TUFTS UNIVERSITY
School of Veterinary Medicine

Department of Clinical Sciences

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CHINOOK GENETIC DIVERSITY STUDY *

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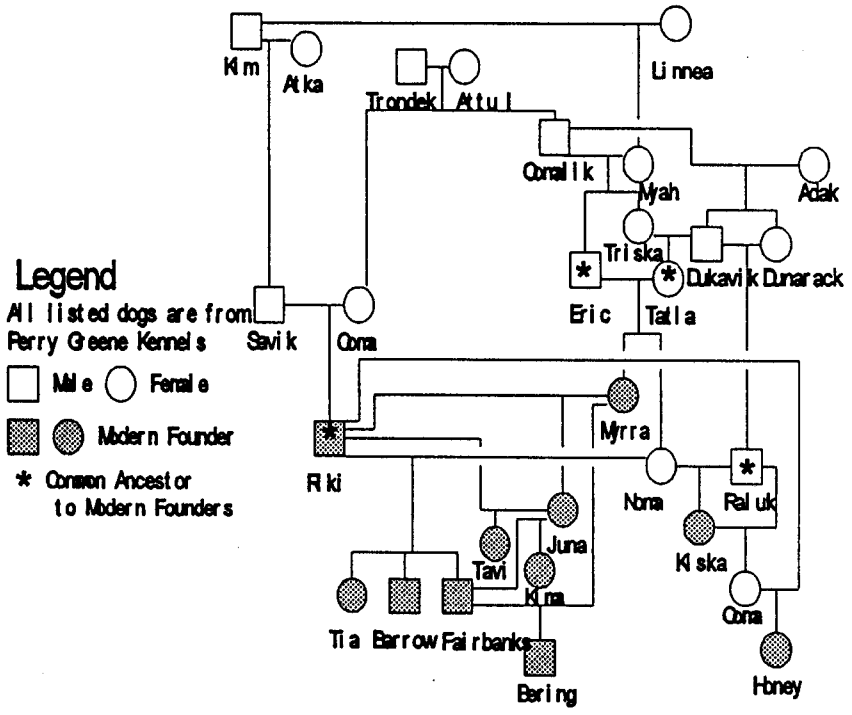
INTRODUCTION

The Chinook breed was originated in the early 1900s by explorer Arthur Treadwell Walden. A foundation female was an offspring of one of Admiral Peary's Husky sled dogs from his North Pole expedition. She was crossed with other breeds to create a sturdy, intelligent, and gentle sled dog. Walden and his dogs accompanied Admiral Byrd on his explorations of Antarctica in the 1930s. The breed was consolidated under the Perry Greene kennel in Maine in the late 1930s. After years of growth and then decline, eleven Chinooks (four males, seven females) were rescued in 1981, and formed the modern foundation for the breed. Concern exists among breeders that though the breed is growing; the genetic bottleneck it went through may have been too narrow for it to survive. The purpose of this research is to conduct a small-scale genetic diversity study, to document measurable heterozygosity in the breed.

PEDIGREE ANALYSIS

The genetic structure of the breeding pool was researched (JSB). The eleven modern founders were closely related (see pedigree). Three (Perry Greene Tia, P.G. Barrow, and P.G. Fairbanks) were full-sibs, and an additional three (P.G. Tavi, P.G. Juna, and P.G. Honey) were half-sibs to them. All of the modern founders trace back to four common ancestors; three males (P.G. Riki, P.G. Eric, and P.G. Raluk) and one female (P.G. Tatla). P.G. Riki was also one of the modern founders.



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Nine out of the eleven modern founders trace back to the four common ancestors within one generation. The remaining two modern founders (P.G. Kima and P.G. Bering) were second generation offspring of P.G. Riki.

Of the four common ancestors; P.G. Raluk was from a full-brother sister mating, P.G. Tatla was a half-sib to P.G. Raluk through P.G. Dukavik, P.G. Eric was an uncle to P.G. Tatla through her dam P.G. Triska, and P.G. Riki shared three grandparents with P.G. Erik. The founding gene pool of the modern breed is significantly inbred.

METHODS

With the assistance of members of the two Chinook breed clubs (Chinooks Worldwide and the Chinook Owners Association) and an independent health entity, the Chinook Health Fund, Chinook dogs were selected to participate in a genetic diversity study (JSB). The object was to identify dogs from as diverse backgrounds as possible in the breed gene pool, who either contributed significant offspring to the breed, or could contribute diversity through their genetic background. Dogs were analyzed based on their relationship coefficients to the eleven modern founders, and the four common ancestors to whom they trace. Eight purebred Chinooks were selected based on having the highest relationship coefficient to certain founders, and the lowest to other founders. Wright's inbreeding coefficients were calculated on all dogs based on five and eight generations.

A Chinook cross-breeding program had previously been established, based on the possibility that there is not enough genetic diversity present in the purebred Chinook gene pool to sustain itself. Two dogs, both third-generation Chinook-crosses (88.5% Chinook/12.5% other breed) were selected. One dog had a Malamute in its background, and the other a Siberian Husky.

Whole blood was collected from the ten selected dogs, and analyzed by molecular genetic methods for six different genes known to be polymorphic in dogs (GSJ). The genes used were the autosomal genes WT, vWF, ACE, TYRP2, TAT, and SA. None of these genes are known to have disease causing alleles in the Chinook breed. Both alleles at the gene pair for each gene were recorded. A heterozygosity factor was calculated base on the percentage of gene pairs found to be heterozygous. This was calculated for each gene among all dogs, and for each dog across all six genes. The number of different alleles present at each of the six genes was recorded.

Ten randomly selected Doberman Pinchers were assayed for the same six gene pairs, and had heterozygosity factors calculated. The number of different alleles present at each of the six genes was recorded. The heterozygosity factor for the six genes across a minimum of 50 dogs from at least 33 breeds was also calculated.

RESULTS

The eight generation Wright's inbreeding coefficients of the eight purebred Chinooks ranged from 25.10% to 47.31%. (A coefficient of 25% is the same as a father to daughter or full-brother to full-sister mating. It takes three generations of continuous full-brother to full-sister matings to achieve a coefficient of 50%.) These were also the lowest and highest inbreeding coefficients found of all Chinooks reviewed for inclusion in the study. The eight generation Wright's inbreeding coefficients of the third-generation Chinook crosses were 18.13% and 27.03%.

The difference between five generation and eight generation Wright's inbreeding coefficients was 9.8% or less in five of the eight purebred Chinooks. In the other three purebred Chinooks, the difference between five generation and eight generation Wright's inbreeding coefficients ranged from 32.2% to 69.6%.

The heterozygosity factor of purebred Chinooks for each of the six genes ranged from 12.5% (1 out of 8 dogs having two different alleles at the gene locus) for the vWF and ACE genes, to 87.5% (7 out of 8 dogs having two different alleles at the gene locus) for the WT and TAT genes. The average heterozygosity for the purebred Chinooks at all six genes was 47.9%. The average heterozygosity for the two Chinook crosses at all six genes was 41.7%. This difference is not statistically significant, based on only two crosses.

The number of different alleles identified at each gene pair across the breed ranged from two (at the ACE and SA genes) to five (at the TYRP2 gene). The average number of different alleles present across the six gene loci was 3.17. No novel alleles could be found in the Chinook crosses at the six gene loci that could have originated from non-Chinook background.

HETEROZYGOSITY	*WT*	*vWF*	*ACE*	*TYRP2*	*TAT*	*SA*	*AVERAGES*
CHINOOKS	87.5%	12.5%	12.5%	62.5%	87.5%	25%	**47.9%**
DOBERMANS	56%	33%	50%	60%	60%	67%	**54.3%**
ALL BREEDS	54%	38%	62%	68%	50%	52%	**54.0%**
#ALLELES	**WT**	**vWF**	**ACE**	**TYRP2**	**TAT**	**SA**	**AVERAGES**
CHINOOKS	4	3	2	5	3	2	**3.17**
DOBERMANS	4	2	4	4	3	2	**3.17**

The heterozygosity factor of the eight purebred Chinooks based on the six gene loci ranged from 16.7% (heterozygous at one of six gene loci) to 100% (heterozygous at all six gene loci). The average heterozygosity of the eight purebred dogs was 47.9%. The average heterozygosity of the two Chinook-crosses was 41.7%. There was a correlation coefficient of -0.495 between the Wright's inbreeding coefficient (homozygosity) and the heterozygosity factor for the Chinook dogs.

The heterozygosity factor of the ten Doberman Pinchers for each of the six genes ranged from 33% for the vWF gene, to 67% for the SA gene. The average heterozygosity for the Doberman Pinchers at all six genes was 54.3%.

The number of different alleles identified at each gene pair across all ten Doberman Pinchers ranged from two (at the vWF and SA genes) to four (at the WT, ACE, and TYRP2 genes). The average number of different alleles present across the six gene loci in Doberman Pinchers was 3.17. The heterozygosity factor of the ten Doberman Pinchers based on the six gene loci ranged from 16.7% to 83.3%. The average heterozygosity of the Doberman Pinchers was 52.7%

For all breeds analyzed, the heterozygosity factor for each of the six genes ranged from 38% for the vWF gene, to 68% for the TYRP2 gene. The average heterozygosity for all breeds at all six genes was 54.0%.

DISCUSSION

The pedigree background of the modern Chinook is limited, and traces back to only four common ancestors directly behind the modern founders. As only one of the four common ancestors is a female, all mitochondrial genes for the breed (assuming accurate pedigrees) originated from her. Baring any mutations in these genes since the bottleneck, the entire breed should carry the same mitochondrial genes. If Perry Greene Tatla carried any defective mitochondrial genes, the whole breed would be affected. (The mitochondria are components of the cytoplasm in the cell that contain their own DNA, separate from the chromosomes in the cell nucleus. The mitochondria are passed

from generation to generation through the egg. Sperm do not pass on any mitochondria in the fertilization process. As the mitochondria are all derived from the mother's egg, this is termed "maternal inheritance.")

The breed carries very high Wright's inbreeding coefficients. These estimate the expected homozygosity across all of the breed's variable gene pairs. Some Chinooks have been linebred on certain founders, and have only recently been crossed back to other founders, producing lower five-generation inbreeding coefficients. If a significant difference exists between a five generation and eight generation inbreeding coefficient, it indicates that the recent pedigree (within five generations) brings together more diverse Chinook breed lines than the closely inbred founding population. In one dog, the five-generation inbreeding coefficient was 19.94%, but the eight-generation coefficient was 33.82%, an increase of 69.6%. As breeds get more populous and their recent pedigree base becomes more diverse, the obvious background inbreeding that created the breed becomes less obvious without deep pedigree inbreeding coefficients. This process is seen in rare breeds, and further back in populous breeds that began with a small population base. As the Chinook breed continues to propagate through the generations, the average inbreeding coefficients based on five and eight generations will continue to decrease.

A study of six autosomal genes cannot attempt to estimate the amount of total heterozygosity in the breed. In spite of this, the results show a significant amount of heterozygosity, with a significant array of alleles present at gene loci. Heterozygosity is caused by the two parents passing down different alleles at the gene locus, and diminishes with increased inbreeding. The number of different alleles present for each gene pair in the breed is a measurement of genetic diversity. Based on four common ancestors, and baring mutation since the recent bottleneck, there could be a maximum of eight alleles present for any gene pair in the Chinook breed.

The Chinooks were compared with randomly selected Doberman Pinchers, a large, genetically diverse breed, with low average inbreeding coefficients. While the average heterozygosity across all six genes was lower than both that of the Doberman Pinchers and all breeds combined, it still showed significant heterozygosity. The average number of different alleles present at the six loci was the same for Chinooks and Doberman Pinchers. This shows considerable diversity for the breed.

The computed heterozygosity factor for the individual Chinook dogs based on the six gene pairs also showed significant diversity. One purebred Chinook was heterozygous at all six loci. The comparison between the Wright's inbreeding coefficient and the computed individual heterozygosity produced a negative correlation; where the heterozygosity factor goes down as the inbreeding coefficient went up. A correlation coefficient of zero indicates that there is no relationship between the two values, and a correlation coefficient of -1 indicates a direct linear relationship. The calculated coefficient showed a correlation trend, but did not provide statistical confidence with only six genes analyzed.

To put these results in perspective, the nature of genetic diversity must be addressed. What constitutes acceptable diversity versus too restricted diversity? The problems with inbreeding in purebred populations concern the fixing of deleterious recessive genes, which when homozygous cause impaired health. Lethal recessives place a drain on the gene pool either prenatally, or before reproductive age. Other deleterious recessives cause disease, while not affecting reproduction.

Problems with a lack of genetic diversity arise at the gene locus level. If there is no diversity (non-variable gene pairs for a breed) but the homozygote is not detrimental, there is no effect on

breed health. The characteristics that make a breed reproduce true to its standard are based on non-variable gene pairs. A genetic health problem arises for a breed when a detrimental allele increases in frequency.

The Doberman Pincher breed is large, and genetically diverse. The breed has a problem with vonWillibrands disease, an autosomal recessive bleeding disorder. Some researchers estimate that up to 60% of the breed may be homozygous recessive for the defective gene, and the majority of the remaining dogs are heterozygous. Therefore, there is diminished genetic diversity in this breed at the vonWillibrands locus. A genetic test and screening program now exists for Doberman Pincher breeders to identify carrier and affected dogs, and decrease the defective gene frequency through selection.

Dalmatians have a high frequency defective autosomal recessive gene controlling purine metabolism. Homozygous recessive individuals can have urinary problems due to urate bladder stones and crystals, and an associated skin condition (Dalmatian Bronzing Syndrome). At one time, the breed and the AKC approved a crossbreeding program to a few Pointers, to bring normal purine metabolism genes into the gene pool. The program was abandoned for several reasons, but it was agreed that the number of individual Dalmatians with two normal purine metabolism genes far exceeded the few Pointers that were being used in the program. The impact of other Pointer genes foreign to the Dalmatian gene pool could have had a greater detrimental effect than the few normal purine metabolism genes being imported through the program.

None of the alleles studied from the two Chinook crosses were novel to the Chinook gene pool. The recently completed Chinook Breed Health Survey showed that Chinook crosses had a higher incidence of both canine hip dysplasia and shyness than purebred Chinooks. None of the disorders appearing at high frequency in the Chinook breed are unique. Cryptorchidism, Epilepsy, Hip Dysplasia, Shyness, Skin Allergies, and Difficulty Whelping are shared by many Northern and working breeds. The need to crossbreed to combat these disorders has not been proven. The fact that these disorders are occurring at a higher frequency in the Chinook breed is significant, and deserves the attention of breeders for greater selective control. The support of research to develop tests for defective genes causing these disorders is also important.

The fact that the Chinook breed has such high inbreeding coefficients, but displays significant diversity, and good vitality as a breed may relate to its diverse origins. Many different breeds were used in the development of the Chinook breed, and selection was based on a working phenotype, not a conformational one. The ancestral dogs linebred on to create the breed may have had significant heterozygosity and multiple alleles due to this background, and could have passed these onto their offspring. The variability and standard deviation in height, weight, color, ear carriage, and eye color also document the genetic diversity in the breed.

Breeders should concentrate on selecting toward a breed standard, based on the ideal temperament, performance, and conformation, and should select against the significant breed related health issues. Using progeny and sib-based information to select against both polygenic disorders, and those without a known mode of inheritance will allow greater control. Breeders should select the best individuals from all kennel lines, so as to not create new genetic bottlenecks. There is a tendency for many breeders to breed to a male who produced no epileptics in matings to several epileptic dams, or to an OFA excellent stud. Too many breedings to one dog will give the gene pool an extraordinary dose of his genes, and also whatever detrimental recessives he may carry, to be

uncovered in later generations.

In spite of the recent genetic bottleneck, and not without its own share of serious genetic disorders, the Chinook breed appears robust, and diverse. By addressing these genetic issues with open eyes, Chinook enthusiasts should be able to enjoy the renaissance of a healthy Chinook breed.

GLOSSARY

Allele/Alleles: One gene from a gene pair. More than one different gene from a gene pair. Ex) The genes for brown (B) and blue (b) eyes are alleles at the same gene pair (locus).

Bottleneck (genetic): A situation where a breed with many breeding stock is reduced to a small number of breeding stock.

Common Ancestors: An ancestor, or group of ancestors through whom all members of the breed are descended.

Cross-breeding: Breeding two different breeds together.

Full-sibs: Two individuals who share both parents. They do not have to be littermates, but can be from repeat matings.

Half-sibs: Two individuals who share one parent.

Heterozygous/Heterozygosity: Having two different alleles present at a gene pair. One of the pair comes from the sire, and the other from the dam. Can be stated as a percentage of all gene pairs showing heterozygosity.

Homozygous/Homozygosity: Having the same allele at both locations of a gene pair. Can be stated as a percentage of all gene pairs showing homozygosity. Homozygosity is estimated through the Wright's inbreeding coefficient.

Inbreeding: Breeding two individuals together who share a common ancestor. The degree of inbreeding is measured through the Wright's inbreeding coefficient. Most breeders consider the term to represent close inbreeding, such as a father to daughter mating.

Linebreeding: Concentrating the genes of a specific individual, through appearances on both the sire and dam's sides of the pedigree.

Locus/Loci: The location of a gene pair (on the chromosomes). Loci is the pleural of locus.

Modern Founder: One of the eleven rescued Chinooks from whom all modern members of the breed descended.

Polymorphic: A gene pair having more than one different allele present in the population.

Wright's inbreeding coefficient: A numerical measurement of homozygosity, based on pedigree analysis.