 1 2 0 15.8

BR/AQ 0 0 1 0 16.0

AQ/BR 0 9 11 0 15.6

(AQ/BR)2 0 2 9 1 15.9

Sq-long= 25 26 27 28 29 Sqm

DAG-PB 0 3 3 0 0 26.5

DAG-AW 1 20 50 6 3 26.8

DAG-BR 2 3 7 2 0 26.6

DAG-MO 0 5 6 1 0 26.7

AW/BR 0 1 8 4 0 27.2

(AW/BR)2 4 0 0 0 0 25.0

BR/AW.BR 0 1 4 1 0 27.0

BR/AQ 0 1 1 0 0 26.5

AQ/BR 0 14 18 8 0 26.8

(AQ/BR)2 0 11 10 1 0 26.6

Variations in counts are very limited in DAG.

Within *Epiplatys*, most species develop marked differences in the throat patterns of the two sexes. Such differences are also found within DAG. In both subspecies these patterns belong to the "permanent type" like in GRA and MAC (more or less). The two subspecies however differ markedly and we may say that in this species two types of patterns occur.

In AQ, BR and MO males the central part of the throat becomes bright orange red after maturing, whereas in PB and AW males this color does not develop. In *E. dageti monroviae* (that is AQ, BR and MO) there is no need for further differences in throat patterns as the females do not develop that orange red marking. The black pattern of the throat (for this reason) does not differ when males and females are inspected. In a few MO females (and probably also in BR and AQ) a certain black marking occurs in the center of the throat and in the line between the corners of the mouth. This marking does not occur in males. In my opinion this particular marking in a few MO females represents traces of the usual dark band that crosses the throat in most species in *Epiplatys*.

In the AW strain most males develop this particular black marking in the center of the throat whereas this marking is absent in the single male of the PB strain. In the PB strain the two females had a black throat

pattern corresponding almost exactly to the pattern found in the AW males. In the AW females the black pattern differs markedly from that of the males. These differences are based on further development of the black markings at the center of the throat. In this way differences in throat patterns are produced in all known strains.

The black pattern of the throat in DAG differs from all other known species in nominal *Epiplatys* by the two distinct black lines that are situated longitudinally and not transversally on the throat. The black pattern found in the AW strain is closer to the main "*Epiplatys* throat pattern" than the patterns found in the other strains. In the hybrid strains these complicated throat patterns break down, but in these strains the human eye still is able to distinguish between the two sexes from their throat patterns.

### **(35) DAG/GRA 1962**

Individuals of these two species differ markedly in their color patterns. They resemble each other in egg type, in the general shape of the body and the fins and in their choice of biotope. Also both species develop similar hemoglobine patterns (the "Aphyosemion pattern" = four-lines). I do not doubt that these two forms together with MAC form a certain taxonomic group in *Epiplatys*.

Both species are found in freshwater swamps near the coast. In Ghana and in Ivory Coast individuals of DAG are sympatric with the larger and more aggressive CHA species that extends its range all over the forests of these countries. In Nigeria individuals of GRA are sympatric with SEX (and LON in the Niger delta area), a close relative of CHA. So we may say that DAG and CHA in Nigeria are replaced by GRA and SEX.

I had twelve eggs in a single spawning. Six of these eggs developed an embryo. Two embryos died from unknown reasons in the eggs and four viable hybrids were hatched, too soon, but they were able to absorb their yolk. These four hybrids were not difficult to raise and three individuals matured. These hybrids developed a behavior corresponding to females, whereas their colors more or less corresponded to males. During display they developed a very broad black lateral band from gill covers to the root of the caudal fin. Such lateral bands occur in females of many species in *Epiplatys*, but they are not known for the parent species. Females of DAG that are very interested in spawning with males that are not interested (in crossings) may develop such a band. In these females the lateral band is not complete (broken) and the normal black crossbars are not reduced. I have not seen this lateral band in my GRA females nor in my MAC females. A close study of some of my close-up photos of GRA females disclose that traces of a dark lateral band are present sometimes. The development of such a band in the hybrids probably is an "ancient" trait.

The hybrids developed the "spoon like" lower jaw that frequently occurs in (aquarium kept) individuals (rarely in males) of CHA and SHE, but not in DAG.

The (probably) most important difference between individuals of DAG and GRA is found in the development of the red pattern on the body sides. In DAG this pattern is an almost regular red reticulation. No rounded red dots, no crescent shaped red dots. In GRA (and MAC) no red reticulation is found, not even on the back, and also no crescent shaped spots developed in my individuals. In these species the red pattern is a pattern of perfectly rounded red dots. In my opinion these two types of

patterns present the extremes in the development of red patterns on body sides in Old World Rivulins. I had developed the idea that the "intermediate red pattern" (between the two extremes) would be crescent shaped red dots. Indeed the three matured hybrids developed this particular red pattern on the body sides.

Also the throat pattern of these hybrids is interesting as the two parent species differ markedly in their throat patterns. The usual throat pattern in GRA (future research on this species may disclose more than one type of throat pattern) correspond to the usual Epiplatys pattern more or less. In (some) males there are two red transverse lines - one red line along the line of lateral line organs just behind the lower lip and another (more or less complete) line between the corners of the mouth. In the female these red lines are replaced by thin dark lines, often absent or not complete. The black pattern of the females does not belong to the permanent type of throat patterns. As I have only seen preserved males of GRA I do not know if males of this species develop a black color all over the lower part of the head during display, but in preserved individuals rather many melanophores are seen all over the lower part of the head.

The hybrids developed a broad red line along the line of lateral line organs behind the lower lip. The two distinct black lines that characterize the DAG species also were present. These lines (see DAG/DAG) do not occur in GRA. At least I have not been able to discover such lines on my individuals. In the hybrids the whole area of the throat in front of the imaginary line between the corners of the mouth had many black pigments, evenly distributed as in most species of Epiplatys (females only).

I made these counts on GRA individuals from Stenholt Clausen's large collection of Rivulins from S Nigeria. I found reasons to divide these samples into three major groups: the extreme west (Badagri and other localities of this area), the Lagos-Ijebu Ode area and the Niger delta area.

D= 07 08 09 10 11 Dm

Badagri 1 6 4 0 0

Lagos-Ijebu Ode 0 31 17 1 0

Niger delta 0 0 2 0 0

GRA 1 39 23 1 0 8.4

DAG (-hybrids) 0 0 31 31 5 9.6

DAG/GRA 0 0 0 3 0 10.0

A= 14 15 16 17 18 19 Am

Badagri 0 1 6 3 0 0

Lagos-Ijebu Ode 0 13 31 3 0 1

Niger delta 0 0 2 0 0 0

GRA 0 14 39 6 0 1 15.9

DAG (-hybrids) 9 36 20 3 0 0 15.3

DAG/GRA 0 0 1 2 0 0 16.7

Sq-long= 25 26 27 28 29 30 Sqm

Badagri 0 0 10 10 0 0

Lagos-Ijebu Ode 0 2 15 25 6 2

Niger delta 0 0 0 1 3 0

GRA 0 2 25 36 9 2 27.7

DAG (-hybrids) 3 31 66 9 3 0 26.8

DAG/GRA 0 0 0 5 1 0 28.1

The DAG/GRA hybrids never spawned and probably they were not able to produce eggs. They reached GRA size, the largest individual measured 33 mm without caudal.

### **(36) DAG/MAC 1964**

GRA and MAC resemble each other in most details and probably they are so closely related that they both belong to one species that is the GRA species. For this reason the DAG/MAC cross was expected to give viable hybrids indeed. As MAC comes from the Lower Congo drainage whereas GRA comes from the Lagos-Niger area certain genetical differences not expressed in the phenotype may occur. I had one individual on MAC onla. A female. I used a DAG-BR male that in genetics may correspond well to the DAG-AQ male used for the DAG/GRA cross.

My MAC female probably was diseased (tuberculosis?) and as it was not willing to spawn with different males in crossings it kept its eggs "too long" and for this reason most eggs first produced in crossings were not viable. After two weeks I have had 37 eggs in this combination. Six spawnings. First, most eggs were not viable. A few however developed a normal blastula but died thereafter. Most eggs of this female had a very large yolk ball and the space between the yolk and the membrane was so narrow that it was impossible to trace the blastula. Later on the eggs were more normal and I had fourteen eggs that developed. Fourteen hybrids were hatched. They apparently were normal and viable. I placed these hybrids in two tanks of their own. First the hybrids were growing normally, but after a few days they started dying one by one. No external disease was discovered, so probably they were not viable after all.

### **(37) DAG/LIN 1958**

LIN is an Aplocheilus from India. As other Aplocheilus also this species has many characters in common with the African Epiplatys. Both individuals used in this cross were of the usual aquarium strains of DAG and LIN.

It took much time to train these two individuals to spawn. At last I had eight eggs in a single spawning. All eggs developed. After nine days the development of the hybrid embryos differed markedly. In some eggs the embryo had a fine blood system and these embryos indeed were very promising. In other eggs the development of the blood system is not promising. The largest embryos had already the heavy black pigmentation that characterizes embryos of Aplocheilus. After eleven days the development of the embryos differs even more. In some eggs severe thrombus occurs and the circulation of blood has come to a complete stop. In other eggs the situation is still promising and these embryos might be hatched if thrombus does not develop. After thirteen days however all embryos are dead or dying from severe thrombus.

For further control I prepared a second cross using the same two individuals. I had seventeen eggs in a single spawning and twelve of these eggs developed. After five days the development of the embryos is not satisfying. After eight days most embryos are dead or dying from thrombus. After twelve days a few are still alive. Preserved.



The result of the DAG/LIN crossing however is the "best" of the Epiplatys/Aplocheilus crossings so far produced. See PAN/FAS, SEN/PAN and SEX/LIN.

### **(38) DAG/SEX 1960 (see also SEX/DAG)**

SEX and DAG represent two "lines of evolution" in nominal Epiplatys, the SEX-LON-CHA-SHE line contra the DAG-GRA-MAC line. The group first mentioned belongs to the "six-line Epiplatys" and the second group to the "four-line Epiplatys" if hemoglobine patterns are considered. Indeed there are other "four-line" or "six-line" Epiplatys which -for the present- cannot easily be linked to these two groups of apparently closely related forms.

I used a DAG male of the common aquarium strain and a SEX female of the Ibadan strain. The crossing was very difficult to arrange as the SEX female did not consider the DAG male as a natural partner in spawning. After a long time of training and heavy feeding the spawning started however. The eggs were not harvested from the nylon, so I have no report on development of eggs. Only one egg gave a viable hybrid however. This hybrid was raised to adult size and was a very slow growing individual. I had this hybrid for eighteen months and used it in several backcrosses to females of DAG and SEX without any fertile eggs from these spawnings. The hybrid acted as a male in spawning, but apparently it was more like an intersex (colors etc.).

The DAG/SEX and SEX/DAG hybrids both resembled each other in most characters. In SEX the individual normally develops six dark crossbars on the body sides: 2P, 2V, 4A. Additional black bars may occur in females (temporary or permanent) rarely in males. The DAG strain used in this cross develops 2P, 0V, 4A very constantly. Both hybrids developed the posterior-most bar in the "A series". Sometimes also the last but one bar was visible on the sides. The SEX/DAG hybrid developed the P bars, whereas these bars were very weakly developed in the DAG/SEX hybrid. Apparently both hybrids were not able to develop any V bar nor did they develop the two anterior-most A bars. Instead of these bars a very broad indistinct and pale lateral band developed on the sides during display (spawning).

The two hybrids did not develop any produced rays at the lower corner of the caudal fin as in DAG and sometimes in old males of SEX. Also the lower edge of that fin did not develop the broad dark edging seen on fine males of DAG-AQ, BR and MO. This black edging is weakly developed in most strains of SEX.

DAG and SEX differ in the development of central lateral line organs on the snout (Stenholt Clausen's system) as individuals of the SEX-LON group in Epiplatys are the only species in Epiplatys (so far known to us) that develop the pattern seen among Fundulopanchax in Aphyosemion. All other species develop the pattern seen in Callopanchax in Aphyosemion. The hybrid SEX/DAG which is in my own collection developed an intermediate pattern of organs on the central part of the snout. The DAG/SEX hybrid is in the Amsterdam Museum.

On the SEX/DAG hybrid I made these counts:

D 11, A 17, Sq-long 28 and 29

These figures correspond well to the mean values for SEX and DAG. The sole derivation from the

morphology of SEX and DAG was found in the length of the head. In percent of standard length this is 32% in the hybrid, 24-26% in SEX (Ibadan) and 25-29% in DAG-AQ.

(39) DAY/LIN 1964 DAY and LIN both belong to the genus *Aplocheilus*. As these species also are used in crossings to African *Epiplatys* I find reasons for the publication of the results of this cross and other crossings in *Aplocheilus*.

DAY and LIN appear to represent a certain group in *Aplocheilus* and also DAY has been considered as a subspecies of LIN. The resemblance however is not (probably not) a question of close relationship. So say the results of the crossing, the study of the hemoglobine patterns and the patterns of lateral line organs on the head.

Just the same could be said about the second "group" in *Aplocheilus*, the PAN-BLO group. Also these species have been considered as closely related forms and BLO has been considered as a synonym for PAN or a subspecies of PAN. A close study of the hemoglobine patterns and the development of scales and lateral line organs on the head does not support such an idea. There are strong indications that the "grouping" in *Aplocheilus* is not DAY-LIN contra BLO-PAN, but DAY-BLO contra LIN-PAN if any grouping of closely related forms occurs in this genus.

Here are some counts that I prepared on preserved individuals of these four species:

D= 06 07 08 09 Dm

BLO 0 1 7 2 8.1

DAY 0 3 3 0 7.5

LIN 0 0 6 7 8.5

PAN 1 5 54 16 8.1

A= 14 15 16 17 18 19 20 Am

BLO 3 7 0 0 0 0 14.7

DAY 0 1 3 0 1 0 15.7

LIN 0 0 0 2 2 7 2 18.7

PAN 0 1 16 38 20 1 0 17.0

Sq-long=24 25 26 27 28 29 30 31 32 33 34 Sqm

BLO 1 7 5 4 0 0 0 0 0 25.7

DAY 0 0 0 0 0 1 1 7 1 0 30.6

LIN 0 0 0 0 0 0 5 8 6 2 32.2

PAN 0 0 0 2 2 29 17 18 3 1 0 29.8

The differences in counts between these four species are marked indeed. LIN and PAN develop simple hemoglobine patterns that are composed by two broad lines only. The two "two-line" patterns however differ in some details. DAY and BLO develop the complicated "six line pattern" found in SEX, LON, CHA, CHE and DUB among nominal *Epiplatys*. We are not -for the present- quite sure that these two groups of six line patterns correspond to each other in all details.

In the development of lateral line organs on the central part of the snout LIN and PAN correspond (more or less) to the species in Nothobranchius, Fundulopanchax, Aphyosemion and the SEX-LON group in Epiplatys, whereas BLO and DAY develop a pattern corresponding to that of Callopanchax and Epiplatys (SEX and LON).

These findings indicate a grouping different from the usual one in Aplocheilus. BLO (most individuals, not all of them) differs from the other species by the scale pattern of the forehead. In this form the rare "reversed" pattern or E- pattern occurs, as in Pachypanchax and in GUI in Callopanchax.

Individuals of BLO, LIN and PAN do not develop any particular throat pattern. Individuals of DAY develop temporary throat patterns corresponding to those found in Epiplatys. Rather complicated and still not known in all details.

DAY and LIN develop dark crossbars in both sexes. Very weakly developed such bars are sometimes seen on young individuals of BLO, but never on individuals of PAN.

I found it very difficult to produce natural crossings between individuals of DAY and LIN. After one month of hard training I had a single egg, after all. After this result they refused to do more about it.

The egg developed a blastula, but apparently the gastrulation was not normal. I was able to trace some heaps of undifferentiated cells here and there on the surface of the yolk. After three days the decomposed. That is all. Compare with: PAN/LIN and PAN/DAY crossings which gave similar results in more eggs.

#### **(40) FAS/CHA 1964**

I used a FAS male of the Kenema strain and a CHA female of a mixed strain (CHA.SHE/KUM strain, see CHA/SHE cross). Two such females were used for this cross. These females were not willing to spawn the FAS males. Two males, separate tanks. Also the males were not interested in these females. During two months of training I harvested a few eggs now and then. Totally I had twelve eggs. Five eggs, at least, probably had been fertilized by the male. Only in one egg I saw a blastula and the multidish. No further development was noticed and soon the eggs died.

#### **(41) FAS/DAG 1964**

A FAS-12.13S male (see FAS/FAS) was used. One female of DAG-BR and one of DAG-AW/BR were used. The individuals were not very willing to spawn. At least the male was not very interested.

DAG-AW/BR female: after some time of heavy feeding this female had very many eggs and was very interested in spawning. The male was not willing to cross. The female now developed a dark lateral band, not very complete nor regular. The normal dark crossbars remained unchanged. I have never seen (or heard of) such a lateral band in DAG. See also PAN/LINL and SEX/LIN for development of such an "unnatural band" in crossings.

I had seven eggs. Only two eggs developed. In one egg the blastula and the multidish were normal, but after this the egg died. The second egg developed an embryo. After five days the embryo measured about 90degrees on the yolk. Next day it measured 180 degrees and a deep groove became visible in the yolk.

Despite of this groove the tail of the embryo turned off at a right angle and the embryo started "curling up". Now further development of the embryo stopped and the egg remained unchanged during the next eleven days. During the first days the heart of the embryo pulsated, but later on the pulse decreased and the heart stopped movements. No blood elements were seen in veins. After twenty days the egg was preserved. The female developed tumors in the gill region and was preserved.

DAG-BR female: as the male had sufficient training it accepted the second female at once. 28 eggs were spawned and 23 of these developed. After the development of the blastula and the multidish ten eggs died. The remaining 13 eggs developed a corda, but from now on further development stopped in some eggs. Some eggs developed a "blister" on the yolk ball. As this blister grew very large in some eggs these eggs burst and were lost. The development of the blister and the embryo on it did not differ from the observations made on eggs in the CAL/SEN and SEX/LIN crossings. The thin vein that crossed the interior of the blister was present and at least in one egg a few blood elements were moving in this vein. The embryo was situated with the head on the blister and the tail on the yolk. The blister was separated from the yolk ball by a plane formed by the inner membrane of the yolk. This plane was slightly moving up and down by the pulsating vein, attached to its center. Some eggs had a working blood system, but this was put out of action rather soon as a thrombus developed, in particular at the end of the tail of the embryos. One embryo recovered from the first development of thrombus and was again circulation the blood for a few days. Then again a thrombus developed and stopped the circulation. Some embryos reached almost the size of a ripe embryo before they were killed by thrombus. An embryo, 20 days old, hatched out of the egg because of the development of a blister. This embryo measured 3.2 mm. After 27 days the last embryo died. No hybrid embryo hatched alive.

FAS is difficult to place within *Epiplatys*. Together with its close relative, the SEN (or SPI) species, these forms do not appear to be closely related to any known species in *Epiplatys*. It may be so that these two species are linked to the *Callopanchax* group in nominal *Aphyosemion* in some or another way.

#### **(42) FAS/FAS 1963-65**

During 1963 E. Roloff in Karlsruhe sent me live individuals of FAS from Sierra Leone. These individuals originated from different localities, but the exact locality was not known for some of the individuals. Two rather distinct forms were present in this collection.

FAS-10S and FAS-10L (I use Roloff's codes, modified) originated from the Freetown area where Roloff has taken his individuals in mountain brooks. This phenotype probably corresponds best to the types for FAS that probably came from that area. The individuals of the FAS-10 strain (strains) were big fishes, robust and deep bodied, colors weakly developed. When afraid they did not hide in the mud at the bottom of my tanks. The two strains from different brooks differed somewhat in size (10L is smaller than 10S). Also males differed constantly (also in their offspring) by the way that in FAS-10S the males developed a double red line near the edge of the anal fin and at the lower edge of the caudal fin. In the FAS-10L strain the males developed a single red line only. The pattern found in males of the 10S strain is the normal (?) pattern for FAS males, at least from Sierra Leone. The two Freetown strains were crossed, but no abnormalities in embryo development or in viability developed in the F1 and the F2 generations. The differences found between these two strains are much too small to be considered in taxonomy of FAS.

Other individuals originated from Kenema, a forest biotope, near the border to Liberia. These two areas are (at least during the present climatic conditions) separated from each other by a savannah wedge that comes in from the north. Rainforest living species probably will not pass through this wedge. FAS from Sierra Leone however is not restricted to the forest and individuals are found also in savannah biotopes together with BIF and *Aplocheilichthys normani* Ahl. For this reason there may be a chain of FAS populations between the Freetown and the Kenema areas. The Kenema male is much more colorful than the Freetown male. The general line of the color pattern however does not differ. Perhaps the Kenema form is a bit more slender (body depth). I have however worked more with a strain that has been coded "FAS-12/13S". The exact locality for this strain is not known. The male and the two females (male FAS-12 and females FAS-13S) originated from different localities, but their phenotypes correspond.

This form is characterized by a much more slender body than the FAS from Freetown and Kenema. In their colors and color patterns these slender individuals correspond almost exactly to the Kenema phenotype, although the slender males probably develop even more brilliant colors. When afraid these individuals dive deep into the mud (I use mud in all tanks) and they will stay there even for a long time. I had to "dig" these individuals out of the mud when they had to be removed to another tank. In this these FAS individuals correspond well to individuals of SEN that however normally soon will come out of the mud. The FAS-12/13S individuals also were "mud divers". These individuals (12/13S) were rather variable in counts, indicating the origin of the parents from different demes.

#### Intraspecific fertility:

The crossing of the two Freetown forms did not disclose abnormalities. The offspring was fertile and viable. Also the cross of the slender male (no 12) to a female of the Freetown phenotype did not disclose abnormalities.

The FAS-12/13S individuals were intended to form the base for a handsome aquarium strain. For this reason a more exact control of their characters was prepared. A pair was spawned under control. I had seventeen eggs and all eggs developed. Ten of these eggs were studied during their development. After five days the embryos had a well working blood system, but in some eggs the tail of the embryo began "curling up". After eleven days one embryo suffered from a thrombus, but the circulation of blood has not come to a complete stop. Other embryos are "hibernating". Their hearts do not move and the blood and the blood elements are not moving in veins. Such hibernating is not common in *Epiplatys* and indicates "annual characters". It is however very easy to "wake up" these embryos. A few seconds or minutes in a strong light will do the job and full circulation of blood will be seen a few seconds later. After 19 days (too late for FAS) the first embryos are ripe and hatch out. One of these was badly deformed (from the "curling up" in one end of the egg) and was not viable. In another fry that had hatched out I found that all circulation of blood has stopped. The elements are heaping in front of the heart (see BIF/BIF). The embryo that developed a severe thrombus lived up to more than twenty days. It did not recover from this abnormality and died. Most fry however were viable and not difficult to raise. These small abnormalities in the reproduction of the FAS-12/13S again indicate that the individuals (lot 12 and lot 13) from which this strain was produced were not from a single deme of FAS. About 30% are not viable.

As I had only a single male of the Kenema strain left I crossed this male to one of my 12/13S females. Individuals from this cross were very viable and colorful. A pair of these KE/12.13S individuals was spawned in spring 1965. I studied the development of 43 fertile eggs. Ten of these eggs developed severe abnormalities that were lethal to the embryos. Most of these were killed by thrombus, but at least in one egg the development of the embryo also was not normal. The heart was situated much too far from the throat. These ten embryos are in Bouin. I hatched 32 apparently viable fry. Only two of these survived. They died one by one from unknown reasons. I was not able to trace any Oodinium or other external disease on their bodies. I do not consider FAS as a difficult fish to raise and I have not had any difficulties in the reproduction of the many strains which I studied.

Here are some counts on preserved material at my disposal:

D= 10 11 12 13 14 15

- 1) Freetown 0 1 24 0 0 0 FAS-10S and 10L
- 2) cross of these 0 1 2 2 1 0 FAS-10S/10L
- 3) Magbenta 0 4 7 0 1 0 nature caught
- 4) Kamlui F.R. 0 0 9 8 1 0 likewise
- 5) Kassewi 1 1 2 0 0 0 likewise
- 6) Kamakwie 0 1 2 0 0 0 likewise
- 7) Kenema 0 0 1 0 0 1 likewise
- 8) FAS-12 male 0 1 0 0 0 0 likewise
- 9) FAS-13S females 0 1 1 0 0 0 likewise
- 10) their offspring 2 4 1 1 0 0 FAS-12/13S
- 11) "slender/deep" 0 1 4 5 2 0 FAS-12/10?
- 12) pair "deep form" 1 4 1 0 0 0 FAS-10/10?
- 13) mixed strain 0 1 5 1 0 0 FAS-KE/12.13S

Lot 10 and 11 represent the offspring of lot 8 male with different females.

Lot 11 and 12 represent the offspring of one female to different males.

A= 15 16 17 18 19 20

- 1) Freetown 0 1 14 11 0 0
- 2) cross of these 0 0 2 3 1 0
- 3) Magbenta 0 0 0 5 6 1
- 4) Kamlui F.R. 0 0 5 9 4 0
- 5) Kassewi 1 0 2 1 0 0
- 6) Kamakwie 0 0 0 1 2 0
- 7) Kenema 0 0 0 1 0 1
- 8) FAS-12 male 0 0 1 0 0 0
- 9) FAS-13S females 0 0 0 2 0 0
- 10) their offspring 0 1 4 1 2 0
- 11) "slender/deep" 0 0 1 2 7 1
- 12) pair "deep form" 1 1 3 0 1 0
- 13) mixed strain 0 0 1 3 3 0

- Sq-long= 27 28 29 30 31
- 1) Freetown 1 6 11 4 0
  - 2) cross of these 0 0 3 7 1
  - 3) Magbenta 1 4 9 3 0
  - 4) Kamlui F.R. 0 1 14 19 0
  - 5) Kassewi 0 0 6 0 0
  - 6) Kamakwie 0 0 3 3 0
  - 7) Kenema 0 0 0 4 0
  - 8) FAS-12 male 0 0 0 1 0
  - 9) FAS-13S females 0 0 1 1 0
  - 10) their offspring 0 1 6 3 2
  - 11) "slender/deep" 0 1 4 9 7
  - 12) pair "deep form" 0 0 1 5 1
  - 13) mixed strain 0 1 3 10 0

This rather large variation in counts is not a result of my different crossings as it can be seen that this variation already exists in nature. Notice that the offspring (different males used) from the "10?" female (cross 11 and 12) had this variation among 18 individuals: D 10-14, ! 15-20, Sq 28-31. Also the two broods of the slender "12" male with different females shows much variation. D 10-14, A 16-20, Sq 28-31.

#### **(43) FAS/SEN 1964-65**

FAS and SEN (or SPI) are very closely related forms and SEN probably originated from some savannah stains of FAS in or near Sierra Leone. I have not seen any individuals of SEN in Sierra Leone samples in which BIF occurs together with *Aplocheilichtys normani* Ahl. These two species are indications of "savanna biotopes". Instead a somewhat different form (color patterns) of FAS occurs in these samples. The Kamakwie individuals of FAS (A. Schiotz 1963) belong to this form of FAS.

Ten eggs were spawned and nine of these developed. After three days the embryos measured 90\_ on the yolk. After five days they measured 180 degrees and pigmentation was visible. Next day the blood circulated in veins. After twelve days the first fry hatched out. All nine eggs gave viable hybrids. These hybrids were rather small. The rate of growth was high and as I had fry from SEN and FAS of the same age I was able to compare. First the rate of growth was intermediate to that of SEN (slow) and FAS (quick), but after some time the hybrids were growing faster than did the young FAS. At an age of six weeks the broad dark lateral band (prematuring "signal" for females of SEN and FAS) was visible. Later on the size of the hybrids differed as some individuals were very slowly growing, whereas other individuals continued their fast growth. Six males and three females (or two females and one inter-sex) developed. The sexual characters were well marked in most individuals and also their sexual display apparently was normal.

Here are the counts for nine hybrids and for the parent strains (FAS-12/10? and SEN-VO/NI).

D= 08 09 10 11 12 13 14 Dm  
FAS 0 0 0 1 4 5 2 12.7  
FAS/SEN 0 0 1 7 1 0 0 11.0  
SEN 1 6 5 0 0 0 0 9.3

A= 15 16 17 18 19 20 Am  
FAS 0 0 1 2 7 1 18.7  
FAS/SEN 0 1 7 0 1 0 17.1  
SEN 1 3 5 3 0 0 16.8

Sq-long= 27 28 29 30 31 Sqm  
FAS 0 1 4 9 7 30.0  
FAS/SEN 1 3 12 2 0 28.8  
SEN 1 9 2 0 0 28.1

The color patterns developed by SEN and FAS are closely related. Generally males of FAS are much more colorful than males of SEN, but the elements of the color patterns are similar.

In both forms the red pattern on body sides is a red reticulation which however tends to develop crescent shaped red spots and rarely also rounded red dots just behind the pectoral fin (resembling the "wound" of species in *Aphyosemion*, all groups). Hybrids corresponded to their parent forms in this pattern. The red throat pattern found in these two forms is not very conspicuous as the heavy concentration of black pigments covers most red pigments. In FAS there are normally two red cross-lines (one behind the lower lip and one between the corners of the mouth), but a third red line may be visible behind the line between the corners of the mouth. The red pattern of SEN is similar, but the development of red pigments is much weaker. In one savanna strain of FAS the red line behind the lower lip was very conspicuous, broad without black pigments.

The black pattern of the body sides of both forms contains narrow oblique dark crossbars that apparently do not belong to the type of crossbars found in other nominal *Epiplatys*. In adult males of both forms these dark bars are rarely visible and also adult females may lose their dark bars. Hybrids corresponded to this pattern also. The black pattern of the throat is the usual one found in *Epiplatys*. There two black bands corresponding to (and converting to) the two normal red lines. However, in FAS and SEN the bands are not very distinct and black pigments may cover the whole area in front of the line between the corners of the mouth (in females) or the lower part of the head (in males). During display the males of these two species (less marked in hybrids) develop a very deep black color all over the lower part of the head.

Fins are uncolored (SEN) or yellow or orange yellow in both species. The hybrids were much closer to the FAS phenotype than to the SEN phenotype in their development of colors of the males.

Males of FAS normally develop a double red line at the edge of the anal fin and the lower edge of the caudal fin. In SEN this line (edge) is not doubled. The hybrid males developed the double red line in anal and caudal, but also in the dorsal fin a red line not found in SEN and FAS was visible near the upper edge



of that fin.

Two hybrid males and two hybrid females were placed in two separate tanks for control of fertility. One female spawned eggs of variable size (1.1-1.7 mm), whereas the second hybrid female spawned eggs of rather constant size (1.20-1.25 mm). I had 10 + 44 eggs. Most eggs developed a true blastula and in some eggs also the multidish was visible some hours later. These multidishes were not normal, they "broke up" into isolated heaps of cells. The blastula was controlled under the microscope and contained many normal cells. No further development was seen and after some days the eggs died.

Both males now were backcrossed to two females of FAS (KE/12.13). These two spawnings gave 79 + 84 = 163 eggs. No blastula could be seen in any of these many eggs. After two days I had however eight transparent eggs left. After one more day these eggs were dead.

The two females were backcrossed to FAS-KE/12.13S males. Only one female produced eggs. I had 21 eggs from that female. Most eggs developed a normal blastula. Seventeen eggs went through the normal gastrulation and a deep groove was visible in the yolk. After four days I had fifteen live eggs. Only two or three of these had a normal corda, whereas the remaining eggs contained "corda like" developments on the yolk. After six days only one egg contained an "apparently viable embryo". This embryo had already a working blood system. The "embryos" found in other eggs appear to be abnormal or deformed. After eight days fourteen eggs were still alive. Some eggs now apparently develop a more normal embryo but only in two eggs circulation of blood is visible. After twelve days all eggs were closely inspected under the microscope: two eggs contained a "more or 180 degrees" embryo. Five eggs contained no visible embryo at all. Five more eggs contained abnormal embryos. After fourteen days I had eleven eggs left. Micro photos of all eggs were prepared. Now more eggs started dying and the live eggs were preserved as there probably would not come any viable offspring from this backcross.

The spawning hybrid female now was backcrossed to a SEN male. I had nine eggs. These two individuals do not cooperate and the female does not produce eggs as it did before. None of these eggs developed. The female reacted more and more as a male.

#### **(44) GUL/NIG 1960-61**

Both species belong to the subgenus *Fundulopanchax* in nominal *Aphyosemion* and are found in southern Nigeria, but as far as I am informed they are not sympatric (GUL and COE are sympatric). The GUL male used for this cross was caught at Ago-Iwoye in SW Nigeria by Stenholt Clausen in 1959. The NIG female was from the Akure strain used for most crossings. I spawned these two individuals on coarse peat and probably they produced very many eggs. However, only one hybrid hatched out of the peat after six weeks of drying. This particular hybrid was very viable and was very easy to raise to adult size. I kept this female hybrid for almost two years. The standard length reached 51 mm. I backcrossed the female to males of NIG and COE (as I had no GUL males). Very many eggs were spawned in these backcrossings, but no zygote developed in any egg. Several eggs remained alive for up to one week. In one egg that remained alive for more than one week I found black pigment cells on the surface of the yolk, but no embryo was visible. I did not control for blastula or gastrulation at that time.

I prepared these counts on preserved material from NIG-AK (Akure), GUL-AG (Ago-Iwoye) and the

single hybrid.

D= 12 13 14 15 16 17 18 Dm  
GUL-AG 0 0 0 3 10 9 2 16.4  
GUL/NIG 0 0 0 1 0 0 0 15.0  
NIG-AK 1 21 11 6 1 0 0 13.6

A= 15 16 17 18 19 Am  
GUL-AG 0 0 4 17 3 18.0  
GUL/NIG 0 0 0 0 1 19.0  
NIG-AK 3 23 12 0 0 16.3

Sq-long= 29 30 31 32 33 34 35 Sqm  
GUL-AG 1 1 4 7 6 4 4 32.6  
GUL/NIG 0 0 1 0 0 0 1 33.0  
NIG-AK 0 2 9 5 5 1 0 31.8

In GUL individuals develop H scales normally. These additional scales are situated below the anterior edge of the large G scale. In the Akure deme of NIG about 50% of the individuals develop H scales, some below and some upon the anterior part of the G scale. The hybrid developed two prominent H scales that were situated on the G scale. Both parent species develop ctenoidy in males. This character is less developed in NIG individuals and in particular in those of the Akure deme. The hybrid female did not develop any spines on scales.

#### **(45) GUL/SPU 1965**

For this cross I used a GUL male of the common aquarium strain called BEA by the aquarist. The individuals of this strain do not differ from Stenholt Clausen's Ago-Iwoye strain and probably the aquarium strain originated from SW Nigeria.

I had 19 eggs. 13 of these developed a blastula and after this four eggs died. probably these eggs did not "close". After the gastrulation further development stopped. Eggs were "resting or hibernating" (Stadium "Ib" of Peters, "Ruhende Eier" of Foersch). After some weeks the yolk of the eggs started decomposition and the eggs died one by one. After 40 days only three live eggs were left. Also these eggs decomposed during the next weeks. So far this cross is the only one among the many crossings between individuals from different West African species which gave this result that is well known from crossings in *Nothobranchius* and *Cynolebias*. As GUL and SPU both belong to the group of "annual Rivulins" in *Aphyosemion* the result is not surprising. I arrange the reciprocal cross SPU/GUL now. Maybe this cross will give away viable hybrids.

GUL males resemble males of FIL in most details of the color pattern. GUL however is a large species, whereas FIL belongs to the small *Fundulopanchax*. Besides the difference in size two marked differences are seen. GUL males develop produced rays in the anterior part of the dorsal fin and in GUL males the hindmost central part of the throat is black, thus producing a very conspicuous dark marking. An alike marking, less marked, but probably related to the GUL marking occurs in SPU males and even much

more reduced in certain NIG males. Not even traces of such markings are seen in FIL males. In these the branchiostegal membrane is red. This membrane is black or blackish in SPU, GUL and certain NIG. Also FIL differs from GUL in the number of scales long.

#### **(46) LAB/CHR 1959**

I had 31 eggs in one spawning. 14 eggs developed. After four days all eggs contained embryos. Eyes had black pigments already. After one week the embryos exceeded 360 degrees on the yolk. The development of the blood system was promising and the body had black pigments. After fifteen days the first sound hybrid hatched out. The remaining eggs did not hatch so they were forced to hatch. All were killed during hatching. The sole hybrid that hatched alive died while maturing. With more care the production of viable hybrids would be possible and is likely because these two species are close relatives.

#### **(47) LAB/CIN 1961**

I had 14 eggs in one spawning. All eggs developed without any abnormality and the first hybrid hatched out after seventeen days. The remaining lot of eggs did not hatch in a natural way and were forced to hatch. These hybrids were bad swimmers, very feeble and soon suffered from an attack of Oodinium. I was not able to keep them alive and after two weeks all were dead.

#### **(48) LAB/COG 1957**

I had 50 eggs in one spawning. 48 eggs developed without any abnormality and have viable fry. After the hatching the fry measured 5.0 mm. Ten weeks after the hatching I had 44 hybrids. Most hybrids developed into males and the remaining individuals probably were inter-sex or sterile females. The LAB/COG hybrid male is a real beauty and after maturing it is indeed a hard fish that will not give you any trouble. In backcrossings these males proved to be sterile and the female like individuals did not spawn. Most of these hybrids are in the Amsterdam Museum, a few are in the Congo Museum and I have 15 left. On these I prepared these counts:

D= 09 10 11 12 Dm  
LAB/COG 3 3 7 2 10.5

A= 15 16 17 Am  
LAB/COG 2 11 2 16.0

Sq-long= 28 29 30 31 Sqm  
LAB/COG 1 9 17 2 29.7

I have not enough material of LAB and COG to prepare statistics. Probably these extremes in counts occur in these forms: COG D 09-11 A 14-16 Sq-long 29-30  
LAB D 12-14 A 16-17 Sq-long 30-3k

This beautiful and very viable hybrid form has been produced in the USA several times since my first report on the crossing in the "Killie Letters". Franz Werner from Detroit sent me beautiful color slides showing his hybrids and his parent species. These hybrids corresponded exactly to the one I produced in 1957. These hybrids belong to the "flame tailed" group in Aphyosemion.

#### **(49) LAB/NIG 1958**

I had nine eggs. Three of these developed and after eight days the eggs contained large embryos. Sixteen days after the spawning all embryos died in their eggs from severe thrombus. See also NIG/LAB.

#### **(50) LUJ/CHR 1961**

The aquarium fish here identified as LUJ (with some hesitation) originated from a locality about 200 km N of Leopoldville in the Leo-Congo area. My identification corresponds to that of S. Weitzmann (in La Corte, R. "Aphyosemion lujae", Aquarium Journal, 1961, pages 64 ff. This article contains a fine color photo showing a male). Poll and Lambert (personal identifications) first identified this form as CAM, later as STR. In my statistics I placed this form in STR, not in LUJ.

"LUJ" belongs to the difficult group of "flame tailed" Aphyosemion (Lambert), but it differs markedly from other forms in this group (NDI, NIA, LAB etc.) by the intense overall yellow orange red color in males. The "flame tailed" Aphyosemion from the Congo drainage spawn eggs which do not develop any trace of reticulation, whereas in NDI a strong reticulation develops.

I had six eggs in one spawning. Four eggs developed. After four days a small embryo was visible in all eggs. After ten days the embryo is promising, but there are indications that the development will not be normal. After seventeen days the first hybrid hatched out. The hybrid is weak and cannot swim. After 23 days another hybrid hatched out. Two hybrids died at the hatching. These hybrids were very slender, but they were able to swim. 40 days after the hatching the two hybrids are still alive. The heads apparently are deformed and the lower jaw is "spoon like" as in hybrids of the NIG/COG cross. These severe deformations of the jaws normally are fatal as the young fish is not able to eat. The hybrids however lived for a few more weeks. At last they lost their "air of the swimbladder" and sank to the bottom. Apparently they had black crossbars on the body sides.

My strain of LUJ became extinct as I was not able to raise most fry to adult size. Most individuals developed into "dwarfs". Such difficulties in the reproduction of Aphyosemion are often reported in literature, but only this species in my collections was found to be difficult to raise. See also LUJ/CIN and LUJ/COG.

#### **(51) LUJ/CIN 1961**

I had three eggs only. Two eggs developed. First the development of the hybrid zygote was very slow. After eighteen days however the two eggs hatched and the hybrids were able to swim at once. One hybrid soon died. The second hybrid did not grow. More than six weeks after the hatching the hybrid was caught for control. No deformations of the head were seen. No external diseases. The hybrid however measured 8-10 mm only (caudal fin included). This hybrid lived for several more weeks, but it did not grow at all. It died in its tank and was not preserved.

#### **(52) LUJ/COG 1961**

19 eggs were spawned in one spawning. Eighteen eggs developed. After eight days the development of the embryos was promising and embryos had developed equal size. After 25 days the hybrids started hatching. Soon after the hatching all hybrids died from unknown reasons. The only deformation of the

hybrids that I was able to trace was a deformed tail in one individual, probably caused by "curling up" in the egg.

*[continued in Scheel Letter No. 53; Part 3]*