Breeding a Royal Line - a cautionary tale

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The ultimate goal of most animal breeders is continual improvement of the breed through careful selection of sire and dam. The "average" alpaca in New Zealand has improved tremendously over the last 15 years through this kind of selective breeding.

When discussing breeding, the terms "bloodline" or "blood" often emerge: animals may have "good blood" or produce a "strong bloodline." Comparisons to royalty then occur, and this kind of thinking can be seen in a quick perusal of the registry where hundreds of "kings," "queens," "prince/princesses," and other such royal titles can be found.

One common tactic in breeding selection is to select the "big names," as these famous animals, usually stud males, have a reputation for throwing superior traits. As a result, many of these famous animals have hundreds of offspring. Given a population base of only 15,000, it is not uncommon to find the same names popping up again and again in a pedigree. This is often used as a promotional point, highlighting how closely related the animal is to its famous ancestor.

This type of breeding system has a close (and very relevant) relationship to many of the royal houses of Europe before the 19th century. There, a pool of a few thousand individuals sought matings (marriages) that would provide close relationships to famous (politically powerful) sires (kings). Over a period of many generations these royal houses would seek to "improve their bloodline" by carefully choosing partners whose pedigrees had the most links to famous ancestors. This practice did not always end well.

Recently a paper by Alvarez et al analyzed the Spanish Habsburg Dynasty (1516-1700) to determine how multiple generations of marrying close relatives (uncle-niece, first cousins, and other consanguineous unions) affected the level of inbreeding of the members of that house. Inbreeding had been suspected in playing a role in the extinction of the dynasty, the Alvarez paper provided numerical/statistical analysis of how the inbreeding coefficient rose with time, and how that negatively affected outcomes. The dynasty ended in 1700 when Charles II, physically and mentally disabled, died after producing no children from his two marriages. (I suggest reading the full paper which is available through the *PLoS ONE*, an international, peer-reviewed, open-access, online publication.)

Royal dynasties have the advantage that accurate marriage records were kept over a widely extended family and many generations: this case involved 3,000 individuals and 16 generations. Likewise records of the health and mortality of many members of the family were available.

The depth of the family tree allowed for analysis of consanguinity based on remote or ancestral relations. These distant relations had a significant influence, with the inbreeding coefficient rising as the family tree was more deeply examined. A shallow study of a pedigree increases

the risk of significantly underestimating the degree to which individuals are inbred. (See Figure 1) To get an accurate estimation of the inbreeding coefficient the pedigree had to be analyzed to a depth of at least 10 generations! So while the marriages to close relations (uncle-niece, first cousins) draws attention towards potential inbreeding, the mathematical analysis showed that the cumulative effect of more-distant consanguineous unions deep in the pedigree had a significant effect on the inbreeding coefficient. Even if you never mate fathers to daughters or brothers to sisters, if there are enough third- and fourth-cousin matings in the tree, you can end up just as inbred.

Direct evidence for the negative health and survival influences on the Hapsburg family from their inbreeding can been seen in a rise in child mortality. 30% of Habsburg children died before the age of 10, whereas Spanish children from mixed backgrounds only suffered about 20% child mortality during the same historic period, a significant difference. The difference is even more significant if you consider that the non-royal children came from a mixture of social classes, and would not have had access to the significant class benefits of the royal children, such as nutrition and medical care. This drop in survival is an example of "inbreeding depression," where even in the absence of a single acute genetic disease, the decreasing genetic diversity results in lower survival.

Any discussion of genetics tends to devolve into the use of jargon which can be opaque and even intimidating to readers without a technical background. Building a complex multi-cellular organism (like a human or an alpaca) obviously requires detailed instructions, and these instructions are in our genes. Each gene can be thought of as a book on a single subject, and a library of about 25,000 of these books (genes) is required to make an organism. Furthermore, our library has two copies of each book, one from the mother and one from the father. Let us suppose (in this completely hypothetical example!) that one of those books was "how to grow roses." As we all know, there are lots of different books on how to grow roses. These different books are the *alleles*—they all deal with rose growing, but each is slightly different and some books are better than others. In fact, some books give exceptionally bad advice ("pour on petrol, apply match"). If you have only one copy of a bad book, then usually the good copy (allele) can step up and provide all the information you need to successfully grow roses (the more effective book is said to be "dominant," the less effective book to be "recessive"- this is only a general rule of course, in actual genetics it can get much more complicated!). If you end up with two copies that suggest petrol and matches, you will not be growing any roses. Suddenly you have the "no-rose" genetic disease! You can get less extreme versions of this where you end up with two copies of books on rose growing that are downright mediocre, so while you still grow roses, your roses are not nearly as colourful or fragrant as your neighbour's. This is the root of *inbreeding depression*. As a library becomes increasingly inbred, more and more of the 25,000 book-pairs end up having two of the same book (allele). Having different versions makes it more likely that one of the two will be a superior version, this is known as *heterosis* (also called *hybrid vigour* and *outbreeding enhancement*).

Inbreeding depression and genetic diseases can be very hard to spot in a small population. Small percentage changes in cria survival rates can easily be masked by some good or bad luck. Likewise even a very common acute genetic disease will, in a non-inbred population, only affect one individual in a few thousand—a rate that would be impossible to spot unless you were monitoring all the cria born every year in the entire country! (Something, incidentally, that the annual Health Surveys are trying to do, keeping a constant eye open for any patterns that might suggest lurking genetic diseases.) For example cystic fibrosis (CF) is one of the most common genetic diseases in Caucasians of European descent, with nearly one person in 25 being a carrier (having one copy of the CF allele and one "normal" allele that makes up for the dysfunction of the CF allele so that the person appears completely healthy and normal). Yet even with this high carrier rate, only one child in 2000 has CF.

Of course, when we are selectively breeding animals, we are seeking to make the target traits (fleece characteristics, conformation, coat colour, etc.) as strong (or common) as possible. That involves finding the best alleles (books) for the genes that drive those traits. Ideally we would find the best possible allele, and have two copies of it to ensure that no problematic alleles are available to pass on. Having two identical copies of the same gene is known as *homozygosity*. You will find this term used most often among Suri breeders, who strive to breed animals that are homozygous for the Suri gene.

Line breeding (aka inbreeding, where the mate selection is usually not very close relations like father-daughter and brother sister, but first-cousin and similar relations) is a perfectly valid tactic for trying to concentrate key alleles in a population. However, you will be increasing the homozygosity of many genes, not just the ones you are targeting for the key breeding traits. (When you "fix" the copies of the rose-breeding books in the library, you may also be "fixing" the fruit books, the veggie books, the lawn books, etc. Are you fixing superior books in those slots, or duds ?) It is nearly impossible to tell at the start of a line breeding experiment how many harmful alleles are lurking in your breeding herd. You cannot predict success beforehand; rather you proceed and hope to avoid failure. You inbreed until a very high consanguinity coefficient has been reached (they are very uniform in character and genetically inbred), and if no terrible genetic diseases emerge and they remain generally healthy and vibrant, you were successful. The danger is that if significant inbreeding depression or acutely fatal genetic diseases do become apparent in the population, you have bred your animals down a genetic cul-de-sac. Restoring the genetic health of your line will be long and arduous. Getting them healthy can be done relatively quickly (only a few generations of out-crosses), getting them healthy but keeping their high-genetic value traits could be very hard. (You swap with a library that has a good veggie book to replace one of your bad pair, but you take a chance of replacing one of your superior rose books that you worked so hard to get two copies of with a mediocre one.)

But hasn't line breeding been done before successfully? Yes. There are modern human communities that practice consanguineous marriages at a similar frequency to the Hapsburgs (the urban Pondicherry in South

India and among army families in Pakistan) without severe genetic consequences. Why? It may have just been bad luck on the part of the Hapsburgs: they may have started with relatively more defective alleles for critical genes, leading to severe inbreeding depression and eventually acute genetic disease. (It is speculated that the last Spanish Hapsburg, King Charles II, had two distinct severe genetic diseases simultaneously, combined pituitary hormone deficiency and distal renal tubular acidosis, which would explain his many medical problems, infertility, and eventual early death.)

But don't panic! Yes, a significant proportion of the alpaca population is consanguineous (inbred) to a greater or lesser degree. The chance of bad outcomes is still probably quite low at this point (I don't have the tools to do the actual calculations of the inbreeding coefficient for our alpacas at present). The goal moving forward should be to reduce the inbreeding-level of your animals. Careful selection of sires can, over successive generations, effectively concentrate the positive traits you seek while simultaneously increasing the heterosis (genetic variability) of your animals.

Base your sire selection on traits, not on names. Look for animals that have the traits you desire—and, better yet, for animals that dependably pass those traits on to their offspring. Check pedigrees, and be wary of sires that share many relatives—the aggregate effects of those seemingly-distant relations can result in a surprisingly consanguineous (inbred) animal. If looking for a tie-breaker among multiple equally-desirable sires, select the animal that is least related to the dam.

Commercial software packages are available that can compute consanguinity using databases like the IAR. These might provide a tool for breeders that would help identify which animals are inbred and which matings would provide the most heterosis.
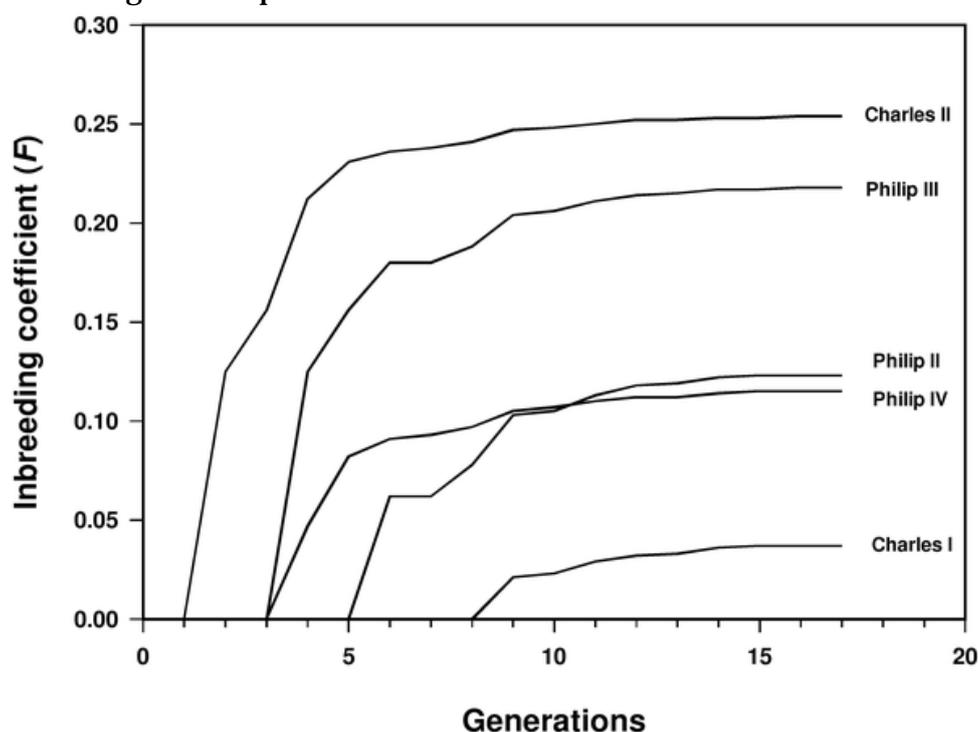
Figure 1: Inbreeding coefficient of selected Hapsburg kings. It requires at least 10 generations of depth before an accurate estimate of consanguinity is calculated due to the aggregate effect of many distant relations deep in the pedigree. Note that a coefficient of 0.25 is what would be expected from a brother-sister mating. Charles II was the product of an uncle-niece pairing.(Alvarez *et.al.*, 2009)

References:

Alvarez G, Ceballos FC, Quinteiro C (2009) The Role of Inbreeding in the Extinction of a European Royal Dynasty. PLoS ONE 4(4): e5174. doi:10.1371/journal.pone.0005174