Preservation Breeding and Population Genetics

(This discussion is based on outline notes for the talk I gave at the 1994 Annual Meeting of the Arabian Horse Historians Association. The timeliness of the topic is underscored by a comment from the outgoing AHHA president, Carol Schulz, that at least 90% of the Arabian foals registered in the last several stud books are of generalized "show horse" lines, representing no particular breeding direction or identity. This does not say anything against the show horses, but makes it clear that all other aspects of the Arabian horse--and that includes straight Polish, Egyptian, Russian and Spanish--must be divided among less than 10% of current US breeding activity.)

What do we actually mean when we talk about "preserving" a genetic stock? The object of the exercise is not simply, or even chiefly, keeping names in pedigrees; pedigrees are merely a tool which may aid in evaluating the structure of a breeding group. It is obviously possible to breed in a preservationist sense with stocks that don't even have recorded pedigrees. It is also perfectly possible to have a name present in pedigrees, while no modern representative carries a gene from the individual in question.

The goal of preservation breeding is to keep in the world the traits, characters, hereditary factors which make one aspect of a breed or species different from another--in short, to preserve genes for the future. Preservation breeding carries the unspoken assumption that the "preserved" genes will benefit a larger population in future; defined breeding groups have value and identity in their own right, but in another sense they are being maintained for future use.

This brings us inescapably into the realm of population genetics: the aspect of the science of heredity which considers the behavior of genes over time, as affected by particular mating systems. Population genetics is a mathematical and highly theoretical discipline--frankly in graduate school I found it the least compelling aspect of genetics--until you have a real problem to which it applies, when the charts and equations suddenly take on life and meaning.

Much of population genetics theory is derived for the special case of "random mating"--defined as a situation in which every individual in a population has equal probability of mating with every other individual of opposite sex. Clearly this is an imaginary construct to simplify the math. Real-life matings are constrained by geography, finance, fashion, etc., any of which will lead to wide use of some lines or individuals, and neglect of others, and so directly to loss of genetic diversity.

Any individual horse standing before us is the product of its genetic makeup interacting with all the environmental factors it has encountered. Nutrition, training, medical care--all these come under the heading of "environment," not just weather and soil conditions. Genetic diversity buffers the population against the effect of environmental change; it is what gives a breed the potential to respond

to new conditions. Diversity includes the physical and mental traits of the traditional Arabian; "new conditions" in our context may include things like an increased appreciation of the traditional using and companion Arabian horse.

A breed is the sum total of all its individual horses. Historically the genetics and veterinary literature has treated members of breeds as if they were interchangeable average mathematical units. Fortunately with the recognition of genetic diversity as a positive good, an alternative approach is gaining currency. Preservation breeding emphasizes that a breed must not be viewed as the average of all its "random mating" individuals--in order to preserve we must identify and try to understand the differing strands of its makeup.

I have referred before to that useful metaphor of "the tapestry you are preserving." One may "preserve" almost anything, from a near-perfect wall hanging which just needs to be cleaned and protected from future damage, down to a scrap of authentic thread which may be very useful for repair or reinforcement of a more complete but related fragment.

A static image of conservation or preservation could be misleading (any metaphor however useful is a comparison, not a description). We do need to remember that in Arabian horse terms there are no perfect tapestries, and clarify one difference between preservation breeding and other kinds of conservation (working with animals even differs from preserving rare plant stocks): *Genes* (DNA molecules) are essentially unchanged over the generations; individual *horses* are transient, ephemeral, fleeting combinations of genes. The tapestry image works so long as we keep in mind that the process is analogous, but the object of the process is quite different.

What classes of fragments might we conserve? All will be arbitrary, defined in some historical terms-- "species" at least in the ideal is a natural, biological classification, but we are not working at the species level. Fortunately we can describe any group in biological terms once we've defined it.

Large closed groups: this is certainly the easiest category if you have one.

Large groups, with fuzzy edges: this has practical advantages but must be defined.

Small closed groups: working with these is challenging but possible.

"Endangered species": this is where we run the greatest danger of "keeping a name in a pedigree" without any associated biological reality; small fragments are meaningful only if maintained in some relevant larger context.

Large closed groups: These are easy to define once we decide how large is "large"? Bottlenecks are relative, the more numbers we work with the better our chance of keeping a major proportion of the genetic variation we're trying to save. We can describe a general picture here, and the other situations can be treated as they vary from it. This is where we need to introduce some population genetics concepts:

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"Gene frequency": a thing, a number, which tells us something about a breeding group; don't worry about how to develop the actual number. All traits are based on genes, and all genes exist at some frequency--it's just harder to measure the interesting ones so we sometimes use "markers." "Effective population size": another informative number, which takes into account the relative breeding contributions of males and females. An effective population of 10 can retain genes existing at frequency of 0.1 or higher; uncommon (below 0.1) and rare (below 0.05) variants will likely be lost. For our purposes, in a typical horse-breeding situation, "effective size 10" means some number much larger than 10. Note: it does not matter whether the population expands in numbers; expansion helps to keep in circulation the genes that you do have, but it does not do anything about ones that were lost when the founders were selected.

"The sire is half the herd"--we all know that maxim. In a preservation breeding context the point is precisely that we don't want any one sire to dominate any program to the extent of half its genes. The more one narrows down the sire selection, the more, and the more diverse, mares must be kept in order to retain the original genetic variation. The most efficient way to maintain diversity is to use multiple sires on several small sets of mares, and rotate the sires. The idea, always of course influenced by real-world considerations, among them the phenotypic suitability of a particular combination, is to equalize breeding opportunity in order to maximize the proportion of genes retained.

Inbreeding and selection pressure are considerations in any breeding situation--they are not specialized aspects of the preservationist approach. Inbreeding, like random mating, simplifies the math, so is overly important in population genetics theory. Inbreeding can be a useful tool, and incidentally is a fact in any closed breeding group--inbreeding operates at the level of breeds, so long as they have closed stud books, not just within limited subsets of breeds. Inbreeding drives genes to fixation and can lead to the loss of alleles from the population, so one goal of preservationist planning should be to minimize the average degree of inbreeding. Inbreeding is not an end in itself.

Once we have a preservation group defined (say for now all the horses, or at least a representative sample, are in preservationist hands, though that is not a trivial assumption) and reproducing, the best way to retain maximum genetic diversity is to spread the horses among more than one program, and let subgroups happen. In theory we want a set of "cooperator breeders" working toward a shared vision. That calls to mind another non-trivial problem: preservation breeders as people will, by definition, be eccentric and... let's say independent minded. Those independent visions are essential, each maintaining its own distinct sample of the horses in question; there still must be enough of the shared vision, and some sort of working definition, to retain the genetic identity of the preserved group.

Part II (CMK Record, XI/3 Fall, 1995)

(*Continued from last issue -- the "to be continued" text block was lost in production. Last time we outlined the basic notions of population genetics, in terms of preservation breeding with a large closed population. Further implications arise when other kinds of genetic entities are to be preserved*.)

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Large blurry groups will maximize the contribution from the founder animals. Generally, by the time any breeding group needs attention at the preservation level, the genetic influence of many founders will be lost among those descendants which qualify for inclusion in a closed group. Whether through attrition of numbers, or use in outcross programs, or most likely both, any set of "straight" pedigree horses carries only a fraction of the founders' genes--compare, for example, the original Blunt or Davenport array, with the sample of those influences represented in modern straight Blunt or straight Davenport breeding.

Gene frequencies among the surviving descendants of anything reflect the action of mutation (negligible over human time scales), chance and selection. The gene frequencies of any modern closed group likely will be very different from the frequencies that would have been calculated among the founders. This effect is apt to be less exaggerated (simply because more of the founders are represented) if we define our modern population so that it descends "largely" (deliberately vague) from those founders. To follow up the previous example, there are Blunt and Davenport genes in modern CMK Arabians which have been lost from their straight Blunt or Davenport relatives.

Philosophically and historically the breeding group with blurry outlines is different from more traditional approaches but it is squarely based on an accurate biological view: species are naturally distinct biological entities with more or less firm barriers against crossing; breeds are artificially maintained subsets of a species. "Breed" is a historical (originally geographic) concept, and acquires biological reality only after the fact; this cannot be overstressed. "Breed" and "species" do not have equivalent implications, in terms of original or maintained genetic differences. In evolutionary terms, the genetic distance between pairs of species is measured by comparing their relative frequencies for marker genes--in making such measurements researchers do not expect to find complete non-overlap between related species. Obviously then this will not be expected between breeds, leave alone subsets of a breed.

Working with a blurry edged pedigree definition is not the same a as maintaining a closed group, and not a substitute where the closed group still exists--the two approaches are complementary. In setting up a blurry group its organizers must neither claim that it is something else, nor allow it to be thought less than it is in its own right. There must be a working definition which sets off a biologically and phenotypically distinct entity from the breed at large.

Few (if any) absolute genetic differences exist between breeds. Still less can there be absolute differences between subsets of a breed, and there simply is no way to tell what caused such differences anyway--they are every bit as likely to have arisen through chance loss of genes from one set but not from the other, as they are to reflect an original difference. Given they were shown to represent an original difference, such still could represent accidents of sampling the original population (in our case the Bedouin horses, which ranged over a large area geographically and were more or less separated in terms of tribal origins).

Working with a blurry-edged definition gives tremendous possibilities in terms of developing subgroups: founder genes of different origin (in Arabian terms, different desert samples) will get

together and produce new combinations not existing in the original animals. This may suit a particular breeder's approach admirably, while it strikes another as highly undesirable. Neither response to this biological fact is "wrong," but this does underline that one must be aware that gene combinations are not static, even in a closed group.

Preservation breeding of livestock is not like working with, say, historical rose varieties. Modern bushes of a rose bred in 1830 are biological clones of the same plant, with exactly the same gene combinations as the ancestor (barring rare mutations). Modern descendants of an individual Arabian horse which lived in 1830 need not actually carry any of its genes, and they certainly carry those genes in different combinations than did that ancestor. To give a simple coat color example from a more recent individual, Skowronek was homozygous for grey and heterozygous for the black and red pigment genes at extension locus. There are modern chestnut Arabians of intense Skowronek breeding--horses bred to maintain a high relationship to this ancestor have lost three (at least) of his detectable genes at these two easily defined loci.

Small closed groups make for the most difficult and challenging and certainly the most intellectually fascinating kind of project. We have already acknowledged that large groups will develop subgroups. Over time these may be selected or defined into their own distinct existence, so eventually the "small group" scenario becomes a concern in almost any preservation breeding context, regardless of your starting level. Keeping to our original examples, the Davenport program is developing an elaborate substructure, and within the English descended aspect of CMK there are a number of possible distinctions, including straight Blunt, Skowronek-Blunt, straight Crabbet, GSB-eligible, Crabbet-Old English, and CMK of high Crabbet percentage. Each of these may be maintained in its own distinctive form, while individuals of the more specialized groups may contribute genes to the more general ones.

The narrowly defined groups exist in their own right but they also serve as a resource of mental and conformation traits, soundness and performance ability, for use in other contexts. This is quite analogous to the position of preservation-bred stock relative to the breed at large. The drawback, at least in theory, to maintaining the maximum number of small sub-groups, is that inbreeding within each subgroup will increase more rapidly than it would if the entire set of horses had been crossed freely among themselves. The other side of the same coin is that crossing sub-groups will later provide a way to increase heterozygosity, and theoretically vigor and fertility, without going outside the original closed definition.

The notion to take home here is that maintaining population substructure is an efficient way to maintain genetic diversity; the modern Thoroughbred, with its history of international exchanged of sires and overall genetic homogenization, possesses far less genetic diversity than does the Arabian, with its history of breeding in national or smaller subgroups.

We all learned long ago that "inbreeding creates uniformity." If you take nothing else away from this discussion, at least cross that off your list of life's basic concepts. Inbreeding drives genes to homozygosity and thereby shows up underlying genetic variance. Inbreeding actually creates phenotypic variability. *Selection* among the results of inbreeding *may* give rise to uniformity. *Is this what you want?*

A program cannot possibly maintain the full range of genetic diversity, and is not likely to maintain representative frequencies, of any founder population, through a bottleneck of two or three or five individuals. "Rare" genes are defined to exist below 0.05 frequency--nothing in a group of five horses (among them possessing a theoretical maximum total of 10 genes at any locus, and in practice there will be fewer) can exist below 0.10. If a "rare" gene from the original population, of which these five horses are a sample, is by chance present, it automatically has gone above its original frequency; if it's not in there it never can come back, so long as the group is bred closed. This effect is not automatically either good or bad, but is simply what happens, and it illustrates that "preservation" operates at different levels. Clearly one can only "preserve" what is still in the world to be worked with, but just as clearly, the more extensive the sample with which one starts breeding now, the more correctly the desired population will be reflected in future generations.

A program cannot achieve flat phenotypic "uniformity" without losing genes; selection for a totally uniform true-breeding group is in fact the opposite of genetic preservation (besides being a highly theoretical construct --biological reality is quite different). A program, or a group of cooperator programs, can maintain or reproduce something closer to the original population by crossing derived lines back together. Sublines will automatically develop when more than one breeder is directing the course of selection, and so far from being disadvantageous, these can be highly useful from many viewpoints. (I am deliberately running this idea into the ground--it is one of the most important things of which preservation breeders must be aware.)

Endangered Species: At this level ("threads and fragments" in our tapestry analogy) a real genetic presence can readily be reduced to "a name in a pedigree" unless the line is maintained in some appropriate biological context. When a breed is evolving rapidly, saving descendants of an uncommon element means nothing, unless the breeder interested in preserving that element is working with some semblance of the breeding background to which it belongs historically and genetically. This point is missed by many people who breed horses--perhaps especially Arabian horses--who boast they have a line to Mare X or Great Sire Y but haven't noticed (or alternatively may be quite proud of) how often the descendant bears little resemblance to the ancestor. No one would try to deny that such resemblances can persist across a breed--but the point of preservation is precisely that more such resemblances may be more predictably maintained if breeders don't depend simply on chance to bring them forward. Chance will tend to swamp the real genetic influence of rare lines, by simple force of numbers, outside the preservation context. [See Ann T. Bowling's "Questioning breeding myths in light of genetics"]

Sire lines tend to be the most rapidly evolving aspect of any breed of any species, except where a closed stud book has been essentially taken over by a line or two and there's no more room for change. The Y *chromosome* is a biological entity and is only handed on from sire to son. It is possible to measure genetic distance by sequencing yDNA. Probably more important for our discussion, old and traditional sire lines are more likely to be maintained in old and traditional breeding contexts; the persistence of a no longer fashionable sire line is an obvious marker for the program directed by a breeder who appreciates the traditional stock. Emphasis on sire lines works both ways then--it definitely helps us to find genes of diminishing frequency, and it theoretically carries them physically (but

remember few genes on the Y are known, except those directly relating to male fertility). [NB: to date (2007), while Y chromosome variation is easily found in most species tested, none has been detected in the horse.]

Dam lines tend to be biologically conservative. Rare and uncommon genes tend to be carried through the bottom of the pedigree--simply because so many more mares than stallions breed actively in each generation. By simple chance, more carriers of any uncommon gene will be used on the female side than on the male. Occasionally a mare will hand a rare gene on to one or more influential stallion sons and a breed experiences a major change in gene frequency. *Mitochondrial DNA* (mtDNA) is associated with the cytoplasm, not the cell nucleus, and thus transmitted almost entirely through the egg, essentially only through the female line. Very little mtDNA is carried by sperm (though such transmission has proven detectable in carefully designed mouse experiments). [See M. Bowling's 1998 article "What's in a Name"] [NB: it has been shown since this writing that sperm transmission of mtDNA does not occur under normal conditions.]

mtDNA carries important genes which interact with nuclear genes; also, like yDNA [which has not proven to be informative in the horse], it can be a tracer for historical and biological change and the interrelationships of lines. Generally populations have more dam than sire lines so mtDNA theoretically is more useful than yDNA; it has also proven more variable in practice. This area is only beginning to be investigated in the horse but it carries exciting potential.

"Middle of the pedigree" elements may readily be overlooked. Historically breeders have thought in terms of sire (west) or dam (east) lines--we often study published charts of sire and dam lines as a shorthand way of handling pedigrees. Sire and dam lines in fact reflect the smallest portion of any pedigree, and certainly of gene transmission--only the Y chromosome and cytoplasmic mtDNA respectively are guaranteed to run along the top or bottom of a pedigree. Except in terms of those two elements, and thus for the vast majority of genetic material, position in the pedigree has nothing to do with potential genetic influence; important horses, still visibly influential, may not have left direct sire or dam lines. Davenport's *Haleb and the Blunt's Bint Nura GSB come readily to mind as examples.

This opens an enormous area for discussion or consideration, and space forbids addressing it in more than this very elementary fashion. The underlying reality is that any ancestor in any pedigree may have contributed genes to any modern descendant--but at the same time any ancestor's genes, once we get back a few generations, may have been lost completely. There is no way to tell by looking at the list of names which is a pedigree, the ancestors that actually are genetically important in the horse to which that list belongs. We must look at the horses and learn as much as possible about the ancestors, in order to make rational judgments on this point.

Mid-pedigree names may become important in developing subgroups. Simply as a fact--with neither negative nor positive associations--breeders may use any name as a marker to define a group (and it may be used by its presence or absence). The bigger and more influential the "name," in fact, the more useful it may be, in terms of future genetic balance, to reserve some lines for crossing back to it--within the large group however defined.

What are we trying to preserve? Genetic diversity buffers the breed against change; genetic diversity interacts with environment to provide the basis for all variation within a breed. Preservationist breeders have one underlying goal: to promote the maintenance of genetic diversity. It should not be necessary to state that the preservationist approach grows out of having observed negative changes in the breed. We are preserving the genes which influence major traits, including disposition, soundness and endurance, which are not necessarily addressed in the show ring.

Different preservationist groups have more in common than they do dividing them; it is to all our benefits to make common cause for *a generally different approach* to breeding the Arabian horse. A listing of preservationist group contacts would be a very useful practical tool in advancing this goal, and the members of the Arabian Horse Historians Association, assembled at their 1994 Annual Meeting, agreed that serving as the clearing house for such information was a valid role for AHHA. Preservation breeders may themselves become an endangered species--no one has any choice without a vigorous preservationist movement.

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(GMB--We've edited Deborah's letter because as we understand her point it's not so much to comment on other preservationist activities, as to caution CMK breeders about mistakes they might be in danger of making. Of course we suspect, too, Deborah would agree if we pointed out that there are many registered Arabians which are not preservationist-bred in any sense, but which also "should not be bred on" for their lacks with regard to conformation, soundness, disposition or breed character. Overall we certainly second her warning and are glad to see such thinking in the CMK ranks: this movement absolutely would lose its identity, its purpose and its point if it did not continue to turn out the beautiful, traditional using Arabian that brought all of us into the CMK circle. Fortunately it is clear that CMK pedigrees continue to produce just that kind of Arabian. We have thought about this quite a lot, over the years, and it strikes us that CMK breeders in particular are not so much in danger of full-blown "preservationist syndrome" as may be the followers of some other lines of breeding. It is easy to be caught up in enthusiasm over the rarity of a particular individual, and obviously we all have our own preferences for some style of horse as opposed to another. That said, very few of us began in CMK Arabians with the idea first and looked for the horses later; a more typical CMK story is learning to appreciate a particular kind of Arabian--we would say practically always starting from a using, riding horse orientation--and then finding that "our kind of horse" belongs with the CMK Heritage. Other major advantages to CMK as a preservation scheme are its avoiding a closed definition and the great genetic diversity it maintains. Large-sense CMK breeders have much more room to operate than do the people working with other narrow closed preservation groups; specialized narrower groups within CMK may be crossed with other CMK lines without losing their CMK identity.

As the CMK preservation movement explores more kinds of promotional efforts, we can expect to hear from more people who actually do set out to see what these CMK horses are about, with no preconceived idea of what kind of horse they're going to find. That is precisely why we need to go cautiously on the promotion front: we must be sure we are attracting people who can understand and appreciate this kind of horse, rather than those who may latch on to the name yet expect to modify the horses to suit some other set of criteria.

Deborah may not have had this next point in mind but many horse activities pursued these days do not place very high priority on the well-being of the horse, whether physical or psychological [the two are very closely intertwined]. [See Rick Synowski's article "POST-TRAUMATIC STRESS DISORDER IN ARABIAN HORSES".] No thinking breeder would care to see any horse exposed to such dangers, but we are convinced the CMK Arabian in particular is ill served by certain aspects of modern training and presentation [and statements by show trainers bear this out]. The CMK Heritage will place more emphasis in future on the actual physical "preservation" of individual horses in this day-to-day safety sense. This must include, almost by definition, the encouragement of alternative systems of use and presentation which do maintain horsemanlike values and do emphasize the well-being of the animal.

We find, too, we can't close without attempting to give a slightly different slant on "preservationist syndrome." The American Livestock Breeds Conservancy distinguishes conservation--the simple maintenance of a stock in existence, without changing it—from improvement, breeding with selection toward any set of visual and functional standards. ALBC advises conservation of very primitive breeding groups, whose *raison d'etre* is to serve as a reservoir of basic genes for health and soundness which may be at risk for loss in high-performance domestic lines. By contrast selection for continued improvement is accounted appropriate in traditional "improved" stocks whose history includes a performance standard.

The using Arabians of the Reese and Dean circles, whose breeders provided the background for the CMK movement, certainly were highly selected. So were those of the Crabbet Stud. The breeders of the CMK Heritage can call on the genetic strength resulting from that selection; at the same time we have, as Deborah pointed out, a grave responsibility to maintain the standards which were achieved by those past breeders. The problem in modern Arabian horse circles, of course, is to recognize "improvement" when one sees it. There certainly are Arabian breeders who see any change that has come about since the horses left the Bedouin tribes as change for the worse, and who think in ALBC's conservationist terms, of maintaining a comparatively primitive stock as little different as may be from the desert war mare. There are many more of us who are not impressed with the way the show horses have changed in this country over the past two decades [the wink of an eye compared to the breed's history in the west, leave alone its prior existence]. There is a place for all of us, but it is essential that we understand the implications of our positions.

Do remember that many of the preservationist programs are operating with minuscule numbers of horses -- all recognizable activity with an identity other than "mixed source show horse" amounts to little more than 10% of the breed combined. We address this not in terms of what level of selection a given program may have room to impose, if they are to breed any horses at all; but of the simple fact that their horses have relatively little impact on the 400,000+ living Arabians in North America. They cannot change the breed's nature, and if such horses fill a place in their owners' lives, that is really all that need be asked of them. There is nothing wrong with conservation breeding, in the ALBC sense, so long as one recognizes one is doing it, and does not make impossible claims for the results.

It's a completely separate subject, of course, but we have never been comfortable with those overarching schemes one occasionally sees put forward, whereby some party or official entity is meant

to "certify" breeding stock--not because we approve of breeding from poor horses, but because we cannot picture how any breed-wide selection scheme could be at once effective, in the sense of doing anything in particular, and sufficiently inclusive to recognize all the range of variation which the breed includes and which must be maintained for future reference.

As to the other-bashing of "preservationist syndrome," we do consider it basic to be civil to one's neighbors. In fact we always think it's a pity when anyone with a preservationist slant doesn't recognize that we are each other's natural allies.)

[For more thoughts on this subject, see M.Bowling's 1997 article "Preservation and Improvement."]

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