

The 2008 Rhodesian Ridgeback World Congress Discussions of the “Ridge Gene”

by Clayton Heathcock¹

I was a participant in the recent Rhodesian Ridgeback World Congress in Ireland. One of the speakers at the Congress was Nikki Salmon-Hilbertz, who also spoke at the 2004 World Congress in Texas. Nikki summarized the results of her doctoral thesis work on genetics of the ridge and dermoid sinus, some of which was published in a recent article in the prestigious journal *Nature Genetics*.² I have summarized this work in a previous article in *The Ridgeback*,³ and I refer the reader to this previous commentary as background reading. However, for those readers who do not have immediate access to that article, I will begin with a very brief synopsis.

The ridge in both Rhodesian Ridgebacks and Thai Ridgebacks is caused by a mutation on chromosome 18 in which a stretch of the DNA that is 133,400 nucleotides long has become duplicated. This duplicated segment of chromosome 18 has been called the “ridge gene,” but the duplicated segment includes four known genes and part of a fifth. It is presumably the presence of multiple copies of one or more of these genes that is responsible for formation of the ridge during embryonic development, by a mechanism not yet known.

I have previously defined the normal and mutated versions (alleles) of the “ridge gene” of chromosome 18 as “*r*” (normal) and “*R*” (mutated).ⁱⁱ The “ridge gene” appears to be fully dominant. That is, dogs with either one copy or two copies of the mutated gene (*Rr* or *RR*) are ridged,⁴ and dogs with no copies (*rr*) are ridgeless.⁵

This knowledge equips breeders with a valuable tool to understand and control the production of ridgeless puppies in their breeding, provided they know the genotype (*RR* or *Rr*) of their bitch and of the various sires they are considering for a given breeding. If a breeder knows the genotype of both partners of a planned mating, it is trivial to predict the expected distribution of genotypes and phenotypes from the mating (see Table I).

Sometimes it is possible to deduce the genotype of a dog or bitch from past breeding history; if the prospective parent has ever produced even a single ridgeless puppy, then he or she must be *Rr* (assuming, of course, the dog or bitch is ridged). On the other hand, if the dog or bitch has been bred and has not produced any ridgeless puppies, one cannot deduce the genotype unless one knows the genotype(s) of the previous mates. For example,

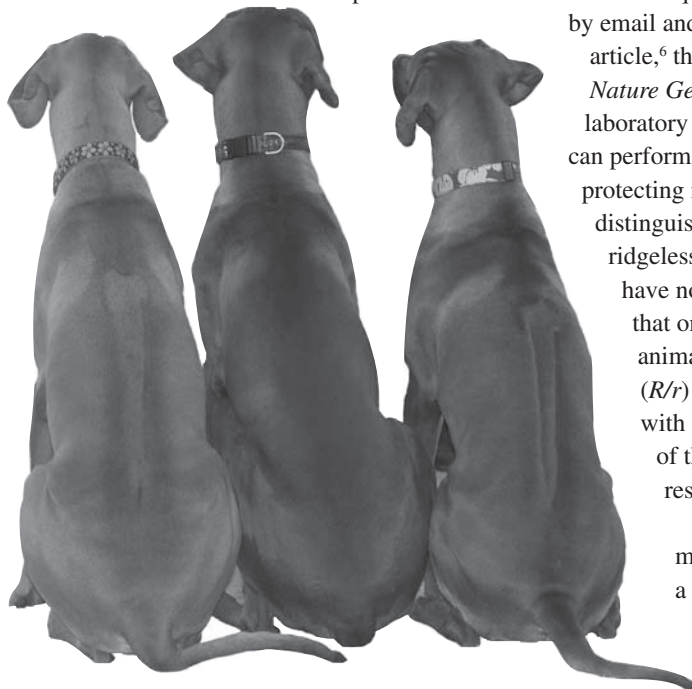
Parental genotypes		Puppy genotype (phenotype)		
Sire	Dam	<i>RR</i> (ridged)	<i>Rr</i> (ridged)	<i>rr</i> (ridgeless)
<i>RR</i>	<i>RR</i>	100%	0%	0%
<i>RR</i>	<i>Rr</i>	50%	50%	0%
<i>RR</i>	<i>rr</i>	0%	100%	0%
<i>Rr</i>	<i>RR</i>	50%	50%	0%
<i>Rr</i>	<i>Rr</i>	25%	50%	25%
<i>Rr</i>	<i>rr</i>	0%	50%	50%
<i>rr</i>	<i>RR</i>	0%	100%	0%
<i>rr</i>	<i>Rr</i>	0%	50%	50%
<i>rr</i>	<i>rr</i>	0%	0%	100%

a given stud dog may be heterozygous (*Rr*) and yet has produced all ridged puppies for one or more litters if his previous mates have all been homozygous ridged (*RR*). I gave an example in my previous article of a heterozygous stud dog who produced only ridged puppies in his first four litters, presumably because all four dams were homozygous.

In her presentation at the World Congress Nikki Salmon-Hilbertz reported to the delegates that she and her coworkers at Uppsala University and MIT have not developed a convenient DNA marker that breeders can use to determine whether a dog is homozygous or heterozygous for the ridge gene. This was a big disappointment, as many of us were hoping for just the opposite announcement—that the important discovery of the genetics of the ridge (and indirectly, of the dermoid sinus) would be followed shortly by an available DNA test that we can use to more efficiently plan litters. In a subsequent comment that was circulated

by email and is printed just before this article,⁶ three of the authors of the 2007 *Nature Genetics* article wrote “Any DNA laboratory skilled in the art of DNA testing can perform this test and there is no patent protecting its use. It is therefore easy to distinguish a ridged dog (*RR* or *Rr*) from a ridgeless dog (*rr*) by the DNA test but we have not yet established a diagnostic test that on a routine basis can distinguish animals that are heterozygous carriers (*Rr*) from homozygous ridged (*RR*) with 100% certainty. The establishment of this test will require some further research.”

I doubt that the authors really mean that it is easy to distinguish a ridged dog from a ridgeless dog by the DNA test since it is



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only necessary to look at the back of the dog to tell if it is ridged or ridgeless. More likely, they mean that “It is therefore easy to distinguish a homozygous ridged dog (R/R) from a heterozygous ridged dog (R/r) by the DNA test.” However, I would submit that although it may be easy for molecular biologists at the Broad Institute to carry out this DNA test, it is far from easy for a breeder to do so. The important part of this statement is its reiteration of what Nikki Salmon-Hilbertz told the delegates of the World Congress—that a routine DNA marker test has not yet been developed. We can only hope that one will be forthcoming soon as a result of the further research that is promised in this statement.

All of this would be of rather academic interest were it not for another result of the 2007 *Nature Genetics* article, namely the identification of the ridge gene as a risk factor for dermoid sinus. The original *Nature Genetics* article stated “Furthermore, 13 of 15 Ridgebacks with dermoid sinus were homozygous for the duplication. Thus, the mutation predisposes to dermoid sinus with low penetrance in duplication heterozygotes and with high penetrance in homozygotes.” This statement is in agreement with the recent statement of Andersson et al., who wrote “Most DS dogs in our study were homozygous R/R (10 of 12) for the Ridge mutation but two were classified as heterozygous R/r .”⁷

The original *Nature Genetics* article stated “Dermoid sinus is closely associated with the ridge phenotype, and no ridgeless dogs with dermoid sinus have been reported.”⁸ While it is no doubt true that Hilbertz and coworkers have not been presented with ridgeless dogs having dermoid sinus, there are reliable accounts of such occurrences.^{9,10,11,12} In addition, in their recent statement, Andersson et al. write “DS or DS-like malformations also occur in humans so it is possible that this type of malformation may occur in dogs in the absence of the Ridge mutation. But its frequency in unridged Ridgebacks should be as rare as it is in non-ridged breeds.”

The bottom line is that the available evidence, based on a rather small sample of 12 Swedish Ridgebacks with DS and anecdotal accounts, it is likely that dogs with RR genotype are more likely to have DS than those with Rr genotype and that dogs with rr genotype are the least likely to have DS. This correlation is far from quantitative and it is hoped that researchers will continue to examine DS-affected animals, both ridged and ridgeless to increase the sample size so that the DS risk of homozygotes can be more accurately stated.

In their recent statement, Andersson et al. wrote “DS does not have such a simple inheritance as the Ridge but our data clearly showed that the Ridge mutation (i.e. the duplication described above) is the major risk factor for the DS malformation in Rhodesian Ridgeback dogs.” This statement is, in my view, not completely supported by facts. It is true, as has been discussed above, that dogs with the “ridge gene” seem to be at greater risk for DS than those

without it, and that dogs with two copies of the gene are more at risk than those with only one copy. However, evidence has not been presented that the ridge gene “is the major risk factor” for DS. As I reported in my previous article,¹¹ three different surveys have shown that the incidence of DS in the United States is 4.7-5.3%. Furthermore, we know from these same surveys that the fraction of ridgeless (rr genotype) is approximately 10%. Using a relationship called the Hardy-Weinberg equation, we can deduce that the breeding pool in the US is 38% homozygous ridged (RR) and 62% heterozygous (Rr). If all of the DS-affected animals were homozygotes, the DS-risk of being homozygous would be about $5/38 = 13.2\%$. However, even the small sample utilized by the Swedish workers showed that about 15% of the DS-affected dogs were heterozygotes. Thus, possession of the ridge gene does appear to be a risk factor for DS, mainly when the animal carries two copies of that gene, and then only to the extent of about 12%. There must be other as yet undiscovered factors that cause DS.

The discussion at the Open Forum section of the World Congress was largely about the ridge gene and on some possible implications with regard to breeding practice. One particularly disturbing suggestion was made by a gentleman representing The Kennel Club (the British equivalent or our AKC). This gentleman is not a Ridgeback owner or breeder, but attended the World Congress at the invitation of The Rhodesian Ridgeback Club of Great Britain. In the Open Forum, he rose to recommend to the delegates that henceforth we should breed only heterozygotes to ridgeless and “accept a somewhat higher incidence of ridgeless puppies in order to eliminate DS.” The position of the representative of The Kennel Club seems to be based on the following sentence in the original *Nature Genetics* article: “The problem with dermoid sinus could be virtually eliminated by allowing ridgeless dogs in breeding and by avoiding matings between ridged dogs.”

The suggestion of a $Rr \times rr$ breeding strategy has also been explicitly made by Andersson et al. in their recent statement under the heading “Recommendations for Breeding:” In matings between a heterozygous ridged dog (R/r) and a ridgeless dog (r/r), 50% are expected to be heterozygous ridged (R/r) and 50% are expected to be ridgeless (r/r). In these matings no homozygous ridged progeny, which are the major problem as regards the incidence of dermoid sinus, would occur. It should therefore be possible to retain the ridge while keeping the incidence of DS to its absolute minimum.”

In the World Congress Open Forum, I responded to the representative of The Kennel Club that although his recommended strategy would indeed lead to “somewhat” more ridgeless (50%) it would not eliminate DS because even the small sample of DS-affected dogs studied by the Swedish group showed that about 15% were heterozygotes. Indeed, since these DS-affected dogs would be removed from the breeding pool, but few or none of the ridgeless dogs would be eliminated, the end result of such a strategy would be complete elimination of the ridge.

Although the subject was not raised in the World Congress Open



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Forum discussion, I was approached privately by two breeders from Germany to solicit my opinion on a different strategy—breeding only homozygous ridged to ridgeless dogs ($RR \times rr$). As shown in Table I, this breeding practice will yield 100% Rr (ridged) puppies and reduce the risk of DS, since about 85% of the DS-affected dogs in the Swedish study were homozygotes. I have already discussed this possible strategy in my previous articleⁱⁱ—it is not practical on a long-term basis because soon there would be no RR or rr genotypes left. In fact, this would happen in only about 8-10 years, by which time all of the dogs of appropriate breeding age would be heterozygotes. At this point, breeding of Rhodesian Ridgebacks would have to cease or else the restriction would have to be eliminated and heterozygotes again used for breeding. But starting with the exclusively heterozygous breeding population, the initial breeding results would be 25% ridgeless, 50% heterozygous and 25% homozygous ridged, with essentially the same DS risk as at present. When I pointed this out, my German friends suggested that the breeding policy be put in place “for a limited period of time.” When I asked how they would monitor such an experiment, they did not have an answer.

Where do we take our new knowledge about the ridge gene and its association with DS? First, we must understand that dermoid sinus, as undesirable as it is, is a condition that can be surgically corrected. At the 2004 World Congress we heard a very informative presentation by Dr. Erik Clough, who routinely carries out DS surgery on neonates. I know several Bay Area veterinarians who are skilled at removing dermoid sinus from five- to six-week old puppies. Furthermore, desexing can be carried out while the puppy is anesthetized for the DS removals. Neonates and young puppies heal very rapidly and by the time they are ready for adoption by their new families, they are fully recovered and make excellent family companions. Finally, the cost of DS-removal surgery is considerably less than the sales price of a pet-quality puppy, so there is not even a financial incentive for the breeder to cull the DS-affected puppy.

This is not to say that we should accept DS as a permanent risk in our breed. As I have written above, although homozygous ridged dogs are more likely to have DS than heterozygotes or ridgeless, it remains that 88% of the homozygous ridged dogs do not have DS. This suggests to me that there are other genes that are the primary cause of DS and that the ridge gene acts to amplify the effect of these other genes. The real goal should be to identify the actual DS-causing genes and develop a DNA marker test for these genes. Then we breeders can work to eliminate the actual DS gene from our breed.

I will conclude this article in a personal way, with an account of my own experience with dermoid sinus. Since I began breeding in 1990, there have been 30 Camelot litters with a total of 194 puppies. None of these puppies have had dermoid sinus. In addition, I have owned four stud dogs who have sired a total of 48 litters (not counting five litters that are included in the foregoing totals for the Camelot litters); these litters comprised a total of 434 puppies, of which 7 had dermoid sinus.¹³

This suggests to me that there are other genes that are the primary cause of DS and that the ridge gene acts to amplify the effect of these other genes. I believe that I had the good fortune to

start with a foundation bitch who lacked most or all of the genes that are necessary for DS to form, and even though I have out-crossed frequently, as luck would have it, I have not picked up a full suite of DS-causing genes in any of my 30 litters. One of my stud dogs produced no DS in a total of 79 non-Camelot puppies. The three who have produced DS were all the result of out-crosses and these three dogs have sired a total of 355 puppies, of which 7 have had DS. I probably picked up one or two of the necessary DS-causing genes in these stud dogs from their sire. Occasionally, one of these stud dogs has been bred to a bitch who supplied the rest of the gene suite necessary to produce a dermoid. However, even this frequency ($7/355 = 2\%$) is less than half the breed average, which is about 5%.

In summary, goal should not be to approach the problem by introducing ridgeless dogs to our breeding population, but to identify the actual DS-causing genes and develop a DNA marker test for these genes. Then we breeders can endeavor to rid our breed of the root cause of DS, while retaining the hallmark of our breed.

(Endnotes)

1 Clayton Heathcock is Chief Scientist of QB3 Berkeley, The California Institute for Quantitative Biosciences. He has bred Rhodesian Ridgebacks for 18 years and has been a member of the Rhodesian Ridgeback Club of the United States since 1993.

2 N. H. C. Salmon Hilbertz, M. Isaksson, E. K. Karlsson, E. Hellmén, G. R. Pielbert, P. Savolainen, C. M. Wade, H. von Euler, U. Gustafson, A. Hedhammar, M. Nilsson, K. Lindblad-Toh, L. Andersson, G. Andersson, *Nature Genetics*, **9**, 1318 - 1320 (30 Sep 2007).

3 Clayton Heathcock, *The Ridgeback*, Issue 1, 77-80 (2008).

4 Dogs with the RR genotype are called “homozygous ridged” and those with the Rr genotype are called “heterozygous.”

5 Recall that an individual has two versions of every gene, one inherited from each parent.

6 L. Andersson, K. Lindblad-Toh, G. Andersson, “A Statement Concerning the Genetic Basis for the Hair Ridge and the Congenital Malformation Dermoid Sinus in Rhodesian Ridgeback Dogs” (24 Sep 2008).

7 This apparent quantitative disagreement is because the *Nature Genetics* narrative statement quoted included data for three Thai Ridgebacks with dermoid sinus, all homozygous.

8 Salmon Hillbertz, N.H.C. *J. Small Anim. Pract.* **46**, 71–74 (2005).

9 Diane Jacobsen has been a California Rhodesian Ridgeback breeder since 1962. In the early 1970s, she evaluated a litter that included a ridgeless puppy with a dermoid sinus. Diane Jacobsen, personal communication.

10 Elizabeth Akers operates Rhodesian Ridgeback Rescue of Northern California. Several years ago she accepted two littermates, a ridged female and a ridgeless male, both with dermoid sinus. The dermoids were removed by Dr. Richard Hoskins of the Santa Rosa Veterinary Hospital. Both dogs are still alive in Northern California and available for DNA studies. Elizabeth Akers, personal communication.

11 Janet Nolan has been a British Rhodesian Ridgeback breeder since 1981. In 1996 she evaluated a litter of 11 Ridgeback puppies. Five of the puppies were ridgeless and two puppies had dermoid sinus, one ridged and one ridgeless. Janet Nolan, personal communication.

12 The fact that the Uppsala researchers did not encounter an example of a ridgeless dog with a dermoid sinus is not unexpected. They report 12 dogs with DS, of which 10 were RR and 2 were Rr . That is, having two copies of the “ridge

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gene" causes roughly a 6-fold greater probability of DS. If this risk factor also applies to *Rr* and *rr*, the sample of 12 DS-affected animals would have had 1/3 of a ridgeless dog. In addition, about 90% of Rhodesian Ridgebacks are ridged, whereas only about 10% are ridgeless. Furthermore, until relatively recently, many breeders still euthanized ridgeless puppies. Therefore, it was unlikely that a

ridgeless dog with a dermoid sinus would come to the attention of the researchers. In other words, the sample upon which the conclusion is based is sufficiently large to show that *RR* genotype carries more DS-risk than *rr* genotype, it is not large enough to conclude that ridgeless dogs do not have DS.

13 I am quite certain of the DS results reported in this paragraph for the Camelot-bred litters. However, for the litters produced to Camelot-bred stud dogs, I did not physically observe the puppies and am relying on information supplied to me by the various dam owners.

Uppsala September 24, 2008

To whom it may concern,

A statement concerning the genetic basis for the hair ridge and the congenital malformation dermoid sinus in Rhodesian Ridgeback dogs.

It has come to our attention that the recent World Congress of the Rhodesian Ridgeback Association has left some uncertainty concerning the genetic basis for the hair ridge and the dermoid sinus in Rhodesian Ridgeback dogs. We would therefore like to clarify our views on this subject and its implications for breeding.

1. The inheritance of the Ridge.

All our data and all data we are aware of indicate that the ridge is inherited as a fully dominant trait. The dominant allele causing the ridge is denoted R whereas the recessive wild type-allele is denoted r. This means that a ridged dog may either be homozygous (R/R) or heterozygous (R/r) for the Ridge allele whereas all ridgeless dogs should be homozygous *r/r* for the normal allele. The result presented in our Nature Genetics paper from 2007 (Hillbertz et al. Nature Genetics 39:1318-1320) provided conclusive evidence that the mutation causing the ridge in Rhodesian Ridgeback as well as in Thai Ridgeback dogs is a 133 kb duplication on dog chromosome 18. [A duplication means that each Ridge chromosome has two copies of this 133 kb fragment whereas a normal chromosome has only a single copy. It is this doubling or duplication of the chromosome region that constitutes the Ridge mutation.] So far all tested dogs with the characteristic dorsal hair ridge have been heterozygous or homozygous for this mutation whereas all ridgeless dogs we have tested lacked the duplication. The duplication contains four complete genes (FGF3, FGF4, FGF19 and ORAOV1) and we assume that it is the higher than normal expression of one or more of these

genes, attributable to their greater number, that leads to the development of the hair ridge.

2. The genetic basis for dermoid sinus (DS).

DS does not have such a simple inheritance as the Ridge but our data clearly showed that the Ridge mutation (i.e. the duplication described above) is the major risk factor for the DS malformation in Rhodesian Ridgeback dogs. Most DS dogs in our study were homozygous R/R (10 of 12) for the Ridge mutation but two were classified as heterozygous R/r. However, DS or DS-like malformations also occur in humans so it is possible that this type of malformation may occur in dogs in the absence of the Ridge mutation. But its frequency in unridged Ridgebacks should be as rare as it is in non-ridged breeds.

3. DNA tests.

In our Nature Genetics paper we described a simple DNA test that can be used to identify the presence of the Ridge mutation (the duplication). Any DNA laboratory skilled in the art of DNA testing can perform this test and there is no patent protecting its use. It is therefore easy to distinguish a ridged dog (R/R or R/r) from a ridgeless dog (*r/r*) by the DNA test but we have not yet established a diagnostic test that on a routine basis can distinguish animals that are heterozygous carriers (R/r) from homozygous ridged (R/R) with 100% certainty. The establishment of this test will require some further research.

4. Recommendations for breeding.

The most straightforward way of reducing the incidence of DS in Rhodesian Ridgeback dogs is to reduce the frequency of homozygotes for the Ridge mutation. This can be accomplished by allowing the use of ridgeless dogs for breeding. While we are aware that dogs with a DS are not usually kept for breeding, matings between homozygous (R/R) ridged dogs (presumably without DS) and ridgeless dogs (*r/r*) would give progeny all of which would

be heterozygous ridged (R/r) and therefore show ridging, and the incidence of DS would be low. In matings between a heterozygous ridged dog (R/r) and a ridgeless dog (*r/r*), 50% are expected to be heterozygous ridged (R/r) and 50% are expected to be ridgeless (*r/r*). In these matings no homozygous ridged progeny, which are the major problem as regards the incidence of dermoid sinus, would occur. It should therefore be possible to retain the ridge while keeping the incidence of DS to its absolute minimum. It would be useful to develop a diagnostic test to distinguish carriers (R/r) from homozygotes (R/R) because this would allow breeders to avoid matings such as R/R x R/r that will produce homozygous ridged (R/R) progeny.

It will of course be up to the individual breeders and to Ridgeback breeding organizations to decide whether they prefer to keep the Ridge and minimize the incidence of DS using the approach described above or whether they would like to completely eliminate the problem with DS by allowing the Ridge mutation to disappear from the population over time. If one decides to eliminate the Ridge mutation it should not be done too quickly (during a few generations) since that will lead to increased inbreeding in the breed as too many potential breeding animals are eliminated.

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