THE STATE OF THE COLONY AT THE END OF 1985

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The Colony staff has undertaken several projects during this past year which will have a significant impact in increasing productivity and improving the health of our axolotls. These projects include the development of effective therapeutic treatment regimens with antibiotics for ill axolotls, the development of facilities and systems to raise live food to replace our traditional pureed liver, and the importation of axolotls from sources outside the USA which are not (closely) related to our stocks for outcrossing into the colony and invigorating our inbred lines.

Since we have experienced recurring problems with health for about seven years, Susan Duhon, Colony Embryologist, has given the development of therapeutic treatment regimens using antibiotics (and several less exotic tricks) priority. Extensive treatment schemes are now underway, but we have only preliminary results. She will present a complete story of our successes and failures and lessons in next year's issue of the Newsletter.

We do know our death rate is significantly lower than a year ago, and we have some evidence that we are now on the offensive side of the battle. After intramuscular injections of gentamicin for five consecutive days (one injection per day), we have noted the complete recovery of ill axolotls insofar as they will spawn for us again.

A possible vector for spreading the pathogen (earlier tests have incriminated Aeromonas) is postulated to be the liver which we hand feed our axolotls. The pureed liver and feeding spatula might become contaminated by contact with an ill animal. By feeding this same food to other axolotls, we may inadvertantly be spreading our health problems. For this reason we are trying to institute live food systems using guppies and tadpoles. As soon as possible, we will be weaning our larvae from live brine shrimp onto live tadpoles or guppies instead of pureed liver.

Our third line of attack against disease problems is to strengthen colony vigor through outcrossing with unrelated imported axolotls. Some of our lines, notably the cardiac stocks, are less resistant to invading pathogens than other

lines, and these axolotls do not grow to large sizes. Perhaps they and others in our colony are immunologically weakened trough generations of inbreeding? Dr. John Armstrong addresses this problem in his contribution to this Newsletter.

In this regard, we have just obtained our first shipment (since 1978) of wild-captured axolotls from Lake Xochimilco near Mexico City. Fourteen healthy specimens were caught in nets by fishermen at the lake over a period of 5-6 weeks. They were brought to us on January 10, 1986 by Miss Maria Guadalupe Delgadillo-Reynoso, a newly arriving graduate student in IU's Biology Department from Mexico City. What a great gift! Finally after two months of effort by George Malacinski, Maria, and myself, they had arrived, but only if we promised the Mexican government to return them when we are finished with them! You better believe axolotls are an endangered species! Miss Delgadillo-Reynoso maintained the earliest-caught ones at her home while checking with the fishermen several times weekly for additional catches. To her credit, and perhaps most remarkably of all, five days after arrival, two of the biggest males gave spermatophores upon overnight mating with prospective mates, and each of the two colony females spawned the next day (January 16, 1986)! These are spawns numbered 6405 and 6406. The male that fathered spawn 6405 mated again, on January 22, 1986, with one of our females heterozygous for the cardiac gene, giving spawn number 6409. This spawn is our first outcross of our cardiac line into this very vigorous wild stock. At this point I named this male "Macho Man." True to his name, he mated again on January 31, 1986 with another colony female to give us spawn number 6412. All quite remarkable fertility for a male axolotl! We have also obtained our first spawn from a mating between two of the new Mexican imports on January 29, 1986, number 6411.

In honor of a job well done, we have designated these fourteen imports as the Guadalupe line, after Maria. We anticipate more exciting developments with our imports as we outcross them into our colony, and as we mate them with each other. We will watch for the appearance of new and interesting genes. As an aside, we are maintaining these home-grown axolotls on axolotl larvae (live) and goldfish. We never want them to develop a taste for pureed liver! And they feed with gusto. I love it!

The Hubrecht Laboratory in Utrecht, The Netherlands, has agreed to send us several adults plus a spawn (embryos) to raise for ourselves. These animals will arrive later this winter. And we have offers of axolotls from laboratories in Denmark, England, and France to further invigorate our inbred lines. All of these

sources are being pursued.

On occasion we find good fortune right below our noses. Everyone knows this can happen, but it is especially true when one passes enough bowls of randomly mated inbred axolotl larvae beneath the old nose, looking for excitement as it were! Lo and behold... sometime late in February of 1985 we were remarking about the small size and peculiar eyes of about 25% of the larvae in spawn number 6291 (spawned February 6, 1985), when we realized we were staring the l gene's phenotype[1] square in the eye. The parents are siblings from spawn number 6083. The host of 6291's which we raised are now almost sexually mature, and with backcrossing to the parents and sib-matings, we should identify several more axolotls heterozygous for l.

In a similar fashion, Susan Duhon and I noted strange eye developments in some of the larvae of spawn number 6365. Susan later nailed it down as the mi phenotype[2]. Two axolotls of the Tompkins stock were mated on October 21, 1985. The phenotype appeared several weeks later. The male parent is some 6 1/2 years old, and, coincidentally, as it were, upon a fleeting quirk of decision making, I decided to mate the same two adults again on December 6, and they mated successfully again, this time spawn number 6387 (identical to number 6365)! The perfect couple! So we are rather over-endowed with potential heterozygotes for mi now! How suddenly boring.

In this issue, Professor Anton (Tony) Neff and Susan Duhon have written short articles on the pinhead, pi, gene. We have increased our number of adults from spawns early in 1985, when several good pinhead matings were obtained. Several of these spawns had at least one parent heterozygous for cardiac, c, genes, so our stock of potential heterozygotes with the cardiac gene is also increased.

The cardiac lines have suffered the most of all of our lines in the wake of our disease problems. But with disease control, live food, and a concerted effort to outcross this line immediately, we feel we have turned the tide on our shortage of cardiac spawns, and we look forward to a higher frequency of these spawns and a more reliable source of cardiac mutants in the future.

In closing, I would like to mention we have also given priority to the increase in number of mature heterozygotes for the e and s genes, and to the recovery of the as gene. Our albino adults' average age is a little old fo optimal reproduc-

tive efficiency, so we have not been able to supply as many albino embryos as requested. We do have a number of maturing young adults now, which should improve our spawn success this Spring of 1986.

We appreciate and encourage your use of and requests for our axolotl materials, and we thank you for your patience in working with both us and these unruly beasts, our axolotls.

1. Humphrey, R.R. and Hae-Moon Chung. 1977. Genetic and experimental studies on three associated mutant genes in the Mexican axolotl: st (for stasis), mi (for microphthalmic) and h (for hand lethal). Journal of Experimental Zoology 202: 195-202.

2. Chung, Hae-Moon, and Robert Briggs. 1975. Experimental studies on a lethal gene (l) in the Mexican axolotl, Ambystoma mexicanum. Journal of Experimental Zoology 191: 33-47.