Profile of Eugenia M. del Pino

Eugenia del Pino does not consider herself a "real" biologist. She cannot abide dissections or fieldwork. She chose her Ph.D. research—her life's work, as it turned out—by a process of elimination. "I didn't want to work with parasites," she recalls. "I didn't want to work with bacteria. I didn't want to do anything in the medical profession." Free-living protozoans seemed safe, but her supervisor at Emory University ordered her to collect samples from the banks of the Altamaha River. "I just imagined myself having to wear boots, collecting those things and that was not for me!"

Fortunately for her and for the field of developmental biology, another professor was studying frog embryos. Harmless *Xenopus laevis* suited del Pino's taste in experimental animals. After her Ph.D., she returned to her native Ecuador to apply her research skills to the study of exotic frogs, which are so prolific in Ecuador that she did not have to leave campus to find wild specimens. del Pino says she fought to advance at every step of her 35-year career, but her tenacity has paid off. She was named to the National Academy of Sciences in 2006 for the perspective her work has brought to developmental biology.

Eugenia del Pino

Exceeding Expectations

The Pontifical Catholic University of Ecuador [Pontificia Universidad Católica del Ecuador (PUCE), Quito, Ecuador] has been the setting for most of del Pino's life. "As a student I was gifted; I could do many things," del Pino recalls. "To choose a career path was very difficult for me." She was interested in the German language and continues to study German even today. However, when del Pino entered PUCE in 1963, the science program at the School of Education, established with aid from the Kennedy administration in the United States, was only one year old. The equipment was brand new, and courses were taught by imported American professors. Impelled by curiosity, del Pino enrolled.

The aim of the science program was to train high school science teachers. Peace Corps volunteers and professors from PUCE's sister university, the University of St. Louis in Missouri, taught the teachers-to-be. "The foreign professors were inspiring," del Pino says, "but in some cases their command of Spanish was somewhat limited." Two who did speak Spanish had tremendous influence on the young del Pino. Candida Acosta, a Puerto Rican affiliated with the University of St. Louis, was head of the biology department. And Peace Corps volunteer Frances Ramirez taught microbiology, instilling an ethic of careful, consistent laboratory work. Both encouraged gifted students to pursue careers in science and took a special interest in del Pino, persuading her to apply for scholarships for graduate training abroad.

Acosta and Ramirez were delighted when the Latin American Scholarship Program of American Universities (LASPAU) awarded del Pino a fellowship that paid for her to study at a U.S. university, on the condition that she return to Ecuador afterward to teach. After a year waiting for the fellowship paperwork to be processed, del Pino found out that she had been accepted to Vassar College (Poughkeepsie, NY). Happy as she was to arrive at Vassar in 1967, an unpleasant surprise awaited.

Vassar considered del Pino's four years at PUCE the equivalent of one year at an American institution and had assigned her sophomore status. del Pino could not believe it. "I went to the dean of studies. I said, 'I think you have a made an error.'" After careful thought, the dean decided to put del Pino on academic probation for a semester. A professor followed her progress and decided that she was indeed graduate student material. Because Vassar is primarily an undergraduate college, there were only two other students in the master's program. However, del Pino realized that the department's small size was to her advantage: She could benefit from much more attention and support from the professors than if she had been at a larger university.

del Pino finished a master's degree in biology in 1969. According to the terms of her fellowship, she needed to return to Ecuador. She was not satisfied, however. She wanted to learn more biology and advanced research techniques. She struggled with the administrators at LASPAU. "I wanted to complete a Ph.D., something that at the time was unheard of," she says. "They said that a master's degree would suffice for the level of scientific advancement in Ecuador. And so I argued and I got everything that needed to be done so they could allow me to complete a Ph.D."

Donald Williams, who had approved del Pino's transfer to the master's program, was a graduate of Emory University (Atlanta, GA). He advised her to apply to Emory because he believed she would receive solid biology training there. Also, Emory's biology department featured a specialist in free-living protozoa, on which del Pino had written her master's thesis. Thus it was that, once accepted to Emory, she approached her target professor to ask him about opportunities for studying the life cycle of ciliates. It soon became apparent that the project he had in mind would involve wading in riverside muck to collect specimens. However, fieldwork had always been anathema to del Pino. She balked.

Luckily, she met Professor Alan Humphries Jr., who was investigating how eggs form in *X. laevis*, the frog equivalent of *Drosophila melanogaster*. For her doctorate, del Pino joined Humphries's laboratory to study the process by which *Xenopus* eggs are fertilized. She found that how susceptible an egg is to fertilization depends on the salt concentration and pH of the surrounding medium. If salt concentration drops, the outer layers of jelly on the egg swell, change structure, and block sperm from entering. After she defended her dissertation in May 1972, it was time to go home.

A Rocky Homecoming

"Once I accepted a fellowship requiring that I return to Ecuador to teach," explains del Pino, "it was my moral obligation to return and try to do my best." However, she did not get the homecoming she expected. She wrote to PUCE ahead of time, telling them that she was finishing her doctorate, in the hope they would have a job for her. On her arrival, the head of the science institute informed her that they could only offer her a position that paid by the hour, she recalls. The going rate was the equivalent of 25 cents

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del Pino and students in her laboratory at PUCE in Quito (credit Micheline Pelletier, L'Oreal).

an hour. Furthermore, PUCE would only allow her to teach the laboratory section of introductory biology because she had no teaching experience in Ecuador. "That was pretty discouraging," she says.

Reluctant to take such an ill-paid, boring job, del Pino sought out the director of the PUCE biology department, Olga Herrera MacBryde. Herrera was happy to see a talented scientist return to Ecuador. "At that moment," del Pino says, Herrera "received a phone call from Guayaquil, our main port. At the University of Guayaquil, they needed someone to come and teach an advanced seminar." Would del Pino be interested?

"That was really an interesting experience," she recalls, "because after you are in these fancy universities in the States" and return, "you are face to face with what it means to be in a developing country. There were groups at the University of Guayaquil that used to fight with one another. The day I was starting my seminar, they had taken over the building so I couldn't go in."

The dean of natural sciences at the University of Guayaquil arranged for del Pino to teach the seminar in a house downtown that served as an administrative center. "The room had no windowpanes," she says. "I had to teach in the evening from seven to nine o'clock, and outside you heard these noises. I asked what it was, and [was told] those were students shooting one another! When I took a bus to go to my uncle's home, you could see the drops of blood on the street."

She found the civil unrest shocking. "But on the other hand, never in my life did I have a group of students that thought what I was saying was so interesting," she says. "They didn't miss a word." At the end of the course, she gave an exam to assess what the students had learned. "I realized that they never had cell biology, they had no training whatsoever for what I was teaching. But they were fascinated, and Guayaquil actually offered me a job." In the meantime, however, the administrators at PUCE had thought things over and decided to hire del Pino after all.

"Now there was a new director of the science institute, and he said, 'I think you are very highly qualified,'" del Pino says. They offered her a full-time position, and she began work in the office of Olga Herrera, the director of the PUCE biology department, who was on vacation. When the director returned, "she announced that her husband had received a job offer in the United States . . . and so this meant that in November I became head of the biology department!" That was November 1972. del Pino had defended her doctoral dissertation only six months earlier. "In the developing world, things change from one day to the next," she says.

The demands of administration and teaching quickly snowed del Pino under. She taught three or four classes for a total of 12 lecture hours a week, along with the many hours it took her to prepare and grade papers. She realized that if she did not keep her research skills sharp, she might soon lose all of the training that she had acquired during her doctorate. Also, she felt that keeping abreast of research would make her a better teacher. In the PUCE laboratory, she found a microtome (a machine for making thin slices of embryos) and various staining agents. "I decided that I could enjoy myself, doing whatever I wanted to do in my free time," she says. All she needed was a research subject.

Fancy Frogs Fuel a Career

"I decided that I needed to look for an interesting frog," she says. She did not have to travel far. "I went to the gardens of the university and found two different frogs. And the most strange was the marsupial frog, which has a pouch and carries the babies inside. The pouch is in the back, like a backpack." Out of the intense competition for reproductive sites in the South American rainforest had evolved over 60 species of these frogs, in which the females double as mobile nests. Their reproduction and development was understood only in barest outline. del Pino had found her subject.

As a natural extension of her graduate work, she examined how marsupial frog eggs are fertilized and how the embryos are maintained in the mother's pouch. "My first idea," she says, "was, maybe in the evening, she will go into the pool and then the eggs will be refreshed with fresh water. Well, take those eggs from the pouch of the mother and place them in fresh water, and they die immediately." She found that, unlike *Xenopus* embryos, marsupial frog embryos develop under saline conditions typically found in the body.

Traditional frogs and marsupial frogs also differ in how their embryos excrete waste. Free-swimming tadpoles excrete ammonia, which would be toxic if accumulated in close quarters. del Pino discovered that marsupial frog embryos excrete urea instead of ammonia. This finding allowed her to devise a urea-based medium for the *in vitro* culture of marsupial frog embryos. Unfortunately, she has never been able to fertilize the eggs artificially.

Before long, the international research community recognized del Pino as an expert on marsupial frog development. *Scientific American* asked her to write a feature article in 1989 (1). Later, in 2000, she won the L'Oreal/United Nations Educational, Scientific and Cultural Organization (UNESCO) Award for Latin American women in science. It did not hurt that the frogs could only be studied at high altitude: Quito, where the frogs evolved, is two miles above sea level, and after trying experiments in the United States and Germany, researchers found that the marsupial frog *Gastrotheca riobambae* refused to reproduce overseas. "This in a way has been to my advantage," she says, "because I had no competition."

She has, however, kept strong ties to scientists and institutions in the developed world and takes every opportunity to attend meetings, collaborate, and learn new techniques. To get up to date on the newest molecular methods during her sabbaticals, she visited the laboratories of Michael Trendelenburg at the German Cancer Center (Heidelberg, Germany) and Joe Gall at the Carnegie Institution (Baltimore, MD).

Conservation in the Galápagos

Over the years, many researchers have visited Ecuador because of its phenomenal biodiversity. In 1972, the same year that she returned to Ecuador, the Charles Darwin Foundation for the Galápagos Islands tapped her to run a program that granted fellowships to Ecuadorian students to conduct research in the Galápagos. "In other parts of the world," she says, "people would pay money to go to the Galápagos Islands. This fellowship program is such a privilege. Galápagos is an important place."

She had no trouble motivating her students to go. She also visited the Galápagos regularly to keep tabs on them and learn about the variety of wildlife—the frigate birds, the native rats, and the iguanas. The Darwin Foundation made her a member of its council, and she became the foundation's vice president for Ecuador in 1992. The administrative work became a chore, however. The same year she that became vice president, fishermen harvesting sea cucumbers for the Japanese markets began to protest restrictions on fishing in the Galápagos coastal waters. They took scientists hostage, and del Pino, sitting in Quito, could contribute little to solve the problem. "I got this feeling in my stomach—what can you do?" she says. "And I decided that that was not for me. The day has only 24 hours, and there are other people who can do the lobbying and fight for conservation." In the end, she decided not to run for office again and to focus on her research instead. However, she maintains interest in the conservation of the Galápagos' unique environment.

One Egg Cell, Many Nuclei

In the years after her return to Ecuador, del Pino kept in touch with her former supervisor Alan Humphries, even visiting his laboratory several times. Back in Ecuador, del Pino had observed that, when viewed under a microscope, some marsupial frog oocytes appeared to have more than one nucleus. "He didn't believe me," del Pino recalls. He said she was seeing artifacts. She continued to mail him pictures from Ecuador until he was persuaded to travel to Quito to see for himself. He brought the materials to detect the incorporation of tritiated uridine into RNA—a way to verify that the extra nuclei were active. They were.

In fact, del Pino discovered that several marsupial frog species have multinucleated oocytes. The most dramatic occurrence is in the Venezuelan marsupial frog, *Flectonotus pygmaeus,* whose oocytes contain more than two thousand nuclei (2). del Pino showed that these nuclei provide embryos with a large store of ribosomal RNA and provide multiple copies of genes, which most likely allow *Flectonotus* to develop quickly. Eventually, all but one of the nuclei break down, and the cell reabsorbs them. In the common *Xenopus* frog that del Pino first studied at Emory, it is known that oocytes have just one nucleus with two million copies of ribosomal DNA in addition to the chromosomes.

Marsupial frogs with multinucleated oocytes are hard to find, so del Pino switched to studying the embryonic development of frogs with ordinary nuclei. She discovered that marsupial frog eggs are very large, reaching a diameter of one centimeter in some species. Moreover, the gills, called "bell gills," envelop the embryo in a sac closely apposed to the mother's pouch. The system resembles the mammalian placenta. Protected in the mother's back, the embryos of the marsupial frogs develop at a very slow pace, like mammalian embryos.

Most Unusual Development

In animals, after fertilization the zygote divides until it is a hollow sphere of cells called a blastula. Then the blastula begins to fold in on itself. Cells migrate across the lips of an opening called the blastopore to form specialized inner tissues that will become organs. This process is termed gastrulation, and the embryo at this point is called a gastrula. In amphibians, including *Xenopus*, the embryo normally develops from the entire gastrula. However, del Pino found that this does not happen in marsupial frogs.

Instead, small cells migrating across the blastopore lip form a wide disk from which the embryo subsequently develops. The rest of the gastrula consists of yolky cells. Before del Pino's discovery, formation of an embryonic disk was known to occur only in mammals and birds. She published her findings in *Nature* (3), accompanied by a full page of illustrations. She notes in the paper that it is remarkable that the early developmental stage in various frog species can be so different, even though the end result is so similar.

del Pino's Inaugural Article (4)—a comparative study of development across seven frog species—epitomizes her research career. Using the 14-hour development of *Xenopus* as a ruler, she and her colleagues observed the events that accompany gastrulation in fast-, medium-, and slow-developing Ecuadorian frogs. They show that in the fastest developers (two species of foam-nesting *Engystomops*, which take 24 hours to progress from fertilization to gastrulation), the future backbone and gut elongate at the same time that cells fold in through the blastopore. However, in several species of dendrobatid "poison dart" frogs, whose gastrulation takes four days, by the time the cells fold into the blastopore to form the gastrula, only the future gut has elongated. The future backbone develops later. In the marsupial frog *G. riobambae*, gastrulation lasts 14 days, and the schedule is even more leisurely: All that is formed by the end of gastrulation is a small gut cavity.

These surprising differences in embryonic development are accompanied by an altered schedule of gene expression in marsupial frogs, which del Pino and colleagues detected at the protein level in whole embryos. They conclude that the process of gastrulation in *Xenopus* appears to be so complex because several events overlap during the rapid development.

"You may think that, being in Ecuador, I am isolated," del Pino says. True, but the isolation does give her the time she needs to focus on scientific problems and the freedom to explore new methods of teaching. Additionally, there is no shortage of exotic frog species to study in Ecuador, every one of which is a new variation on the theme of embryonic development. There is no doubt that del Pino is a "real" biologist, even if she tries her very hardest to avoid getting muddy feet.

Kaspar Mossman, *Science Writer*

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