An Overview of the Backcross Project – Part One

By James E. Seltzer, Ph.D.

The uric-acid stone problem is a principal genetic defect in the Dalmatian breed. Since at least 1938 we have known the inheritance pattern of this defect. It behaves like a simple autosomal recessive. This is the same type of genetic trait as the one that determines whether a Dal will have black spots or liver spots.

It is essential that we keep in mind that the defective trait we are talking about is the very high urinary uric acid (UUA) concentration in Dals. The relationship between UUA and the actual formation of stones is not linear. It is important to observe that: *The mode of inheritance of the uric acid defect in Dalmatians is not in dispute.*

First, we need to understand the fundamentals. A good place to start is at the web page of the American College of Veterinary Surgeons, ACVS.[1]

Overview

Urolithiasis (urinary stones) is a common condition responsible for lower urinary tract disease in dogs and cats. The formation of bladder stones is associated with precipitation and crystal formation of a variety of minerals (magnesium ammonium phosphate hexahydrate, calcium oxalate, urates, and others).

Causes and Risk Factors

What causes urinary stones? Several factors are responsible for the formation of urinary stones. The understanding of these processes is important for the treatment and prevention of urinary stones. In general, conditions that contribute to stone formation include:

- a high concentration of salts in urine
- retention of these salts and crystals for periods of time in the urinary tract
- an optimal pH that favors salt crystallization
- a scaffold for crystal formation
- a decrease in the body's natural inhibitors of crystal formation.

The Backcross project is primarily concerned with the first of these since this is directly related to the uniquely Dalmatian genetic defect mentioned above (Trimble, HC, and CE Keeler. 1938. The inheritance of "high uric acid excretion" in dogs. *J. Heredity*, 29, 280-289.)

The other contributing factors to the urinary stone problem are important as well, but these are not what the Backcross project is about.

In this overview I will explain:

- How one introduces a normal version of the gene into the Dalmatian breed.
- How one identifies and isolates that gene in the progeny.
- How one insures that the normal gene is passed on to succeeding generations.
- How one validates that the Dalmatian uric acid defect has been corrected.
- Current status of the Backcross project and location of the defective gene in the Dalmatian genome.
- Alternative approaches for dealing with the uric acid defect (such as pedigree analysis and selective breeding).

How one introduces a normal version of the gene into the Dalmatian breed.

To anticipate and avoid arguments about inviolability of pure breeds and racial purity, I need to observe that pure canine breeds exist primarily in the minds of the dog fancy and are simply paperwork exercises codified in the registries of the various national kennel clubs. They do not exist in the flesh and blood reality of dogs living in the real world. Dog registries and closed stud books are a recent invention of today's dog fancy – originating only a little more than a century ago. The partnership between man and dog reaches back much further. Robert K. Wayne of the University of California, Los Angeles and his colleagues now have evidence that dogs could have been domesticated 100,000 years ago – if not earlier.[2]

What comprises a breed is not a unique set of genes, neatly packaged with clear boundaries that identify what is and what is not a member of the breed. AKC registration is not especially meaningful for defining the attributes of the Dalmatian. A quick visit to Sue MacMillan's Dalmatian coat-color web pages will quickly shatter such an illusion.[3]

Purebred Dalmatians, presumably AKC registrable, can be found in brindle, lemon, orange, blue, tri-color, and sable. Dals share these genes with other pure breeds. In Dals, these alleles are fairly uncommon; in other breeds they are both common and in many cases desired. There is no doubt that genes that control other conformational attributes (e.g., ears, height, tail set, etc.) are also shared with the other so-called pure breeds.

What distinguishes one breed from another is the relative allele frequencies of the aggregate set of genes that serve as blueprints for the breeds of dogs. Dalmatians, for example, have a higher frequency for the extreme white piebald allele (s_w) and the ticking allele (T) than the cocker spaniel – but Dals do not have exclusive ownership of either of these alleles. Dals just have these alleles in greater abundance.

Most breeds of dogs have a normal gene for uric acid excretion, and, compared with Dals, rarely have problems with urate stones. The ancestor to the Dalmatian also had such a normal gene, but that gene got lost in the shuffle as the breed was propagated and artificial selection was taking place. The normal gene may have been closely linked (on the same chromosome) with another gene that was considered a desirable characteristic by the early breeders. On the other hand, the normal gene may simply have become victim

to random genetic drift and got lost along the way, which is not unusual when the number of dogs being bred is small. However it occurred, to the best of our knowledge, *there were no Dalmatians anywhere that still carried the normal uric acid excretion gene prior to the Backcross project.*

Since that normal gene did not exist within the Dal breed, it was not possible to use breeder selection methods to increase the normal allele frequency and thereby diminish the incidence of urate stone disease in Dals. We can't reestablish the normal gene in the same way that we can establish, for example, a true-breeding, liver-spotted line of Dals.

To find the normal gene it was necessary to turn the clock back to the point in time before the Dalmatian breed branched off from its kin at their common origins and followed its own path. We don't know exactly what the common ancestor was at that early branch point, but we can surmise what its progeny probably look like today even though they followed different selection paths during the intervening generations. Considering a broad array of phenotypic attributes, and the likelihood of a not-too-distant common ancestor, Dr. Bob Schaible selected the Pointer as a probable descendent of that closest common ancestor.

When a Dalmatian was mated to a Pointer, all the cross-bred pups carried one copy of the normal uric acid excretion gene that it got from its Pointer sire. Since, according to the early work by Trimble and Keeler, we already know that the uric acid defect is a simple autosomal (not sex-linked) recessive gene, all the first-generation pups excreted normal levels of urinary uric acid (UUA) as was predicted by the autosomal recessive model. The first-generation pups, of course, did not much look like Dalmatians.

In order to refine the line it was necessary to cross-breed back to a purebred Dalmatian, hence the name Backcross project. The second generation pups, although they began to look more like purebred Dalmatians, did not all carry a gene for normal UUA. Only about $^1/_2$ of these pups got the normal gene. The best of those carrying a copy of the normal UUA gene, i.e., those that most closely resembled Dalmatians, were selected for further breeding in the Backcross project.

The process continued to select pups 1. for normal UUA, and 2. for proper Dalmatian conformational attributes. The Backcross project has continued to the point that the latest generation pups are tenth generation descendents of the one original Pointer. The lucky one's still carry that Pointer's genetic bequest: a gene for normal UUA. Most of their other genes are derived from their Dalmatian dam, their Dalmatian grandam, their Dalmatian great-grandam, etc.

These pups are still heterozygous for the normal UUA gene. The decision not to breed a homozygous-normal UUA line (yet) has been intentional and relates to the necessity to avoid a genetic bottleneck and all the concomitant headaches that ensue when a line is closely line-bred.

How one identifies and isolates the normal UUA gene in the progeny.

The Backcross project started with a Pointer that had normal uric acid excretion (10-60 mg of uric acid in his urine per day) that was mated to a Dalmatian dam with high uric acid excretion (400-600 mg of uric acid per day). There is no overlap in these numbers; there is no mistaking one for the other. A veterinary lab technician provided an unlabeled urine sample from the sire and a urine sample from the dam could easily tell you which sample came from the Pointer and which sample came from the Dalmatian. (*Canine and Feline Nephrology and Urology*, Osborne & Finco, 1995, p824)

As noted above the hereditary pattern for the Dalmatian defect is transmitted as an autosomal recessive. Trimble and Keeler (1938) crossed Dalmatians to Collies and through subsequent crosses determined that the genetic defect in Dalmatians was an autosomal recessive trait.

When a carrier for the defect (one normal gene and one defective gene) from the Backcross line is mated to a purebred Dalmatian (two defective genes), the expected ratio of carriers to defectives in the resulting litter is 1:1, i.e., we expect approximately ½ of the pups to be UUA normal and ½ to be UUA defective. This is the distribution of the defect that could be expected by the second generation and for all subsequent generations of puppies.

As early as 1968 a method for screening for abnormal levels of uric acid in humans had been published: *J Pediatr.* 1968 Oct; 73(4):583-92., "Urine uric acid to creatinine ratio [UUA:CR] – a screening test for inherited disorders of purine metabolism. Phosphoribosyltransferase (PRT) deficiency in X-linked cerebral palsy and in a variant of gout."

Another paper that was published many years after the Backcross project had been initiated questioned the use of the UUA:CR ratio test to estimate the 24-hour total uric acid excretion in healthy Beagles. *Am J Vet Res.* 1994, 55:472-476, Bartges, JW; CA Osborne; LJ Felice; LK Unger; KA Bird; LA Koebler; M Chen, "Reliability of single urine and serum samples for estimation of 24-hour urinary uric acid excretion in six healthy Beagles."

The authors of the 1994 paper found that some spot samples of urine and creatinine taken during the day did not correlate well with the 24-hour UUA excretions, and they attributed that "to differences in urinary uric acid and creatinine excretions after digestion, absorption, and metabolism of the diets."

Yet another paper published in 2004, questioned the use of single 24-hour urinary uric acid excretion measurements in healthy humans since uric acid excretion levels fluctuate widely over even longer periods. [*Rheumatology* 2004 3(12):1541-1545; doi:10.1093/rheumatology/keh379, K.-H. Yu, S.-F. Luo, W.-P. Tsai and Y.-Y. Huang "Intermittent elevation of serum urate and 24-hour urinary uric acid excretion."]

The authors of the 2004 paper conclude: "The data presented here demonstrate individual variations in serum urate levels and 24-h urinary uric acid excretions in healthy men with serial measurement. Transient hyperuricaemia and hyperuricosuria are more common than expected, and both transitory and monthly variations are important factors to consider when evaluating the influence of other factors upon serum urate levels and urinary uric acid excretion."

Needless to say, this puts the veterinary clinician who is trying to manage urinary uric acid problems in his patients in a quandary. The UUA:CR test, it is claimed, is invalid, because of diurnal fluctuations. The 24-hour urine collections are no good because urinary uric acid excretions are found to vary widely when monthly measurements are compared. Further, these monthly variations are not insignificant.[4]

Fortunately, the fluctuations in UUA excretions are of far less concern to the geneticist who is armed with foreknowledge that the

pups produced in the Backcross line will segregate into two distinctly different classes according to their levels of UUA excretion. If he can demonstrate that whichever test he uses differentiates between a normal UUA level and a high UUA level, and that the two classes do not overlap, then his objective of matching the pups to the class carrying the normal gene and the class of those that are homozygous for the defective gene is solved.

The Dalmatian Backcross project has used and continues to use the UUA:CR ratio test for puppy classification purposes. Typical results are given below.

More recent studies have also used UUA:CR ratio tests. [*Urology.* 2003 Sep; 62(3):566-70. Carvalho M, Lulich JP, Osborne CA, Nakagawa Y. "Role of urinary inhibitors of crystallization in uric acid nephrolithiasis: Dalmatian dog model."]

The role of urinary crystallization inhibitors is also discussed below. This is relevant since such inhibitors have been postulated as a reason why, though all Dalmatians excrete high levels of uric acid, not all Dalmatians form urate stones.

How one insures that the normal gene is passed on to succeeding generations.

I have already mentioned the use of various urinary uric acid testing procedures and briefly discussed their weaknesses. I observed that the job of the geneticist working on the Backcross project is considerably easier than that of the veterinary clinician treating stone forming Dalmatians. Nonetheless, the Backcross geneticist must select with a high degree of confidence only those Dals that carry the normal UUA gene for further breeding.

Let us assume that there are 8 puppies produced in a litter where the sire carries one copy the normal UUA gene and the dam is homozygous for the defective UUA gene. The pups should segregate into two classes: a low-UUA class and a high-UUA class, and the most probable split is 4 of each. Of course, getting that exact ratio is not guaranteed. In fact, all 8 pups might fall into the one class or the other – though that outcome is unlikely (about 4 chances in a thousand for either extreme).

The Backcross breeder will use the computed UUA:CR ratios for each pup in the litter and can plot these values as points along the x-axis on a graph. Examining such a plot generally identifies the puppies that belong to each of the two classes since the human eye has the ability to recognize patterns in data. Further, a statistician can analyze the data using a simple algorithm that defines each class on the basis of minimum variance. Listed below are the UUA:CR ratios as they were measured for one set of Backcross pups, Topper X Twyla litter, Aug, 2005, 8 pups:

UUA:CR ratios (mg/dl uric acid per mg/dl creatinine)

0.266
0.282
0.294
0.319
0.376
2.03
2.34
2.77

It is not difficult to identify the high and low UUA classes. The class boundaries are readily apparent.

This concludes part one of a discussion of the Backcross project. Part two will pick up the thread and examine the validation, project status, and alternative approaches for solving the Dalmatian uric acid defect.

Reference URLs:

1. http://www.acvs.org/AnimalOwners/HealthConditions/SmallAnimalTopics/Urolithiasis(UrinaryStones)/

2. http://www.sciencenews.org/pages/sn_arc97/6_28_97/bob1.htm

3. http://www.geocities.com/~paisleydals/color.html

4. http://rheumatology.oxfordjournals.org/cgi/content/abstract/43/12/1541

Ed note: Part two of this article will appear in the next Spotter.