Report to Rocky Mountain Horse Association

Genetic Survey of the PMEL17/ Silver Mutation Which Causes Multiple Congenital Ocular Anomalies (MCOA)

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Acknowledgements:

Our understanding of MCOA results from the efforts of many people. This disorder has been a part of our breed since the beginning. When reading this report, it would be all too easy to forget that the first study happened over 20 years ago with the cooperation of many bold members of the RMHA and the RMHA itself. Rather than letting politics or popularity of their decisions paralyze them, they chose to face MCOA head on. We owe those people a debt of gratitude for doing the right thing by the horse.

This study could have stalled out before it even began if it wasn't for the dogged determination of David Swan and Steve Autry. David and Steve kept us (the genetics committee) corralled until we understood how important understanding MCOA is to the future of our breed. Steve developed the proposal and laid out the experimental design for this project, then carefully shepherded the project until it was near completion. We are fortunate that Steve is continuing to be a guiding light in our genetics committee, as we work to continue the tradition of doing the right thing by the Rocky Mountain Horse. David passed away while this study was being conducted; we will miss his leadership.

Mik Fenn kept me swimming in data from our pedigree database of which he is the expert custodian. The database is a tremendous resource. Thank you for helping put the results of this project into an appropriate context.

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Genetics Committee Members:

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Past Members and Significant Partners:

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Mik Fenn
Judy Brummer (President RMHA)
Natalie Hodges and Wittier Foundation

Drs. Kathryn Graves, Philip Sponenberg, and Gus Cothran provided crucial feedback on experimental design.

Dr. Amy Runck of Winona State University graciously reviewed and provided thoughtful comments on this manuscript and analyses.

A Note to Readers:

I have attempted to make this report as readable to the layperson as possible, with a minimum of jargon and a summary of the most important ideas you need to know at the beginning of each section. For those who "just want the facts," I have included these important ideas into the Executive Summary. The glossary is organized into topics with the more important ideas first and grouping of ideas rather than a random alphabetical list.

Glossary of Terms:

MCOA: Multiple Congenital Ocular Anomalies; a heritable eye disorder that affects both eyes of an affected horse; caused by a mutation, PMEL17, also known as the silver mutation.
PMEL17: The genetic mutation that causes the Silver Dapple/Chocolate coloration in all breeds of horses. Abbreviation for the *premelanosome protein*.
Silver mutation: the PMEL17 mutation- these horses would be silver dapple or chocolate in color.
Phenotype: The outward appearance of a horse; in this case, coat color, i.e., Silver Dapple, chocolate, bay or black, etc.; eye defects resulting from PMEL17 would also be a phenotype.
Genotype: The genetic makeup of a horse designated Z/Z, Z/N, or N/N,
Homozygous: having two of the same copies of an allele, i.e., Z/Z or N/N
Heterozygous: having two different copies of an allele, i.e., Z/N
Dominant: An allele that is expressed preferentially over other alleles, e.g., Z
Recessive: An allele that is masked in the presence of a dominant allele, e.g., N.
Z: The genetic shorthand designation for presence of a PMEL17 or Silver mutation.
N: The genetic shorthand designation for absence of the PMEL17 or Silver mutation.
Z/Z: The genotype of a horse with two copies of PMEL17, which would have a chocolate coat color and likely have eye defects.
Z/N: The genotype of a horse with one copy of the PMEL17, which would have a chocolate coat color and most often no eye defects, but could have cysts in the eye.
N/N: The genotype of a horse lacking PMEL17. These horses have two functional alleles for producing a black coat color.
Gene: A segment of DNA that codes for some aspect of a horse's phenotype, e.g., hair color.
Allele: A different version of a gene, e.g., Z or N
Mutation: An altered version of a gene, also known as an allele.

Executive Summary:

What do we know about MCOA?

1. It is 100% caused by a gene called PMEL17, which also causes the silver (chocolate) coat coloration (Andersson et al., 2013).
2. The eye abnormalities are more common and severe in homozygous silver (Z/Z) horses. These include but are not limited to: retinal detachment, retinal dysplasia, large, protrusive corneas (pop eyes), dysfunctional pupils that lack typical pupillary response, cataracts, and myopia (near-sightedness: Ramsey et al., 1999).
3. Horses that are heterozygous (Z/N) for silver typically have no major eye abnormalities, instead, they present with fluid filled cysts within the eye. Studies have not been able to determine what effect, if any, cysts have on the eyesight of the horse. Currently, they are thought to be benign.
4. To date, only myopia (near-sightedness) has been shown to be progressive component of MCOA; Evidence of which has been found in silver Icelandic horses older than 16 years (Johansson et al. 2017). Other defects have been found to be stable over the horse's lifetime (Bellone, 2017).
5. Lastly, it is important to remember that not all eye problems are caused by MCOA. Horses, just like humans, can acquire or develop eye problems.

What do you need to know about the results?

1. 63% of foals produced in 2006 and 2016 are silver in color. Color data gathered from 1986-2018 confirms this very consistent trend.
2. On average, 5.2% of silver based foals are misidentified as non-silver at the time of registration.
3. Breeders prefer to cross silver to non-silver horses. This pattern can be detected genetically. This cross is happening much more often than expected if crosses where happening at random.
4. Silver x non-silver crosses are more common in 2016 than in 2006. This shift in breeding is likely explained by more breeders crossing homozygous silver (Z/Z) horses to non silver (N/N) horses.
5. As a result of crossing silver to non-silver horses, there are fewer homozygous silver horses being produced, which means fewer MCOA foals.
6. Certainly, there are other crosses happening, but in particular, there appears to be no detectable effort to increase the number of non-silver horses in our breed.
7. These results highlight how important the non-silver segment of our breed is for future breeding efforts.

What are the most important conclusions from this study?

1. Breeders have shifted their breeding practices to avoid producing MCOA foals. However, a low percentage of MCOA foals are produced each year.
2. Trends to watch for:
    a. Are homozygous silver foals being raised and promoted as breeding stock? In other words, will the Z/Z to N/N cross become more common?
    b. Because 63% of our foal crop each year is silver, it is likely that some silver to silver crosses will continue out of necessity.
3. The production of foals has been declining over time.
4. Fewer foals produced means that quality, non-silver breeding stock will become extraordinarily rare.
5. Because non-silver horses will become rare, the current strategy of crossing silver to non-silver horses will be difficult to continue in the future.

Recommended Action on Silver Horses the RMHA Should Take:

1. Encourage voluntary reporting of silver status on registration paperwork of all foals.
2. Explore creation of a "Breeding Group," where quality foals, especially non-silver foals can be offered to other breeders prior to sale to the general public (non RMHA members).
3. Encourage breeding efforts by trail riders who own mares by offering reduced registration incentives.

Background

In a large study of 514 Rocky Mountain Horses, a group of congenital eye defects was first described in 1999 (Ramsey et al., 1999). Originally, these defects were lumped under the term *Anterior Segment Dysgenesis* (ASD). However, this syndrome of congenital defects has been re-termed *Multiple Congenital Ocular Anomalies* (hereafter referred to as MCOA) to more accurately represent the range of changes to the equine eye. It is beyond the scope of this report to summarize all of the eye defects present in MCOA, but rather, I give a brief overview (below).

Genetic testing has demonstrated that MCOA is caused by a mutation called PMEL17 located on the 6th chromosome of the horse genome (Andersson et al., 2013). It has also been demonstrated that PMEL17 is the mutation that causes the silver dapple/chocolate coloration found in horses. Since that original 1999 study, MCOA has been found in several more breeds (e.g., Kentucky mountain saddle horse, Mountain pleasure horse, Icelandic horse, Exmoor pony, Belgian draft horse, Shetland pony, American miniature horse, Comtois, Quarter horse, Morgan horse and any other breed that inherited PMEL17 mutation from its ancestors; Bellone, 2017) The widespread distribution of this gene in different breeds suggests that is has been present in the gene pool of horses since ancient times (Andersson et al., 2011).

The genetics of MCOA behaves in an incomplete dominant fashion—in other words, there is a gradual effect of the gene, depending on how many copies a horse possesses. Horses sort into three categories (affected, cysts only, normal). The letter Z is used as genetic shorthand for the presence of the PMEL17 mutation. The letter N is used for horses with a functional allele. That means that animals that are homozygous for the PMEL17/silver mutation (Z/Z) are affected with pronounced eye defects. Horses that are heterozygous for the silver mutation (Z/N) have only been found to have fluid filled cysts. Horses that lack PMEL17 (N/N) have normal eyes.

It is important to remember that while MCOA accounts for a range of eye defects, it is distinct from several other eye problems commonly found in horses. Common eye disorders that are NOT associated with MCOA include: primary glaucoma, equine recurrent uveitis, other forms of megaloglobus and acquired retinal detachment (among others; Premont and Knott 2020).

What do we know about MCOA?

1. It is caused by a gene called PMEL17, which also causes the silver (chocolate) coat coloration (Andersson et al., 2013).
2. The eye abnormalities seen in MCOA are more common and severe in homozygous silver (Z/Z) horses. These include but are not limited to: retinal detachment, retinal dysplasia, large, protrusive corneas (pop eyes), dysfunctional pupils that lack typical pupillary response, myopia (near-sightedness) and cataracts (Ramsey et al., 1999).
3. Horses that are heterozygous (Z/N) for silver typically have no major eye abnormalities, instead presenting with cysts within the eye. Studies have not been able to determine what effect, if any, cysts have on the eyesight of the horse.

4. To date, myopia (near-sightedness) has been shown to be the only progressive component of MCOA. This is true at least in heterozygous silver Icelandic horses older than 16 years (Johansson et al. 2017). Other defects have been found to be stable over the horse's lifetime (Bellone, 2017).
5. Lastly, it is important to remember that not all eye problems are caused by MCOA. Horses, just like humans, can acquire or develop eye problems.

Goal of this study:

By surveying for the presence of PMEL17 in our herd, we can determine how often MCOA foals are being produced, what typical crosses breeders appear to be conducting, and how these two factors are changing over time. *It is imperative to understand that while we are using a genetic survey that can be used to determine color, we are primarily concerned with what effect this is having on the eye of the horse.*

Methods

Genetic Survey for PMEL17:

The RMHA genetics committee teamed up with Dr. Kathryn Graves at the University of Kentucky to survey the presence of the silver mutation (PMEL17) in our herd of Rocky Mountain Horses. We selected horses registered in 2006 and 2016. Most of these horses were foals from that year, with some horses being registered when two years or older (e.g., born in 2004 or 2005 but registered in 2006). For simplicity's sake, I termed all horses registered in a given year as "foals", since the majority were foals. In 2006, 1149 horses were available for study, while only 501 horses were available for study from 2016. Dr. Graves extracted DNA from hair samples that were submitted for registration with the RMHA. The DNA was genotyped using a technique called Single Nucleotide Polymorphism or SNP. A fluorescently marked probe is designed to bind to DNA sequences located near the mutant PMEL17 allele (Z) or the normal allele (N). Each hair sample was tested twice to ensure that no errors in genotyping were made. Each horse was categorized according to their genetic makeup (i.e., Genotype). For example, non-silver horses, such as black or bay, were categorized N/N, single silver gene carriers (chocolate horses) were assigned Z/N, and Z/Z was assigned if they had two copies of the silver dapple gene.

Color phenotypes were able to be generated from the genotypic data without needing to reference pictures. For the purposes of this survey, we lumped all horses into silver (e.g., chocolate, red chocolate, etc.) vs non-silver (e.g., black, bay, roan, etc.) horses and ignored any other genetics of color.

Using the observed allele frequencies derived from the genotypes, we were able to calculate the "expected genotype frequencies" using the Hardy-Weinberg formula ($p^2+2pq+q^2=1$); where p=Z and q=N. Given the allele frequencies for a population, the Hardy-Weinberg formula will generate what genotype frequencies we would expect if all breeding of

horses was random. In other words, if all mating between horses was random, the herd size was very large, no genotypes were preferred over others, there was no mutation of the DNA and there was no gene flow from other breeds into our breed, then we should find that the proportion of genotypes (Z/Z, Z/N, N/N) we observed in our herd should match those calculated by the formula. I compared the expected genotype frequencies generated from the formula to those observed in our dataset and was able to statistically compare them using a Chi-square Analysis (α=0.05). I employed Bonferroni corrections to the alpha level (α =0.0167) on all repeated Chi-square analyses; it did not change the interpretations of significance of any test.

Data Collection from the Pedigree Database:

Additional data on silver foals and foal crop size was collected from the RMHA pedigree database by Mik Fenn and myself. Because the database is constantly being updated with newly registered horses, there is the possibility that if you search the database today, it might yield slightly different results than those presented here. I present these statistics not to definitively report the absolute number of foals produced on a given year, rather to illustrate the overall trends of color production and foal crop production.

Results:

What do you need to know about the results?

1. 63% of foals produced in 2006 and 2016 are silver in color. Using the pedigree database, color data gathered from 1986-2018 confirms this very consistent trend.
2. Breeders prefer to cross silver to non-silver horses. This pattern can be detected genetically. This cross is happening much more often than expected if crosses where happening at random.
3. Silver x non-silver crosses are more common in 2016 than in 2006. This shift in breeding is likely explained by more breeders crossing Z/Z horses to N/N horses.
4. As a result of crossing silver to non-silver horses, there are fewer homozygous silver horses being produced, which means fewer MCOA foals.
5. Certainly, there are other crosses happening, but in particular, there appears to be no detectable effort to increase the number of non-silver horses in our breed.
6. These results highlight how important the non-silver segment of our breed is for future breeding efforts.

Phenotype (Color) results:

Using the genetic data to predict phenotype, we found that the percent of silver (chocolate) and non-silver horses has not changed during the 10-year time period of this study. In 2006, 63% of the horses were silver and in 2016, 62.5% of horses were silver. Similarly, in 2006, 37% were non-silver and in 2016, 37.5% were non-silver (Figure 1). Essentially, there is

no difference in proportion of phenotypes between the years. While not much seems to have changed with the colors of horses, there are significant trends happening genetically (Figure 5).

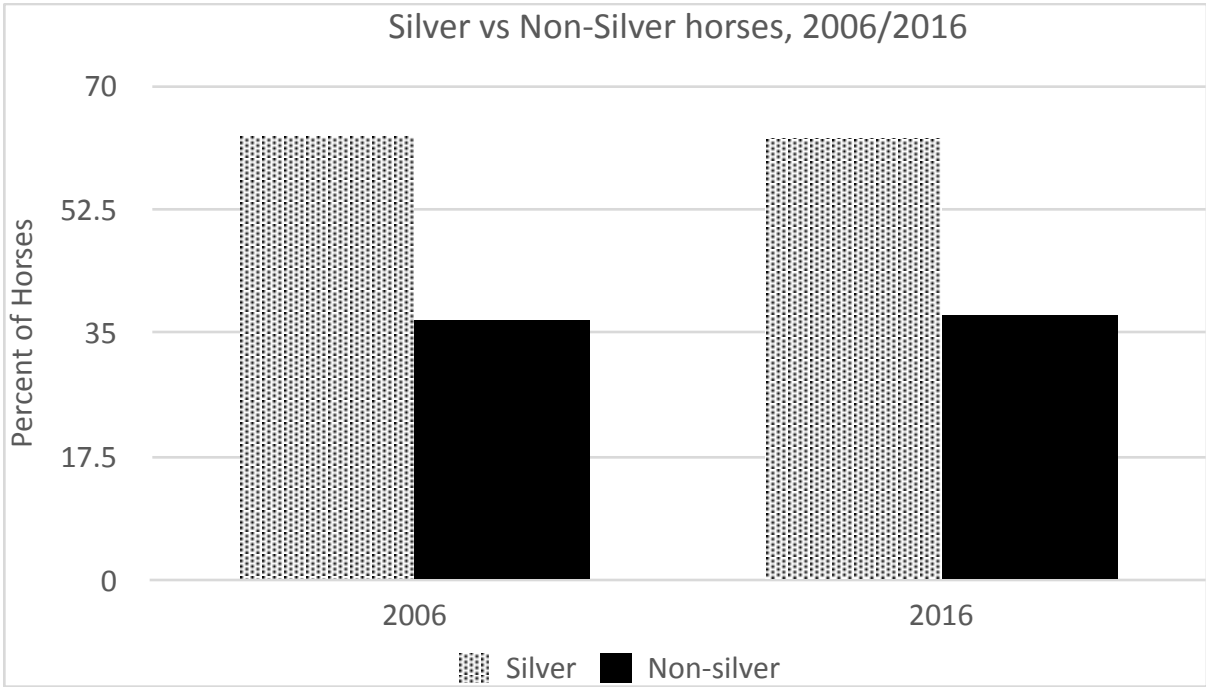

Figure 1. Comparison of phenotypes of foals produced in 2006 vs 2016. These data were generated from the genetic data. Bars of silver horses are stippled, non-silver horses are in black. The proportion of foals produced is virtually identical.

Color of horses over time, from RMHA Pedigree Database.

If we turn to the database, we can fill in the gaps between these years we were not able to sample using the genetics survey (Figure 2). The database underestimates the number of silver-based horses slightly, when compared to the genetic data we collected. For example, in 2006, 59% of horses from the pedigree database were silver, whereas 63% of horses sampled in the genetics study were silver based. Similarly, in 2016, 56% of horses from the pedigree database were silver, whereas 62.5% of horses sampled in the genetics study were silver based. On average, our registration process misidentifies 5.25% of silver horses as being non-silver. This is not too surprising, given that some chocolate horses can be very dark and hard to identify, especially as foals. What is interesting is how consistent the proportion of silver foals has been over the years: it hovers right round 60%. From conversing with other breeders, there is some disagreement on the proportion of silver horses early in the formation of the RMHA, as in their memory, it was much lower than 60% (S. Autry, personal communication). Indeed, when we look at the database, the first year of registration only 38% of horses were silver. However, some caution should be exercised when interpreting the early years when foal production was in the low 100's.

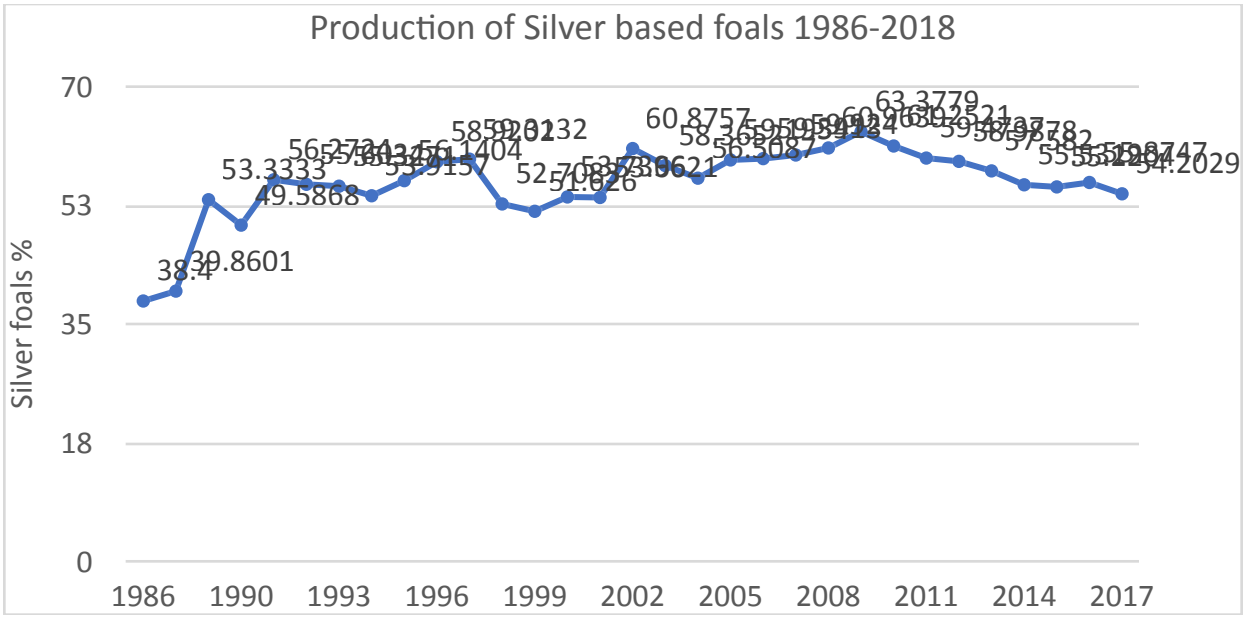

Figure 2. Proportion of silver foals from 1986 (first year of the registry) to 2018, data collected from the RMHA pedigree database. Numbers above and below lines indicate the percentage of silver foals on peak (2009) and low (1986, 1999) years.

Genetics results:

When we look at the genetic data, we can start to understand:

1. The breakdown of the genotypes that result in 63% of the foals being silver in color.
2. The types of crosses that produce these results.
3. How many foals likely have MCOA.

In 2006, 11.4% of foals were homozygous silver. In terms of actual numbers—this was 130 foals. Heterozygous silver horses represented 51% of the foal crop and non-silver horses represented 37.3% of the foal crop. By looking at the genetics, we can see what is happening to the 62.5% of silver colored horses—most are heterozygous, but a good number of them are homozygous silver. Overall, these proportions are significantly different from random (X^2=11.86, p=0.0026).

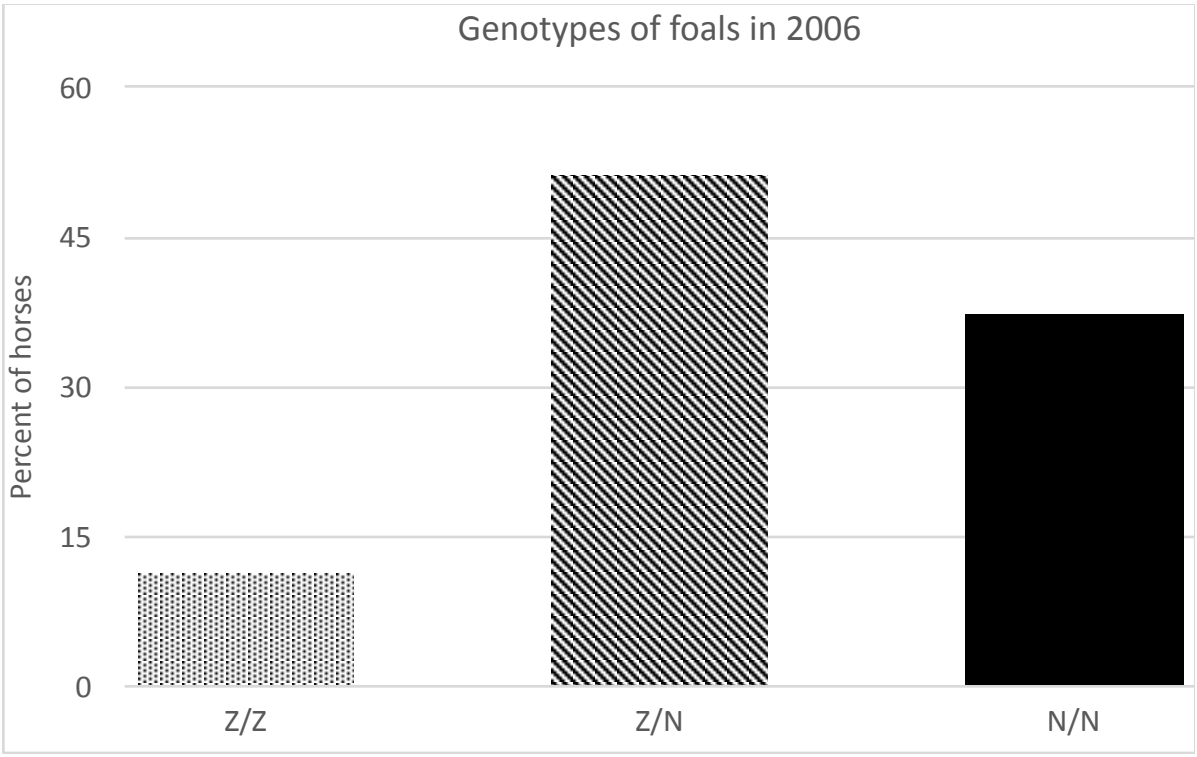

Figure 3. Comparison of genotypes of foals produced in 2006. Homozygous silver (Z/Z) is stippled, heterozygous (Z/N) is slashed, and non-silver (N/N) is in black.

In 2016, the overall proportion of non-silver foals is similar to 2006 (37.5% are non-silver). However, there has been a subtle, but significant shift in our silver foals: 7% are homozygous silver, 55% are heterozygous silver. Relative to 2006, there are fewer homozygous silver horses, slightly more heterozygous horses and no change in the proportion of our non-silver horses. These proportions are highly statistically significantly different from what would be expected from random breeding (X^2=22.49, p=0.000013). There are 4.8% fewer homozygous silver horses than would be expected at random, 9.6% more heterozygous horses than we would expect and 4.8% fewer non-silver horses than we would expect. This is good news, breeding trends are reducing the number of homozygous silver foals produced. However, the production of our non-silver horses has not changed; we need to consider what effect this has for breeding in the future. Overall, it probably isn't very surprising that our breeding trends are different from random breeding. Later, we will explore what types of crosses breeders appear to be employing.

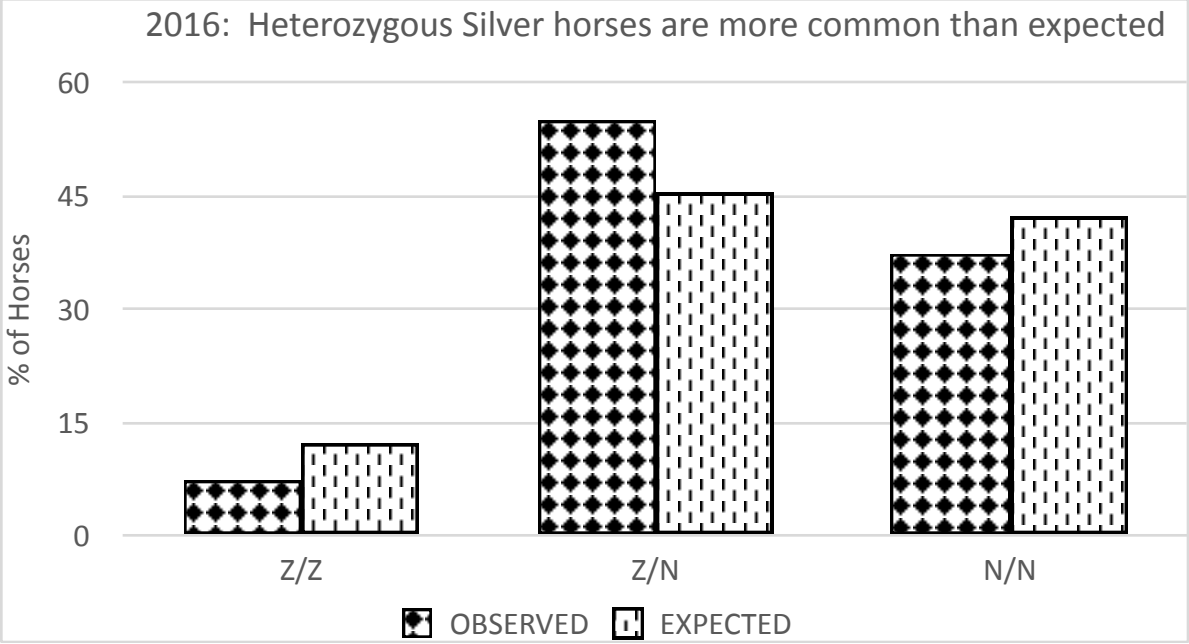

Figure 4. This graph compares the observed genotype ratios in 2016 to what we would expect the ratios to be if breeding was occurring randomly. Checkered bars are the observed genotypes generated from SNP DNA data and dashed bars are the expected genotypes generated by the Hardy-Weinberg formula. Statistical analysis shows that there are more heterozygous silver animals being produced than we would expect. There are also fewer homozygous silver foals and fewer non-silver foals.

If we compare 2006 to 2016, can directly observe the shift in the genotypes within the silver colored foal crop- with fewer homozygous silver horses being produced and more heterozygous silver horses being produced. In order to find out if this result was significant, I statistically compared these two years. Specifically, I used the observed genotype ratios of 2006 to generate the expected genotype ratios for 2016. In other words, are breeders in 2016 conducting the same types of crosses as they were in 2006? The answer is NO; they are statistically different from each other (X^2=8.49, p=0.014). What this means is that collectively, breeders appear to be changing the types of crosses they perform, with a distinct shift away from producing homozygous silver (i.e., MCOA) foals.

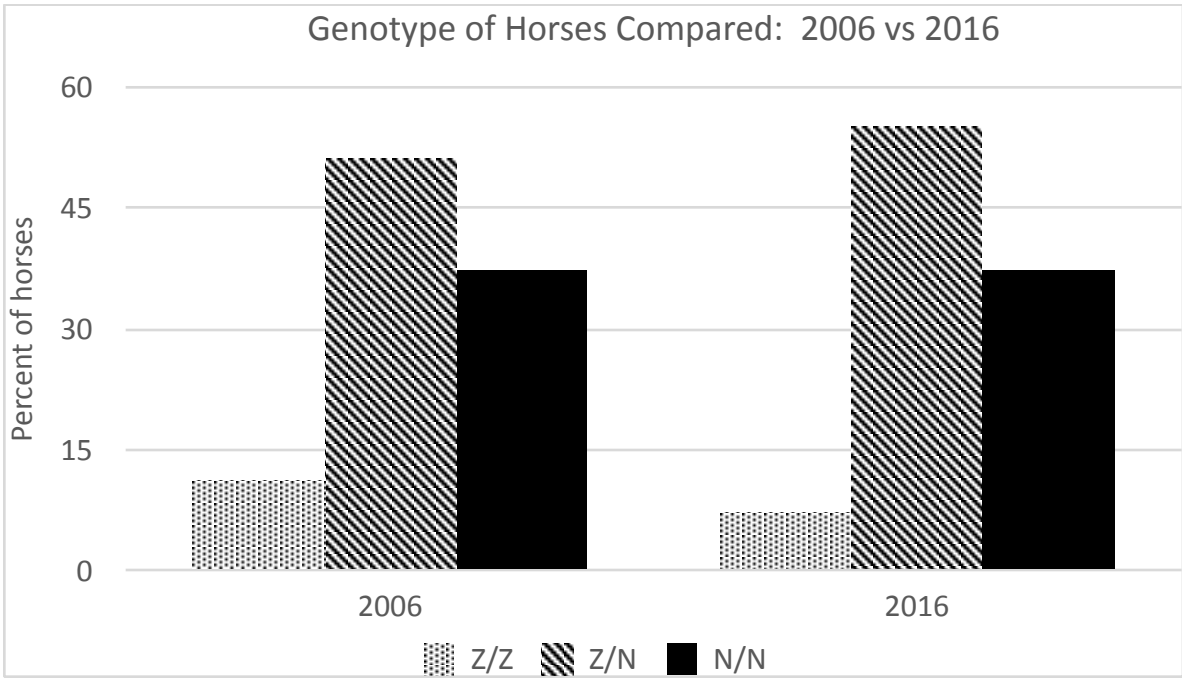

Figure 5. Observed proportion of genotypes the 2006 vs 2016 foal crops. Genotypes were determined from SNP data. Homozygous silver (i.e., likely MCOA) horses are stippled, heterozygous silver (i.e., likely cysts only) horses are slashed and non-silver horses are in black. Note: there are fewer homozygous silver horses in 2016, and more heterozygous silver horses as well.

Results: What types of crosses to most breeders appear to be making?

What we need to know is: how have breeding trends changed? What crosses do breeders appear to be conducting? As a starting point, I considered two crosses: Heterozygous silver to Heterozygous silver (Z/N x Z/N) and Heterozygous silver to non-silver (Z/N x N/N). I chose these two crosses because it seemed like the simplest way to account for our current genotypic data, and anecdotally, they appear to be the most popular crosses. Certainly, there are breeders conducting the other crosses: non-silver to non-silver, homozygous silver to non-silver, and homozygous silver x silver. Given the way we designed this study, there is no way to 100% separate out all these crosses and see how common they are. Rather, I was interested in answering the question: Which crosses appear to be the most common?

I generated the expected data from the proportions of foals you would expect from each of the two crosses (Z/N x Z/N: 25% Z/Z, 50% Z/N, 25% N/N and Z/N x N/N: 50% Z/N, 50% N/N). Further, I assumed that these two crosses were employed 50% of the time and accounted for 100% of the foal production.

When I compared these expected ratios to the 2006 genotype dataset, and there was no significant difference (X^2=1.72, p=0.42). We can roughly interpret this to mean that these two crosses account for most of the foal production in 2006. Interestingly, I ran the same test against the 2016 data, and the genotype data for that year was significantly different from the expected ratios (X^2=13.08, p=0.0014). Exactly what is going on is hard to nail down. However, here is an educated guess:

1. The proportion of non-silver foals has not changed over time. This indicates that the pairing of Z/N to N/N horses has not changed appreciably (as 50% of their foals would be non-silver and tend to raise the proportion of foals above 37%)
2. The number of crosses that produce homozygous silver foals has gone down significantly. There are fewer Z/N x Z/N and Z/Z to Z/N crosses, etc.
3. The number of heterozygous foals has increased.
4. The only way to account for an increase in Z/N foals above 50%- without increasing the N/N foals is by crossing a homozygous silver horse to a non-silver horse. In other words, there is likely an increased number of homozygous silver breeding stock being used as time goes on.
5. What is the effect, if any, of using a homozygous silver horse as breeding stock on a foal's eyes? We simply don't know.
6. The important common denominator to remember here is that non-silver horses are necessary to achieve both of these forms of crosses.

Discussion

What do you need to know about the discussion?

1. Breeders have shifted their breeding practices to avoid producing MCOA foals. However, a low percentage of MCOA foals are produced each year.
2. Trends to watch for:
    a. Are homozygous silver foals being raised and promoted as breeding stock? In other words, will the Z/Z to N/N cross become more common?
    b. Because 63% of our foal crop each year is silver, it is likely that some silver to silver crosses will continue out of necessity.
    c. Non-silver stock out of Z/N to Z/N crosses are rare, potentially important breeding stock.
3. The production of foals has been declining over time.
4. Fewer foals produced means that quality, non-silver breeding stock will become extraordinarily rare.
5. Because non-silver horses will become rare, the current successful strategy of crossing silver to non-silver horses will be difficult to continue in the future.

Overall, we now have concrete evidence that breeding has shifted away from crosses that would tend to produce foals that suffer from MCOA. Our analyses indicate that silver-silver crosses are becoming less common and silver to non-silver crosses are becoming more common. Specifically, it is most likely that heterozygous silver to non-silver (Z/N to N/N) and homozygous silver to non-silver (Z/Z to N/N) are the most common crosses today. Both crosses would eliminate the chances of obtaining an MCOA foal. It is important to consider that *these two crosses work well when there is a large annual foal crop* where many N/N horses are available as future breeding stock. Silver to silver crosses are still happening at a low level and we still see homozygous silver foals being produced. There will be two trends to keep an eye on in the future. First, is there evidence that homozygous silver foals are intentionally being produced and promoted as breeding stock to continue the trend of crossing Z/Z to N/N? Second, because 63% of our foal crop each year is silver, it is likely that silver to silver crosses will continue out of necessity. To understand what this signifies, we need to explore what this means in light of recent population trends in Rocky Mountain Horses.

Context of Genetic Results: How our breeding trends need to change.

It is important to recognize that the steady trend of 60-63% of our herd being silver should be evaluated in the context of the size of our overall herd. The nucleus of actively breeding Rocky Mountain Horses has never been very large. Further, given the recent economic events surrounding the pandemic of 2020, the trend in foal production does not seem likely to reverse. In the past, when foal production was over 1000 foals a year, finding quality stock was relatively easy. Furthermore, because of the abundance of non-silver horses produced, the

practice of breeding silver to non-silver horses was a successful strategy for preventing MCOA foals. However, our foal crops have been declining for nearly 20 years now.

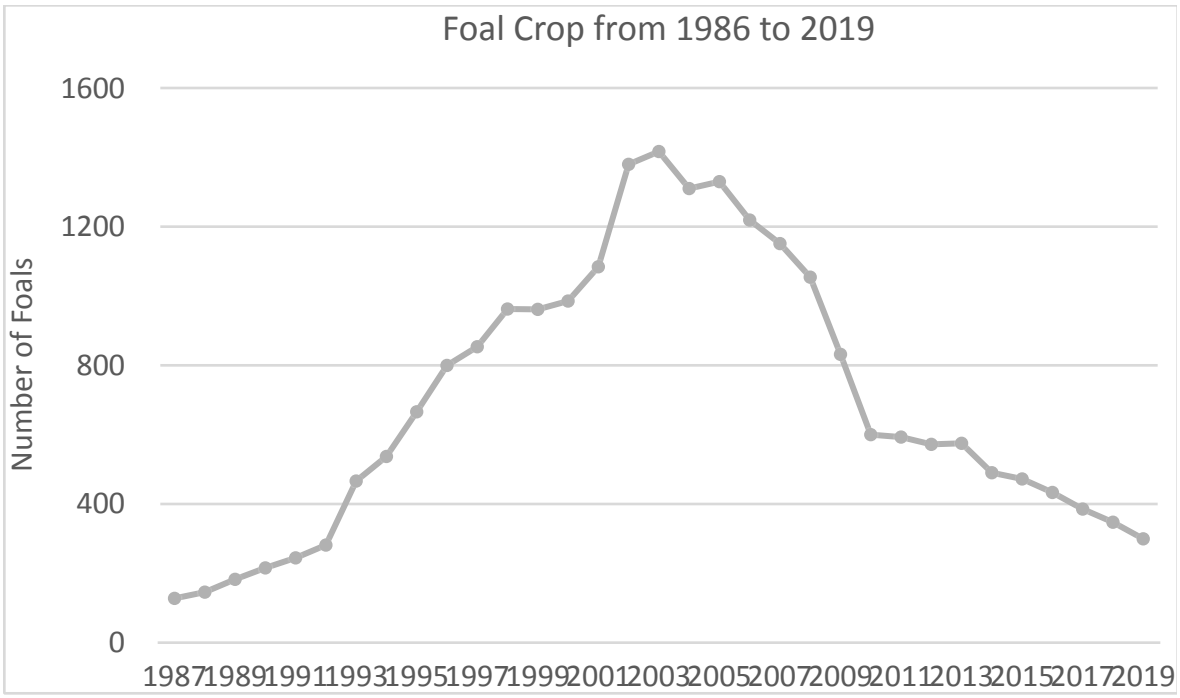

Figure 6: Number of foals registered by year (1986-2019), data pulled from the RMHA pedigree database. Production peaked in 2003, with 1416 foals produced. Production dropped prior to the Great Recession and has been on a slow, yet steady decline since 2010. Average foal production from 2009-2019 was 455 foals per year.

The Effect of Low Foal Crops on Available Breeding Stock:

Let's explore a hypothetical scenario so we can understand what this means for breeding Rocky Mountain Horses in the future. If we use the average number of foals produced (455 foals) per year, we can expect 50% of those to be male/female. However, not every foal produced will be suitable as quality breeding stock. Granted, quality is difficult to define and measure. We're thinking about temperament, gait, and conformation. Further, we all have our own opinion of what a high-quality foal is or is not. I'm not here to debate any particular definition of what a quality horse is—however, I think we can all agree that there are great horses and horses that don't meet quality standards.

If we think statistically about the law of averages, we should expect that roughly 50% of the foals produced by a horse will be better than their parents (and ancestors), and roughly 50% will be inferior relative to their parents/ancestors (Bennett 1988). If we look to nature as a guide, around 60% of mares and stallions do not reproduce or maybe produce one foal in the wild. It is the top 10% of mares that carry a foal every year and only 7% of the stallions that produce many of the foals (Berger 1986). Of course, we can all point to horses in our breed that seem to

produce better than themselves and are renown for their top-quality foals. For example, anyone sufficiently familiar with our breed's history can name of a few of the famous mares that produced several outstanding sons that later became great stallions. But we are all also aware of many horses that never produce foals as good as themselves and a great many mares that never produce a stud quality colt.

Returning to the law of averages, a great many of these foals will be around that 50% mark; they are an average foal. What we should be hoping for is that our breeding stock, our mares and especially our stallions, is better than average. For illustrative purposes, I assumed that the top 50% of mares would be kept back and certified as breeding stock. For stallions, I adopted the old horseman's rule of thumb that a stallion should be in the top 10% of the foal crop. Below you can see a table that walks us through this hypothetical foal crop. In it, I assume that 63% of the foal crop will be silver based, as our genetic survey suggests.

455 foals

228 fillies	**227 colts**
114 mares (top 50%) certified	**23 stallions (top 10%) certified**
Of these mares:	**Of these stallions:**
72 will be silver	**14 will be silver**
42 will be non-silver	**9 will be non-silver**

These are sobering numbers. All of the genetic data we gathered suggests that breeders are indeed preferring to cross silver horses to non-silver horses in an effort to avoid any complications with MCOA. However, because of the decline in our foal crops we are dramatically limiting our ability to continue to use this strategy in the future. There will be too few quality non-silver mares and stallions produced and that will limit our breeding options. *What this means is that many breeders will be forced with a very tough decision: breed silver to silver and risk an MCOA foal; or breed to an inferior quality, non-silver animal.* Clearly, something needs to be done to promote and certify quality non-silver horses for the future of our breed.

Someone might want to argue that this hypothetical scenario is unrealistic. Granted, I was concerned only with biology, I did not take into account any economic or other factors. However, in this scenario, I estimate that, on average, 30% of the foal crop would be certified to breed. If we look to the pedigree database, in 2006 (one of our peak years of foal production with 1218 foals registered), 36% of the foal crop was certified to breed. I also have some numbers from the 2016 foal crop, when 13% was certified to breed.

Whether you agree or disagree with this hypothetical analysis, one thing is true: the current method of crossing silver horses with non-silver horses can only happen if we have quality non-silver horses available. Declining foal crops mean that there will be fewer non-silver foals available in the future. At some point, this cross will become self-limiting—that is to say there simply won't be enough non-silver horses to breed to. If we begin to take action now, to

actively work to increase the number of non-silver foals, we can prevent this from becoming a problem in the future.

Recommendations:

Possible Management Strategies for the Future:

1. Place the Silver genotype status on all foals' papers at time of registration.
2. Develop differential registration fees for homozygous silver horses resulting from a silver to silver breeding.
3. Education: Develop a comprehensive article and infographic on Silver Dapple and MCOA. Place on RMHA website, handed out at horse expos, available to public, included with registration paperwork, stallion owners can make available to prospective mare owners etc.
4. Develop a breeder's group open to RMHA members on the website, which can offer quality non-silver foals to other breeders first, as these foals have the most value to RMH breeders. This would increase visibility of these foals and likely increase their monetary value as well.
5. Explore ways to incentivize breeding among horse owners whom do not regularly breed (e.g., trail riders). This would expand the effective breeding population, aid in loss of genetic diversity and increase the number of outside breedings for stallion owners.
6. Increase promotion of the other aspects that make Rocky Mountain Horses popular: Gait, Temperament, Versatility, to reduce perception that it is a color breed.
7. Develop a series of pictures on the "Ideal Stallion", "Ideal Mare", "Ideal Gelding" and "Ideal Gait."

The genetics committee reviewed (and will continue to review these recommendations, and others as we develop them) prior to making a final report to the RMHA Board. However, we did conduct one vote and so have a recommendation thus far—"The genetics committee recommends having owners voluntarily placing results of Silver Dapple testing on their registration papers (any non-silver horses: black, bay, etc. would be exempt)." This motion received unanimous approval.

Recommended Action on Silver Horses RMHA Should Take:

1. Encourage voluntary reporting of silver status on registration paperwork of all foals.
2. Explore creation of a "Breeding Group," where quality foals, especially non-silver foals can be offered to other breeders prior to sale to the general public (non RMHA members).

3. Encourage breeding efforts by trail riders who own mares by offering reduced registration incentives.

Future of Genetic Testing in Our Herd:

Over the past few years, the RMHA as conducted three genetics projects. One was a widespread survey of the genetic diversity of our herd and two studies targeting specific genetic mutations found within our herd. The genetic diversity surveys are essential to protecting the integrity of our breed and the results of these surveys are largely beneficial. However, while targeted genetic testing for specific mutations has benefits, it also incurs substantial costs. Therefore, it is worth thinking about the general benefits and costs of these tests in a general sense.

Genetic testing is a powerful tool, but its use should be weighed very carefully. There are three main ways we use genetic testing in human and veterinary medicine. In this section, I argue that we should employ the first two, and as much as possible, avoid the third form of genetic testing.

1. Diagnostic genetic testing
2. Carrier genetic testing
3. Predictive genetic testing

Diagnostic genetic testing:

Diagnostic genetic testing is when a horse develops outward disease symptoms and a veterinarian performs a genetic test to aid in diagnosis and treatment of the animal. This type of genetic testing is beneficial and may be necessary for the lifelong care of the animal. Using genetic tests for diagnosis should be in the toolkit of every horse owner. It is important to remember that in this case, the disease (i.e., phenotype) is noticed first, then we apply a genetic test to understand the cause of the disease.

Carrier genetic testing:

Carrier genetic testing is used to determine the genotype of a healthy individual, with no known outward disease. It is important to notice that these tests can conducted without any knowledge of the phenotype, we're just interested in the gene itself. The RMHA's surveys for the PMEL17 and PSSM1 mutations fall into this category. Here, we were randomly selecting individuals to understand how common these mutations were in our gene pool. In the course of these studies, we made no attempt to diagnose any diseased state in an individual. More importantly, the results are reported anonymously, and no individual horse is identified. We have been interested in understanding how common these mutations were in our herd, if they are becoming more or less common and then deciding what we should do about those trends. Conducted in this fashion, these genetic surveys are largely beneficial, and the social costs of these tests are not a burden on any one or group of owners. However, there can be the wrongheaded concern that if it is uncovered that a particular mutation is common in a breed, then that breed is somehow inferior.

Predictive genetic testing:

Predictive genetic testing is used when an otherwise healthy individual is tested to determine if they might develop a disease later in life. This type of genetic test is done with no evidence of a disease process or perhaps with only limited knowledge that the disease runs in the family. It is important to note that here, we're looking at the gene first then the phenotype—the opposite of diagnostic genetic testing. From a general standpoint, we might seem as if this as the most powerful use of genetic testing, because it holds the possibility of preparing for the problem before it arrives. However, genetic tests have limited predictive power and can also incur an equally large cost. Let's take an example from human medicine. It is fairly common to test for one of the genes that causes breast cancer. For some, knowing they have a gene that might cause breast cancer might lead to greater surveillance later in life, or perhaps a preemptive surgery, in the thinking that it might prevent cancer. For others, this knowledge can lead to life-long anxiety over the possibility that they might develop cancer. For a healthy person with this gene, a surgery is likely unnecessary and costly, with no guarantee that it would prevent cancer.

Predictive genetic testing was employed by several owners after the initial discovery of PSSM1 in our breed, to dramatic consequences. Horses that were outwardly healthy their entire lives, had never displayed symptoms of PSSM1, but were related to horses that developed PSSM1 were tested. Some of these horses tested positive and overnight their value as breeding stock or riding stock plummeted. However, *nothing about the horse had actually changed.* It was the same horse before and after the test.

Overall, it is my recommendation that predictive genetic testing be avoided by the RMHA. What we should care about most is the outward health- the phenotype- of our horses. Certainly, genes do affect the health and phenotype of our horses, but genes do this by interacting with other genes and innumerable environmental factors. For these reasons, genes often have limited predictive power and as we have seen, a predictive genetic test has tremendous costs.

Recommendations for Future Efforts of the Genetics Committee and RMHA:

1. *Develop a position statement on the use of genetic tests and a policy to guide our response as new genetic tests emerge.* As time goes on, more and more genetic tests will be developed. As new genetic tests are employed in the process of diagnosing horses, and previously unknown traits are uncovered within our herd, the public perception becomes that "the RMHA should do something about this." I think it would be useful to get out in front of these issues before they arise, develop a position statement and make it available somewhere highly visible on the RMHA website.
2. *Newly developed genetic tests could be reviewed and evaluated in advance of finding a genetic "defect" in our herd.* Similar to above, a short one-page educational handout could be developed and made available to inquiring owners, regardless of if the trait is found in the RMH or not. This would allow us to get out ahead of any diagnostic testing done by individual owners. When they turn to the RMHA with questions, we can supply them with our own educational materials. In doing so, we can perhaps, mitigate any future "genetic panics."

3. *Conduct a biannual "State of Herd" Report for membership.* We need to be kept up to date on breeding trends, foal production, popular stallions, genetic and phenotypic surveys on a regular basis. We have a fantastic database that Mik Fenn put together. We have committees surveying owners for traits found in their horses (e.g., horse height), we should capitalize on all this work, and communicate those significant efforts to our membership.
4. *Develop a series of pictures on the "Ideal Stallion", "Ideal Mare", "Ideal Gelding" and "Ideal Gait."* The genetics of our herd is the result of selection on the phenotypes of our horses. Without a clear communication of what those phenotypes should be, some educational materials to help new owners recognize horses above and below average, our small breed will experience genetic drift until it is no longer recognizable.

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