# Two New Genes Discovered In Recently Imported Stock of *Ambystoma mexicanum*

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This short communication describes two new genes in the F3 of recently acquired stock of *Ambystoma mexicanum*. Both of these apparently new genes display phenotypes which mimic the effects of mutations that have previously been described in the axolotl. They also, however, differ from them in some respects. There is evidence to suggest that these newly discovered genes are probably not allelic to the existing counterparts established for some years in the Axolotl Colony.

In 1978 the Axolotl Colony received 18 new axolotls from Lake Xochimilco near Mexico City. This new stock was collected and imported by G. Malacinski and W. Hansberg (University of Mexico City) and subsequently named the Humphrey stock in honor of R.R. Humphrey who founded the I.U. Axolotl Colony and to whom we owe the description of the majority of the mutant genes in the Axolotl Colony. From the 18 axolotls imported, a total of five spawnings were obtained. Four of these were either artificial or naturally produced outcrosses to existing colony stock made to improve the breeding vigor of colony stock. Only one of the many matings made between the imported pairs resulted in a viable spawning, #4755.

The $F_1$ stock (#4755) was then sib-mated to obtain nine separate $F_2$ spawnings. Most of these proved difficult to raise or were sent out to interested researchers, and only spawning #5005 ($F_2$ progeny) reached maturity in our colony. (Other researchers may have received some of the other $F_2$ spawnings.) In one partcular mating between $F_1$ sibs (4755.2 and 4755.13) we noticed the typical pear-shaped body and fluid accumulation in the coelom that

is characteristic in part of the cardiac gene originally described by Humphrey (1). Of 60 pre-hatch larvae, 15 exhibited this syndrome but had not been studied at an early enough stage to distinguish whether the presumptive new gene was cardiac or ascites in nature.

Sib-matings of #5005 then provided the F3 of the Humphrey stock. In spawnings of this type, which we were now alerted to monitor carefully, we have found both a cardiac and a spastic-like mutation (see Table I [A and B]).

The most striking and potentially useful of these new or mimicked genes is the cardiac-2 mutation. (The already-existing cardiac gene is one of the genes most heavily in demand by our user group.) The new cardiac mutants become obvious at stages 36-37. At that time the normal sibs in the spawning have initiated pulsation in the heart and circulation of blood has begun. In the mutants, very little if any pulsation is evident and blood tends to pool in and around a blood island on the flank. As in Humphrey's original description, the affected members of the spawning will continue to develop and even reach hatching stage. Yet their bodies take on a pear shape with a somewhat squared head and poorly developed gills. They develop considerable fluid accumulation due to lack of circulation. The only obvious difference in appearance that could be noted under the dissecting scope between the Humphrey stock cardiac gene (c-2) and the originally described cardiac (c) is the slight pulsation of the heart tissue in the Humphrey stock cardiac gene (c-2) at the early stages when the cardiac gene is first detectable. This slight pulsation was no longer detectible after 24 hours, and from then on the two genes appeared identical in phenotype. It is likely that more sophisticated investigation such as organ culture, SEM, or biochemical techniques, will reveal important differences in these two cardiac mutations.

After several more spawnings made from matings between the $F_2$ sibs in which c-2 again was seen to segregate, we then proceeded to cross the known carrier (c-2) from the Humphrey stock to a known cardiac (c) carrier from the colony stock. In two such spawnings, no evidence of cardiac segregation was present (see Table I [D]). This result suggests that the cardiac mutation seen in the F3 of the Humphrey stock could be a separate gene from the cardiac mutation which has been in the colony since Humphrey first described it in 1971.

The original cardiac gene arose in stock also imported from Mexico City by Dr. Delanney in 1961.

We now have mature c-2 stock, some of which have been identified as heterozygote carriers of the new cardiac gene, c-2. There is also mature breeding stock that carry the double cardiac mutant; i.e., known heterozygotes for both the established colony cardiac gene (c) and the new non-allelic cardiac mimic (c-2). Both of these lines should be of considerable interest to biologists interested in heart development and heart function. Note in Table I (E) that the double heterozygous cardiac mating (+/c, +/c-2 x +/c, +/c-2) gave results close to the 9:3:3:1 ratio expected for two genes segregating independently. The two cardiac genes are virtually indistinguishable from one another under the dissecting scope and therefore were simply classified as cardiac.

In the process of testing other members of the 5005 stock for additional c-2 carriers, we found several pairs which segregated 25% of what appeared to be a spastic mutation (see Table I [B]). The affected individuals could be seen floating on their backs just post-hatch. When stimulated by pinching their tails, these back-floaters displayed the wild coiling swimming motions as described by C. Ide and R. Tompkins (2). Unlike the original spastic gene

was classified as a semi-lethal gene, this newly discovered mutation is fully lethal. The affected individuals never eat and eventually all die within a week of hatching. To date no detailed analysis has been attempted on this new spastic gene. Neither is it known whether this gene is allelic or non-allelic to the already described spastic gene since the original stock of spastic animals has long ago died out in the Axolotl Colony. The fact that the new gene shows full lethality would seem to indicate that this is probably a new and non-allelic gene to the originally described mutation.

It is hoped that investigators interested in either cardiac or spastic-like mutants will take advantage of our new findings and research these genes more fully than the colony is presently equipped to do.

References

1. Humphrey, R.R. (1972). Genetic and experimental studies on a mutant gene (c) determining absence of heart action in embryos of the Mexican axolotl. (Ambystoma mexicanum). Dev. Biol. 27: 365-375.

2. Ide, C.F. and R. Tompkins. (1975). Development of locomotor behavior in wild type and spastic (sp/sp) axolotls. (Ambystoma mexicanum). J. Exp. Zool. 194: 467-478.

TABLE I. SPAWNING RECORDS HUMPHREY STOCK $F_2$

| | Spawn # | Female | Male | Relationship | Total # | # Normal | # Cardiac | # Cardiac$^2$ | # Spastic-1 | # Other |
|---|---|---|---|---|---|---|---|---|---|---|
| A Spawnings →$C^2$ only | 5253 | 4755.2 | 4755.13 | $F_1$ sibs | 79 | 56 | --- | 23 | --- | --- |
| | 5392 | 5005.6 | 5005.4 | $F_2$ sibs | 35 | 24 | --- | 11 | --- | --- |
| | 5513 | 5005.6 | 5005.1 | $F_2$ sibs | 12 | 7 | --- | 5 | --- | --- |
| | 5724 | 5253.1 | 4755.13 | backcross | 12 | 10 | --- | 2 | --- | --- |
| | 5895 | 5513.1 | 5399.1 | $F_3$ + outcross | 147 | 108 | --- | 39 | --- | --- |
| EE | | | | | 285 | 205 | --- | 80 (28%) | | |
| B Spawnings →sp-1 only | 5412 | 5005.1 | 5005.4 | $F_2$ sibs | 33 | 20 | --- | --- | 13 | --- |
| | 5512 | 5005.5 | 4755.13 | backcross | 38 | 26 | --- | --- | 12 | --- |
| | 5593 | 5005.10 | 4755.13 | backcross | 65 | 46 | --- | --- | 19 | --- |
| EE | | | | | 136 | 92 | --- | --- | 44 (32%) | |
| C Spawnings →Both Cy and sp-1 | 5846 | 5399.4 | 4755.13 | backcross | 61 | 31 | --- | 16 (26%) | 12 (20%) | double mutant 2 (3%) |
| D Allele Tests | 5399 | 4928.3 | 5005.4 | +/C x +/$C^2$ | 31 | 31 | --- | --- | --- | --- |
| | 5980 | 5253.1 | 5417.3 | +/$C^2$ x +/C | 61 | 57 | --- | --- | --- | various developmental defects (4) |
| E Double Cardiac Cross | 5986 | 5399.4 +/C +/$C^2$ +/sp | 5399.3 +/C +/$C^2$ +/sp | sibcross | 135 | 72 | --- | 61 cardiac-like (45%) | 24 (17%) | db mutants 4 (3%) |
| | | | | | actual ratios ----------→ | | | | | |
| | | | | | expected ratios 9 (56%) | | | 3:3 38% | | 1 (6%) |