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How the *Xiphophorus* Problem Arrived in San Marcos, Texas

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The Genetic Stock Center can trace its origin to Myron Gordon's doctoral research at Cornell University. He began his scientific work in 1924 while still an undergraduate student with 6 platyfish of a domesticated stock, and the following year he added some swordtails. Platyfish and swordtails were not new to him because already as a teenager he had kept many tropical fish. But a great enthusiasm for genetics was aroused by Professor A.C. Fraser in the Department of Plant Breeding. Other professors at Cornell who took a great interest in his work were H.D. Reed (Zoology), who was his official sponsor, and G.C. Embody (Limnology) and R.A. Emerson (Plant Breeding). Dr. Gordon concentrated first on platyfish, working out the genetics of various pigment patterns, and then on swordtails. He coined the terms micromelanophore and macromelanophore (Gordon, 1926).

It is an incredible coincidence that at the same time, Dr. Curt Kosswig in Germany also started research on swordtails. Neither Gordon nor Kosswig knew of the other's existence. But their initial contributions to the *Xiphophorus* melanoma problem were not the same (Atz, 1941). Kosswig (1928) pointed out that the melanoma was hereditary and in some way associated with melanophore patterns from the platyfish, while Gordon (1931) clearly identified the macromelanophore gene of *X. maculatus* as being responsible for the tumor.

The complementary relationship between laboratory and field research that has made the study of *Xiphophorus* so exciting and successful was initiated early in Gordon's career, and one hopes it will be continued in the future. Whereas the development of melanoma in certain platyfish-swordtail hybrids was predictable, the sporadic appearance of such growths in some platyfish presented a problem. While working on his thesis at Cornell, Gordon reasoned that platyfish were known only from the hobby and had been bred for many years under domestication and hybridized with swordtails. He could not determine whether platyfish of natural populations also developed pigment cell abnormalities or whether the occurrence of melanoma in the domesticated platyfish was due to the introgression of *helleri* genes. The sample of 83 platyfish from Mexico in museum collections was too small to provide the answer. He also thought it possible that platyfish and swordtails might hybridize in nature, because Meek (1904) had found them together in one location. He must go to Mexico himself!

None of the modern collecting paraphernalia were available to Gordon. There were no maps or rental cars, and only a few paved roads. He went in a model T Ford, loaded with camping equipment, shotguns, letters of introduction to every conceivable agency, and with milk and oil cans for shipping fish back to the United States.

In 1930 only 6 of the 22 presently described species of *Xiphophorus* were known, and virtually nothing was known about their habitat and distribution. Platyfish were assigned to *Platypoecilus* and swordtails were known as *Xiphophorus*. Gordon knew that 3 scientific collections of platyfish had been made. The first consisted of 2 fish collected by La Salle prior to 1866 in "Central America," a locality description utterly useless if you are trying to find the fish (Gordon,

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1947a). A second collection of 13 fish in 1867 by Dr. Francis Sumichrast, an ornithologist, came from Cosamaloapan on the Rio Papaloapan, Veracruz, Mexico, where the Smithsonian had maintained a field station for several decades. The third collection of 68 fish was made by Seth E. Meek in 1902 (Meek, 1904), near the railroad station of El Hule, Oaxaca, Mexico, the last stop of the Transisthmian Railroad before it passed over the Rio Papaloapan into the state of Veracruz. Meek, an ichthyologist, had traveled by railroad from Chiapas in the south to the state of Nuevo Leon in the north. He detrained every other stop to collect fishes and then continued his journey the next day. Myron, in his search for platyfish, made the most sensible decision: he headed for the Rio Papaloapan.

Gordon's 1930 Mexico Expedition was financially supported by the National Research Council and by the Museum of Zoology of the University of Michigan, which wanted to enlarge its fish collection. The expedition consisted of three men: Gordon, Edward Creaser, a malacologist from Cornell, and Ricardo Ostos, M.D. They drove to Laredo and had to ford the Rio Grande, then proceeded through Monterrey along the foot of the Sierra Madre to Ciudad Victoria. The section from Monterrey to Ciudad Victoria was unpaved and every stream had to be forded. They learned to wrap their socks around the sparkplugs to keep them dry. This entire distance of 267 km took 10 hours to drive. From Ciudad Victoria they continued to Ciudad Valles and then headed southeast to Tantoyuca, crossing the Rio Moctezuma at El Higo. Here they stayed for several days on Ricardo Ostos' ranch and explored the surrounding area on horseback (Figure 1). One day, 20 km south of Tantoyuca, they collected 4 immature swordtails near the Rio de los Hules and Rio Calaboza, which Gordon identified as *X. montezumae.* Actually, these fish represent the first specimens of *X. birchmanni,* a species not described until 1987. No further collections of *Xiphophorus* were made in this region until 1985. From Tantoyuca the expedition headed east to the Estero Cucharas, a small stream flowing into the Laguna Tamiahua, about 75 km south of Tampico. The road conditions were so horrendous that they had to rest for half an hour every 3 km. From there they turned south toward the Rio Cazones and then climbed up over terrible roads across the headwaters of the Rio Tecoluta to the Mexican Plateau. The Rio Tecoluta watershed is characterized by steep canyons and waterfalls several hundred meters high. In small streams on a narrow plateau above the falls at 1200-m altitude Dr. Gordon discovered a platyfish, somewhat similar to *X. variatus,* many years later described as *X. evelynae.* A series of hydroelectric power plants and dams had been constructed in this rather inaccessible region, and undoubtedly Gordon was aided by Claudio Martinez, an alumnus of Cornell and an engineer with the Mexican Power Company. The expedition headed for Mexico City, where they rested, and then drove to Veracruz.

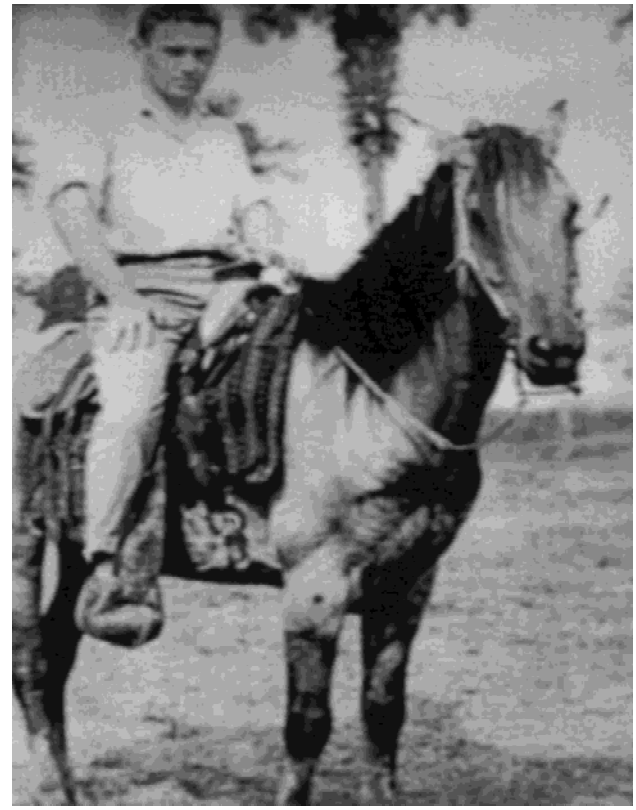

Figure 1. Dr. Myron Gordon at Tantoyuca, Veracruz, Mexico, 1930.

On the way they collected swordtails, *X. helleri,* at Jalapa and were puzzled by the many large males with incompletely formed gonopodia and undifferentiated gonads (Gordon, 1937a). The only record that I can find about their next move states that they hired a boat at Alvarado and headed up the Rio Papaloapan toward Cosamaloapan and El Hule, 45 and 85 km, respectively, to the south, but more than twice that distance by river. A railroad spur from Veracruz ends at Alvarado, but I do not think they took the train when they could have traveled on another one to El Hule. It is also unlikely that they took the Ford there, because the track to Alvarado on the narrow high ground between the high dunes to the left and the marshes to the right must have been blocked by the ever-present sand drifts. Gordon placed Alvarado only 20 miles from Veracruz; however, it is actually twice that far. I think he may have hired the launch at Boca del Rio, 20 km south of

Veracruz, and followed the coast to Alvarado, because he wrote many years later, "we sailed up the ocean-like mouth of the river" (Gordon, 1940). This is an apt description if you enter the river from the Gulf.

The quest for *X. maculatus* soon came to an anticlimactic end. The current of the river was swift and the motor of the little launch was too weak. The river meandered endlessly and appeared to follow the contour of a pretzel. For every mile they covered in a straight line, they had to travel three. The marshes on both sides of the river seemed to extend forever. Finally they reached Cosamaloapan, but then the rains came, and they had to turn around without having seen a single platyfish. They returned to Mexico City and made the slow trip north via the Mexican Plateau as far as San Luis Potosi. There they picked up a track through the Sierra Madre, passing through Tula (elev. 1400 m), still today a rather small, isolated desert town, to Ciudad Victoria (elev. 300 m). The section from Tula to Victoria was so horrible that Myron declared he would never make this trip again. On the way back the expedition collected *X. couchianus* near Monterrey, and introduced live specimens for the first time to the United States. Although no *X. maculatus* were caught, the trip was nevertheless a success. Over 100 species of fishes were collected, of which 10 were new to science ("Introducing Dr. Myron Gordon, *The Home Aquarium Bulletin,* March 5, 1935).

On March 6, 1932, the model T Ford was again heading south. Gordon was accompanied by John Ross and Joe Whetzel, sons of Cornell faculty members. According to Gordon (1940), the great business depression had been good for the tropical fish hobby, inasmuch as people stayed at home and became interested in tropical fish keeping. The Cornell University Mexico Expedition 1932 was supported by private funds from hobbyists, commercial tropical fish breeders, and the Heckscher Research Foundation at Cornell. The Shedd Aquarium in Chicago and the New York Aquarium also promised to buy exotic fish that the expedition hoped to bring back. The expedition arrived at the border in Laredo on March 12th, where it was unexpectedly delayed. Myron had shipped by Railroad Express Agency 120 one-gallon cans to the border, to be reshipped to various small Mexican railroad stations near areas where the expedition hoped to collect fish. The cans, painted on the inside with asphaltum varnish, were the shipping containers. A requested permit to bring the empty cans across the border free of customs duty, because they would eventually be returned to the United States, had not arrived. The customs officer demanded US $1.00 per can ($12.49 in current dollars), and this was far more than Gordon could afford. Frantic telegrams exchanged between Laredo and Mexico City elicited sympathetic advice but led nowhere. Why don't you buy goldfish, Myron was told, and place them into your jars? There is no duty on importing goldfish into Mexico, and your cans then become the shipping containers, which are not taxed. But Gordon eventually paid. He also complained about the price of gasoline, which was US $0.35 per gallon ($4.38 in current dollars), and remarked that nobody trusted Mexican gas pumps. Gasoline was first pumped into well-marked cans to measure accurately the amount purchased (Figure 2).

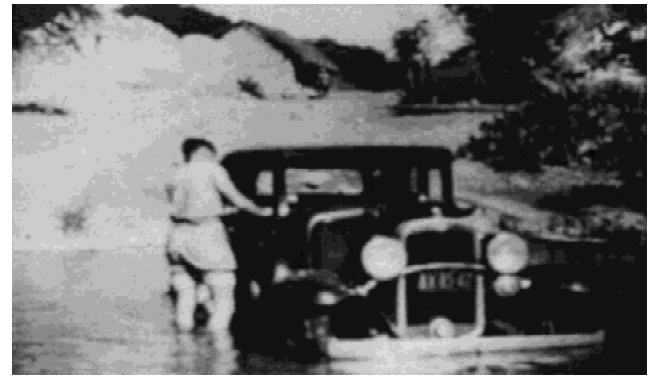

Figure 2. Dr. Gordon's model T Ford in Mexico, 1930.

Once beyond Monterrey the spirits of the expedition soared. The road was paved now with macadam or consisted of packed gravel and the many streams had all been bridged. Five hours later they arrived in Ciudad Victoria. The expedition was not eager to drive again the Victoria–Tula segment, and they had heard about a new road being built to Mexico City via Tamazunchale. The more they inquired from the locals about road conditions ahead, the more contradictory the information became. Finally they settled on going via Tula. They left early in the morning and arrived totally exhausted in Tula, long after nightfall, with an empty gas tank, having covered the 140 km in 15 hours. Afterwards Gordon (1940) wrote he could not understand what made him drive this route again. I presume he chose a known quantity, even if it was difficult, over something completely unknown. They experienced every imaginable road hazard: huge rocks strewn across the track, hidden steep ravines, axle-deep ruts, narrow mountain passes, and enormous climbs and descents. The road crossed seven ranges. The remainder of the drive across the Mexican Plateau to Mexico City, which took 8 days, was a "diabolical trial: parched, barren, dusty and death dealing". But they survived and rested up in comfort in Mexico City.

Today, Tula is not considered such a bad place for *Xiphophorus* researchers. Only 15 years ago we learned of a level path through the barrier range that leads to a valley at 1000-m elevation, only 20-km distant, with a small stream teaming with swordtails, *X. nezahualcoyotl.*

Early in April the expedition arrived in Veracruz, where they stored the model T. From there they proceeded in relative comfort by local train to El Hule, which had been renamed Papaloapan. For a belated impression of the joys of riding this train, the reader is referred to Theroux (1979). Papaloapan lies near the fall line. To the east of the railroad track stretches the low coastal plain with its enormous swamps, muddy lagoons, and oxbows, and to the west we find the beginning of the Piedmont of the Sierra Madre del Sur. Today, a narrow vehicle bridge crosses the river at Papaloapan with a toll station at its southern (Oaxaca) side. Truck traffic on the road is as busy as on the New Jersey Turnpike because it provides the least mountainous access to Puebla and Mexico City from the southern states. A new superhighway built through the marshes 30 km to the east is barely used because of its excessive toll rates.

But in 1932 the area was a sleepy backwater. The Standard Fruit Company had banana plantations in this area with wonderful facilities for its administrative staff. With letters of introduction to military and civil authorities, the second Cornell expedition was taken in by the Fruit Company and installed like royalty. After camping out for nearly a month, it felt wonderful. "The perfect service and comfort of our headquarters meant more to us than a paid-up, de luxe suite of rooms at Sun Valley Lodge" (Gordon, 1940). The company's officers must have looked on the three New Yorkers as strange little fish, indeed, to have traveled 3000 miles to catch 1-inch-long fish! Every facility of the company was placed at Dr. Gordon's disposal: vehicles, boats, horses, and guides. Three days' fishing effort yielded not a single platyfish. Gordon had not yet discovered its exact ecological niche. He thought platyfish, being rather broad and somewhat deep-bodied, could not be a stream fish, but must prefer quiet waters without current. So he concentrated on the nearby oxbows and swamps. From my own experience, I know how utterly depressing it can be to have come so far and fished for many days without catching a single specimen. Myron must have felt likewise, but he merely wrote they were "quite discouraged." Eventually, they headed upstream by boat from company headquarters and entered the Rio Tonto and one of its little side streams. And there Gordon caught his first *X. maculatus!*

Excitedly, and no doubt with satisfaction, he telegraphed his success to Cornell. On April 17, 1932, the *New York Times* carried a long article with the headlines: "Cancerous Fish Found in Mexico," "Oaxaca Jungle Pierced." A direct quotation from the telegram reads, "We found by actual count ninety-nine platyfish, some of which are suspiciously suffering with a small degree of melanosis. There is one in which the condition is well seen."

And of course the publicity hounds of Cornell sprang into action and added that the Cornell expedition attained its objective, "deep in the jungle recesses of the Mexican state of Oaxaca." In reality, the jungle had been long gone and the only *Xiphophorus* person, if we may call him that, to rhapsodize about the luxuriant vegetation was C.B. Heller, a 21-year-old German botanist who landed in Veracruz in 1845 (Siemens, 1990). One of his objectives had been to scout for areas suitable for German colonists, and while doing so he collected in the Rio Blanco drainage the first swordtail, which now carries his name. This river is the northernmost tributary to the Rio Papaloapan estuary, and the general area where Heller found the swordtail is approximately 100 km northwest of the village where Gordon caught his first platyfish.

The next day, the platyfish and also some swordtails were placed in the cans and successfully shipped by railroad to a commercial fish breeder in New Orleans. Later in the summer when it was warm up north, they were reshipped to Cornell. "Melanotic" platyfish, however, were not to be. Closer examination later showed that the darker fish merely had a well-expressed spot-sided pattern, and the single most heavily pigmented one exhibited a new pattern, called spotted-belly, Sb (Gordon and Smith, 1938a), or black-bottomed (Gordon, 1948), but there was no melanosis. It remains the only *Sb* fish that has ever been caught in the Rio Papaloapan drainage, although fish with somewhat similar patterns have been taken in some of the river systems to the east (Kallman, 1975). Next day the expedition members packed their bags and retraced their steps to Mexico City to collect goodeid fishes on the Mexican Plateau to satisfy their sponsors in the aquarium trade. Later, before the return trip north, Myron inquired at the Mexican Automobile Association about the new road being constructed beyond the old towns of Zimapan and Jacala to Tamazunchale at the foot of the Sierra Madre; they were not going to pass through Tula again. Assured that on average 12 cars per day traveled over this road, the expedition opted for the new highway (Gordon, 1933).

For 160 km beginning just beyond Zimapan the road passes from 2000-m elevation through lush forests of pine,

then cloud and deciduous forests reminiscent of the Smokies, to the tropical lowlands at Tamazunchale at about 300 m. The road crosses not a single stream along its entire length; it follows closely the top of the long divide between the Rio Amajaque to the east and the Rio Moctezuma to the west because this minimizes the ups and downs across mountain ranges. In several places the road straddles the very top of the narrow divide. There were long stretches where the road was still under construction. Not a single piece of mechanical equipment was used, only dynamite, crowbars, and pickaxes.

The expedition reached the Rio Moctezuma at Tamazunchale, but was unable to fish. The main river is too deep and swift as it comes out of a gorge, and Myron could not locate any side channels or small tributary streams. They pushed on another 30 km and were stopped at the Rio Axtia. Men used mules to pull the cars through this river, but it was late in the day and the men had stopped working. Seining in the river near their campsite yielded Montezuma swordtails, *X. cortezi,* and beautiful *X. variatus.* Many of these fish were preserved. The next morning they rushed to the railroad station in Ciudad Valles, 75 miles to the north, to claim their cans, which had been waiting for them for well over 6 weeks. The expedition returned to the Rio Axtla and filled 12 cans with 20 fish each. The next day the fish were successfully shipped by railroad to New Orleans. Farther north other successful collections of *X. xiphidium* were made. The expedition returned to Cornell by the end of May. Sorting through the preserved collections, Gordon was eventually left with a single fish that he could not identify; it turned out to be the first pygmy swordtail.

As early as 1931, Gordon had been wondering how many genes were involved in the exaggerated expression of the macromelanophore genes in hybrids with *helleri.* But he considered it an almost impossible task to determine: "While it is probable that many modifying factors are operating in *Xiphophorus* which influence the degree of melanosis, it is hopeless to establish the exact number without increased facilities" (Gordon, 1931). Like every investigator after him, Gordon needed more tanks! Between 1932 and 1938 Gordon made good use of the fishes he had brought back; he crossed *X. maculatus* from the Rio Papaloapan with 3 other species, *X. variatus, X. xiphidium,* and *X. couchianus.* In each of these crosses the expression of the macromelanophore gene of *X. maculatus* was enhanced, but to different degrees. The *maculatus* × *couchianus* hybrids were especially interesting because they exhibited the neoplastic disease at birth. Thus the modifier genes of these species could not be the same, but the question of whether they were different alleles at the same locus, or different genes altogether, was not addressed (Gordon and Smith, 1938a). These experiments also established that the occurrence of melanoma and pigment cell abnormalities was not restricted to *maculatus* × *helleri* hybrids, but was a general phenomenon in *Xiphophorus.*

Gordon (1937b) employed the concept of multiple factors to explain the formation of melanoma in the hybrids. Closely following Kosswig (1931) in Germany, he hypothesized that 2, but perhaps many more, modifier loci were contributed by *X. helleri.* But it is not clear from his data how he arrived at this conclusion, and a year later Gordon and Smith (1938a) stated the the 2-factor hypothesis was really a somewhat arbitrary construct. Later in the same year, Gordon and Smith (1938b) wrote that the swordtail contributes apparently more than one dominant modifier. Gordon thought that the two species, *maculatus* and *helleri,* possessed different alleles at the 2 loci, aa bb and AA BB, respectively. Those hybrids exhibiting the most advanced state of melanosis were thought to have inherited, in addition to the macromelanophore factor, all 4 dominant modifiers. But there was no independent confirmation that this interpretation was correct.

Detailed histological descriptions of the abnormal macromelanophore patterns were provided by Reed and Gordon (1931) and Gordon and Smith (1938b), who classified the melanotic overgrowths into 3 stages. The first stage is characterized by a macromelanophore hyperplasia in the corium, the second stage by an invasion of the muscular tissues by macromelanophores along the myoseptae with some tissue destruction of fin rays and the soft tissue between them, and the third stage by the appearance of invasive spindle-shaped cells, significant tissue destruction, and melanotic overgrowth. No metastases were observed. They emphasized that there is no sharp separation between the 3 stages and that the development of the pigmentation can stop at any stage. The histological description, of course, was important not only in its own right, but also because it served to make the *Xiphophorus* melanoma system highly pertinent to the medical community at large. "The spindle cells of the melanotic overgrowths in hybrid fishes histologically resemble the cells of mammalian melanosarcoma. They also are infiltrative and destructive to adjacent tissue" (Gordon and Smith, 1938b). This was followed 3 years later by a report of growing the *Xiphophorus* melanoma in tissue culture. "In its morphology and behavior, including its property of clasmatosis, it is identical with

the melanoblast of the mouse and human melanoma" (Grand et al., 1941). The fish melanoma had established its credentials.

In 1937 Professor Reed, who had been Gordon's sponsor for more than 12 years, died. Latent opposition to Gordon's project now manifested itself. Not only was Myron forced to relocate his laboratory, he also lost his financial support from the Heckscher Foundation at Cornell. Gordon, perhaps introduced by Professor G.M. Smith of Yale, then turned to Dr. Charles M. Breder, Jr., the director of the New York Aquarium. Breder had his own ideas of what a public aquarium should do. It should not be only a place to exhibit aquatic life and perhaps provide some education to the public, it should also carry on its own unique research. At the Aquarium scientists were already experimenting with electric eels, and the research on *Xiphophorus* melanoma seemed unique and important. Gordon was invited in.

In 1938 the New York Aquarium was located at the Battery in Castle Clinton, at the southern tip of Manhattan. The building had been constructed in 1807 as a fort in New York Harbor, known as the South-west Battery. Fourteen years later, after the fort became obsolete, it was ceded to New York City and given a new name: Castle Clinton. It was connected to Manhattan by a bridge and later by landfill and remodeled to serve as a place of entertainment until the mid century. Between 1855 and 1890 New York City served as the main landing place for immigrants, and a total of 7,690,606 passed through the building. After 6 years of extensive renovation, Castle Clinton reopened in 1896 as the New York Aquarium.

Gordon built his new laboratory with several hundred aquaria on the second floor above the larger exhibit tanks of the exhibition hall (Figure 3). He had also become a Fellow of the John Simon Guggenheim Foundation and secured a generous grant. The 101 platyfish he collected in 1932 were not sufficient. A new trip to Mexico was in the planning stage, one that he had been thinking about since 1932 (Gordon, 1940). He hoped to stay at Papaloapan for several weeks to study the fate of platyfish populations as the dry season progressed and the lagoons shrank in size and small arroyos dried out. In the meantime platyfish had been found at several places in the Rio Usumacinta system in Guatemala, indicating that *X. maculatus* might have a wide distribution. He had also heard about lakes in Chiapas near the border with Guatemala, and that was where he was going to search for platyfish. He still thought that platyfish preferred quiet waters, notwithstanding that he had caught none in the lagoons near Papaloapan. He must check out the lakes!

Figure 3. Dr. Gordon and his laboratory setup above the main exhibition tanks in the old New York Aquarium at the Battery, Manhattan, 1939.

In January 1939, Gordon drove south again, with Mr. J.W. Atz of the Aquarium. The car was again placed in storage in Veracruz, and they rode the train across the Isthmus of Tehuantepec to Arriaga near the Pacific coast. This was a daylong ride. From there they took a bus through the hot canyons and bone-dry mountains to Tuxtla Gutierrez, capital of the state of Chiapas, where they stayed overnight. The next day they continued by bus the slow agonizing crawl up the Sierra. The landscape certainly changed. They were now on the lush Atlantic versant of the mountains. The temperature dropped not only because the road kept climbing, but also because of the cloud cover and frequent fog. There were still magnificent forests covering the mountains. Upon their arrival at San Cristobal, well over 2000 m above sea level, they must have realized that their quest for platyfish had turned into a wild goose chase. They never made it to the lakes. The following morning, with the help of a local guide, they found a stream and seined it, but they collected only *Profundulus.* The area was too high for poeciliid fishes.

There are no records of how Gordon had learned about the lakes, and obviously he knew few details. The only lakes of any size in this region are the Lagunas de Montebello, now in a national park, a little bit more than 125 km beyond San Cristobal, and at much lower altitude. The lakes are within the range of *X. alvarezi.* Was it really such an outrageous idea to look for platies near San Cristobal? Taken out of context, it was a failure because none were brought back. But, in retrospect, I think Gordon was right to go there. He was interested in platyfish distribution. They had

been reported from Guatemala. He had found *X. helleri* and what is now known as *X. evelynae* at an altitude of 1200 m, and we now know that *X. cortezi, X. malinche,* and *X. xiphidium* may occur at similar elevations. "World Aeronautical Charts" of southern Mexico, which Gordon consulted, carried the following warning: "reported elevations could be off by several hundred meters." There was also a very important psychological factor involved. If it is really important to you to find out what *Xiphophorus* might occur in a certain region, you have to go there yourself and look; otherwise, the uncertainty is going to prey on your mind for years. It took also courage to go to Chiapas in 1939. It was one of the least developed areas of Mexico, and the natives were even more hostile to outsiders than they are today.

After picking up Evelyn Gordon, Myron's wife who had arrived in Veracruz by boat, they headed for Papaloapan, where they were again welcomed by the Standard Fruit Company. For an entire week they again searched for platyfish in the wrong location in the broad lagoons east of the railroad track, not realizing that their fish were close by. A small part of the swamp is isolated between the railroad and the higher ground to the west from which small springs were flowing. This part of the swamp drains through a few openings in the railroad embankment to a gradually widening lagoon (Zacatispan). Not much has changed since 1939 except that the swamp has been divided once more by Mexican Highway 145, about 100 to 200 m to the west of the railroad. It was in the springs and in the small shallow pools that formed in the swamp that Gordon caught his fish. On February 20, in a pool near railroad marker 149 km, they collected 954 platyfish. From another pool 500 m away, 1937 platyfish were collected between March 4 and 10. Over several weeks the expedition caught more than 3310 platyfish. Even though none of the fish exhibited *Sb,* they had hit the platyfish jackpot (Gordon, 1940).

More importantly, Dr. Gordon now recognized the precise habitat of *X. maculatus:* "Four species of platyfish, *P. couchianus, P. xiphidium, P. variatus* and *P. maculatus* have this point in common: all of them may be found in relative large numbers in regions of springs" (Gordon, 1947a). I may add this also holds true for 4 species of platyfish that have been discovered since then.

The most important platyfish turned out to be the strains Jp 163 A and 163 B. They have been distributed to many laboratories in the United States and Europe and form the baseline for many *Xiphophorus* studies. Their origin was a mere accident. In March 1939, after lengthy discussion at Papaloapan, it was decided that Atz would return to New York by steamer from Veracruz with a live subset of the Papaloapan platyfish. While he was waiting in Veracruz for passage to New York, disaster struck. The platyfish in the shipping cans developed "Ich" and died. Desperately, Atz hit upon a plan. If platies are so common in the Rio Papaloapan, perhaps they also occur in the Rio Jamapa drainage, which is the next, albeit small, river system to the north? He took the train out of Veracruz to Plaza de Agua (El Tejar), near today's Veracruz airport, where the railroad crosses a small tributary of the Rio Jamapa. This looked like a likely spot, and platyfish there were. Several hundred were collected and preserved, and several dozen were brought alive to New York. The experiment designated number 20, a mating of a spot-sided female, *Sp*/+, with a spotted-dorsal, stripe-sided male, *Sd/Sr,* gave rise to pedigree 163 (Gordon, 1947b). These fish and their descendants have been inbred by brother-to-sister matings ever since. After 9 generations the strain was split into 2, carrying *Sd* and *Sp* on their X chromosomes, respectively. Jp stands for Rio Jamapa. Strains Jp 163 A and B were born!

In 1971 I revisited the site. An abattoir, a mere roofed-over cement slab, stood next to the small brook, which received blood and other offal. There was nothing here but stinking mud. Seining was futile. But 3 km away in a small roadside stream I caught platyfish, some of which I brought back alive to the stock center in New York. One fish exhibited the spotted-dorsal pattern, Sd. For a number of reasons we maintained this gene and noticed minor, yet consistent differences in phenotype as compared with Sd in Jp 163 A. It turned out that two *Sd* factors exist in Jamapa. When *Sd* of Jp 163 A is introduced into *X. couchianus,* its expression is suppressed whereas that of *Sd* from the 1971 collection is enhanced. This illustrates that when patterns within a population appear identical, it does not necessarily mean they are caused by the same gene.

The Genetic Stock Center stayed at the New York Aquarium at the Battery for only a short time. Coney Island, the big amusement center of Brooklyn, which had its heyday in the early part of the 20th century, and the surrounding neighborhoods were steadily deteriorating. The New York City administration thought the area could be upgraded by relocating the New York Aquarium to Coney Island. Today only the outer shell of Castle Clinton remains. Inside, there is a ticket booth for the Statue of Liberty ferry, and at night rock concerts are held here.

The Genetics Laboratory found new quarters on the

6th (top) floor of the American Museum of Natural History, where three rooms of the bird department were not being used. The rooms had high ceilings and a roof that consisted of glass panels sloping somewhat towards the west. Each room had a western exposure that consisted almost entirely of glass. During the summer, the roof and windows were repeatedly whitewashed and a sprinkler system ran cold water over the roof. Nevertheless, during heat waves the water in the aquaria sometimes climbed to 32°C. At these temperatures the fish had brood intervals of 18 to 19 days. The fish did well.

In 1948, Gordon organized the first of a series of Pigment Cell Conferences. In his contribution to it, he analyzed "the effects of five primary genes on the site of melanoma" (Gordon, 1948). This paper is important, because all 5 macromelanophore genes of *X. maculatus* are listed here together, and it gives the impression, and certainly Gordon thought, that there are only 5 alleles at the macromelanophore locus: *Sd* (dorsal-spotted), *Sp* (spot-sided), *Sr* (stripe-sided), *N* (black-sided), and *Sb* (black-bottomed). In reality, the situation is much more complicated: *X. maculatus* exhibits 5 or 6 basic macromelanophore patterns corresponding largely to Gordon's 5 alleles. But there are consistent differences within each pattern between the 7 major river systems, and these differences are not caused only by genetic background, but rather reside within a particular macromelanophore factor (Kallman, 1970, 1975). Thus, instead of a single *Sd* factor, there are at least 7, perhaps even more because some populations may have more than one. The same holds true for the other patterns. Thus within this species at least 35 macromelanophore factors exist. There are additional macromelanophore factors in other species of *Xiphophorus.* One should also not forget that each collection in nature is only one tiny snapshot in place and time. I do not believe that Gordon and I have found every macromelanophore gene. Only a small fraction of these factors has ever been tested to determine how they are expressed in interspecific hybrids.

In 1948, Gordon still believed in his artificial scheme of at least 2 unlinked modifier loci involved in melanoma formation, but he seemed to lose confidence in it. He was puzzled why in crosses with *helleri* the *Sd, Sp,* and *Sb* factors readily gave rise to melanomas, whereas few tumors developed in *Sr* and *N* hybrids. In his opinion, different macromelanophore factors responded to different modifier systems, similar to gene interactions at the complex tailspot locus (Gordon, 1956). For example, the modifier locus *E* interacts with one, and only one, *Co,* of the 9 tail spot alleles, and a second modifier locus, *Cg,* changes only the expression of *T.* In other words, 2 unlinked modifier loci may interact with *Sd, Sp,* or *Sb,* while 2 other loci may modify *N* or *Sr.* Dr. Gordon (1951a) expanded on this idea: "Apparently, the platyfish *Sr* gene requires a number of genic modifiers in homozygous state to induce the formation of the melanotic tumor." These ideas are somewhat similar to those expressed by Kazianis et al. (2001), who found that the *CDKN2X* gene seems to interact with *Sp* and *Sd* of Jp 163 in crosses with *helleri* but that other factors are responsible for melanoma formation in *variatus* × *helleri* and *maculatus* (Jp 163 A *Sd*) × *andersi* crosses.

Unfortunately, Gordon rarely presented a decisive cross that provided solid data as to the number of modifiers. His *helleri* × *maculatus* crosses in the 1930s were conducted with domesticated stocks of doubtful genotypes, and those listed in 1948 were also compromised by the introduction of a domesticated swordtail stock with the albino gene. Only one cross is free from all these defects (Berg and Gordon, 1953). It involves a cross of Jp 163 carrying *Sp* and *Sd* with *X. helleri* from the Arroyo Zacatispan. The first hybrid generation was then backcrossed to either parental strain. On face value, the range of macromelanophore expression of the *Sd* genotypes seems to point to a single modifier factor and that of the *Sp* genotypes to two. But one should note that of the 64 first-generation hybrids, 16 were listed as "normal." Unfortunately, the paper failed to define "normal," and I do not recall ever having seen such a hybrid with a macromelanophore pattern that was identical with that of the *maculatus* parent (i.e., "normal").

The observation that the expression of the macromelanophore gene of *maculatus* was also enhanced when introduced into other platyfish species (Gordon and Smith, 1938b) made Gordon wonder whether a similar reaction occurs in interpopulation crosses of *X. maculatus* (Atz, 1948). He again went to Mexico in 1948 with Atz and F.G. Wood, Jr., a graduate student from Yale University, and they headed from Veracruz by train for the Rio Coatzacoalcos. They got off at Almagres and Jesus Carranza, where in 1939, unbeknown to Gordon, Coronado had collected platyfish. They obtained 485 fish from these two sites and then headed toward the Rio Tonala, where more fish were collected. For the first time, live fish from Gordon's field trips were sent by air from Mexico City to New York. Subsequently, he discovered that *Sd* of the Rio Coatzacoalcos exhibited increased expression in a Jamapa genetic background.

In 1950 Gordon attended the International Cancer

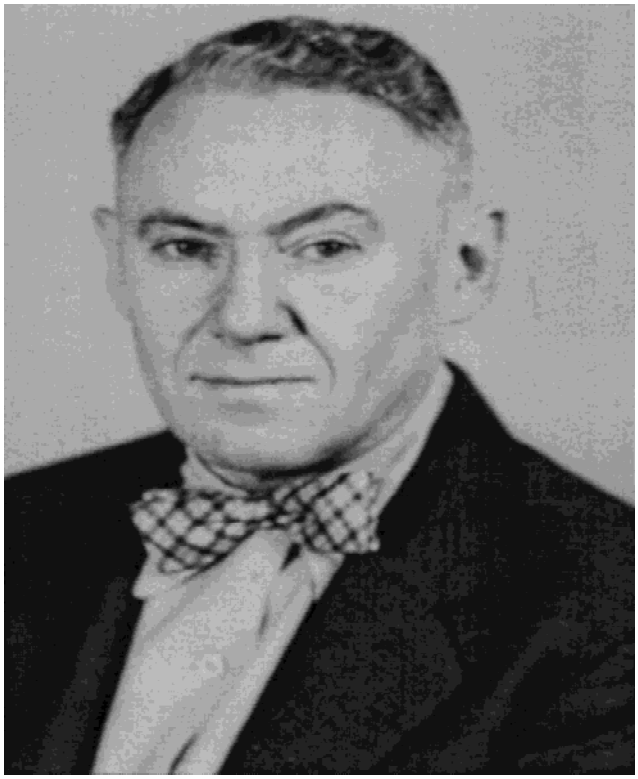

Figure 4. Dr. Myron Gordon, photograph taken in the late 1950s.

Congress in Paris, lectured on *Xiphophorus* melanoma before the Royal Society of Medicine in London on July 4, then flew to Turkey. Finally, 25 years after *Xiphophorus* research began in Germany and the United States, Gordon (Figure 4) and Kosswig met on July 5, 1950, in Istanbul (Gordon, 1952). Dr. Kosswig had invited him to speak at the university.

There are in the literature references to the "Gordon-Kosswig melanoma model" and to the "classical cross." These terms are poorly defined. I feel the term Gordon-Kosswig melanoma model should only be used to describe the rather general fact that an *X. maculatus* macromelanophore factor, when introduced into an *X. helleri* genotype, gives rise to melanoma. The term "classical" refers to something authoritative, or of long standing, or to a standard. The term classical cross is applied to matings involving Jp 163 A of *X. maculatus* with *Sd* and *X. helleri.* But because these crosses are not always made with the same *helleri* stock, the results cannot always be replicated. We are dealing with not one cross but many. The crosses with Jp 163 A and various *helleri* stocks only date back 25 to 35 years after this stock became widely distributed. Kosswig never made this cross, and Gordon only once (Berg and Gordon, 1953).

Strains Jp 163 A and B of *X. maculatus* have become the workhorses in *Xiphophorus* melanoma research. But how representative of *X. maculatus* are these stocks or the Jamapa population? The phenomenon of enhanced pigment gene expression in *maculatus* × *helleri* hybrids is also observed when the genes from other *maculatus* populations are introduced into the Jamapa stocks, except that the frequency of malignant melanoma is not as high (Gordon, 1951b; Kallman, 1970, 1975). Not enough crosses between *maculatus* populations that do *not* involve Jamapa have been made to determine whether changes in gene expression are of general occurrence in such hybrids. Preliminary evidence suggests that this may not be the case.

A gene sequence called *Xmark* (or *Xmark-2*) has been identified that maps closely to the macromelanophore locus (Schartl, 1990). It is present in all *Xiphophorus* fish with macromelanophore factors that may give rise to melanoma after hybridization, and it is absent from wild-type fish and fish with macromelanophore factors that do not change in expression after hybridization. *Xmark* has also been equated with the macromelanophore gene, and the macromelanophore gene has been called the oncogene or tumor gene, but this is misleading. There is only one *Xmark,* and it plays an important role in melanoma occurrence, but more than 50 macromelanophore factors are known from *X. maculatus* and other species. DNA sequences other than *Xmark* must control these patterns. Note that *X. variatus P-1* individuals lack *Xmark-2,* but they develop a macromelanophore pattern.

Atz (1962) applied to *Xiphophorus* the model of Fisher (1928) and Ford (1957) showing that a population with a potentially deleterious locus accumulates modifiers that will eventually mask its harmful effects. Whereas Kosswig (1931) and Gordon (1948) had always thought that the swordtail contributes specific genes that enhance the expression of the macromelanophore genes, Atz (1962), following Dr. D.E. Rosen, proposed that *helleri* and other species lack genes that control the expression of the macromelanophore factors of *maculatus.* This view was also more in line with Gordon's (1959) characterization of the melanoma cell as an incompletely differentiated pigment cell. Later, Anders (1967) in Germany expressed a similar view and presented a more detailed model. The expression of the macromelanophore gene is controlled by a regulatory gene, *Diff,* and both are species specific. Hybrids that inherit two *Diff* alleles of *helleri* develop malignant melanoma, whereas those that are heterozygous, one *Diff* factor from *helleri* and the other from *maculatus,* exhibit melanosis or benign melanoma (Anders et al., 1979). How could such an expla-

nation, so attractive in its simplicity, have escaped Gordon and Kosswig?

In a recent paper, Kazianis et al. (2001) found a highly significant correlation of the CDKN2X genotypes with pigmentation phenotypes and melanoma formation. They had tested the *Sp* gene of Jp 163 B in *couchianus* and *helleri* genetic backgrounds. In both crosses, there was a very high association between fishes with melanoma and the presence of two CDKN2X alleles inherited from either *couchianus* or *helleri.* Most fishes that did not develop melanomas were heterozygous at the *CDKN2X* locus. Similar results were obtained when the *Sd* gene of Jp 163 A was introduced into *X. helleri.* These results were interpreted as suggesting that *CDKN2X* might be the *Diff* tumor suppressor gene. Not mentioned in this paper was the well-known fact that the expression of *Sd* gene of Jp 163 A is suppressed in a *couchianus* background. Obviously, the structures of *CDKN2X* of *X. couchianus* and *X. helleri* cannot be identical. One should also recall Zander's (1969) report that the pigment gene of *maculatus* is kept constant, the degree of its expression is dependent upon the particular strain of *X. helleri.* If *CDKN2X* is one of the important players in melanoma formation in *Xiphophorus,* it may exist in different allelic states even within the same species.

Although *CDKN2X* is a likely candidate for *Diff,* the question of how many loci play a role in pigment cell proliferation after hybridization is still unanswered. The *Sd* phenotype of Jp 163 A exhibits little variation and is markedly different from that of F_1 hybrids, which show a 100-fold increase in pigmentation. A wide gulf in phenotype separated the most heavily pigmented *Sd* fish of Jp 163 A from the least pigmented F_1 hybrid. If only a single gene, e.g. *Diff,* were responsible for the change in expression, then a cross of F_1 to Jp 163 A should result in two nonoverlapping phenotypes: one identical with Jp 163 A and the other indistinguishable from that of F_1 hybrids. Why is such a simple cross not performed?

Myron Gordon died unexpectedly in 1959 at the age of 59 while Dr. Rosen and I were in San Salvador. Three weeks before, both of us, together with J.W. Atz, had received our doctorate degree from New York University under the sponsorship of Myron Gordon. Eventually I took over the laboratory, which stayed at the American Museum until 1968, when it moved to a newly built science facility, the Osborn Laboratory of Marine Sciences, at the New York Aquarium in Coney Island. I took the genetic research in a somewhat different direction focusing not only on pigment cells, but also on tissue transplantation, sex determination, and the hypothalamic-pituitary-gonadal (HPG) axis. The collaboration with the researchers in Texas dates back to 1975. The field work in Mexico, which continued at an ever-increasing rate, often with colleagues at Science Park, Smithville, and more recently, Southwest Texas State University, turned out to be extremely successful and led to many new discoveries. It was only natural that after my retirement in 1992, the laboratory would be moved to Texas.

There may be more to the segment of DNA that makes up the macromelanophore gene. The *P* locus controls the activity of the HPG axis, and its most obvious phenotypic effect is that it determines the age and size at which the fish become sexually mature (Kallman, 1989). Each species may have from 1 to 10 alleles and, as far as we know, these alleles are species specific. Depending upon its genotype, *X. maculatus* may mature as early as 8 weeks or as late as 2 years. Introduced into *helleri,* a *P* allele that causes maturity in *maculatus* at 12 weeks causes maturity in *helleri* at 3 years! Conversely, *P* alleles of *helleri* cause accelerated maturity in platyfish at 5 weeks. I find this change in *P* phenotype analogous to what happens to macromelanophore genes after hybridization. Moreover, is it a mere coincidence that the *P* locus is so closely linked to the macromelanophore gene that not a single crossover has yet been discovered? A third locus, again closely linked to the macromelanophore gene, controls pterinophore patterns. It, too, shows changes in phenotype after hybridization that range from no expression to erythrophoroma formation, albeit rarely (Gordon, 1950). I wonder whether there is not a common denominator responsible for all these changes. Perhaps, one should not focus exclusively on the macromelanophore gene, but on this entire interesting segment of DNA.

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