GYNOGENESIS: A MEANS OF RAPIDLY CREATING INBRED LINES?

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The value of inbred lines in eliminating genetic variability as a step in achieving uniform and controlled experimental conditions has been recognized for many years. The laboratory mouse was the first experimental animal in which there was a systematic attempt to create inbred lines, and is the one for which the most strains exist. However, such lines exist for a number of other laboratory animals (1).

The "inbreeding coefficient", F, is used to measure the progress of inbreeding. F is the probability that both alleles at a locus are identical by descent, and has also been loosely interpreted as the proportion of the genome that is homozygous. In plant species where selfing is possible, F exceeds 0.9 on the fourth generation. However, in the animal world, full-sib matings have normally been the fastest way of increasing homozygosity. F reaches 0.886 after 10 generations of full-sib mating and 0.986 after 20. The convention for declaring a strain inbred has generally been 20 generations of full-sib mating. Beyond that point, the potential gain has not been considered worth the additional effort because of the effect of new mutation. Of course many lines in the mouse have now been inbred for far more than 20 generations, but, if maintained by separate labs, they may actually diverge because of new mutation and should be considered separate sublines.

The problem of inbreeding depression

The phenomenon of inbreeding depression has been recognized in many species of plants and animals. That is to say, the growth rate, viability, and/or reproductive success of highly inbred individuals or lines is often noticably reduced. The reasons for this are not completely clear. One reason is that inbreeding makes individuals homozygous for deleterious recessives carried in the population. However, it is equally true that some individuals will be made homozygous for the "good" alleles, and if we select from a large enough pool, we should be able to find individuals homozygous for no deleterious recessives. There are two problems. The first is that animal breeders in particular often do not have a large enough pool to select from. This should not be a problem for the axolotl, where progeny number in the hundreds per spawning. The second problem is that deleterious recessives with relatively small effects on fitness may not be easily recognized. This argues for making our selective criteria as rigorous as possible and working on several inbred lines simultaneously so that we will have a choice from which to pick the best.

Unfortunately, deleterious recessives may not be the only reason for inbreeding depression. Some geneticists have suggested, mainly to explain the amount of genetic variation observed in natural populations, that heterozygosity per se is advantageous. There are few actual examples of this, and Lewontin (2) argues persuasively that if a significant proportion of genes showed such a heterozygous advantage, inbreeding depression would be much more severe than actually observed. Nevertheless, if this factor comes in to play for even a few critical genes, then inbreeding will always be a losing proposition. However, the fact that we are dealing with a laboratory population, maintained under relatively constant and less competitive conditions than a wild population, may make this less critical than for a wild population. Thus, we may be able to excuse (and get away with) maintaining a less than optimally fit line on the grounds of the usefullness of reduced genetic variability to our experiments.

Inbreeding by gynogenesis

In species with long generation times, 20 generations of full-sib matings may not be practical. Purdom (3), Nace et al. (4), and others have suggested using gynogenesis as a means of rapidly producing homozygous lines. In principle, one may suppress either first cleavage or second polar body release in an egg that has been activated in some way. In the first case, the embryos will be completely homozygous. This method has not been pursued to a great extent. However, a few years ago my colleague Laura Gillespie and I worked out optimum conditions for suppressing first cleavage in the axolotl by both heat shock and hydrostatic pressure (5). In both cases the survival to hatching was very low (less than 5%). We suggested that this was likely due to a combination of trauma caused by the treatment and homozygosity for deleterious genes carried by the parent. One might reasonably expect that the rare survivors are those embryos that fortuitously received no deleterious genes. However, we had limited success getting these animals to spawn. It would seem that carrying good genes for survival is no guarantee to carrying good genes for reproduction.

An added complexity to producing a homozygous line by first cleavage suppression is propagating the line. As the female axolotl is heterogametic, we will expect both WW females and ZZ males, but each animal, though homozygous, will be genetically different. To get a number of genetically identical animals (a clone, in other words) we would have to do a second gyno- or androgenesis. All these animals would be the same sex and, to propagate them further, we would have to resort to hormonal sex reversal.

Suppression of second polar body release has been used successfully to create gynogenetic diploids in the axolotl (6). However, some proportion of the progeny remain heterozygous because there is crossing over between the centromere and distal genes. This is useful for mapping genes relative to centromeres, but complicates the calculation of the inbreeding achieved, as

it is not the same for all genes. Different mathematical approaches have been taken by Nace et al. (4) and Nagy and Csányi (7). Nace et al. make certain assumptions regarding the total map length and the degree of interference in Rana pipiens, and have concluded that the "average" inbreeding coefficient for the entire genome approaches unity somewhat more rapidly than with full-sib mating. In contrast, Nagy and Csányi derive a formula for calculating F, for any gene, based on the observed proportion (r) of the progeny that remain heterozygous after a single gynogenetic generation. For the i-th generation:

$$F = 1 - r^i$$

If there were no interference, then r would be 2/3 for genes distant from the centromere and F would reach 0.983 after 10 generations. However, because of interference, the proportion is even higher for some genes. [The reasons for this are discussed in Nace et al. (4) and Armstrong (8).] Of the genes mapped to date, the situation is worst for melanoid, which is 59 map units from its centromere (8). Following gynogenesis, 83.7% of the progeny remain heterozygous. Employing the formula of Nagy and Csányi, we calculate that, by the 10th generation, F would have reached only 0.831, which is less than that achieved by full-sib mating.

If this calculation is not sufficiently discouraging, then I suggest the reader take a serious look at the conclusions drawn by two groups working with trout (9,10). There, some genes remain heterozygous in nearly 100% of the progeny, indicating very high interference. Thorgaard et al. (9) have suggested that chromosomes shorter than a certain length may have only a single obligate chiasma. Genes distant from the centromere on such a chromosome will almost always be subjected to crossing over, making homozygosity through gynogenesis impossible to achieve. This situation could apply to some of the shorter axolotl chromosomes.

To inbreed or not to inbreed?

That is one of the questions. The others may include: Can we avoid it, given the restricted gene pool from which laboratory axolotls originated? If we do inbreed, what is the best route?

The last may be the easiest to answer. In theory, gynogenesis by second polar body suppression could be used as a means of rapid inbreeding if there were no interference. In practice, high interference makes it difficult to achieve homozygosity for some genes, such that there is no advantage over

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* The key word is "obligate". My equations (8), and those used by others for gene-centromere mapping, predict that very short chromosomes may have no chiasmata. Mather's data (11) indicates that, at least in some species, even the shortest chromosomes have a single chiasma.

conventional full-sib mating in using gynogenesis by itself. However, Nagy and Csányi (12) have recently proposed that alternating generations of gynogenesis and full-sib mating are the formal equivalent of a serial selfing scheme of double generation time. That is, after 10 generations (5 gynogenetic alternating with 5 full-sib), F should reach 0.969 (4), the equivalent of 5 generations of selfing.

Suppression of first cleavage in a gyno- or androgenic haploid egg leads to immediate homozygosity. However, survival is very low and there are some practical difficulties in maintaining a line that is either all male or all female. Nevertheless, we are continuing to investigate this route in our lab.

The answer to the second question is that it may be difficult to avoid inbreeding completely unless we can outcross to fresh wild stock from Mexico. Even then, the wild stocks have been reduced to a remnant of what they once were and are likely themselves inbred. With luck, they may homozygous for different alleles from the lab stocks.

The value of an inbred line will not be the same to each researcher. In our lab, we note interspawning variation in developmental parameters such as the developmental rate, the response to refrigeration, and the number of somites at a particular stage, to name only a few. We mostly use random matings and repeat our experiments with a number of spawnings in an attempt to eliminate artifacts. An inbred line might make life simpler.

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