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## Special Newsletter Contributor

Dr. Pedro León Azofeifa is the special contributor for this issue of our newsletter. A steadfast member of the Nectandra Institute Board of Directors since 2007 and a former classmate of Alvaro Ugalde in Biology at the University of Costa Rica (UCR), he obtained his PhD (Biology and Chemistry) from the University of Oregon and returned to Costa Rica to join the faculty of the School of Medicine in 1974. His team, together with collaborators from UC Berkeley and U of Oregon, went on to investigate, identify, and publish the genetic mutations of numerous heritable Costa Ricans conditions. At the same time, and as far back as the 1960s, Pedro was an active participant in the formation of the Costa Rican national parks system and a collaborator of Alvaro Ugalde in the Costa Rican environmental movement, culminating in his position as Coordinator of President Oscar Arias' Peace for Nature Initiative in 2008. Notwithstanding the above multi-track career, Pedro participated as board member of various academic and scientific institutions (UCR, Organization for Tropical Studies, EARTH University, National High Technology Center among others). He is a member of the US Academy of Sciences and the current President of the Costa Rican Academy of Sciences.

Advising the CR government on Covid-19 has kept Pedro very busy, but he consented to recount below his seminal work on a uniquely Costa Rican condition affecting the hearing of many generations of a large extended family. Part of the following article is excerpted from Pedro's book titled *Journey into Silence* (Editorial Technológica de Costa Rica, 2017).

## From Deafness to Auditory Choices

*by Pedro León Azofeifa,*

It all started with a visit to my office by my childhood friend Susana Roberts, who taught at the Education Faculty of the University of Costa Rica, where I had just been hired to teach cell physiology at the Medical School. I completed my dissertation late in 1974 at the University of Oregon, where I learned molecular techniques to locate genes on chromosomes with labelled RNA probes, using the large chromosomes of salamanders. Why salamanders? Because of their huge chromosomes, more than 10 times the size of those of humans. However, I soon realized the medical school did not seem too impressed with this line of research,

On her visit Susana told me about a large, medically intriguing family in Cartago, the M-family, with many deaf members, who were apparently born with normal audition, but slowly became deaf, onset at primary school age progressing to profound deafness by adolescence. They were discovered by a graduate student, Grace Jara, who found many large nuclear families with several deaf members, both males and females alike. They all claimed to be related by ancestral grandparents. I was dumbfounded; how can a normal ear at birth, soon became dysfunctional? Susana added, "*They want to know why they are deaf*. Can you help? Didn't you study genetics in Oregon?" Totally unaware of the consequences, knowing nothing about inherited deafness, I agreed to help and start a project to answer the question. Surely, the Medical School would like this project more. Little could I imagine that our quest to find the answer would take two decades.

Thus began my acquaintance with the very large M family. Piecing from various historical records, we now know that the grand conquistador progenitor, Señor M-0, sailed from Spain in 1604, wandered in Peru, eventually settled and started a family in Costa Rica. His hearing status is unknown. Six generations later, at least three sons of nine children born to Señor M-5 were among the first recorded hearing impaired in the M family. Two of the three sons, A and B, eventually generated the family tree below:

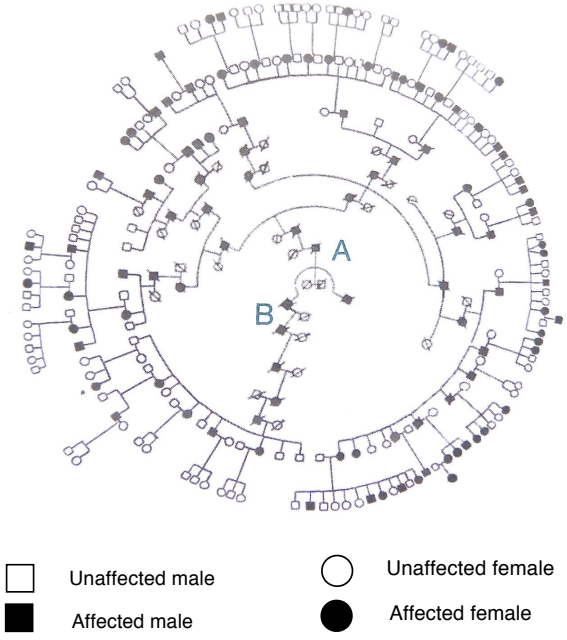
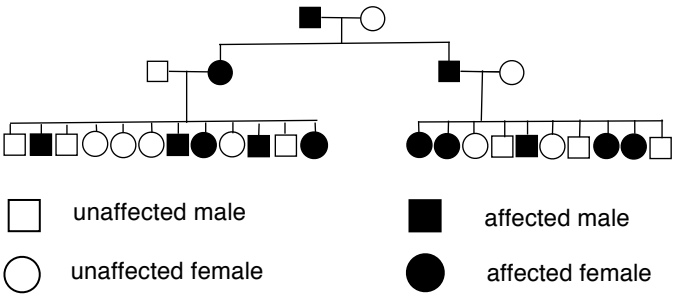



Fig. 1 The M family tree,1988. The larger branch was derived from son A of señor M-5 and the smaller branch from son B.

Let's analyze one nuclear family of the A branch:




Deafness is a *dominant* genetic trait. That is, only one of the two inherited parental genetic copies are necessary to affect hearing. This is in contrast to recessive genetic trait, where copies of both parental genes are necessary for the manifestation of the disease condition. In addition, the affected gene can be from either parent, indicating therefore that the affected gene is not linked to the sex chromosomes. Statistical analyses showed that the trait can be inherited by either daughters or sons at a ratio of 1:1, hence there are no silent transmitters, *i.e.,* only affected members can transmit the trait.

The M family was remarkable in another aspect — their use of lipreading exchanges and accentuated facial signals without emitting voice. The affected and unaffected members were equally skilled in this form of silent communication, a proficiency instilled by the senior family members and supported by the unaffected members. This novel "silent speech" was a highly effective channel even in a room full of people! In working with them, we soon learned to do the same.

Hearing loss began anytime after five years of age, when children were already able to talk. Because of the slow, progressive nature of the hearing loss, most affected individuals could master language by the time deafness affected the mid-frequency range which impacted verbal communication. This, we eventually realized, had a profound effect on the way deaf family members perceive the world and themselves. Given their predicament, they were very committed to vocalization and lip-reading and shunned Sign Language openly. More on this later.

Part of our task was to put together a research team that could test each family member for their hearing status, perform medical examinations and clinical tests of basic physiologic functions, and determine if they were all related by a common ancestor. This was the basis for a small grant from the recently created research council (CONICIT), for a three-year period and a total of some $10,000 dollars. It covered the cost of an assistant and an audiometer to be located at the School for Deaf Children in Cartago. Much of the team members were volunteers, including a group of medical residents under the direction of Dr. Roger Vanegas, a professor of cardiology at the medical school.

Family M enthusiastically endorsed and participated in the study, with the condition that they were kept abreast of the research. Every December, the research team invited the family via local radio announcements. These meetings began usually with a general discussion of the medical and genetic study, followed with more personalized and detailed discussion using non-technical language and finally a Q & A session. At the meetings we always emphasized the knowledge learned but not a cure for the deafness. Many unaffected also participated. Unaffected siblings were the best baseline controls, as they shared 50% of the gene with test subjects, as well as similar environments.

This well attended Christmas gathering became a two-decade long popular tradition and an M family reunion, with children activities such as piñatas and raffles. In addition to listening to the presentations and asking questions, the adults visited with each other over refreshments and traded jokes, a favorite Costa Rican pastime.

The first surprising finding came from the audiologic profiles obtained by José Raúl Sánchez, from the Education Faculty, that showed an initial loss of low frequency perception, which progressed through adolescence to involve all auditory frequencies. In comparison, most other forms of progressive deafness usually starts with high frequency loss.

At onset, some of the affected members report a high pitch *tinnitus* (ringing in the ears), which disappears as a profound deafness ensues. Initially, hearing loss involve sound tones around 250 Hertz (~ middle C on piano), which does not affect verbal communication, so that early signs of deafness are easily overlooked. However, as deafness slowly advanced, the mid-tones are inevitably affected, hindering oral communication. Older affected individuals show profound loss in the entire audible spectrum (250Hz – 8 kHz) and are unresponsive to sound over 100 decibels, the noise level of a nearby train or jet.

Hearing tests soon showed that the deafness was not due to damage of the mechanical parts of the middle ear, but rather the sensory components of the cochlea of the inner ear and of the brain signaling via the auditory cranial nerve. Protusions on our cochlea are "tufts" of hair-like receptors.
The deflection of the hair by the sound vibrational energy ultimately leads to release of neurotransmitter which is key to passing signals to the cerebral cortex of the brain. Loss of the receptors at the base of the cochlea result in gradual hearing loss in the upper frequency range (as in age-related deafness). This loss can be exacerbated by chronic

exposure to loud sounds. Loss of the receptors at the apex of the cochlea results in deafness in the lower frequencies — as in the Costa Rican M family deafness.

Knowing the physical lesion in the ear was only the very beginning of understanding the loss of hearing in the affected individuals. The wide gap in knowledge of how inherited genes affect the auditory signals took decades to unravel, made possible only with the gradual advancement of molecular biology. In 1992 we finally had the technology to map the gene involved in the M-family deafness to a specific human chromosome, named chromosome 5p31, in a collaboration with Mary-Claire King, at UC/Berkeley at that time, and our students: Henriette Raventós in San José and Eric Lynch and Jan Morrow in Berkeley. Identifying the mutation in a gene designated as *Diaphanous* took several more years, till 1998. Remarkably this gene has been found mutated, since then, in other families around the world (England, France, Korea and Japan), with damage that also results in deafness. It appears that different mutations produce the same type of protein damage. Hence, the M-family has a unique mutation, but shares phenotype (*i.e.,* deafness) with other mutations that affect the *Diaphanous* gene.

At that time cochlear implants were just emerging as a possibility for them, and we invited an expert from UC/San Francisco to talk to the family. However, implants were then new, expensive, with few channels and the members that attended were not impressed.

Things have changed since then, implants are now available through the social health system, and many family members have received them with immense success, according to colleagues that have implanted them and followed their progress, particularly Drs. Julian Chaverri Polini and José Raúl Sánchez.

Aside from the scientific and medical fronts, there are the social and communication fronts involved in deafness.

Curious about the social consequences of the hearing loss, a University of Costa Rica graduate student Ana Victoria Quesada, with minimal support, interviewed a group of 77 deaf family members evenly divided among males and females. Questionnaires were also completed on participants from 49 nuclear family, made up of 245 related individuals, including unaffected siblings and those married into the family. In general, many affected individuals had low level schooling, worked in unskilled labor and jobs in private sectors. One large family branch worked together as teams, involving both affected and unaffected family members. About half of the participants interviewed felt that their hearing loss limited their job opportunities and felt uncomfortable in unfamiliar environments. Eighty-five percent held jobs that required little social interaction. On the other hand, half of the deaf participants claimed that deafness did not significantly affect their communication skill and only with unfamiliar people and places. Most (81%) agreed that they received emotional and economic support from their families. Most of them (72%) did not wear hearing aids, complaining that they were bothersome, expensive and useless after the deafness became profound. Sociologist Ana Victoria did not find drug, alcoholic abuse or prostitution in the family cohorts, but recommended informational and educational assistance as part of the public health support system, especially around onset of the hearing loss.

I alluded at the beginning that the M family shunned Sign Language. In the 1970's, my limited knowledge about the world of Sign Language was mostly wrong. I had ignorantly assumed that Sign Language was a short-cut form of communication imparting crude factual information with little emotion, essentially a sort of pantomime. I had no understanding at the time that Sign Language is a complete idiom able to convey complex statements full of information, emotion and even poetry.

I was caught off-guard when a group of concerned but unaffected parents proposed Sign Language for the M family. I was perplexed by the reaction to the proposal relayed to the M family. It was met with lengthy silence, confusion and slight annoyance. One elder family member explained: "*Pero es que nosotros sí sabemos hablar*" ("But we already know how to speak"). Of course, they were equating use of Sign Language with the inability to speak, and felt proud of their own achievements with successful communication by lip-reading. Ultimately, respect for other's right to decide is truly the basis for conviviality.

I finally saw the uniqueness of the world inhabited by the congenitally deaf when I met a man with hearing loss who asked me: "My wife and I were both deaf children of hearing parents. We have a hearing child. What are the odds that our second child will be deaf?" "Well," I responded, "there is always a chance, but since the first one was unaffected, there are no reasons to fear that the next one will be affected". I reasoned that, as their parents were unaffected, both he and his wife were homozygous for a recessive mutation. Since they already had a normal child, their mutations had to be on different genes. To my surprise and dismay, he expressed his disappointment with my prediction, since he wanted a deaf offspring. That incident made me rethink many of the premises I held about the world of deaf people confronting life in a hearing world, which had never entered my emotional consciousness with such startling clarity. I can only be grateful for all the lessons they have taught me at so many different levels.

As I look back at many years of research, I wonder whether the original questions that Susana posed in 1975 have been

clearly answered. Very concrete results have, of course, been obtained in genetic terms, albeit complex and incomplete, as new questions, unimaginable at the beginning, have emerged. Science research seems to open doors into space with yet more doors into a never-ending labyrinth. In 1997, the study concluded. We decided to organize a gala event with the family and our collaborators from the US giving a power-point presentation while I translated into Spanish, taking care to pronounce the words very clearly. Despite the complexity of the subject matter and time limitations, I perceived a real sense of closure as they understood that there was a concrete, coherent explanation for their inherited loss, as well as a simple assay to detect the mutation. I personally experienced a true sense of completion as decades of periodic visits with the family drew to an end.

## News Highlights

As in the rest of the world, Covid-19 shut down our Nectandra public operations completely as of March 2020. From the start, Costa Rica issued clear and firm mandates for all to lockdown, to set curfew and driving restrictions, to socially distance and wear masks at all public places. As new information became available, restrictions were gradually withdrawn, one step at a time. Limited businesses hours and driving were gradually allowed to resume but under restrictions, even after vaccination began. Currently, masking and social distancing in public places are still in place. School only opened two weeks ago.

With wide distancing among them, our ground crew at the Reserve resumed full time field and outdoor work, but fully masked as of May 2020. We stepped up our patrolling due to the increasing presence of poachers, who now had more time on their hands to pursue their illegal deeds, and took the opportunity to repair and maintain our fences, trails, and buildings.

At the same time, our Nectandra Institute office staff began to engage with the communities virtually, and resumed their contact work and held conferences from home. Outdoor work at the nursery also resumed. However, many activities could not.

For the past first 12 of 18 months, we canceled:

- All reforestation and field work associated with the eco-loans.
- All volunteer activities
- All visitations to the Nectandra Reserve
- All face-to-face and indoor meetings

As of this April, our naturalist guides received full vaccinations. The garden guided tours have resumed as of May 2021. In the meantime, our office staff is still working from home, but is able to hold virtual meetings with our community partners. Vaccination in Costa Rica is now available to adults 20 or older. We expect to gradually return to "normal" schedule when our staff is fully vaccinated.

**March** — To commemorate Día Internacional del Agua (International Water Day), Manrique Esquivel from our staff posted a short video to celebrate the critical role of water to life on earth.
https://www.youtube.com/watch?v=eyooZCQ7ZOQ

While water covers 70% of the earth's surface, only 2.5% is fresh water. Of that, only a fraction is accessible while the rest is locked up in glaciers and snow. In total, just 0.007% of the planet's water is available for human consumption. While the volume of drinkable water has remained static, total human population is now 6.8 billion and growing. In spite of water's importance to our life and health, at the present rate and usage, only one in three persons on our planet will have access to adequate potable water by 2025.

Potable water distribution in Costa Rica is largely managed by the federal Institute of Water and Sewers (AyA). In many of the country's rural watersheds, potable water management is distributed by some 1500 semi-autonomous volunteer water management associations.

**June** — Joel Nitta, featured in the 2020 -1 issue of our NI newsletter, uploaded photos of many Nectandra ferns on Ferns of the world . Joel hopes to include photos of all of the ferns written in his paper titled, *A taxonomic and molecular survey of the pteridophytes of the Nectandra Cloud Forest Reserve*, *Costa Rica*, published in PlosOne (https://doi.org/10.1371/journal.pone.0241231). The paper summarizes Nitta's years of collecting and molecular studies on the exceptionally diverse fern species (176 total) at the Nectandra reserve.

Greetings from *Sceloporus malachiticus*

For additional information, please visit:
Website — nectandra.org
Blogsite — serendips.net