Comments on the Ridge Gene, by Clayton Heathcock; February 15, 2008

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"The hallmark of this breed is the ridge on the back which is formed by the hair growing in the opposite direction to the rest of the coat. The ridge must be regarded as the characteristic feature of the breed."

Official Standard of the Rhodesian Ridgeback
Approved by The American Kennel Club on August 11, 1992

The recent publication in *Nature Genetics*[i] of the discovery and deciphering of the gene for the ridge has captured the attention of the Rhodesian Ridgeback community. The "ridge gene" turns out to be a mutation that resulted in the duplication of a region of chromosome 18. Such duplications sometimes occur when the DNA-copying machinery makes a mistake and re-copies something it has already copied once. In the case at hand, the duplicated section of chromosome 18 is 133,400 nucleotides long and includes the codes for four known genes and part of a fifth. Three of the genes in the duplicated region are fibroblast growth factors, which have been implicated in angiogenesis (development of blood capillaries), wound healing, and embryonic development. While it is not yet known how this mutation results in the characteristic ridge, the evidence that it does is very compelling.

The genetics of the ridge are simple autosomal dominant. Ridgebackers are very familiar with this mode of inheritance because it is the mechanism that gives us "black-nose" and "liver-nose" varieties. We are all familiar with the fact that a black-nosed Ridgeback can have either two copies of the "black" gene (*BB*) or one of "black" and one

of "liver" (*Bb*), but a liver-nosed Ridgeback must have two copies of the "liver" gene (*bb*). In other words, black is dominant to liver; a dog just needs one copy of the "black" gene to have black-nose phenotype. It turns out to be the same with the ridge gene. The two alleles[ii] of the "ridge gene" are a version of chromosome 18 without the duplication (*r*) and a version of chromosome 18 in which the 133.4 kilobase segment is repeated (*R*). If a dog has one or two copies of the duplicated region (*Rr* or *RR*), it will have a ridge. If it has no copies of the duplicated region (*rr*) it will be ridgeless. In other words, the *R* allele is dominant over the *r* allele.

This part of the story seems to be very strong; the Swedish group sequenced the appropriate region of chromosome 18 of a total of 54 Ridgebacks and determined whether they had 0, 1, or 2 copies of the mutation. The results are summarized in Table 1. It was also noticed by the Swedish workers that dogs with the *RR* genotype (two copies of the duplication) are predisposed to have dermoid sinus. However, this part of the story, while suggestive, is not nearly as quantitative as the correlation of ridge phenotype with genotype because the conclusion is based on a rather small sample. Nevertheless, there is a strong suggestion from this study that the mutation on chromosome 18 that gives us the ridge also gives us the dermoid sinus.

Table 1: Genotype-phenotype relationships in regard to 133-kb duplication

	Number of dogs	**0 Copies (*rr*)**	**1 Copy (*Rr*)**	**2 Copies (*RR*)**
Rhodesian Ridgebacks				
Ridgeless (no dermoids)	10	10	0	0
Ridged without dermoid	20	0	16	4
Ridged with dermoid	12	0	2	10
Thai Ridgebacks				

Ridged without dermoid	9	0	3	6
Ridged with dermoid	3	0	0	3
Other Breeds	37	37	0	0

This study provides us with a simple view of ridge genetics. Each Rhodesian Ridgeback is either of genotype *RR*, *Rr*, or *rr*. The ones that are *RR* or *Rr* have ridges and the ones that are *rr* are ridgeless. The described "ridge gene" only determines whether a dog has a ridge or doesn't have a ridge. It doesn't determine the other morphological characteristics of the ridge—length, breadth, number and placement of crowns, etc. These characteristics are also under genetic control, but other genes are at work. The chromosome-18 "ridge gene" is like an off/on switch. The switch must be on to order up the ridge, but other genes determine what the ridge looks like.

What are the implications for the breeder? If you breed a dog and a bitch that both have genotype *Rr* (this is called "heterozygous"), each puppy has a 25% chance to be *RR*, a 50% chance to be *Rr*, and a 25% chance to be *rr*. That is, heterozygous parents will produce, on average, 25% ridgeless, and 75% ridged offspring (the ridged offspring will be two-thirds *Rr* (heterozygous) and one-third *RR* (homozygous dominant). However, if one of the parents in a mating is *RR* and the other is *Rr*, the puppies will be, on average, half *RR* and half *Rr*. In other words, if just one parent is homozygous dominant, it is impossible for a ridgeless puppy to result.

How do you know the genotype of a dog or bitch that you are considering for a breeding? Of course, you can easily tell by observation if it is *rr* because it will not have a ridge, but this is not very relevant since we normally do not consider ridgeless dogs as possible breeding stock. However, dogs with ridges can be either homozygous dominant (*RR*) or heterozygous (*Rr*)—how can you tell which? If the dog or bitch has been bred

previously and produced even one ridgeless puppy, then the genotype of that sire or dam must be *Rr*. However, the simple fact that a dog or bitch has not produced a ridgeless puppy is not proof that it is genotype *RR*; you need to know something about the genotypes of the breeding partners in the previous litters to make that call.

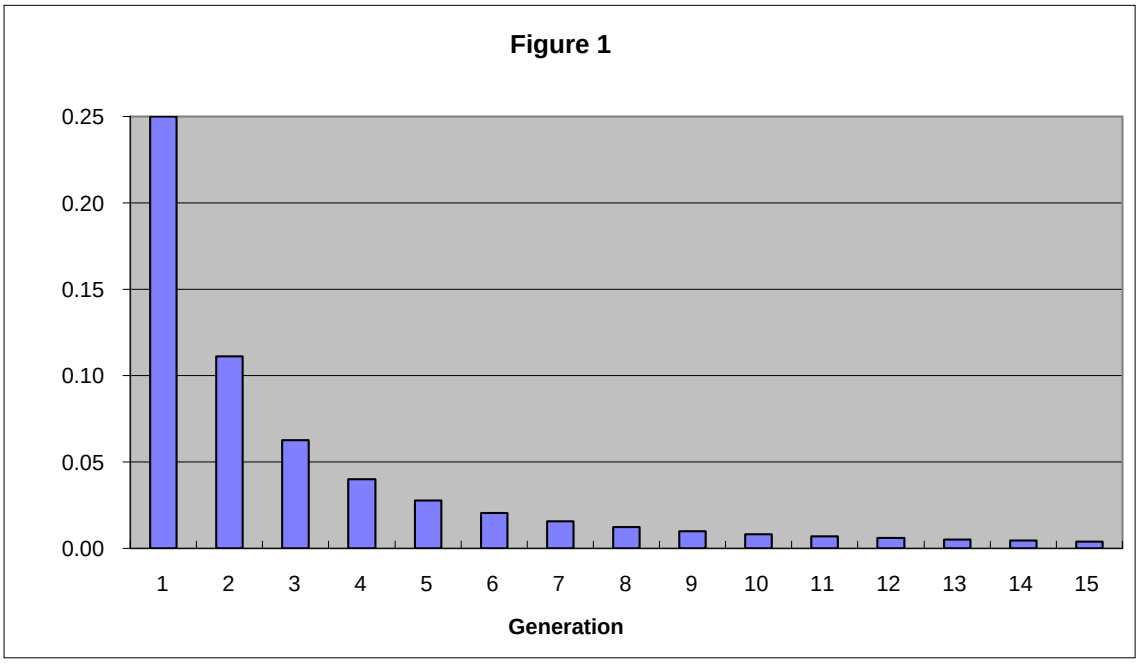
I am going to illustrate this with three of my own stud dogs, Mojave,[iii] Ruger,[iv] and Hadley.[v] Mojave's breeding career began while he was in the care of Lisa Barrenger in Australia in 2003-04; his first four litters in Australia included no ridgeless puppies out of 38 total. However, there were two ridgeless in his 5th litter and three more in his 6th litter. The fact that Mojave eventually produced ridgeless puppies proves that his genotype is *Rr*. It is very likely that the first four bitches he was mated with are all *RR*, which is why there were no ridgeless in those litters. Through his entire breeding career, Mojave sired 14 litters that had no ridgeless puppies (128 total puppies) and 8 litters that had one or more ridgeless puppies (94 puppies, of which 76 were ridged and 18 were ridgeless). It is quite likely that all of the dams of the 14 litters that had no ridgeless were *RR* and the dams of the 8 litters that had ridgeless were *Rr*. His total ridgeless rate for all 22 litters is 9%, mainly because most of the dams were *RR*. If all of the dams had been *Rr*, the ridgeless rate would have been about 25%. Indeed, Mojave's litter-brother Ruger has been bred 15 times and his total ridgeless rate is about 30%. Ruger, like Mojave, is *Rr*, but unlike Mojave, most of the bitches he has been mated with are also *Rr*.
Hadley has been bred six times in all. His first two litters were with Marley,[vi] and all 13 puppies in these two litters were ridged. This is a strong suggestion that Hadley is homozygous dominant (*RR*) because Marley had produced a ridgeless puppy in a previous breeding with Chip,[vii] and therefore, we know that she was *Rr*. If Hadley was

also *Rr*, the chance that all 13 Hadley-Marley puppies would be ridged is only about 1 in 50. Hadley was subsequently bred to four other bitches and all of the 53 puppies he has produced have been ridged, consistent with the fact that he is homozygous dominant. [That Hadley is *RR* was independently determined by Dr. Mark Neff at UC Davis, who determined his genotype experimentally.]

Since Rhodesian Rigeback breeders never use ridgeless dogs for breeding and since there have been more than 30 generations since the studbook was closed in 1922, it is remarkable that we still have as many ridgeless puppies as we do (about 10% according to three separate surveys).[viii]

To understand this, we need to review a few basic points. Every Ridgeback has two alleles at the ridge locus on chromosome 18, one from each parent. Therefore, every Ridgeback is either *RR*, *Rr*, or *rr*. Assume that there are equal number of *R* and *r* alleles, distributed randomly throughout the entire Rhodesian Ridgeback population. Statistically, we would have 25% *RR* genotypes, 50% *Rr* genotypes, and 25% *rr* genotypes. If all of these dogs are mated randomly, without any consideration of their genotype, this genotype distribution would continue indefinitely—every generation would simply result in reshuffling the equal number of *R* and *r* alleles. However, this isn't the way we operate; we systematically exclude all of the dogs with *rr* genotype and essentially remove them from the beeding pool; the *rr* genotype is effectively a lethal genetic defect. So when we breed the first generation from our starting pool of 25% *RR*, 50% *Rr*, and 25% *rr*, we only use the *RR* and *Rr* genotypes. In essence, we remove half of the *r* alleles but none of the *R* alleles. If one continues this practice, generation after generation, systematically removing all of the ridgeless (*rr* genotype) from the breeding

population but randomly mating *RR* and *Rr* genotypes, the fraction *rr* approaches zero. This is illustrated in the Figure 1, which shows what happens if you begin with 100% *Rr* genotype and select mating partners at random, continuing generation after generation, except that no ridgeless are ever used for breeding. The results are striking—in only 9 generations, about 20 years, the ridgeless rate should drop to 1% and after 15 generations it should be be only 0.4%! At the same time, the fraction of the population that is homozygous dominant (*RR*) increases and would be approximately 87.5% after 15 generations.



However, this isn't what has happened. The only possible explanation is that there is a "heterozygous advantage." What this means is that dogs with *Rr* genotype are more successful at passing their genes on to succeeding generations than dogs with *RR* or *rr* genotype. It is obvious why dogs with *rr* genotype are not successful—they are ridgeless and are rigorously excluded from the breeding pool. But why are dogs with *RR*

genotype discriminated against, especially in view of the fact that breeders cannot tell from simple observation whether a dog is *RR* or *Rr*, since both are ridged? One possible answer to this question is found in the second important conclusion from the Hilbertz et al. article—Ridgebacks with *RR* genotype have a predisposition to dermoid sinus, relative to dogs with *Rr* genotype. Examination of Table 1 shows that 10 of the 14 Rhodesian Ridgebacks in their study group with genotype *RR* also had dermoid sinus, whereas only 2 of the 18 genotype *Rr* dogs had dermoid sinus. Another way to look at the data in Table 1 is that 83% of the dogs with DS were genotype *RR* compared to only 20% of the dogs without DS.

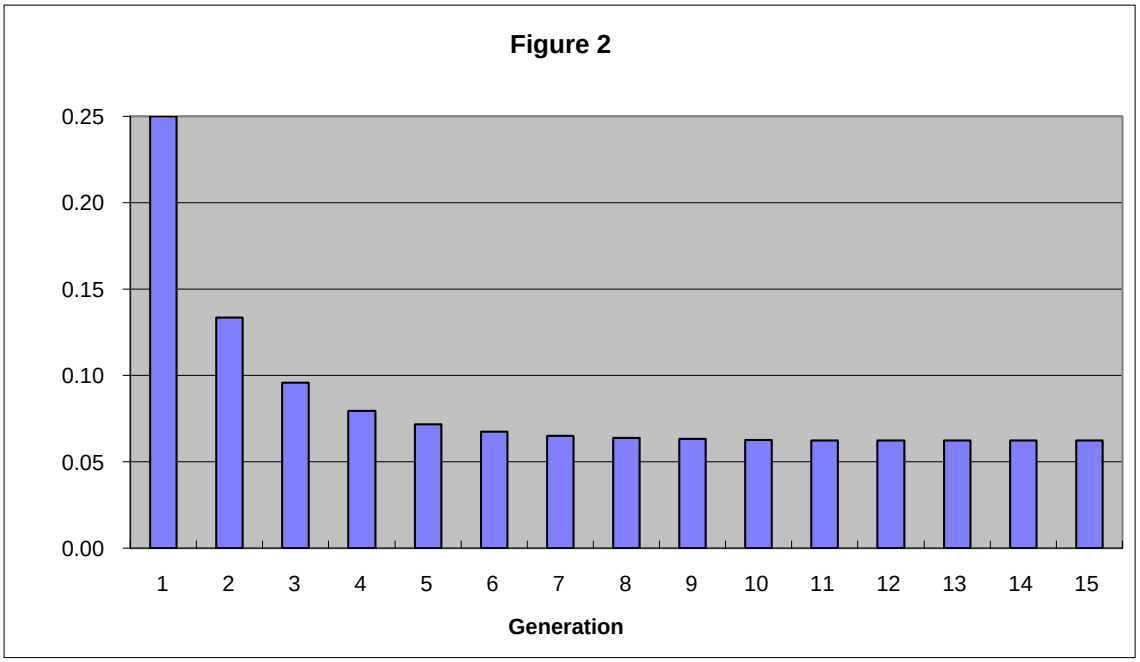
This high correlation of DS with ridge genotype is suggestive but not quantitative. Another way to ascertain the degree to which *RR* genotype predisposes to DS is by statistical analysis of survey data. A convenient relationship called the Hardy-Weinberg formula can be used to estimate the fractions of *RR* and *Rr* genotype in the breeding pool. For our case, the Hardy-Weinberg formula is $(R + r)^2 = 1$, which is the same as $R^2 + 2Rr + r^2 = 1$, where R^2 is the expected frequency of *RR*, $2Rr$ is the expected frequency of heterozygotes, and r^2 is the expected frequency of *rr* (ridgeless). Since we know the frequency of *rr* (10%) in the population, we can use the Hardy-Weinberg equation to work backwards and deduce the frequency of *RR* (38%) and *Rr* (62%).

According to the three surveys previously cited, the US incidence of DS is 4.7-5.3%. If all of these had *RR* genotype, then the DS risk would be 0.05/0.38 = 13.2%. However, it is shown by even the small sample reported in the Hilbertz article that *Rr* genotype animals can also have DS, so the actual DS-risk of *RR* genotype is probably more like 12%, or one in eight. What this means is that an average litter of *RR* puppies

(which would result from mating a sire and dam who are both *RR*) would have, on average, one DS-affected puppy.

Dogs that have DS are also removed from the breeding pool and if a disproportionate number of these DS-affected dogs are *RR* genotype, this might be the reason we have been systematically discriminating against *RR*, relative to *Rr*. This can be modelled. Figure 2 shows the situation that would result, starting with a breeding pool that is uniformly heterozygous and carrying out random matings, except that at each step 100% of the *rr* (ridgeless) and 12% of the *RR* (dermoid sinus) are eliminated. The results are again striking; the ridgless numbers fall, as before, but now they level out at an incidence of approximately 6%, while the fraction of the breeding pool that is *Rr* falls from 100% to about 43%.

Figure 2



This is similar to our current situation in the United States, except that our ridgeless number is about 10%, rather than the 6% predicted in Figure 2. To achieve a

stable ridgeless rate of 10% (and a corresponding heterozygous fraction of 0.6), we need to remove about 23% of the *RR* genotype at each generation. There are several possible explanations for the discrepancy. First, we might question the accuracy of the survey data. The number of dermoids may be under-reported, which would mean that the DS-risk of the *RR* genotype is greater than 12%. Alternatively, the number of ridgeless may be under-reported; this would decrease the fraction of *RR* genotype in the population and also lead to an increased DS-risk for *RR*. I think that a more plausible explanation is that breeders have also been discriminating against breeding stock, mainly sires, who have a record of producing dermoid puppies. Since a *RR*-genotype sire will produce more *RR* puppies than a *Rr*-genotype sire, and since *RR*-genotype puppies are more prone to DS than *Rr*-genotype puppies, this is another mechanism for selection against *RR* and in favor of *Rr*.

It is interesting that a recent survey of breeders carried out by the National Rhodesian Ridgeback Club in Australia indicates that the Australian Ridgeback breeding pool produces fewer ridgeless (6.3%) and more dermoid sinus (6.7%) than in the United States. This is in qualitative agreement with the foregoing analysis; if you breed to minimize ridgelessness, dermoid sinus should increase. Australian breeders may be discriminating somewhat against heterozygotes by avoiding breeding dogs who have produced ridgeless offspring.

Now for a little speculation. We will probably have access soon to a DNA test that will reveal the ridge genotype. Even without such a test, we can make some assignments; any ridged dog or bitch who has produced even one ridgeless puppy must

be *Rr*. What choices will breeders make, equipped with this knowledge and eventually with a genotype test?

What would happen if breeders generally elect to completely avoid *RR* breeding stock, so as to minimize the risk of DS? This would, indeed, have the effect of lowering the DS rate, but it would return the breeding pool to one that is overwhelmingly heterozygous and the overall ridgeless rate would climb back to 25%. What about the suggestion, made by Hilbertz in her article that "The problem with dermoid sinus could be virtually eliminated by allowing ridgeless dogs in breeding and by avoiding matings between ridged dogs." This is tantamount to breeding against the ridge; if every mating was between a ridgeless dog and a ridged one that is either *RR* or *Rr*, in only a dozen or so generations, the ridge would virtually disappear.

Another strategy that has been been suggested is to carry out only *RR* x *rr* breedings; that is, to exclude all heterozygotes. It is true that in the short run this would assure no ridgeless and minimize (but not completely eliminate) DS. However, this would work for only a short time because there would soon be no more *RR* or *rr* genotypes left!

Personally, I will probably steer a middle course and try for *RR* x *Rr* matings. This will result in 100% ridged puppies, but the DS-risk will be about half of what would be expected from *RR* x *RR* matings. Using the Hardy-Weinberg analysis of our survey data, *RR* x *RR* matings will produce about one DS puppy per litter wheras *RR* x *Rr* matings will produce about one DS puppy every other litter. Of course, the DS-risk is even lower for *Rr* x *Rr* matings (about one DS-puppy every four litters), but this will produce, on average, 25% ridgeless in each litter.

[i] 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).

[ii] Alleles are alternate forms of a gene that may be found at a given locus; *R* and *r* are alleles.

[iii] Mojave is BISS MBIS Au/Am Ch Camelot's Code Red, ROM.

[iv] Ruger is MBIS DC Camelot;s Promise to Bakari, ROM.

[v] Hadley is Ch Camelot's Galahad of Avalon.

[vi] Marley is BIS Ch Camelot's Follow the Sun, ROM.

[vii] Chip is MBISS MBIS Ch Kimani's Blue Chip Image, ROM.

[viii] The three surveys are the 1996 *RR*CUS Health and Genetics survey (1263 dogs, 11.0% ridgeless), the 2001 *RR*CUS Health and Genetics Survey Update (1763 dogs, 10.6% ridgeless), and an informal survey of US breeders that I carried out with the use of *RR*-FOLK in 1997 (1204 puppies, 8.9% ridgeless).