In the February 2008 issue of this magazine, Rod Humphries wrote a personally agonizing account of losing nine of his beloved Doberman home breeding stock – six in a span of just 21 months – to dilated cardiomyopathy (DCM). The sad irony is that while Rod was writing this article about the breakthrough of genetic tests for DCM, he lost his 10th victim of the disease, a female who just reached her 8th birthday and had long battled advanced congestive heart failure. Although he wishes he didn't…Rod definitely knows dilated cardiomyopathy.

Humphries describes himself as a self-taught amateur "genetics junkie" who believes we are living in arguably the most exciting time in history as genome sequencing of all organisms has opened up an incredible world of discovery from treatment of disease to mapping the first humans out of Africa. He has a special fascination for evolutionary genetics and the scientific validation 150 years later of Charles Darwin's evolutionary theories on the origin of species. He says the word "mutation" mostly has a negative connotation for those unfamiliar with genetics, but if it wasn't for good genetic mutations we would not have the incredible variety of species on the planet today. Arguably, he says, humans and canines evolved from a common source many millions of years ago and today continue to have similar genomes where half of the 400-plus known genetic diseases in canines have a human equivalent.

Also, as a journalist and the author of several books including a labor of love on the Doberman Pinscher -- and being a successful breeder since 1966 under the Bikila/Marks-Tey prefix in Australia and the United States -- he is in a unique position to analyze the new landscape of genetic tests and what they mean for the breed and the breeders.

EUROPEAN TEAM CLAIMS SECOND DCM MUTATION IS IMMINENT…

Raises Question of Serious Genetic Complications in Efforts to Eradicate Disease from the Breed

By Rod Humphries

A crack European research team with $16.5 million in grant money from the European Union government has dropped a bombshell with announcement of the imminent identification of a **second genetic mutation** for Dilated Cardiomyopathy (DCM) in the Doberman. The Europeans have warned that dogs which test negative in America may still have the disease and suggested to Europeans directly that they put breeding decisions on hold until the second test is available. The recommendation is obviously also applicable to American Doberman breeders and owners.

The research team headed by veterinary cardiologist Dr. Gerhard Wess of the University of Munich in Germany and geneticist Dr. Tosso Leeb of the University of Bern in Switzerland is involved in a major EU research project which traces human disorders using the canine genome as a model system. The mutation may also be applicable to humans.

A second mutation would seriously complicate the fight against DCM in Dobermans and the news hits at a time when the Doberman fancy is already confused and questioning the tests for the first mutation recently discovered in America. They are also reeling from the high incidence of DCM which is likely to be between 50 and 75 per cent of the Doberman population.

No sooner had Dr. Kathryn Meurs of Washington State University and her American team announced the historic discovery of a DCM mutation which seriously diminishes protein production in the heart cells -- 16 missing DNA bases in the massive three billion base genome -- than the Europeans told me emphatically that they were only a few months away from a test for a second mutation.

Dr. Meurs was prophetic when she stated at her presentation in Topeka, Kansas on October 4: "I wish I could stand here and tell you that this (American test) is it. All you need to do now is test and you are good to go. But I can't. I don't think we ever will because cardiomyopathies are complex diseases with multiple causes," she added.

Dr. Meurs warned repeatedly that she did not think that the mutation she had isolated would be the only cause of Doberman DCM. She said that a "small number" of dogs which she tested negative for DCM had later contracted cardiomyopathy. Also, the vagaries of the American test were highlighted when Dr. Meurs announced in her study report to the American College of Veterinary Internal Medicine that a surprisingly **high 21 of 57 apparently unaffected Dobermans also had the gene deletion which causes DCM**. The scientists have yet to figure that out but Dr. Meurs suggested "their health status may reflect a later onset or variable penetrance of the disease."

The American test is a major first step toward controlling DCM in Dobermans but unfortunately it is certainly far from the complete answer. But as Dr. Meurs said: "Even if there is another cause then you will (by this American test)

have gradually chopped off a large chunk of it (DCM)." She also said that any time the scientists look at the genetics of cardiomyopathies — "even in human beings where they know much, much more about it, there are things that are not very well understood…. There are still numerous things we have to figure out over the next couple of years."

Doberman breeders and fanciers, many of whom have never been concerned with genetics despite the fact that they use its tools every time they breed, have received a large dose of reality on the multiplicities and complexities of the science. Some diseases and traits are triggered separately by multiple genes, and one gene alone can often trigger multiple diseases and traits.

There is absolutely no doubt in this writer's mind that there are multiple causes and/or a separate form of cardiomyopathy in the Doberman, and the European test is expected to help solve some of the mysteries. It is interesting to note that both Dr. Meurs and Dr. Wess recommended on-going echocardiograms and holter monitoring on top of the genetic tests, obviously to catch any additional problems.

The European scientists reluctantly made a premature announcement of their research to alert breeders before they made important breeding decisions. They felt compelled to recommend that serious breeding decisions should be put on hold until the second test is available because dogs which test negative in America may still have the disease.

The ramifications are enormous for this second mutation which has been pinpointed on a separate chromosome to the mutation discovered on canine chromosome 14 in America. The first mutation has the relentless autosomal dominant mode of inheritance and Dr. Wess believes that the mutation he is fine mapping at this time is also almost certainly autosomal dominant.

Autosomal dominant diseases which are in-your-face every generation and do not skip generations because there are no recessives, are probably the easiest of all to eliminate because, as geneticists say, it simply requires eliminating dogs which test positive for the disease from any breeding program.

But that is highly impractical and mostly unmanageable in Doberman DCM where there are two major problems which will seriously impede attempts to breed it out:

1. The extremely high incidence of positives (heterozygous and homozygous which both manifest DCM) – likely to be between 50 and 75 per cent of the entire population judging by early numbers from the American test -- which will force breeders to use dogs with the mutation in the early generations of revamped breeding plans. Eradication of all dogs testing positive would upset the balance of the breed in type and temperament and could elevate another major disease.

2. The genetic complications from having two separate mutations for the same disease, or maybe even two different forms of cardiomyopathy, which will necessitate difficult juggling acts to even make a dent on the disease. There is the likelihood of different results from the two tests, e.g., a dog may be negative in the American test and heterozygous in the European test which means it would still have the disease.

Dr. Gerhard Wess of the University of Munich, Germany

Breeders, if they are earnest and make ethical decisions, may still be looking at many generations and possibly 10-20 years before an acceptable level of incidence can be achieved.

It will be an unwieldy nightmare trying to incorporate the results of two mutation tests into a breeding program. The number of probabilities jumps substantially with two mutations and it will be a daunting task for a breeder to find a genetically compatible stud. What does a breeder do with a bitch which is heterozygous for the American test and clear for the European test and wants to breed to a stud which is clear in the American test and heterozygous in the European test?

Breeders will need to have a crash course in **dihybrid** Punnett (crossing) squares which illustrate two mutations instead of the simple **monohybrid** for a single mutation. I have provided an illustration later in the article of a dihybrid square in which both parents were heterozygous (one copy of the mutant gene) for both the American and European mutations.

When two mutations are calculated together there are four possible gene combinations from each parent and thus 16 permutations among the offspring. It is suffice to say that in my illustration, 15 of the 16 offspring would be homozygous or heterozygous for one or both of the mutations and therefore have the disease – and only one dog, or 6.25 per cent of the litter, would not have either mutation. These numbers are true over a large sample size.

That is just one example and it will not get any easier as breeders grapple with probabilities from multiple pairings in the two mutations.

The existence of a second cause or a separate cardiomyopathy is backed by clinical evidence in Dr. Meurs' study and some early anecdotal evidence from Doberman breeders who were first in line for the American test. She openly admitted that there were dogs in the early study which tested negative yet later developed DCM. She said there were six or eight dogs – "a very small number" – which tested negative and later manifested heart disease. Dr. Meurs was quick to point out that it was unknown if they were direct cases of classic DCM. She said it could have been something else – "a valve

disease, a viral disease or **maybe a second mutation,** we don't know," she added.

Also, owners of a handful of Dobermans who claim their dogs definitely have clinical DCM have tested negative for the disease in the new American test – further evidence that it could be triggered by the imminent European mutation.

Dr. Meurs also told her Topeka audience that she had discovered cardiomyopathy mutations in the Boxer and the Maine Coon cat only to find over time that there was a second cause in both breeds. Animals testing negative were getting the disease and Dr. Meurs said that to this day the mystery of the genetic basis of the second causes has not been solved.

It is sheer speculation on my part, but two mutations could be an explanation for the two distinctly different manifestations of DCM in Dobermans – lingering congestive heart failure with an enlarged heart and sudden death not always with an enlarged heart. Dr. Meurs said that 20 per cent of Dobermans with the disease have the latter manifestation. "That's what makes us wonder if there might actually be two variants of the disease or two different diseases," she said just days before the European announcement.

What is truly scary is that it may just be the beginning of a number of mutations for Doberman DCM unearthed by the international scientific community. Super fast genome sequencing machines have brought an explosion of scientific discovery since the human genome was first sequenced in 2001 and the canine genome in 2003, providing evidence of great similarity between the two. Scientists are literally churning out disease mutations in humans and animals.

In a few short years scientists have uncovered 24 causative genes for Human DCM (only one is needed to trigger the disease) which is further complicated by different modes of inheritance.

Dobermans have an extremely limited gene pool compared to the vastness of the human race, but it would be dangerous and naïve to believe that Dobermans have only one or two mutations for DCM. And then there are the mysteries of the other genetic tenets of modifying genes, incomplete penetrance and variable expressivity which can wreak havoc with conventional thinking on diseases and traits.

So Doberman fanciers, welcome to the explosive new world of biological science in the 21st century!

One wonders if current breeders will have the stomach for the fight over the long haul and what long term impact it will have on the popularity of the Doberman breed. Breeders are going to have limited stock to breed, acerbated by the likelihood of two separate tests and the possibility of split results, and it could take 10-20 years to cut the incidence to acceptable limits.

The days when there were no genetic tests and a breeder merely put dog to bitch and the only difficult decision was whether an animal was show or pet quality, have long faded in the rear view mirror.

European Team Forced to Make Premature Announcement

Obviously the European team felt that to be fair to Doberman breeders worldwide they needed to make a premature announcement of their pending mutation confirmation and test to alert the fancy that one test may not be all they need to make proper breeding choices.

There is absolutely no doubt about the credentials of the European team which has made me comfortable to write about and analyze the pending mutation. Dr. Wess is from the storied Ludwig Maximilian University of Munich, and his partner, leading geneticist Dr. Leeb is from the Institute for Genetics at the University of Bern. The head of the team, Dr. Wess, graduated from the University of Munich and did part of his residency at the University of Georgia and the University of California as well as stints at the University of Zurich. He is a Diplomate of both the American College of Veterinary Internal Medicine (as is Dr. Meurs) and the European College of Veterinary Internal Medicine.

Dr. Wess has received a huge grant of 12 million Euros ($US16.5 million) from the European Union to conduct a research project at the University of Munich entitled: "Unravelling the Molecular Basis of Common Complex Human Disorders Using the Dog as a Model System (LUPA)." The University of Munich is one of Europe's most celebrated institutions, having been established in 1472.

He said that the Doberman mutation was pinpointed during research work for the LUPA project. "It just seems to be a new defect that might also be interesting for humans as it is not known there (in humans). That is my last information (on human discovery)," he added.

Dr. Wess confirmed to me that they have isolated a mutation on a chromosome different to that on which Dr. Meurs and her team discovered the first mutation. "We are currently doing the fine mapping so we expect to have the test available in a few months," Dr. Wess reported.

He said a second mutation means "that either there are several defects – which may explain the different forms of DCM in Dobermans –or that European lines and American lines have different mutations – maybe they work together. We will see in the future but I would think that the lines are quite related."

Dr. Wess created a controversy when he told me: "We currently recommend to not rely on the American genetic test for breeding at this time." He stated "it may be possible that a European (or American) dog is negative for the test from Dr. Meurs, but still has the disease."

In my opinion it makes perfect sense for breeders to mark time until they have results from both tests.

In any type of research there is often fierce competition between competing groups, especially international teams, and some friction seems to have surfaced between the American and European teams over the separate discoveries. It is not unusual in the cutthroat world of scientific research where attracting grant money is the essence of survival. Universities provide facilities but mostly it is up to the researchers to pull in the money from corporations and foundations, etc. to finance long term projects which obviously pay their salaries. It is a constant battle as researchers are not only trying to focus on their work, but are practically begging for money. In this case Dr. Wess is heavily financed by the European Union and Dr. Meurs chases her own grant money.

Dr. Wess's comment for breeders to not fully rely on the American test so that they could obtain a full picture after two separate tests, obviously did not sit well with Dr. Meurs who has a history of serious opposition from a British source over her test for Boxer cardiomyopathy.

"I am afraid that at some point Dr. Wess has decided that we are competitors as he has previously tried very hard to discredit one of our earlier studies where we identified a genetic mutation that causes Hypertrophic Cardiomyopathy in the cat. Dr. Wess and I are both veterinary cardiologists so you would think we could be united in our work for the betterment of animal heart health but somehow this is not the case. I regret this and would hope that someday we could work together," she wrote on line.

Dr. Wess made no personal comment to me about the American team or its test except to say that he had recommended to breeders to not completely rely on the American test until his test was available, ensuring that they were fully informed from both tests.

Disturbing High Numbers from Early Testing

The disturbing backdrop to the confusion over testing is the high numbers being returned on Dobermans testing positive for DCM. In Topeka, Dr. Meurs announced that a stunning **150 of the first 212 tests, or 71 per cent, returned a positive result** in the phase before public announcement of the mutation and testing.

That correlated with the **71 per cent of positives (25 of 35 dogs)** in an extended family studied by Dr. Meurs over an eight year period which identified the mode of inheritance as autosomal dominant.

However, Dr. Meurs is now reporting that after **318 tests there are 157 positives (134 heterozygous and 23 homozygous) for a percentage of 49.4, statistically half the breed population.** She does not expect to have an accurate percentage until her laboratory has tested 1,000 dogs.

I expect the percentages will rise when the European test is in operation.

Dr. Wess told me that a recent comprehensive study in Europe using physical and not genetic testing put the percentage of dogs with DCM at 58.2 percent.

Genetic Tests a Long Time Coming

The Doberman breed may well have been harboring these mutations from days long before Herr Louis Dobermann began his breeding experiments in Apolda, Germany in the 19th century. In America it had a huge launching pad when at least three, and maybe four or even five, of the second wave of America's Doberman foundation, the exalted Seven Sires of the 1940's, died of "heart attacks." The first alarm bells were sounded in the original mimeographed *Doberman Pinscher News and Views* magazine in December 1964 when there was a plaintiff cry for help from the Doberman fancy on the increasing number of "sudden deaths." And who will forget the shocking claim in 1986 by celebrated DCM pioneer, Dr. Clay Calvert, that between 8,000 and 10,000 Dobermans a year were dying from the disease? Dobermans had reached their peak registrations of just under 82,000 in 1978 and eight years later the warning bell was beginning to toll very loudly. And over all this time the disease has continued its deadly march, mostly unchecked.

The full force of the spread of the disease, and the devastation it has caused, has been softened by genetic tenets such as "incomplete penetrance" and "variable expressivity" (see the section on Wildcards: Incomplete Penetrance and Variable Expressivity) which lulled many in the Doberman fancy. And because it takes time for the heart to break down, the late onset often masked the true depth of the severity of DCM in the breed at large. However, in recent years there was growing concern that more and more Dobermans were dying at a much younger age.

There is absolutely no doubt now that the high ratios of the unyielding autosomal dominant mode of inheritance have allowed the disease to march unchallenged from generation to generation without a single break and has taken an enormous toll on American and European Dobermans.

There is no skipping of generations in this mode and affected dogs with only one copy of the mutant gene (heterozygous) pass it to at least 50 per cent of their offspring; and affected dogs with two copies (homozygous) pass it to 100 per cent of their offspring, both regardless of the genetic makeup of their mates (over a large sample size).

Because of the merciless nature of the autosomal dominant mode in a small gene pool closed since the early 1900's, I have long argued that at least 50 per cent -- and possibly as high as 75 percent -- of the Dobermans in this country have a mutant gene and therefore the disease.

Genetic diseases in small, closed gene pools can spread like wildfire and a comparison can be made with von Willebrands Disease (vWD), an hereditary blood coagulation disease, which had 60 per cent positive test results in the initial wave after the discovery of the mutation for the recessive gene. But that is where the comparison ends. Unlike the brutal DCM which is killing our Dobermans en masse, vWD has not lived up to the advanced hype of researchers. It is a laboratory phenomenon and not a clinical phenomenon in Dobermans which I continue to treat as a low priority backburner issue.

It is also interesting to note that almost 1700 Boxers have been genetically tested for Arrhythmogenic Right Ventricular cardiomyopathy and 41 per cent were positive heterozygous; 6 per cent homozygous positive and 53 per cent negative.

Puppy Buyers Will Dictate the Future of Dobermans in the New Landscape

The American test and the pending European test will forever change how Dobermans are bred and how breeders do business. And over time it could raise life expectancy from the current average of about 7-8 years to double figures, hopefully in the teens.

But in a free market society the buyer holds the key to business success – so how puppy buyers react to this fight with DCM will dictate the future of the breed. It is natural to me that they will demand dogs which are fully clear of this life shortening heart disease -- and it is going to be hard for breeders to deliver with two mutations. I expect negative test results

from America and Europe will be needed to fully satisfy most puppy buyers.

If there is any doubt that tests will be demanded one has only to look at the unnecessary paranoia for testing of the back burner von Willebrands disease. That ought to tell you that a front burner issue which really kills Dobermans in great numbers will be a priority when it comes to testing demands.

Breeders know that they cannot just eliminate all the dogs testing positive because the breed would lose type and cause escalation of some other major disease. So by necessity affected animals are going to be used in breeding programs. The difficulty will come when a breeder tries to produce not only quality dogs within the framework of the breed standard, but dogs with the ideal type and temperament of the family bloodline. And while they are trying to accomplish these ideal physical and mental traits, they will also be juggling health concerns beyond DCM. Breeders obviously do not want to delete DCM on the one hand and escalate another major problem on the other.

...everybody including breeders selling pups and stud dog and brood bitch owners — will be pressured to test all animals circulating in the system.

Because of the limited number of clears – especially finding one that fits a particular breeding program -- I envisage the lesser of two evils, the heterozygotes with only one copy of the mutated gene, will have to be used for possibly more than one generation. The homozygotes are more seriously affected, having two copies of the mutant gene and far less protein being delivered to the heart cells. They produce 100 per cent affected pups and are likely to die younger than heterozygotes. But as mentioned earlier, serious complications emerge when results from the second test in Europe are inserted into the genetic equation.

Will the bulk of buyers now shun the breed and look elsewhere in the vast canine world?

I certainly would not entertain a pup with a positive result from either the American or European tests for a companion and/or breeding animal because of a likely early death. I lost several five year olds when the disease practically devastated my kennel by taking 10 victims.

Pups testing positive for DCM will be a major liability for a breeder. Do they euthanize? Do they give them away? How cost efficient is it to put all that time, effort and money into a litter for little or no return on pups?

I realize it is only anecdotal evidence, but I do know long time breeders who feel overwhelmed by the heart problems and cropping and docking issues which are an ominous cloud over America following bans in Great Britain, Continental Europe and Australia. These breeders are retiring; contemplating retirement; or simply moving to other breeds with less serious complications. And puppy producers, who are less motivated by the good health of the breed than they are by the money, are facing hurdles big enough to force them out of the breed.

And will young enthusiasts become the breeders of tomorrow or will they baulk at the difficult landscape and opt out of breeding Dobermans altogether?

Tests will Lay Everything Bare

Doberman fanciers, many of whom have been apathetic to DCM or lulled by the insidious nature of this late onset disease, now have to face the reality of a world of full testing in America and Europe.

Whether people like it or not the tests lay everything bare. And in this very litigious world, breeders will have to test and tell and will no longer be able to sell an affected puppy or contract a stud dog without informing the people involved in the transaction of the test results. Failure to do so could bring lawsuits quicker than you can say: "Show me the money."

Breeders or stud dog owners who in the past have paid only lip service to DCM; either did no physical tests such as ultrasound or holter monitor; or paid no heed to the results if they did test; and brushed it off with "they have to die of something" and "there are no definitive genetic tests;" are now on the hot seat.

I know dog breeders. While I expect some will attempt to push the envelope with all kinds of "ifs" and "buts," there will no longer be an option to cloud the issue of DCM. They will not get away with arguing that the breed might have another cardiomyopathy in play or that it might have a wildcard factor such as "incomplete penetrance" or "variable expressivity."

With the option of publicly releasing results on the Data Base at Washington State University, I expect that those with negatives will flood the base and those who have the multitude of dogs who tested positive will decline listing at this time. It is the nature of the beast but it is about to be changed because at some point – if not publicly but certainly privately between people buying a pup, offering a stud, or seeking a stud – they are going to have to release details of their testing in both America and Europe. No tests, no business.

More Repercussions

Following are repercussions for a dog show culture where silence on family breed problems has forever been an integral part of the game:

- Everybody — and I mean **everybody** including breeders selling pups and stud dog and brood bitch owners — **will be pressured to test all animals circulating in the system**. And they will be pressured to make the results public, either in the public Data Base compiled by the testing authorities; advertisements in breed publications; or directly to those involved in the transactions of studs, brood bitches and puppy sales. I expect that there will be no tolerance and no business for those puppy producers who do not test and tell… and litigation for those who try to corrupt the results at somebody else's expense. Having the ability to test at any time after a sale; and knowing the mode of inheritance for tracing; will quickly upend anybody who tries any funny business.
- As mentioned earlier, breeders should expect that all educated puppy buyers will demand full testing – almost certainly for both tests once the European test is up and run-

ning. I expect that the vast majority will reject a pup with a life shortening disease for personal and/or breeding reasons and will request animals which test negative both here and in Europe. **I read recently where one breeder was told that she did not have to test puppies because the Punnett Squares dictated absolutely that a negative (clear) bred to a homozygous (affected) dog would automatically produce ALL heterozygous (also affected) animals. That is a dangerous assumption. These numbers are only applicable over a large sample size and it could be that in the independent assortment of the genes that, for example, some homozygous positives might be among the pups. Somebody who assumes that the genetic process is always going to produce the Punnett square results in a single litter is asking for trouble, and/or litigation. <u>You will always have to test all pups for both mutations!</u> Only when the all clear is sounded on both tests for two dogs in a breeding will they no longer be needed.**

• Because of the expected long term fight against DCM, there will unquestionably be countless puppies, generation after generation, which will test positive for DCM. So what does a breeder do with pups for sale which test positive, especially the high risk homozygous positive? Will this dilemma alone prompt breeders to shut down their whelping boxes?

• Stud dog owners will also now be forced to test and make the results public on both the American and European tests or quietly retire the dog from public offering. **<u>One test result in America will not cut it.</u>** Because of the high incidence of the disease many of our current advertised dogs will test positive in either test and become non-factors. Some specimens testing positive (heterozygotes only) may be used for a short time – mostly within a family breeding program --because of the high quality they possess. But because of the pressures from puppy buyers who will want clear (testing negative) animals, it will not be long before top dogs testing negative for DCM both here and in Europe will be the only marketable studs. Heterozygotes can certainly produce clear offspring but only in restricted numbers…and the ramifications from two mutations are daunting to say the least. There are still going to be affected pups. So who is going to use an affected stud into the future if they cannot sell his affected offspring? Of course, current top stud dogs with good health and breed type who test negative on both continents will be an immediate prize…and a rare animal indeed.

• Brood bitch owners will have their own dilemmas if they have an affected animal in either the American or European test that they plan to breed. Is the bitch good enough to take the risk; will the resultant puppies be good enough quality to continue breeding; what to do with the puppies which are pet quality and test positive in either of the two mutation tests and will be hard to sell? Just as importantly, will a stud dog owner with integrity think the bitch is of sufficient quality to consider breeding to an affected animal?

They discovered a 16-base chunk of missing nuclear DNA (deoxyribonucleic acid) on Chromosome 14 which is coded to produce a protein in the mitochondria for operation of the cardiac cells.

• Breeders will have to provide test certificates and a contract with a disclaimer clause if somebody agrees to purchase an affected puppy. They will also have to provide a full explanation of the life-shortening expectations. Failure to do so could produce future legal action.

• The market will in time force quality control to ensure the integrity of the individual DCM test results before a puppy sale is made or stud contract is signed. I would personally adhere to a strict regimen in which all dogs to be tested -- puppy, stud or brood bitch – would be microchipped and the blood for the test be drawn by a veterinarian. I would then ask the veterinarian to personally verify the match between the microchip and the blood on the DCM test submission form. There would be nothing left to question.

• I expect that the general fancy will also in time demand similar quality control over the tests so that there is no confusion, or malpractice, in the test results, especially in a litter of puppies. For obvious reasons I do not trust unmonitored tests of cheek swabs swiping the mouths of a litter of puppies. Also, if you don't believe that some people switch results, and animals, and cheat on tests that are not monitored – or even pedigrees – then you are very, very naïve. Just follow the money trail and the show ribbons…and check the public lists of people who are banned or suspended from AKC activities. The obvious counter now is that if an unscrupulous puppy producer switches tests to sell a "positive" as a "negative" on either of the two tests, it will not be hard at any time for the new owner of the dog to do his or her own test and check on the seller. Litigation would almost certainly follow.

• And what will the fancy tolerate as far as time lines and boundaries for ongoing use of affected stock? Obviously some affected stud dogs and brood bitches will have to be used for some generations, but where do you draw the line? I suspect the free market – primarily puppy buyers – will answer that question.

• The extra layer of costs for the breeder from both the American and European tests will be burdensome in a business/hobby that already has costs spiraling out of control. If a breeder wants to survive, he or she will be forced to test every single puppy in a litter until the "all clear" is declared many years into the future. Will breeders be able to sustain a breeding program – and will potential buyers want to pay the much higher costs associated with our cropped and docked breed – as financial outlays keep rising? Costs now include, but are not limited to, thousands of dollars for ear cropping, tail docking, Vwd Tests, thyroid tests, hip testing for dysplasia, etc. and now the probability of two tests for DCM. And as I always point out: there is the unaccounted for and unpaid time a breeder spends in raising a litter! A litter of 8 puppies will cost $408 at a discounted rate for American DCM testing alone. Professional breeders will obviously be hurt; but so too will hobby breeders. They already face sharply rising costs to produce a litter which is acerbated by the high cost of showing a dog. And all this in a depressed economy.

THE SCIENCE

CAUSE OF THE AMERICAN MUTATION: A DNA DELETION AFFECTING PROTEIN PRODUCTION IN THE CARDIAC CELLS

Humans and canines have evolved with similar genomes and therefore have equivalent skeletal structures and organs. They also have many similar mutations and more than half of the 400-plus known canine hereditary diseases have equivalent human diseases. Both have trillions of cells and both are one huge mass of proteins. In fact, about 45 per cent of the human and canine body is protein.

Proteins are macromolecules constructed from chains of amino acids and every function in the body depends on proteins. They are quite simply the physical basis of life. Some are structural building blocks of cells which construct skin and muscles; others are enzymes; information carriers; they provide immune responses; and they generate locomotion of cells and therefore muscles etc. The list goes on and on. A European study two years ago determined that while humans have roughly 25,000 genes, the proteins encoded by those genes interact in about 650,000 ways.

It is a tangled web that proteins weave and one protein source that promotes proper function of the heart has been deceiving us for many, many years and killing our Dobermans at an alarming rate. Thankfully the scientific team headed by Dr. Meurs has uncovered a source of the problem and fashioned a test to allow the Doberman to fight back in future generations. The Europeans know they have pinpointed a mutation but at this time they are not certain of the science.

After many years of searching in the canine genome, the American scientists determined that dilated cardiomyopathy in Dobermans can be caused by **"a mutation in a gene encoding a mitochondrial protein."** They discovered a 16-base chunk of missing nuclear DNA (deoxyribonucleic acid) on Chromosome 14 which is coded to produce a protein in the mitochondria for operation of the cardiac cells. The mitochondria, the battery pack of all cells, have been compromised in the cardiac cells and cannot operate at full capacity, thus interrupting the normal function of the heart.

An analogy in the case of Doberman DCM: there are code buttons missing on key activation panels at the central control station and the power grid cannot be switched on for full essential services. A low capacity emergency generator can operate for a time but it cannot sustain the entire power system over the long haul.

Mutations, simply a change in a gene or genes which produces a different trait or a disease, are often caused by a copying error during meiosis, the process of cell division which replicates one set of genes from the father and one set from the mother in sexual reproduction. Deletions and insertions of bases are a well known factor in replication and more than likely caused the missing 16 bases in the Doberman DNA. Mutations can also be caused by free radicals; viruses and mutagens such as radiation and chemicals.

The end result for Dobermans is that dogs which test heterozygous positive (one copy of the mutated gene) have a significant loss of protein working the cardiac cells; while dogs which test homozygous positive (both copies of the gene are mutated), operate on dangerously low levels of protein.

So how do these dogs survive for any length of time? The body is a wonderful machine which heals and adapts under dire circumstances. Dr. Meurs said that dogs with two copies of the mutated gene "make a little protein through an extra pathway but still much less than the normal dog."

"But in cardiomyopathy, for whatever reason, regardless of the species, when you have these genetic mutations you can then compensate and deal ok with them until you are a middle aged adult. So these dogs deal with it; show no signs; don't have abnormal echoes or holter monitors until they get to maybe four, five, six, seven, eight years of age and then their heart muscle can't deal with it anymore," she added.

Each cell in the body contains numerous mitochondria enveloped in a separate membrane which help produce and control the energy that cells need to function. The number of mitochondria varies according to the work load of the cell and can run into many hundreds, even thousands, in cells which need high energy such as the heart muscle.

Mitochondria have their own small section of DNA which contains instructions for making 13 different proteins, but there are more than 1,000 proteins in mitochondria which are coded from the nuclear DNA, which is the library of instructions encased in the nucleus of each cell. It is only in recent years that scientists have discovered that the vast majority of mitochondrial disorders are actually due to mutations in the nuclear genome. Doberman DCM in the American mutation is one of them.

Gene Search is a Difficult Undertaking

The enormity of a gene search is often lost on those who are unfamiliar with the genome; the complete DNA code for making everything that is human, animal, plant and bacteria. I mentioned earlier that it has speeded up since the sequencing of the human and canine genomes, but it is still a daunting task.

The entire human (and canine) genome sequence is likened to a collection of hundreds of books with over three billion letters (or bases). And unlike our 26-letter alphabet, the genome has only four letters -- A, C, G and T – jumbled in different sequences with no chapters, paragraphs, sentences or punctuation. The letters stand for adenine, cytosine, guanine, and thymine which are the nitrogen rich bases. There is no definitive number of genes because scientists are still deciphering the code, but those 3 billion bases now seem certain to break into about 25,000

THE AMERICAN TEST

Genes come in pairs (one from each parent) and "hetero" means different and "homo" means the same. The American gene detectives were able to isolate a single mutation and develop a test to inform breeders and owners whether a dog is:

Negative: which means it has two normal genes and is therefore clear of the DCM mutation.

Heterozygous positive: it is affected with DCM with one copy of the mutated gene and one clear copy or,

Homozygous positive: it is affected with DCM with mutations in both copies of the gene.

For the purpose of breeding under the autosomal dominant mode of inheritance it is important to note that in this single American discovered mutation:

A heterozygous affected dog produces considerably less protein for heart function than a normal dog but much more than a homozygous affected dog which has very weak production.

A heterozygous positive dog will, under this mode which does not skip generations, transmit DCM to at least 50 per cent of its offspring over a large sample size.

A homozygous positive dog will transmit DCM to 100 per cent of its offspring over a large sample size.

Dr. Meurs said that she did not encounter many homozygous positive dogs in her testing, but added that may be because they die young. It stands to reason if a dog is getting a minimal amount of protein for the heart that it would succumb much earlier.

Dr. Meurs warned that breeders should only use homozygous positive dogs as a last resort, as in the case of one last brood bitch of a breeding program. A homozygous affected dog produces a double whammy: its chances of survival are greatly diminished because of so little protein being produced for the heart cells; and it transmits the disease to every single offspring (over a large sample size).

-30,000 protein-coding genes in the human genome. It is an incomprehensibly massive tome which scientists are deciphering piece by piece.

Because the human and canine genomes are similar and so many diseases are found in both species, the first brush for the American research team was to analyze the known mutations and the proteins involved in human DCM. There are 24 causative genes in human DCM.

After analyzing and discarding the human causes as not being pertinent to Doberman DCM, they painstakingly analyzed nearly 50,000 pieces of DNA in "regions of interest." They matched gene sequences from 48 affected and 48 unaffected Dobermans and then performed additional genome sequencing of 121 Dobermans (64 affected, 57 unaffected) and pinpointed a 16 base (letters) deletion in the DNA of affected dogs.

The mutation has been handed down over time and is now a relentless killer in the Doberman breed. Mutations can be beneficial, as in the basis for evolution of the species; have no effect at all; or be disastrous as in Doberman DCM.

(The Scientific team included Dr. Meurs and S. Lahmers of Washington State University College of Veterinary Medicine; B.W. Keene of North Carolina State College of Veterinary Medicine; E. Mauceli of the Broad Institute of MIT and Harvard; G. Ackland of the Baker Institute, Cornell University, and K. Lindblad-Toh of the Broad Institute of MIT and Harvard and Uppsala University, Sweden).

WILDCARDS: INCOMPLETE PENETRANCE AND VARIABLE EXPRESSIVITY

Two genetic wildcards which have thrown scientists and breeders for a loop in Doberman DCM and so many other human and animal diseases are **incomplete penetrance and variable expressivity**. Dr. Meurs spoke of penetrance as a factor in Dobermans whereby some animals with DCM do not manifest the disease.

The origin of these genetic tenets is completely unknown. It is theorized that some other modifying gene, or combination of genes, hidden anywhere in the genome, could either block or change manifestation of the disease.

I have been asked how a breeder can capitalize on a dog with the actual mutation which lives a full and a happy life because of incomplete penetrance. Unfortunately, a dog which has manifestation of the mutation blocked by a gene or genes, will still pass the disease onto its offspring and may not pass along whatever is helping the animal because it is not permanently linked and is likely to have different genetic tenets.

Identifying a mutation where bases are obviously missing from the genome sequence is one thing. To find a gene or genes among the 25,000 in the genome which trigger reactions from a galaxy far, far away on some distant chromosome, is a near impossible task. So if you are waiting for another test to pinpoint the causes of incomplete penetrance or variable expressivity, I would not hold your breath.

Incomplete penetrance simply means that a percentage of a group -- humans or breed of dog etc. -- which carry the mutant gene do not actually get the disease. They will pass it on to offspring, but they will not have any clinical manifestation. If say, 10 per cent of a breed of dog has the gene and doesn't display the disease, then that mutation would technically be described as having "90 per cent penetrance" or "slight incomplete penetrance of 10 per cent."

Huntingdon's Disease (HD) in humans has a late onset like Doberman DCM; is also autosomal dominant in transmission and has slight incomplete penetrance. There has been a genetic test for HD for many years and it has been determined that the mutation has slight incomplete penetrance of 5 percent….so the mutation has 95 per cent penetrance in the human population.

I would expect that no more than 5-10 per cent of our Dobermans are being kept alive by incomplete pen-

etrance. That is an educated guess after looking at the ratios in other diseases such as HD in humans.

Variably expressivity is somewhat of a mate of incomplete penetrance and is used to describe a disease or trait displayed in different degrees of strength from individual to individual within a given group or breed, etc. Unlike incomplete penetrance where dogs with the mutation don't ever display the disease, variable expressivity definitely displays the disease but in varying degrees.

I use polydactyly -- a trait which produces extra fingers and toes in humans and extra dewclaws in dogs -- as a perfect example of variable expressivity. Believe it or not, but extra fingers and toes are genetically dominant over our normal complement. Variable expressivity in polydactyly decides whether a person has a fully functional additional digit; a mere nub or a raised skin tag. Longtime Major League pitcher Antonio Alfonseca has six fully functional fingers on each hand.

There is very little known about the genetics but it has been speculated many times that **modifiers** top the list. Modifying genes are involved in many major diseases such as Huntingdon's Disease, cystic fibrosis, muscular dystrophy and all forms of heart disease. Human DCM is affected by a bundle of modifiers.

As mentioned earlier, modifiers are known to react with the primary gene to alter the final product of a disease or trait. They can vary the effects and mask certain factors. They are mostly scattered throughout the enormous genome and could have different modes of inheritance to the mutant gene –all of which makes it almost impossible to track them down.

Dr. Meurs said that with Dobermans it is possible that while a dog has the protein mutation, there may be some other gene hidden in the background which blocks manifestation of the disease. She said that there is also speculation that diet and exercise may also be modifiers.

COMPARISON OF BREEDING WITH ONE OR TWO MUTATIONS

There is no better way to illustrate the formidable task facing breeders as they combat two genetic mutations for DCM than to draw Punnett Squares for one and two diseases (traits). In both illustrations I have used only animals which are heterozygous.

Monohybrid Punnett Square for Single Mutation

Dominant genes are always designated a capital letter. In this case I have illustrated it as a "C" (for cardio) while the lower case "c" would be the normal gene. So it would be heterozygous (Cc-affected) mated to heterozygous (Cc-affected).

	C	c
C	CC	Cc
c	Cc	cc

Genotype Frequencies (over a large sample size):
CC 1 (25%) Homozygous affected.
Cc 2 (50%) Heterozygous affected
cc 1 (25%) Clear of the mutation.

This combination projects for 75 per cent affected animals. It often surprises those unfamiliar with genetics because **two affected animals can produce a clear....and this will be an important factor in clearing DCM out of the breed.**

(Note: Other Punnett Squares for one mutation can be found in the February, 2008 issue).

Dihybrid Punnett Square for Two Mutations

I have again designated the capital "C" (for cardio) and lower case "c" to represent the dominant and normal gene respectively in the American mutation – and capital "M" (for myopathy) and lower case "m" to represent the dominant and normal gene respectively in the pending European mutation.

This Punnett Square represents two Dobermans who are heterozygous for both the American and German tests. When two mutations are calculated together there are four possible gene combinations from each parent and thus 16 permutations among the offspring. The gene combinations for heterozygous for both mutations is CcMm, which translates to CM, Cm, cM and cm for the crossing square.

	CM	Cm	cM	cm
CM	CCMM	CCMm	CcMM	CcMm
Cm	CCMm	CCmm	CcMm	Ccmm
cM	CcMM	CcMm	ccMM	ccMm
cm	CcMm	Ccmm	ccMm	ccmm

Genotype Frequencies (over a large sample size):
CCMM: 1 (6.25%) Homozygous for both mutations.
CCMm: 2 (12.5%) Homozygous for the American and Heterozygous for the European.
CCmm: 1 (6.25%) Homozygous for the American and clear in the European.
CcMM: 2 (12.5%) Heterozygous for the American and Homozygous for the European.
CcMm: 4 (25%) Heterozygous for both mutations,
Ccmm: 2 (12.5%) Heterozygous for the American and clear for the European
ccMM: 1 (6.25%) Clear for the American and Homozygous for the European.
ccMm: 2 (12.5%) Clear for the American and Heterozygous for the European.
ccmm: 1 (6.25%) Clear for both mutations.

This projects 15 of 16 offspring as homozygous or heterozygous for one or both of the American and European mutations. Therefore all 15 would be affected by DCM. Only 1 of the 16, or 6.25 %, is clear of both mutations.

A Herculean challenge for the Doberman Fancy? I rest my case....