Everything You Wanted to Know About Creating A Strain of Axolotls Carrying a Tiger Salamander Gene But Were Afraid to Ask

By

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In the summer of 1962, a high school teacher in Minnesota found a female tiger salamander, Ambystoma tigrinum, with special characteristics. After keeping the animal for about a year, he gave her to Joe Gall who was then at the University of Minnesota. Joe recognized the potential of this animal and sent her to Rufus Humphrey at Indiana University where she was very carefully nurtured. In the winter of 1964 she had reached enormous proportions (by tiger salamander standards). By popular vote she was the most conspicuous animal in the axolotl colony. Normally, tiger salamanders are dark brown with scattered yellow spots of xanthophores. This fat salamander was yellow and pale pink. The pink color was due to blood in the cutaneous blood vessels in epidermal regions which should have been dark brown. Beauty is in the eye of the beholder, a developmental geneticist. The yellow and pink salamander was an albino.

Humphrey knew that it was possible to hybridize a tiger salamander with a Mexican axolotl by artificial insemination. So, it should have been possible to obtain F_1 hybrids and, through selection and breeding of neotenous descendents, produce a strain of albino axolotls. In theory this could have been a fairly straight forward series of breeding experiments. In actuality, the chain of events was quite different.

The fateful day arrived. Dr. Humphrey fertilized ovulated eggs from the albino female tigrinum with sperm from a white (dd) axolotl. As expected, the eggs from the albino were white as the driven snow. We all trouped into Dr. Humphrey's lab to see them. Bets were placed regarding the color of the F_1 hybrids carrying the tigrinum gene for albinism and axolotl gene for white color. It turned out that the character of interest, albinism, presented problems. The complete absence of pigment in the eggs made cleavage extremely difficult to follow. As time progressed, Dr. Humphrey began to suspect that very few of the hundreds of inseminated eggs had actually cleaved. By the next day his suspicions were confirmed. There were only two blastulae and their survival seemed uncertain. He refrigerated the blastulae to retard their development and conveyed the bad news to the rest of us. The obvious course of action was to use the two blastulae as donors for nuclear transplantation in hopes of obtaining clones of F_1 hybrids and, at the same time, increasing their numbers. Dr. Humphrey went back to his lab to induce ovulation in some white axolotls. In the labs housing the nuclear transplantation equipment there was a flurry of activity.

It was early February and no one had transplanted nuclei in axolotls since the previous spring. All the solutions had to be made up and the supply of glass

threads for plugging holes in the egg membranes replenished. Everything had to be sterilized, including the instruments and micropipettes. By the time the female axolotls had ovulated in mid-afternoon of the following day, the nuclear transplantation crew, consisting of Bob Briggs, Dennis Smith, and myself, was ready.

Bob had the job of activating and U.V. irradiating the eggs since he was the only one of our crew who had extensive experience with this aspect of the procedure. Dennis and I were to dissociate the donor cells and carry out the nuclear transfers. Dennis had some experience transplanting nuclei in axolotls. I had none. The first nucleus was transplanted at 5 p.m. We worked on through the evening with Bob providing ample supplies of activated, irradiated eggs. Some time around 10 p.m. we realized we were very hungry. Bob slipped out and re-appeared shortly thereafter with hamburgers from a nearby restaurant. The last nucleus was transplanted at 12:15 a.m. We checked for cleavage in the first group of recipient eggs. The results did not seem promising.

The next day was one of disappointment. None of the eggs receiving nuclei from the first donor had cleaved normally. Very few from the second donor had formed normal blastulae. In subsequent days it was clear that only six embryos would survive beyond early tailbud stages and of those six, only one would be viable. The efforts of the nuclear transplantation crew did not reap the anticipated rewards. Rufus Humphrey, however, was determined to get that albino gene into the axolotl colony.

Dr. Humphrey found a solution to the problem. He used the six nuclear transplant embryos as donors for orthotopic grafts of their gonad forming areas into eight white axolotl recipients. The rest is recorded history (Humphrey, 1967). One gonad forming area of the viable nuclear transplant embryo was replaced with ectoderm and mesoderm from the flank of a white axolotl in order to keep the only surviving F_1 hybrid alive. This hybrid was called "Junior." Junior, a dark phenotypic male, was a favorite of the lab and received several visitors. There were high hopes for him as a breeder until he underwent metamorphosis at about 4 1/2 months of age. After that he was kept, more or less, as a pet and a reminder of that frantic evening in February when he was created. It is a bit ironic that the gonad transplant (an ovary!) from him was not responsible, initially, for introducing the tigrinum gene for albinism into the axolotl colony. The father of the first F_2 generation had received his gonad graft from one of Junior's clonal sisters. Her graft had been sex reversed in the axolotl host. She had been a stunted, microcephalic tailbud embryo and had long ago gone to her salamander reward.