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Crossings Part 3: Rivulinae Myers in Cyprinodontidae

(53) MIL/NIG 1964
In 1964 Dr. Sick from the Genetical Institute of the Copenhagen University discovered that individuals of Aphyosemion and Rivulus develop similar hemoglobine patterns. Indeed in all details these four line patterns corresponded one to the other. As also these two groups of Rivulins correspond in other details I made up my mind to try a crossing.

As some of the old strains of Rivulins from South America still were kept in Copenhagen I got a fine male of Rivulus milesi Fowler ("Golden Tail Rivulus", Scheidnass strain) and prepared a cross to NIG. Before that crossing I "trained" the male in a tank containing FAS individuals and by accident a single female of CIN. The MIL male did not pay any attention to the FAS females that probably (to the human eye) are closest to the "Rivulus" phenotype, but after some time it was very interested in the CIN female. Often these two individuals spawned in that tank. At that time I had a NIG-PH/AK female (see NIG/NIG) which has been trained in crossings. First I tried it in combination with a PAN male (Aplocheilus panchax, Djakarta strain from Thung Kim Tek). Both individuals were interested in the crossing and they swam to the nylon at the water surface and stood side by side for a long time. These movements were repeated again and again but the final coordination of movements and stimuli probably could not operate. At last the female grew "angry" and attacked the male. She won. Now I had the MIL male ready for crossing and I took out the PAN male and at once placed the MIL male in the tank. The female NIG at once attacked, but soon it realized that this male was different. At once they swam to the nylon and a fully coordinated spawning took place. Indeed in this crossing there was no hesitation that the different movements and stimuli worked just as well as in infraspecific spawnings.

I got 26 eggs from this spawning. Eight of these eggs developed a true blastula that was very high and concentrated. After sixteen hours the gastrulation extended normally over the surface of the yolk. After 48 hours the gastrulation has finished without abnormalities. Three days after the spawning masses of cells formed a "broken corda like" body on the yolk. Twelve days after the spawning the eggs had not changed and they were preserved in Bouin. Probably these two species differ in chromosome number (see C. Kosswig "Evolutionsphoenomene bei Knochenfischen, genetisch betrachtet" in Verueff. Inst. Meeresforsch. Bremerhaven. 1963, pages 178-196) and also conspicuous differences are fund in the development of scales and lateral line organs on the head. It is surprising however that the hemoglobine pattern and the spawning behavior have been conserved through all these years since the South American

continent separated from the African continent. 150 millions of years is an estimate of this separation. Both forms are restricted to pure freshwater and do not occur in brackish waters (aquarist's literature contains much information which indicates that species of Aphyosemion are found in brackish water. Indeed I have seen no information in the zoological literature that supports this strange idea. Indeed most species are found in freshwater which is so pure that it could be compared to distilled water).

If African Rivulins are compared with South American Rivulins indeed the species in Callopanchax comes closest to Rivulus, whereas (I have had no Austrofundulus!) Nothobranchius comes close to Cynolebias.

(54) NDI/CIN 1951

NDI is an unidentified species in Fundulopanchax that Stenholt Clausen brought to Denmark in 1959. This species lives in the Ndian River drainage in Nigeria-Cameroon. NDI has the characters of a Fundulopanchax including well-developed ctenoid scales in males, but also this species comes close to CAM which is not a true Fundulopanchax. NDI is a close relative of NIG, so say the morphological study and the crossing between these two forms. As I have not yet studied live individuals of GAR I am not sure if NDI is closer to this species or to NIG. As an intermediate form between Fundulopanchax and the true Aphyosemion NDI is an interesting species. The strain NDI became extinct a few months ago as we had already tried this fish in most important crossings and because it could not be introduced to the aquarist because it has no scientific name.

NDI is the northernmost member of the "flame tailed" Aphyosemion. I have been trying and trying to cross individuals of NDI to my NIA individuals (also a flame tailed Aphyosemion) but without any result. The individuals are not interested, so what can I do?

I had 31 eggs in one spawning. 22 eggs developed and gave twenty viable hybrids. In two eggs the development of the embryo was poor. A 180 degree corda was produced, but the hybrids were not able to build up a blood system and after twelve days they died. Hybrids hatched after sixteen and more days. First these hybrids were difficult to raise, they were attacked by Oodinium again and again and rather many were lost. After five months I had nine individuals left. Eight of these probably were "males", whereas the ninth probably was an intersex female like. The "males" however did not react as males in Aphyosemion. They were indifferent towards males and females of the parent strains. Probably all individuals were non-sex or inter-sex.

Their hemoglobine patterns did not correspond to that of their parents. Four lines.

Here are some counts on the six adult hybrids in my collection and on individuals of NDI and CIN raised in my tanks or caught in nature (including the types for CIN).

D= 13 14 15 16 Dm
NDI 0 1 3 8 15.6
NDI/CIN 0 1 4 1 15.0
CIN 8 9 0 0 13.5

A= 16 17 18 Am
NDI 1 9 2 17.1
NDI/CIN 2 4 0 16.7
CIN 12 5 0 16.3

Sq-long= 30 31 32 33 34 35 Sqm
NDI 0 0 0 4 16 1 33.9
NDI/CIN 0 1 2 6 2 0 32.8
CIN 6 10 7 1 0 0 31.1

Photos showing the hybrids ("male" and "female") and the males of the two parent species are in Aquarium Journal 1964, Scheel: A. cinnamomeum.

I have not discovered any development of H scales within NDI and CIN. My six hybrids also have no H scales. In both parent species the males develop ctenoid spines on scales on body sides above the root of the anal fin. In these species the development of these spines is not conspicuous and they may be difficult to trace. Only few of the spines are provided with long filamentous "sensory papillae". The male like hybrids developed ctenoidy as in NDI in which the ctenoidy of males is more pronounced than in CIN. Both parent species develop sensory papillae on the upper ray (rays) of the pectoral fins, but I found no sensory papillae on anal fin rays in these species and their hybrids.

Males of CIN do not develop red dots on body sides. A very diffuse red marking is seen behind the root of the pectorals (the "wound" of Aphyosemion) and on gill covers. Males of NDI develop very prominent red markings on body sides. Indeed in the keel these dots confluent forming (in most males) a broad red lateral band as in CAM. The male phenotype among the hybrids developed small and not very rounded red spots on body sides and in this they correspond well to the NIG/CIN male hybrids.

Males of CIN do not develop any red markings on the throat. When not activated the throat is pale and does not differ (much) from the throat of females. When activated the male develops a very dark black color on the lower part of the head including the whole throat. NDI males develop a very prominent red throat pattern belonging to the normal Aphyosemion type of throat patterns. The male hybrids developed a much reduced red patter, but as they could not be activated (fight, spawning) I do not know if they were able to develop any black throat color at all. Apparently they were not able to develop this colour that was not seen on NIG/CIN males when activated.

CIN males are characterized by their brilliant deep orange color in pectorals. No male of NDI developed any yellow color in these fins. The hybrid males developed yellow orange fin edges in all fins. CIN males develop a deep orange color at fin edges of ventrals, dorsal, anal and caudal. In the caudal fin this orange edge exceeds all around the fin edge like in MEI. Old males of NDI do not show any yellow or orange fin color at all, whereas young males may develop a lemon fin color.

(55) NDI/COG 1961

I had 19 eggs in one spawning. Eight eggs only developed an embryo. After nine days the development (size) of the embryos differed markedly, but all eight eggs in time gave viable hybrids. These hybrids were extremely difficult to raise and they almost always were suffering from at least one aquarium disease. After four weeks (after the hatching) I had only five hybrids left. At an age of five weeks the maturing started and all hybrids developed "males' colors". Very weak colors indeed, no brilliant shine at all. A very weak yellow color developed in dorsal, anal and caudal fin. In their phenotype these hybrids were very close to COG and quite different from that of NDI (see Aquarium Journal 1964, photo under A. cinnamomeum). The hybrids differed from COG by their small red dots on body sides (big dots in COG). I have two preserved hybrids left.

D= 12 13 14
NDI/COG 1 0 1

A= 15 16
NDI/COG 1 1

Sq-long= 30 31 31
NDI/COG 2 1 1 No ctenoid scales, no sensory papillae on fin rays. SL= 28 mm. One individual has one H scale. The last hybrid died after ten months.

(56) NDI/NIG 1962 and 1963
Females of the NIG-PH/AK strain were used (see NIG/NIG). These PH/AK females of NIG were not very fertile when spawned with their brothers or sons. Their fertility improved much in crossings. This is a remarkable fact indeed.

During 1962 I kept some NDI and NIG-PH/AK individuals as reserves in a large tank (250 liters). Both sexes were present from both species. When these fishes had been removed I noticed two juveniles in the tank and raised them in a separate tank. These two individuals developed into two males that were intermediate to NDI and NIG in the color patterns. Both were sterile in backcrossings. Next year I prepared the NDINIG cross, using the same two strains. I had sixteen eggs in one spawning. Twelve eggs developed an embryo. If these eggs had been spawned from a male and a female of NIG-PH/AK I would expect that most eggs would give non-viable hybrids.

After four days the eggs had developed a corda each. In some of the eggs the corda measured 90 degrees, in other eggs it measured 180 degrees. Four eggs (33%) did not develop a viable embryo and these died at different phases of their development. Eight eggs developed viable hybrids that were not difficult to raise. After four months I had six hybrids that were all males. These hybrids corresponded exactly to the two hybrids produced in 1962. The growth of the hybrids after maturing differed markedly as some grew fine, whereas others did not grow much. Apparently they were not diseased. I backcrossed one male to the mother. Fifteen egg. No egg developed a blastula and soon all died. Three hybrids were taken over by Pr. C. Kosswig in 1964 for further study.

The phenotype for NDI/NIG (or NIG/NDI for two hybrids) is very close to that of NIG. Indeed it is difficult to distinguish between the hybrids and the NIG males. The hybrids however developed a dark

lower edge on the anal fin (as in GAR) and this particular (small) marking does not occur in NIG males (AK, OW and PH). Also the red pattern in the center of the caudal fin tended towards a "flame pattern" as in NDI (very marked in this species). These "flames" are produced mainly by red streaks situated on the fin membrane in between the fin rays.

Males of NIG do not produce any regular "lyre pattern" in their caudal fins, whereas in NIG all males develop a very prominent lyre pattern. The hybrids were intermediate as their lyre patterns were broken and incomplete (most males). All hybrids developed that bright orange yellow fin edge color seen in NIG (the "yellow" phenotype). Hybrids developed ctenoidy and pectoral fin sensory papillae as in the parent species. Anal papillae do not occur in these forms (in my opinion).

Here are some counts on the hybrids and on their parent forms:

D= 14 15 16 Dm
NDI 1 3 8 15.6
NDI/NIG 1 1 2 15.2
NIG-PH/AK 7 1 0 14.1

A= 16 17 18 Am
NDI 1 9 2 17.1
NDI/NIG 1 2 1 17.0
NIG-PH/AK 2 6 0 16.8

Sq-long= 29 30 31 32 33 34 35 Sqm
NDI 0 0 0 0 4 16 1 33.9
NDI/NIG 0 0 0 5 3 0 0 32.3
NIG-PH/AK 1 2 6 4 0 0 0 31.0

Like in the NDI/CIN cross also these hybrids are almost intermediate. In NDI individuals H scales have not been observed. In the NIG-PH/AK strain H scales occur frequently. One of four hybrids had two prominent H scales situated on the anterior edge of the G scale.

(57) NIG/CIN 1961
For this cross a NIG-PH male was used. The code PH stands for Port Harcourt, situated in the eastern part of the Niger delta. This male (and one more) was caught by Ulf Hannerz from Sweden in 1961 at the Wokocha River. This is where the ARN strain originated together with a most beautiful strain of Aplocheilichthys macrophthalmus (or a relative of this species).

I hatched 13 very viable hybrids from this cross. I lost one egg that developed a small embryo which did not grow and which died in the egg. After four weeks only the first male hybrids started maturing. First yellow color develops at the lower edge of the anal fin. Next this color also occurs at the top of the dorsal fin. At last the yellow color spreads all over the outer edge of the caudal fin, starting from the fin root. After some time the yellow color changes into an orange yellow color, more orange than in NIG, but less than in CIN: This means that at a certain time a male may have orange-yellow color in his dorsal and

anal fins, whereas in the caudal fin the color is lemon. Some weeks later the males developed the guanine shine on the body sides. First this shine was not evenly distributed as in NIG, but more as brilliant spots as in CIN. Later the shine became evenly distributed as in NIG. The color of this shine was intermediate to the parent species (greenish blue in NIG, violet blue in CIN, and blue in hybrids).

The first spawnings were observed when the individuals were eight weeks old. At that time I had eight males and five females. No intersex developed. The eggs from the hybrid females varied somewhat in size, most eggs measured 1.6-1.8 mm, but some were smaller. These eggs could not develop in backcross to NIG males nor in spawnings with their brothers. Not even a blastula was observed. The egg membrane pattern corresponded in the marked reticulation to the parent species (marked reticulation in CIN, weak reticulation in NIG). Also the hybrid males were sterile in backcross.

The phenotype of these hybrids came much closer to the NIG phenotype than to CIN. Photos are in Aquarium Journal 1964. They differed from NIG by their pattern of the caudal fin. NIG develops a regular lyre pattern as do most species in Aphyosemion that develop "separation" bands in this fin. In CIN the separation band (which separates the bright yellow color of the fin) does not form a lyre but continues all along the inner part of the fin, forming an unbroken band all the way round. This red pattern also developed in the hybrid males. The hybrids also differed from NIG by their smaller and less regular red dots on body sides. They developed much more yellow-orange color in pectorals, but the inner part of this fin was not colored as in CIN males. The throat color pattern corresponded to NIG, however the pattern was somewhat reduced. Females resembled NIG females. Their red dots on body sides however were much smaller than in NIG. The handsome lemon fin color of CIN females was not seen in the hybrid females.

I prepared these counts on the hybrids in my collection and on NIG-PH/AK and CIN females:

D= 13 14 15 Dm
NIG-PH/AK 0 7 1 14.1
NIG/CIN 1 7 2 14.1
CIN 8 9 0 13.5

A= 16 17 Am
NIG-PH/AK 2 6 16.8
NIG/CIN 3 7 16.7
CIN 12 5 16.3 (hemoglobine pattern: "four line type" in NIG, CIN and NIG/CIN)

Sq-long= 30 31 32 33 Sqm
NIG-PH/AK 1 2 6 4 31.0
NIG/CIN 2 12 4 1 31.2
CIN 6 10 7 1 31.1

In five individuals (NIG/CIN) that were used for hemoglobine study the frontal scales have been lost or are replaced. In the remaining five individuals two have doubled G scales (i.e. lateral row) and one individual has a single H scale below the G scale. The frontal scales are rather irregularly arranged in

these hybrids. Ctenoidy varies much among hybrid males, some have many ctenoid spines, whereas these spines are rare in other males. Sensory papillae developed on the upper pectoral fin ray, whereas anal papillae were absent.

(58) NIG/COE 1958

For this cross the NIG-AK (Akure) strain was used. COE is the genotype for Fundulopanchax. It occurs in southern Nigeria from the Lagos-Ijebu Ode area to the Niger delta. Loennberg's SJO is similar. As this form comes from the Ndian River drainage and from a locality near the Cameroon Mts. the Ndian River form may differ from the Niger form in genetics. See NIG/NIG and BIV/BIV.

I had a hundred eggs from three spawnings. 89 of these eggs developed and 85 hybrids were hatched. 27 of these however were killed when they were forced to hatch. No abnormalities were discovered during the growth of these hybrids that were strong fishes. Indeed these hybrids were less feeble than juveniles of COE and equal in viability to juveniles of NIG.

Both sexes developed among these many hybrids. The largest males exceeded the size of full grown NIG males and came close to the average size for COE in aquaria. No intersex individuals developed.

Males were sterile in backcross to NIG and COE and their sisters. Females spawned eggs of very variable size. The smallest eggs measured less than 1.0 mm (NIG-AK = 1.0 mm, COE = 1.4-1.5 mm) whereas the largest eggs measured 1.8 mm. Only the largest eggs developed in the backcrossings. In the backcross to COE males I had many eggs that developed but no egg gave viable fry. In the backcross to NIG-AK males I had more than a hundred developing eggs. The embryos however died at various phases of their development and only one viable fry was hatched. This sole F2 individual hatched from a 1.7 mm egg. After hatching: 5.0 mm. The backbone of this individual was badly deformed, resembling a "Z" (upright standing or swimming "Z"). First this individual was not able to swim, but after some days it was indeed swimming, not very well. I raised this individual with much difficulties and it matured as a male. Five months old this male measured 30 mm only (caudal fin included). The deformation of the vertebrae did not change and the young fish was not a good swimmer. It matured as a "yellow male" (see NIG/NIG) and was very aggressive and fought much larger males that were afraid of this "monster". Not preserved, not photographed.

Most hybrids were delivered to Hoedeman, but I have 25 left in my collection. On these and on individuals of NIG-AK and COE-AQ I prepared these counts:

D= 12 13 14 15 16 17 18 Dm
NIG-AK 1 21 11 6 1 0 0 13.6
NIG/COE 0 0 4 10 11 0 0 15.3
COE-AG 0 0 0 0 1 8 2 17.1
COE-SA 0 0 1 2 5 1 0 15.6
COE-AQ 0 0 0 2 1 2 0 16.0

A= 15 16 17 18 19 Am
NIG-AK 3 23 12 0 0 16.3

NIG/COE 0 7 8 9 1 17.2
COE-AG 0 0 4 6 1 17.7
COE-SA 0 2 5 2 0 17.0
COE-AQ 0 2 3 0 0 16.6

Sq-long= 30 31 32 33 34 35 36 37 38 Sqm
NIG-AK 2 9 5 5 1 0 0 0 0 31.8
NIG/COE 0 0 0 0 1 12 14 19 3 36.2
COE-AG 0 0 0 3 8 5 2 1 0 34.5
COE-SA 0 2 7 4 1 0 0 0 0 32.4
COE-AQ 0 0 1 6 2 0 0 0 0 33.1
AG = Ago-Iwoye, SA = Sapele-Ughelli and AQ = aquarium strain
A detailed study of live COE from various localities is needed.

In the NIG-AK deme about half the number of individuals develops H scales that are situated on or below the anterior edge of the G scale. In COE all individuals that I have seen developed prominent H scales which always were situated below the G scale (as in GUL). Among the hybrids I found out that about 75% of the individuals had H scales. In order not to destroy the scale pattern I did not lift up the G scale in order to see if small H scales might be hidden under the scale. This indicates that in this character the hybrids are intermediate to the parent strains. In 1/6 of the hybrids the H scales were situated on the G scale, whereas in 5/6 of the individuals these scales were situated below the anterior edge of the G scale.

The hybrids have been preserved in formol and the ctenoidy for this reason cannot be studied in details. In some strains of COE (and in particular in the type for Loennberg's SJO) the ctenoidy is prominent and almost all spines support a long filamentous sensory "papillae".

(59) NIG/COG 1957
Also for this cross the male originated from the NIG-AK strain. I have no report on the development of eggs from this early cross. The first crossing gave nine hybrids out of 34 eggs. Only two of these hybrids could be raised to adult size. One of these hybrids had badly deformed lower jaw and could not close the mouth. The lower jaw was "spoon like". This hybrid however was able to catch live food and even Daphnia. It used some organs in the inner part of the mouth to "hold" the prey. Ninety days old this hybrid measured 29 mm total length, whereas the second hybrid measured 38 mm.

In NIG and also in COG (and various other forms) two phenotypes of males occur, even within micropopulations. For these phenotypes I have used the names "yellow" and "blue". Yellow males develop yellow or orange yellow fin edges, whereas in the blue males these fin edges have no yellow color, at least not in full grown males. When young blue males may develop a brilliant lemon fin color indeed. For this cross NIG/COG I used a yellow NIG male. My strain of COG developed both types of males. One hybrid developed brilliant orange yellow fin edges (indeed most parts of the ventral fins were yellow), whereas the second (largest) hybrid did not develop even traces of yellow fin color at any age.

The hybrid phenotype resembled COG more than NIG. There were no separation red bands in fins. On

the body sides red dots developed in double number as found in NIG-AK males, but far less than in COG males. Indeed these hybrids resembled the CHR phenotype much.

In order to have more information on the deformation of the lower jaw that was observed in the first brood I prepared a second brood of eggs. This time I used a blue NIG-AK male to the same COG female. I had 59 eggs and all these eggs gave viable (more or less) hybrids. After a few days it was evident that also in this brood deformations of the lower jaw were seen. I prepared a third brood that gave ten hybrids. After five weeks I had 37 of these 69 hybrids left. 25 of these still surviving hybrids had badly deformed lower jaws, whereas twelve only had no deformations. All hybrids that had deformed jaws died before maturing. I raised the twelve sound hybrids. All developed into males and all were blue males.

Apparently the development of the yellow and the blue phenotype in males of these two species, and probably also in other species in Aphyosemion and Nothobranchius, the genetics for these characters are simple. If we assume that the COG female had one gene for "yellow" and one for "blue" and that the blue NIG male had no genes for "yellow" (which blue males indeed often have) then the results of these crossings correspond to the supposed distribution of genes. Yellow males in NIG-AK always had two genes for "yellow" in my crossing experiments in NIG.

Several of these hybrid males (no female or intersex developed) were backcrossed to females from the parent species. No developing eggs were had. Most of these hybrids are in the Amsterdam Museum. I have none.

(60) NIG/LAB 1957

The first spawning gave 24 eggs, but only four eggs developed. The second spawning gave 21 eggs and only two eggs developed. These six eggs developed normally and viable hybrids were hatched after less than three weeks. The hybrids still had rather large yolk sacks and could not swim. The yolk was rather transparent. The hybrids soon died all without being able to swim. Ed Seligman in the USA later on prepared the similar or reciprocal cross. He raised viable hybrid males to adult size. He sent me a color slide showing a blue hybrid male. The phenotype of that hybrid resembled my NIG/COG individuals.

(61) NIG/NIG 1961-64 population cross

In summer 1961 Ulf Hannerz from Sweden collected live Rivulins all over Nigeria. After his return to Stockholm he kindly offered me most of his material. Among these Rivulins there were two yellow NIG males that he caught at the Wockoch River near Port Harcourt. Ulf considered NIG as very rare in this area and he was only able to find these two small males. These two males differed from my 1957 strain of NIG from Akure (caught by Birket-Smith) by their more "Fundulopanchax appearance" and by having three times as many red dots on the body sides as had the NIG-AK individuals. I spawned both males with NIG-AK females and did not expect any abnormalities to develop.

I had 55 eggs and only 22 of these developed. No abnormalities were seen during the embryo development. In order to concentrate on this strain of NIG I preserved my individuals from the NIG-AK strain and the two PH males. I had eighteen apparently viable "hybrids". These individuals differed much in the rate of growth. One male developed extremely quickly, whereas some individuals were very slow. These individuals were isolated, but this did not improve their growth. These smaller individuals were

difficult to keep alive. I never had any difficulties in the reproduction of my 1957 strain of NIG-AK, so these first difficulties surprised me. Both "blue" and "yellow" males developed among NIG-PH/AK males. In "yellow" males of NIG-AK and NIG-OW (Owo) the anal fin has a broad complete red "separation band" in the middle of this fin. Below this band there is a bright warm yellow color and above this band the fin is colored as the body sides. In the "blue" male of this strain (these two phenotypes in males are sympatric in the AK strain, probably not in the OW strain), there is no such broad red separation band. Instead the fin has a row of big red dots. The color of the body sides exceeds just to the lower fin edge and no trace of yellow color is seen in the fin in adult males.

As the PH males had all these red dots on the body sides (both were of the normal "yellow" type, as far as fin patterns are concerned) I made up my mind to produce a new strain of "blue" males that had an unbroken red separation band in the anal fin. Indeed the quickly growing male of the PH/AK brood came close to that "ideal". However in this male the red line was broken up into segments. The PH/AK males developed about twice as many red dots on the body sides as do the AK males. In this the PH/AK males were intermediate to the parent strains as 3+1/2 = 2. Except for the quickly growing male no further unexpected changes of the color patterns were seen among the PH/AK individuals.

I bred the most viable of the PH/AK individuals in the usual way. Spawning on coarse peat. Drying up of the peat for four to eight weeks. Upon wetting and hatching, I found far too few fry hatching from these samples. For this reason I spawned two pairs of PH/AK on nylon for control of embryo development.

During this I had the second surprise. Eggs of my strain of NIG-Ak spawn small eggs - about 1.0 mm only. Eggs of these PH/Ak females spawned eggs of rather variable size (1.25 to 1.40 mm) indicating that probably two different species have been crossed. First control gave 22 eggs. Seventeen eggs developed a normal blastula and after this five eggs died. Seven days after the spawning two more eggs were lost from unknown reasons at the phase when the corda measured about 180 degrees on the yolk. After eleven days one large embryo died from severe thrombus. After fourteen days the embryo development stopped in two more eggs. The embryos in these eggs at that time were far behind in their development, compared with other embryos of that brood. During the next few days the number of viable embryos was reduced to three only. Next control gave the same sad result from two different PH/AK individuals. The average result of the different spawnings was about 10 percent viable just hatched fry from fertile eggs. Also rather many eggs were not normal and probably not viable. These eggs have not been considered in the statistics.

The F2 individuals or (PH/AK)2 individuals were very feeble and very difficult to raise and I had to keep these individuals under control through their development. After maturing they developed into hard fishes however. First I had only males from the few F2 individuals that I was able to raise to adult size. These males were backcrossed to the F1 or PH/AK females. I expected the results of this backcrossings to give improved results. This was not the case. Indeed the number of "viable" fry that could be hatched from fertile eggs in this combination was a bit worse than from the inter-F1 spawnings.

After some months a female developed among the few F2 individuals.

As no F1 males were left at that time that female was spawned with F2 males. I had 57 eggs from these

spawnings. However only five of these many eggs produced a "viable" fry. All five fry however died from unknown reasons. So after all they were not very "viable" indeed.

After this sad result in my efforts to produce a new strain in NIG, I gave up and Pr. Kosswig took over all remaining live individuals of the "PH/Ak" strain. As I had preserved all individuals of the old NIG-Ak strain, Stenholt Clausen gave me individuals of his NIG-OW strain for further research in crossing of NIG. The NIG-OW strain is very close to the AK strain in most details.

The color patterns which developed on the few F2 males or (PH/AK)2 males did not differ from males of the F1 generation, except for one male. In this male no yellow color developed in the anal fin. Whereas in the upper part of the dorsal fin and at the upper and lower edge of the caudal fin the normal warm yellow color developed. In the anal fin the normal unbroken red separation band developed in this male.

The results from this "crossing" between individuals which apparently were belonging to the NIG species indicates that in this species different populations are about to develop into different genetic species without much change in the visible characters of the phenotype. NIG extends over large areas of Nigeria and is found in the forest and in the savannah. Apparently NIG is restricted to the bed rock and is replaced by BIV on the sediments. The type for the name originated from Arum, situated on the southern slope of the Jos Plateau in northern Nigeria. Stenholt Clausen has many individuals of NIG from localities on this plateau and from many more localities in Nigeria. From the Jos plateau NIG (Riyom, Vom and Arum) extends southwards (Lafia and Akwanga) and westwards (Keffi and Abuja) and reaches Igbetti in the far west. Another population of NIG is found in the Akure area (Esa-Oke, Ondo , Foriku, Ilesha, Idanre, Akure, Ekinrin and Owo) in the drier parts of the forest. East of the Niger drainage NIG apparently is replaced by GAR, in the (northern?) Cross River drainage and by NDI in the Ndian River drainage. The Cross River representatives of the NIG-GAR group apparently do not represent one species. Some forms from this river system grade into CAM, but still these individuals develop traits that indicate NIG derivates. Probably the SPU form of Ghana and Ivory Coast represents a more distant member of a NIG-GAR group, but SPU also has traits that indicate a close relationship to such different forms as ARN and GUL.

The throat pattern of NIG males differs rather much through the range of distribution and also within demes. Normally NIG males develop the "usual" Aphyosemion pattern, composed of many red lines and dots. In the Jos males the red pattern often is much reduced and replaced by an overall weak red color. The branchiostegal membrane color also varies. In some males the red pigments dominate, in other males the black pigments (SPU and GUL) take over the dominance.

The development of H scales also differs much. Strangely enough large differences exist when individuals of the Akure strain and the Ondo demes are compared. In the Akure individuals almost half of the number of individuals develop one or two H scales, whereas in the Ondo deme (geographically very close, but belonging to a different river system) individuals very rarely develop H scales.

Here are some counts that I prepared on the large collection of NIG in Stenholt Clausen's collections.

D= 12 13 14 15 16 Dm

Igbetti 0 1 1 0 0 13.5
Keffi 0 2 6 8 0 14.4
Abuja 0 3 13 2 0 14.0
Lafia 0 4 1 1 0 13.5
Arum 0 0 8 4 0 14.3
Vom 0 0 0 1 1 15.5
Riyom 0 0 5 5 2 14.8
Esa-Oke 0 2 0 0 0 13.0
Ondo 0 6 4 3 0 13.8
Akure 0 17 9 4 0 13.6
Owo 2 15 1 0 0 13.0

Jos area 0 9 33 21 3 14.3
Akure 2 41 15 7 0 13.4

A= 14 15 16 17 18 Am
Igbetti 1 0 1 0 0 15.0
Keffi 0 3 6 6 0 16.2
Abuja 1 8 7 2 0 15.6
Lafia 0 2 3 1 0 15.8
Arum 0 1 3 7 1 16.7
Vom 0 0 1 1 0 16.5
Riyom 0 1 9 2 0 16.0
Esa-Oke 0 2 0 0 0 15.0
Ondo 0 1 5 7 0 16.5
Akure 0 3 22 5 0 16.1
Owo 0 6 9 3 0 15.8

Jos area 1 15 29 19 1 16.0
Akure 1 12 37 15 0 16.0

Sq-long= 29 30 31 32 33 34 Sqm
Igbetti 1 1 0 0 0 0 29.5
Keffi 0 2 9 13 7 0 30.8
Abuja 0 4 11 13 7 0 31.5
Lafia 0 2 5 5 0 0 31.3
Arum 0 1 2 9 7 1 32.3
Vom 0 0 1 5 0 0 31.8
Riyom 0 5 6 21 4 0 31.6
Esa-Oke 0 1 0 1 0 0 31.0
Ondo 0 4 7 22 7 0 31.8
Akure 0 3 14 12 6 0 31.5

Owo 0 2 18 13 2 0 31.4

Jos area 0 14 34 66 26 1 31.8
Akure 1 11 39 48 15 0 31.6

The corresponding counts for the NIG-PH/AK strain are published under the NDI/NIG cross.

The "Jos population" differs somewhat from the "Akure population" in a higher count for dorsal fin rays. Also in the general appearance, the northern males are more "Fundulopanchax like".

For comparisons some counts that I prepared on four "groups" of GAR-CAM like individuals from the Cross River drainage:

D= 12 13 14 15 16 Dm
Ogoja 0 7 5 0 0 13.4
Obudu 5 22 3 0 0 12.9
Mamfe 1 0 0 15 15 5 14.7
Mamfe2 1 8 14 4 0 13.8

A= 14 15 16 17 Am
Ogoja 0 3 6 4 16.1
Obudu 3 17 8 2 15.3
Mamfe 1 0 5 21 10 16.1
Mamfe2 2 9 13 3 15.7

Sq-long= 30 31 32 33 34 Sqm
Ogoja 2 3 7 5 2 32.1
Obudu 0 9 21 12 0 32.0
Mamfe 1 0 5 28 19 1 32.1
Mamfe2 5 13 9 5 0 31.3

It is evident that in these counts the Nigerian NIG (Niger drainage plus western smaller river systems) and the Cross GAR-CAM do not differ. The differences in mean figures for counts are more variable in the Cross River samples than within NIG.

Arne Schiotz, the director of the Copenhagen Public Aquarium, caught the Ogoja and Obudu samples. Schiotz told me that although these two localities are geographically very close the climates of the localities differed much. The Ogoja fauna belongs to the lowland fauna, whereas the Obudu fauna resembled the fauna of the higher parts of the Cameroon Mts. Both samples contained "blue" and "yellow" males, thus indicating another affinity to NIG. These two samples however differed not only in their counts but also in the shape of the body. The Ogoja individuals were deeper also in the shape of the body. The Ogoja individuals were deeper than the Obudu individuals. I consider the Ogoja individuals to belong to GAR (types came from Okwoga, not far from Ogoja), whereas the Obudu individuals probably belong to a different but closely related species. The two Mamfe samples originated from five localities

and were collected by Stenholt Clausen in 1957. Three localities are situated in the lowland near Mamfe (Mamfe 1) and contain individuals which come close to GAR, but which also are somewhat different from the Ogoja individuals. The "Mamfe 2" samples (2 samples) originated from localities south of Mamfe and contain individuals that are very close to the Obudu individuals. Indeed these individuals also originated from the upper reaches of small rivers that join the Cross River near Mamfe. Schiotz presently is visiting Obudu and I do hope that juveniles of the two important demes might be brought home alive (he did not catch any, too much water in July). The systematics of the Cross River GAR-CAM species probably will give us the same difficulties as we are presently faced with in the systematics of Sierra Leone ROL-LIB like Callopanchax.

The fact that within NIG and related species (SPU and GAR) two phenotypes of males occur not only in different demes, but also within a single deme, is highly interesting. In my opinion these two phenotypes may reflect the interacting of two opposing forces: sexual stimuli (males which develop yellow fin edges are more easily seen by females) and camouflage ("yellow males" are more easily seen by animals or birds). According to E. Mayr however the opposed forces of selection sooner or later would eliminate from the deme one of the two phenotypes if the heterogenous form, the "blue" male which has a single gene for "yellow" did not develop advantages in the life compared with the two homogenous forms (pure blue and yellow).

(62) NIG/SPU 1965 see also SPU/NIG

I spawned a NIG-OW male to a female of Arnoult's strain of SPU. I had eleven fertile eggs. During the first three days six of these eggs died from unknown reasons. Five eggs however developed normally and as they did not hatch in time they were forced to hatch 23 days after the spawning. Soon after I lost one hybrid. The remaining four individuals were very viable and quickly growing hybrids. After three weeks only the males started maturing. Two males and two females developed. The males have been preserved as they have been backcrossed to NIG and SPU females. With these females they spawned, but the eggs did not develop and soon died. Probably these males are sterile. In SPU no red "separation lines" develop in dorsal, anal and caudal fin. The yellow outer area however is rather well separated from the inner green blue area. In the hybrids (which did not differ in any detail from the SPU/NIG males) the yellow areas are separated from the green blue areas by a very thin red line that often is very complete. I used a "yellow" NIG-OW male (only yellow males occur in this strain) and as the SPU strain develops yellow males only it is not surprising that both hybrid males were "yellow males" (also all SPU/NIG males were yellow males so far). These two males developed a rather marked ctenoidy in scales, but only in the mid rows of scales. Only a few spines are seen in front of a vertical line through the base for the ventrals. Pectoral sensory papillae however are present and well developed. No such papillae are found in dorsal and anal fin. Most ctenoid spines in the mid rows of scales support a long filamentous "papilla". One hybrid had one large H scale, whereas the second hybrid had two such H scales. All H scales rested on the anterior part of the G scale.

Females are large now, they have been tried in spawnings several times, but they did not produce eggs and probably they are not able to do so. They are still alive because they need one more chance to prove that they are real females. Counts for these two males will given in connection with the SPU/NIG report.

(63) PAN/DAY 1964

I had seventeen eggs over two weeks of spawning. Eggs were divided into two broods. The third day the corda was visible, but after six days the corda measured 90 degrees on the yolk only. Now one egg died from unknown reasons. After ten days the tail of the embryo left the surface of the yolk and was "curling up" into a spiral. From now on no further development of the body of the embryos was noticed. After twelve days a few blood elements were circulating in a very poorly developed blood system. After fourteen days three more eggs died. In the remaining eggs the blood was still circulating. After nineteen days the circulation of blood had stopped in all eggs. The bodies of the embryos were very slender and densely covered by black pigments. In between the black pigments a few red pigment cells were visible. After 21 days I had only five live eggs left. Three of these now were preserved and the remaining two eggs died at an age of 29 days. PAN and DAY both are Aplocheilus, but they differ in counts, in development of black crossbars, in hemoglobine patterns and in the development of lateral line organs on the head.

PAN and LIN might be more closely related. However look at the result of the PAN/LIN cross (no 65).

(64) PAN/FAS 1964
A Freetown female of FAS was used together with a PAN male from the usual aquarium strain. I was surprised to see that the two individuals did not discriminate and in short time I had 25 eggs. 20 of the eggs developed and after 24 hours the gastrulation was already passing the equator of the yolk forming a big groove. In one egg a "formation of undifferentiated cells" however was left at the animale pole, resembling a "blastula". After 36 hours the gastrulation had finished and one egg was about to die. After 48 hours the corda measured 120_ on the yolk. After five days red pigments developed on the yolk, the embryo moved in the egg, the heart pulsates, but apparently the heart is situated too far from the throat. The development of the tail of the embryo is indistinct, shapeless. From now on no further increase in size of the embryo was noticed.

Embryos did not differ in size. On the yolk and the embryo the pigmentation increased. After seven days only three of eighteen embryos still circulated their blood. After ten days no circulation of blood occurs in any egg. Embryos developed a thrombus, often at the extreme end of the tail, and the heart was still moving. After 22 days no changes have been noticed. Preserved.

(65) PAN/LIN 1964
Apparently these two species in Aplocheilus form a small "group" of more closely related species in this genus. In scales, in hemoglobine patterns (not quite similar) and in the development of lateral line organs on the head these two forms are close. PAN has no crossbars, whereas such bars develop in both sexes of LIN.

The two individuals did not pay any attention one to the other. After many weeks of training the ripe LIN female developed a very broad and very conspicuous black lateral band, not unlike the band that develops in pre-spawning display within Epiplatys and in SJO from Sierra Leone. In the African species this band does not continue into the caudal fin. In LIN (see also SEX/LIN) this band also extends into that fin. Probably the development of very many eggs in these females induced the development of this stimuli which has not been reported for LIN (DAY females may develop such a band indeed in pre-mating display).

From now on a few eggs were spawned now and then. I had seven eggs. Only two eggs developed an embryo, whereas one more egg developed a real blastula and died thereafter. After three days the embryo measured 90 degrees on the yolk. In one egg the tail of the embryo now lost its connection with the yolk and turned off. In the other egg a blister developed. See also CAL/SEN, FAS/DAG, SEX/LIN and others.

As usual the head of the embryo was situated at the surface of the blister and the tail rested (more or less) on the yolk. This embryo did not develop a blood system. The first embryo developed no blister. It lived for seventeen days and never developed any blood. It did not exceed 120 degrees on the yolk.

(66) PET/COG 1958
I had seven eggs. All eggs developed. After eight days the development of the blood system was not surprising. Within the next three days however the development of the blood system improved much. The first crisis was over. After fourteen days all embryos were dead or dying from thrombus. Preserved.

(67) PET/DAG 1958
A DAG-AQ female was used. During the first seven days, the development of the embryos was promising. The size of the embryo was rather large, the circulation of blood was fine and the heart was not too far from the throat. After thirteen days the situation was changing. The development of veins on the yolk apparently was not sufficient for the size and the age of the embryo. After twenty days I preserved two embryos that hatched and died. Also the remaining fifteen eggs containing dead or dying embryos were preserved. Indeed these hybrid embryos reached rather large size before they died. PET and DAG are sympatric in Ghana. Roberts took both species at Elubo, Nzima, SW Ghana on the oxysols and in areas of high rainfall. Also SHE was present in this sample.

(68) ROL/NIG 1964
I used a ROL male of the robust phenotype from the Taylor Town deme near Freetown. This form belongs to the many phenotypes of ROL that Roloff caught in Sierra Leone. See SL4/ROL. A NIG-PH/AK female was used. See NIG/NIG for fertility of these females.

I had 23 eggs in one spawning. Many of these eggs probably were not normal and not viable. After four days only five live eggs remained. No embryo was visible (several eggs were controlled blastula on the second day, all had a normal blastula). After six days only two eggs remained alive. Both had a corda. After this no further development took place. The yolk started decomposition and soon the eggs died. This is a Callopanchax/Fundulopanchax cross. See also SL4/NIG and CAL/ROL which represent similar crossings and which gave similar results.

(69) SEN/MAC 1964
I used a SEN-VO/CH male. These males were absolutely fertile. I had sixteen eggs in one spawning, most eggs were not normal and not viable. After 24 hours only a few apparently viable eggs were left. Only one of these eggs developed an embryo and an apparently viable fry was hatched. The hybrid was able to swim at once and during the first days I observed this hybrid resting at the water surface in the usual way of Epiplatys. The size of the hybrid increased, but after about one week it died from unknown reasons.

(70) SEN/PAN 1962
I used a female of PAN of Thung Kim Tek's Djakarta strain to a male of SEN of the Volta strain.

From these crossings I had eight eggs only. All eggs developed. After six to twelve hours a very large blastula developed. I am not quite sure that these formations indeed represented real blastuals as -at that time- I was not aware of "pseudo-blastulas". I found no division into several cells in these big "blisters". The blastula or blister decreased during the next ten hours and in some eggs this formation apparently was about to "separate" from the yolk. After 36 hours a distinct corda was visible in the egg. From now on no further development took place. The "head" of the embryo was quite distinct, whereas the central parts of the "body" and the tail were indistinct. Shapeless mass of cells. I stored the eggs for more than 20 days and inspected the eggs every day. I was however unable to see any changes, so at last the eggs were preserved.

(71) SEN/SEN 1961-65 population cross
Three strains of SEN were used or these crossings of individuals belonging to different populations:
SEN-CH = strain from Maiduguri, Chad drainage. Caught by Ulf Hannerz in 1961 (Chad strain)
SEN-VO = strain from SE Ghana (same locality as BIF-VO, see BIF/BIF). Caught by Stenholt Clausen in 1962 (Volta strain)
SEN-NI = strain from Kontagora, N Nigeria. Stenholt Clausen in 1962 (Niger strain)
This means that for these crossings I had individuals from three important and isolated river systems of West Africa. Two hybrid strains first were produced:
SEN-VO/UH: seven males, no females
SEN-VO/NI: nineteen individuals, both sexes represented
"UH" stands for Ulf Hannerz. This particular strain was produced from SEN-CH females and a male of unknown origin that Ulf sent to me after the death of the single SEN male from Chad. This particular male (SEN-XX) might belong to the SEN-CH deme, but as this is not known with certainty I prefer to use a special code. Oddly enough, the SEN-UH strain contained females only.

I spawned two pairs of the combination VO.UH/VO.NI in two separate tanks. One pair gave 48 eggs, whereas the second pair gave five eggs only. In their egg type the eggs of the SEN-VO/NI females did not differ from those produced by the VO, NI, CH and UH females. Most eggs developed without any abnormalities and gave viable fry. In about 15% of the eggs heart difficulties as described for BIF/BIF developed. However in SEN/SEN this abnormality was not fatal and all embryos recovered and hatched as sound fry. After the hatching of the hybrids rather many had deformed backbones, but this abnormality also occurs frequently in Stenholt Clausen's SEN-VO strain at the Zoological Museum (I never had such individuals in my part of that strain). As the VO strain occurs (genetics) in both strains used for this crossing the result is not surprising. This new VO.UH-VO.NI strain was also rather feeble and I have only six individuals left after one year.

The results from the BIF/BIF and the SEN/SEN crossings indicate that post-mating isolating mechanisms do not act when individuals from different large river systems are crossed. This does not correspond to the results found for different species of the rainforest in which the hinderances against wanderings of individuals from one deme to another apparently are less marked. At the present time the populations of

BIF and SEN in the Volta River probably are not able to swim into the areas drained by the Niger River and between this river and the Chad drainage there also is no easy way for individuals to wander. Also the populations of BIF and SEN inside these huge water systems probably contain many more individuals compared with the demes of NIG, BIV etc. of the rainforests. Here the reasons for the "stabilization" of the BIF and SEN genotypes probably are found. The gene pools of such large populations change less rapidly than the gene pools of very small populations. This however is counteracted by the fact that a few invaders (brought in by birds as eggs) from one river system into another probably would not be able to produce much change in the gene pool of the population into which they are introduced.

I prepared these counts on preserved material at my disposal. Co stands for Congo drainage. Individuals belong to the collections of the Congo Museum and originated from a locality near Leopoldville. In all details these individuals correspond to SEN from Volta, Niger and Chad.

D= 07 08 09 10 Dm
CO 1 0 2 0 8.3
CH 0 1 2 1 9.0
NI 0 6 20 8 9.0
VO 0 3 2 0
XX 0 0 1 0 9.0
XX/CH = UH 0 0 3 1 9.2
VO/UH 0 1 6 1 9.0
VO/NI 0 1 5 6 9.4

A= 15 16 17 18 19 Am
CO 0 2 1 0 0 16.4
CH 0 1 3 0 0 16.7
NI 3 9 19 3 0 16.6
VO 0 1 1 3 0 17.4
XX 0 0 1 0 0 17.0
XX/CH = UH 0 0 2 1 1 17.7
VO/UH 0 4 3 1 0 16.6
VO/NI 1 3 8 0 0 16.6

Sq-long= 26 27 28 29 Sqm
CO 1 7 3 0 27.2
CH 1 5 1 0 27.0
NI 7 16 37 4 27.6
XX 0 1 1 0 27.5
UH 0 2 0 2 28.0
VO/UH 0 7 7 2 27.7
VO/NI 0 2 10 0 27.8

The NI individuals originated from Kontagora, Pategi, Jebba and Agenbode in Stenholt Clausen's

collections.

(72) SEX/DAG 1959
A SEX male of the Ibadan deme and a DAG female of the common aquarium strain were used. This crossing was difficult to produce and the individuals did not spawn during the first weeks. I have no report on the number of eggs that were spawned as these were not removed from the nylon. The development of the eggs for control differed and some embryos "curled up" in eggs. These embryos did not develop sufficient blood systems and died rather soon in their development. After some time I had three eggs only. All these contained an almost ripe embryo. Two of the embryos apparently were not viable, as they died before hatching. From the third egg a small embryo (2.5-2.6 mm only) hatched alive, but also this small embryo soon died.

A second spawning was produced. In this brood of eggs there were five eggs that developed better than any egg in the first spawning. Two fry only hatched alive and soon one died. The last hybrid was raised with much difficulty. Six weeks after the hatching the hybrid measured 14-15 mm only. At that age the dark crossbars were already visible on the body sides. Nine weeks after the hatching the hybrid measured 25 mm total length and was about to mature as a male. Like the DAG/SEX hybrid also this hybrid did not develop any red color on the throat. For further details of the color pattern see DAG/SEX. The SEX/DAG hybrid acted as a male in backcrossings, but no fertile eggs were harvested. This hybrid was less "intersexual" than the DAG/SEX hybrid. At an age of twelve months the hybrid was sent to Dr. W. Foersch (who at that time kindly prepared color photos of some of my fish) for photographing. After this the hybrid was preserved and sent to Dr. J.J. Hoedeman.

These two hybrids, the DAG/SEX and SEX/DAG individuals, were the only viable hybrids prepared from species that differed in their hemoglobine pattens (SEX = six lines, DAG = four lines). As Dr. Sick had not started the hemoglobine study on Cyprinodonts at that time, the hybrids were not tested for this character. The two parent species also differ in patterns of lateral line organs on the forehead.

(73) SEX/GRA 1961
Both species were present in the very first shipment of live aquarium fishes that arrived from West Africa to Germany in 1905. These two forms at that time were exported from the Niger delta and the aquarists (most) were considering these species as very closely related. GRA at that time (this form was not described until 1911) was considered as belonging to the SPI species. In order to clear up the affinities Dr. Zimmermann in Germany (see "Wochenschrift f. Aquarien- und Terrarienkunde 5. Jahrgang, 21. April 1908, page 200) prepared a crossing and found that the eggs developed, but also that the hybrid embryos died in the eggs.

For this crossing I used individuals also from the Niger Delta area (Benin City) both of which were caught by Ulf Hannerz in 1961. I had two broods of fertile eggs. Totally 25 fertile eggs developed embryos. The hybrids from the first brood (five) developed without visible abnormalities in their eggs and hatched. A few days after the hatching all were dead from unknown reasons. The twenty hybrids of the second brood also developed without visible abnormalities. Nineteen hybrids however died in their eggs and one hatched and died. Indeed Zimmermann was right, no viable hybrids develop from such a cross.

Juveniles of GRA and SEX are so similar in shape and colors that it is almost impossible to tell them apart. Preserved individuals are easily sorted as these two forms develop different patterns of lateral line organs on the head. For the sorting of juvenile and semi-adult individuals of GRA and SEX I used the differences in the shine of eyes. GRA individuals have very brilliant eyes (grass green color) whereas the eyes of SEX individuals have much less brilliance. Also small but constant differences in the development of the dark crossbars might be used after some training.

GRA and SEX are sympatric in southern Nigeria. GRA individuals apparently are not found outside the swamps and in the swamps where individuals of SEX and GRA occur together, only juveniles and semi-adult individuals of GRA occur in samples. Stenholt Clausen's collections contain adult individuals of GRA from a single locality only, a certain type of swamp forest. Perhaps the juvenile GRA individuals are found together with the young SEX individuals because of protective resemblance of their color patterns, whereas the adult and mature GRA individuals for some reason live in other biotopes. SEX individuals are not restricted to swamps and this species extends its range all over the forest areas of southern Nigeria.

(74) SEX/LIN 1963

Encouraged by the promising results of the DAG/LIN cross I made up my mind to try the SEX/LIN cross because I thought that these two forms were more closely related. Both species develop similar patterns of lateral line organs on the head, similar patterns of crossbars and similarities in shape and counts. Individuals however will not crossbreed. Many times I tried the SEX/LIN and the LIN/SEX combinations. Much fight took place and apparently the individuals were not considering each other as partners for spawnings. I used a SEX male of the Umuahia strain for the crossing that resulted in eggs. The two individuals were stored in a well-planted tank and after some weeks I noticed that the LIN female had developed a very marked and very broad black lateral band from the gill covers to the root of the caudal fin and also in that fin. Such "pre-spawning" bands have not been described for LIN (which indeed is a very common aquarium fish), whereas such bands frequently develop on females of SEX. Probably the LIN female from her feeding on black mosquito larvae had developed very many eggs. Now the spawnings started and I had 21 eggs from two spawnings. During the first 24 hours I noticed very large blastula to develop in most eggs. These blastuals in my opinion were abnormal and also their cells apparently were larger than usual for Rivulilns. After some hours the blastula suddenly disappeared in some of the eggs as if it had "burst" (which is impossible). In other eggs big blisters developed where the blastula previously were situated. The nature of these blisters was as described for CAL/SEN, FAS/DAG, PAN/LIN etc. No egg burst because of the development of the blisters, although some blisters almost reached the size of the yolk ball. During the next days embryos developed on the surface of the blisters in a few eggs. In other eggs no corda was visible. As usual the anterior part of the embryo was situated on the blister and the tail of the embryo was on the surface of the yolk. Microphotos were prepared showing the blastula and the embryo on blister. From the throat of the embryo a thin vein crossed the transparent interior part of the blister and attached itself near the center of the "separation plane" (inner yolk membrane) between the yolk and the blister. As usual the movements of the vein forced the separation plane to move up and down. No blood elements were visible. In the anterior part of the embryo the gill arches and the eyes were visible, but apparently these organs were not symmetrically arranged to the corda of the embryo. From now on no further development of the embryo took place. The

only visible changes were produced by the development of more and more black pigments on the embryo and on the blister and the surface of the yolk. Also in eggs in which no embryo developed the concentration of black pigments on the yolk increased.

Three weeks after the spawnings I preserved all eggs as the eggs were about to decompose. SEX and LIN probably are not close relatives.

(75) SEX/LON 1961-63
The resemblance of these two sympatric species is conspicuous and people who are not accustomed to the SEX species probably will not be able to distinguish clearly between individuals of SEX and LON.

SEX is a widespread species as this form occurs all over the forest areas along the coast from SE Dahomey in the North to Gabon (at least) in the south. LON probably is restricted to the Niger Delta area only. Within the huge range of distribution the SEX species undergoes certain (rather small) changes in the phenotype. If the LON species is compared with the SEX species the demes of SEX from the Niger delta come closest to LON. This is true for morphology as well as for colors.

The differences in counts are small, but rather constant. I made the following counts of 44 aquarium raised individuals of LON originating from our three strains of this species: Benin (Ulf Hannerz in 1961), Benin on the road to Asaba (Stenholt Clausen in 1962) and Ologbo River (Stenholt Clausen in 1962) and 22 aquarium raised individuals of SEX, also from the Benin-Ologbo area. Further data for SEX are published under SEX/SEX.

D= 07 08 09 10 11 12 12 Dm
LON-Delta 1 6 31 6 0 0 0 8.9
SEX-Delta 0 0 2 9 10 0 1 10.5
SEX/LON 0 0 0 0 2 0 0 11.0
SEX.LON/SEX 0 0 1 4 5 0 0 10.4
(SEX.LON/SEX)2 0 0 1 4 4 0 0 10.3

A= 15 16 17 18 Am
LON-Delta 6 19 15 4 16.2
SEX-Delta 0 9 8 4 16.7
SEX/LON 0 0 1 1 17.5
SEX.LON/SEX 0 2 7 1 16.9
(SEX.LON/SEX)2 0 0 8 1 17.1

Sq-long= 25 26 27 28 29 30 31 Sqm
LON-Delta 2 26 38 11 2 0 0 26.8
SEX-Delta 0 0 4 6 13 7 1 28.8
SEX/LON 0 0 0 2 0 0 0 28.0
SEX.LON/SEX 0 0 0 1 0 0 0 28.0
(SEX.LON/SEX)2 0 0 4 4 7 1 0 28.4

If the main figures for the position of the anterior-most and posterior-most fin rays of the dorsal and anal fin are calculated for LON-Delta and SEX-Delta it is evident that the only important difference exists in the position of the anterior-most dorsal fin ray. This ray is more backwards on the back in LON than in SEX, thus indicating that the reduction in number of dorsal fin rays in LON has taken place in the anterior part of this fin only. Generally individuals of LON are more slender than those of SEX. This difference however is not present when old males are compared. Indeed the resemblance of SEX and LON individuals fades away with age. This is also true for color patterns. Juveniles from SEX and LON differ rather much in the shape of the body and in particular in the shape of the head.

In SEX individuals normally develop six black crossbars on the body sides. Females frequently develop additional bars, but some of these are temporary bars only. Additional bars occur rarely among males. In LON individuals develop many black crossbars on body sides. up to thirteen are frequently seen on females and on young males. Old males develop more than six black crossbars. In SEX males tend to loose their dark crossbars with age, but this tendency is more pronounced in CHA. The crossbars however will appear at once if these old males start fighting. Old males of LON normally show their dark crossbars, but also in this form a certain reduction of the distinctness of the bars is seen. The delta populations of SEX develop smaller and less conspicuous red dots on the body sides in males than do other demes of SEX. In LON males some strains have no red dots at all and in other demes small red dots are seen. Other small differences in morphology and in behavior have been seen in between our strains of SEX and LON.

Our strains of SEX and LON from the Niger Delta area were breeding true to the differences in characters mentioned above. No intermediate individuals developed. I have forgotten why I did not use a male of the SEX-Delta strain for this cross. Probably I had no male of sufficient size at that time. I used a male of my old mixed strain in SEX, IB/BA male (see SEX/SEX). This male however was absolutely fertile as all males from the SEX/SEX crossings.

The behavior of the individuals during the crossing is interesting. I had three LON females (Ulf Hannerz' strain) in a tank of their own. My LON male had died and I was expecting a new male from Ulf. When I placed the SEX male in the tank all three females lost their dark crossbars at once. They were resting motionless at the surface of the water. After a few minutes one of the LON females developed a very distinct dark lateral band from the gill covers to the root of the caudal fin. No traces of any dark crossbars were visible. The SEX male reacted at once on this stimuli and swam towards the female and very soon the spawning started. During the spawning the female LON did not loose her dark lateral band (as SEX females usually do if they develop any pre-mating lateral band at all). After some spawning the female swam in front of the male and stopped. She lost her dark lateral band and instead the many dark crossbars developed on the body sides. The male stood motionless watching the female. After some seconds the female again changed her color pattern. The crossbars faded away and the dark lateral band developed. The male reacted at once and more spawnings were produced. These sudden changes of color patterns of the LON female took place several times during the crossing. The two inactive LON females were resting at the water surface motionless, no dark crossbars, no dark lateral band.

In my opinion the SEX male reacted even more promptly to the stimuli of the LON female than normally SEX males do on stimuli from SEX females - if these develop any stimuli at all in pre-mating display.

SEX females do not loose their dark crossbars when they develop a dark lateral band or (Barombi strain) the dark crossbars are reduced in size only.

I had sixteen eggs from this crossing and all eggs developed. No abnormalities were seen during the embryonal development. The hybrids hatched in time, but all but five died during their first weeks. In my opinion they did not die because of a particular hybrid weakness but because of certain unidentified aquarium diseases that also ruined some of the fry of the pure LON and SEX strains at that time.

The five hybrids that survived were not at all feeble individuals and their rate of growth exceeded that of the pure LON and SEX strains. One hybrid was very quickly growing and developed into a male. The four remaining hybrids developed into females. At an age of five weeks the young hybrids developed their first dark crossbars. At that time only the normal six bars found in SEX were visible. Also juveniles of LON started with the development of these six bars. 2P, 2V, 8A. After some days the young hybrids doubled their dark crossbars as also young LON individuals do at that age. Up to twelve crossbars were counted at that time. When the hybrid male matured it lost but six dark crossbars and after this time it never developed additional bars. These six bars corresponded to the normal bars in SEX. Females kept their many bars during their life. As the hybrid females matured slowly I backcrossed the hybrid male to a SEX female of the Ibadan strain. Individuals of this strain (and deme) develop rather many dorsal fin rays and also individuals of the mixed strain used for this cross (Ibadan(Barombi) develop rather many dorsal rays. The eggs of this backcross developed without any abnormality and very viable F2 individuals were hatched and raised (SEX.LON/SEX individuals). Also these F2 individuals were absolutely fertile among themselves and so were their offspring.

Before the F1xF1 spawning could be prepared the male jumped out of its tank and died. Later on, the four females were placed in a tank containing too acidic water and all were killed.

The SEX (many individuals from different demes), the LON and the SEX.LON/SEX individuals were tested for hemoglobine patterns. All developed similar six line patterns. The status of LON as a distinct species has not been supported by the crossings so far prepared in SEX and LON. I do not doubt however that LON is a distinct species sympatric with SEX in the Niger Delta area. If LON was not a distinct species isolated in reproduction from the sympatric individuals of SEX indeed no individual of LON would exist in nature. Also the counts for D and Sq-long support a division of the SEX like individuals from the Niger Delta into two species: SEX and LON.

(76) SEX/SEX 1960-64

Very few species among West African Rivulins extend their range over such a large area as to the SEX species. The types originated from Gabon and this country probably is the southern limit of the range of SEX. There are indeed reports of SEX from localities south of Gabon, but the samples of such individuals (British Museum) that we have seen contained no individual of SEX. From Gabon SEX extends northwards along the coast. It does not occur in the Congo, nor in the Chad drainage. From the Cameroon Mts. SEX extends westwards along southern Nigeria. In central and eastern Dahomey some isolated (?) populations of SEX also occur.

In the west SEX is replaced by its closest relative, the CHA species. In the south and in the Congo

drainage SEX apparently is replaced by MUF which may or may not be considered as a close relative also.

I have prepared counts and also studied other characters on the huge collections of SEX in Stenholt Clausen's collections from Dahomey, Nigeria and Cameroon. I also had the opportunity to study individuals from the British Museum, the Philadelphia Museum (some of the types) and a few individuals caught by J. Lambert. For about 800 individuals these counts were prepared:

D= 09 10 11 12 13 Dm
Gabon 0 2 5 2 0 11.0
South Cameroon 0 1 7 4 0 11.3
North Cameroon 0 4 12 6 3 11.3
Niger Delta area 2 9 10 0 1 10.5
Ijebu Ode creeks 8 97 109 7 0 10.5
Ijebu Ode swamps 4 46 77 8 0 10.7
Lagos area 0 8 12 5 0 10.9
Abeokuta area 0 56 80 11 0 10.7
Meko area 0 4 31 6 0 11.0
River Zou, Dahomey 1 1 2 0 0 10.2
Ibadan area 1 8 51 16 0 11.1
Ife-Ilesha area 0 1 10 8 1 11.5
Owo area 0 1 0 4 0 11.6
Non Nigeria 0 7 24 12 3 11.6
Nigeria-Dahomey 16 234 419 72 3 10.8
E. longiventralis 8.9

A= 14 15 16 17 18 19 Am
Gabon 0 0 4 4 1 0 16.6
South Cameroon 0 0 7 4 1 0 16.5
North Cameroon 0 0 7 13 4 0 16.9
Niger Delta area 0 0 9 8 4 0 16.7
Ijebu Ode creeks 1 9 94 99 19 0 16.6
Ijebu Ode swamps 0 2 37 80 15 1 16.8
Lagos area 0 0 13 12 0 0 16.5
Abeokuta area 0 2 39 78 27 1 16.9
Meko area 0 0 8 32 2 0 16.9
River Zou, Dahomey 0 0 1 3 0 0 16.8
Ibadan area 0 1 20 45 12 0 16.8
Ife-Ilesha area 0 0 2 6 10 1 17.5
Owo area 0 0 3 1 1 0 16.6
Non Nigeria 0 0 18 21 6 0 16.7
Nigeria-Dahomey 1 14 233 393 90 3 16.7
E. longiventralis 16.2

Sq-long= 27 28 29 30 31 32 Sqm
Gabon 0 0 1 5 4 1 30.2
South Cameroon 0 0 3 8 5 1 30.2
North Cameroon 0 0 8 11 6 4 30.2
Niger Delta area 4 6 13 7 1 0 28.8
Ijebu Ode creeks 4 33 113 54 6 0 29.1
Ijebu Ode swamps 0 6 26 15 3 0 29.3
Lagos area 0 6 33 6 1 0 29.0
Abeokuta area 2 14 98 35 6 1 29.2
Meko area 1 7 38 25 2 0 29.2
River Zou, Dahomey 0 0 3 4 0 0 29.5
Ibadan area 6 20 59 20 1 0 29.0
Ife-Ilesha area 0 5 12 4 0 0 28.8
Owo area 0 1 4 2 2 1 29.8
Non Nigeria 0 0 12 24 15 6 30.2
Nigeria-Dahomey 13 99 417 183 28 3 29.2
E. longiventralis 26.8

Scales long: apparently the number of scales in a lateral series is rather high in the eastern and southern parts of the range for SEX. In the Niger delta area a certain drop in the figures for this character takes place. This decrease in scale numbers remains rather unchanged west of the delta area.

Dorsal rays: just the same change for figures exists if the number of dorsal rays is studied. Through Gabon and Cameroon rather high figures were obtained from this count. A sudden drop in the average figures for this character takes place when we enter the Niger Delta area. Even more westwards this figure remains rather constant within the humid parts of Southern Nigeria, whereas in the northern drier areas the figure again increases (Ibadan, Ife, Ilesha and Owo).

Since 1959 I have been able to study rather many nature caught strains of SEX from Nigeria. My first strain originated from Ibadan (IB) and was taken there by Birket-Smith. The next strain originated from Lake Barombi near Kumba in Northern Cameroon. Also this strain was caught by Birket-Smith. On this base the handsome IB/BA strain was produced. During 1961 I received from Ulf Hannerz a strain of SEX from Benin City in the western part of the Niger Delta area. In 1962 Stenholt Clausen sent home individuals from many localities: Meko (on the frontier between Dahomey and Nigeria), Lagos, Ibadan, Ijebu Ode, Benin City, Ologbo River and Umudike near Umuahia (situated at the watershed between the Niger and the Cross drainages). Fourteen different pure strains were produced on this material.

These strains might be divided into three major groups:

Western group in Nigerian SEX: Meko, Lagos (two demes), Ijebu Ode and Ibadan (three demes) belong to this group in Nigerian SEX. In the males no yellow color develops on body and fins. The guanine brilliance is a cold blue to violet blue. The red dots on the body sides are rather large, rounded (except for the back) and often edged with black. Ventrals produce and develop streamers in all males. In females the dark lateral pre-mating band rarely develops during display. Females are rather dark and the dots on

their body sides are dark brown.

Delta group in Nigerian SEX: Benin City (two demes) and Ologbo River. Also the Owo strain that does not belong to this group if counts are studied resembles this group in colors. In males much yellow to grass green color develops on the body sides and in the fins. The guanine brilliance is a warm green to yellow. The red dots on the body sides are smaller and less conspicuous. The concentration of dark pigments in these dots is low or nil. Ventrals produce much as in the Western group. Females develop a warm brown color and their dots on the sides are more red than among western females.

Eastern group in Nigerian-Cameroon SEX: Umudike and Lake Barombi demes. In males the bright yellow color on the body sides and in the fins is replaced by red to orange-red color that is much more concentrated on the outer parts of the fins. The guanine shine is bronze. The red dots on the body sides are larger than among Delta males and in the Barombi strain they are even larger and more conspicuous than among western males. The red dots are not mixed up with black pigments. Ventrals are conspicuously shorter than among western and Delta males. They probably are very short in the Barombi deme. Females develop a warm brown color. Their red dots on the body sides are larger than in the western group and they are red, not brown.

West/East cross = IB/BA cross I had a single female from the Barombi deme only. No male. I had one pair of the Ibadan strain. Birket-Smith for a long time considered the BA female as a male because of the bright colors.

The BA female used her broad black pre-mating lateral band during spawnings. At the same time the upper parts of the dark crossbars disappeared, whereas the lower parts (below the dark band) remained. The "hybrids" developed without any abnormality. At an age of one to 1 1/2 years these males developed much brilliance and were very brightly colored. In their shape of the head and the caudal fin these males differed somewhat from other males of SEX that I had. The snout was more rounded and the caudal fin developed symmetrically to the median line through that fin. In Nigerian SEX the male has a larger lower lobe. The rounded snout also developed in SEX/LON hybrids that were produced from one of these IB/BA males. Old males from this strain did not develop dark crossbars and even during a fight these bars were not seen.

In order to improve this handsome phenotype a male was backcrossed to the old BA female. The F2 individuals developed without visible abnormalities. These individuals were rather feeble and many died from unknown reasons. I had a single pair left after some time. The females stopped the production of eggs and gradually changed into a male phenotype. Such sex reversal is unusual among West African Rivulins although sex reversal is known in Nothobranchius and Cynolebias.

In order to preserve the rare BA genes I had to spawn the last male (IB/BA.BA) to a female of the Ologbo strain. The IB.BA-BA/OL individuals developed without visible abnormalities. During 1965 I bred one pair from this strain under control. The first brood of eggs developed abnormally and most embryos died in eggs. The second brood developed normally (one of thirteen embryos developed abnormally and died). The third brood also developed normally.

West/Delta cross = IB/BE cross A male of the Ibadan strain was crossed to a female of the Benin strain. The "hybrids" developed without visible abnormalities. They were fully fertile and no abnormalities were noticed to occur among the F2 individuals that were also fully fertile. Males of the F1 generation developed very long streamers in the ventral fin. Longer than in any strain of SEX which I have seen. The warm yellow color which characterized the BE males was not seen in IB/BE males.

These few crossings indicate that within Nigeria the different demes of SEX have not developed post-mating isolating mechanisms. Exchange of genes between the IB and the BA demes that are separated by a distance of not less than 650 km is still possible, but certain small abnormalities occur among the offspring.

The Delta demes show certain traits that point towards some gene exchange between SEX and LON in this area. These traits also occur in demes living west of the Delta area and in particular in those living in the humid southern areas. Filter???

(77) SEX/SHE 1962

As I have already mentioned in connection with the CHA/SHE cross SHE probably does not deserve the "rank" of a full species and might be considered as a subspecies in CHA. SEX and SHE correspond in morphology, in color patterns (not exactly similar patterns) and in the development of the "rare" six-line hemoglobine pattern. They differ markedly in their patterns of lateral line organs on the head. I consider these two forms as close relatives and CHA-SHE as the most "ancient" forms.

A male of the Benin strain in SEX was crossed to a female of the Tikawbo strain in SHE. I had thirty eggs in one spawning. 26 eggs developed. After twelve days the development of the hybrid embryos differed somewhat, but all embryos were considered as normal and viable. After 17 days the first viable hybrid hatched out. All eggs gave viable hybrids. During their first weeks rather many hybrids died from unknown reasons, but this also may happen to fry of the pure species. After three months I had ten hybrids left alive. Some individuals apparently were much more robust than other individuals. Two individuals developed into strong males whereas the remaining individuals developed into females (or non sex, the smallest). At least three individuals were real females. The two males differed markedly in their color patterns. After maturing one male did not show any dark crossbars, not even during fight. The second male had the six "normal" crossbars of SEX.

The development of dark crossbars differed also markedly among the "females". Three females (the largest ones) had the bar system of SHE (2P, 0V, 8A). One female developed the bar system of SEX plus one bar (2P, 2V, 9A). One more "female" had a bar system resembling that of CHA (0P, 0V, 5A). In life the female like individuals often developed temporary additional bars.

The two hybrid males were tested for hemoglobine patterns. They both developed the expected six-line pattern. As the patterns of lateral line organs differ markedly in the parent species the development of this pattern in the hybrids is important. The DAG/SEX hybrid that represents a similar problem developed a pattern that could be considered as "intermediate". Not so among the SEX/SHE hybrids. Two hybrids developed the CHA-SHE pattern almost exactly. Three individuals developed a pattern that was somewhat "intermediate", but still this pattern was much closer to the CHA-SHE type than to the

SEX type. The three remaining individuals in my collection have a stronger "intermediate" character, but still much closer to CHA than to SEX. These individuals corresponded well to some individuals of LAM (E. lamottei Daget) from Upper Guinee. Generally however LAM belongs to the CHA-SHE type as far as lateral line patterns are concerned.

The pattern of frontal scales corresponded to the two parent species that develop similar patterns. No decomposition or replacing of these scales were noticed.

D= 09 10 11 12 13 Dm
SEX-Delta 2 9 10 0 1 10.5
SEX/SHE 0 0 8 0 0 11.0
SHE 1 11 41 5 0 10.9

A= 15 16 17 18 Am
SEX-Delta 0 9 8 4 16.7
SEX/SHE 1 2 4 1 16.6
SHE 17 34 6 0 16.0

Sq-long= 26 27 28 29 30 31 Sqm
SEX-Delta 0 4 6 13 7 1 28.8
SEX/SHE 0 6 8 0 0 0 27.6
SHE 10 87 18 2 0 0 27.2

These figures show that in their counts SEX and SHE do not differ much and that the counts for the hybrid also do not differ much from the parent species.

The hybrid females did not spawn and probably did not produce eggs. Males were backcrossed to SHE females. No egg developed. Probably the males also were sterile. The broad lateral "pre-mating" band of SEX and SHE developed on the sides of both sexes among the hybrids when they were afraid.

[continued in Scheel Letter No. 53; Part 4]