PURPOSE AND METHODS FOR THE MORGAN FLAXEN COLOR RESEARCH STUDY

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Overview

The manes and tails of some horses of the Morgan breed show a lighter color than the body coat color, analogous to the blond trait in humans. These horses with very light hairs in the mane and tail are said to have the flaxen trait. Our preliminary findings were based on simple classification as flaxen if the horse's mane or tail had primarily light hairs which are primarily lighter than what is the primary body color of the chestnut horse. Our pedigree analysis (available online for horses in our data set) will help breeders to determine the likelihood of being able to produce flaxen by their planned matings.

The genetic mechanism which produces the flaxen color trait in Morgan horses is not yet understood. Several possible hypotheses have been published. Methodology herein described has been developed to quantify the degree of the trait from color photographs .

We have collected a data set including approximately 375 inter-related Morgan horses, many of which show the flaxen trait. Our objectives are to: (1) produce a rigorous data set for DNA analysis, (2) to compare the observed visual expression of the inheritance patterns with predictions from simple Mendelian genetics as well as more complex inheritance models, and (3) to identify the most parsimonious explanation for the mode of flaxen inheritance in this group of horses. One interesting finding early in the study is that for the 17 flaxen sires and 38 flaxen dams, all of their 121 offspring are flaxen with the exception of 3 non-flaxen offspring from one deceased sire whose flaxen phenotype is anomalous (photographs showing him flaxen but becoming non-flaxen as he aged). This preponderance gives a strong likelihood that the flaxen trait is the result of a homozygous recessive genotype, under the preliminary assumption of simple Mendelian inheritance within this group of horses.

The pedigrees reveal two non-flaxen horses that produced only non-flaxen offspring with a flaxen mate, indicating that these two horses likely have homozygous non-flaxen genotypes. The pedigrees also include three non-flaxen horses having only non-flaxen parents and grandparents. These horses with presumptively "known" genotypes, deduced from visual expression of the inheritance patterns, may be good candidates for DNA analysis.

The pedigrees, mating node graphs, legends, and reports are viewable online at http://www.thesokolows.com/colorresearch/pedigrees . The Mozilla Firefox 2 web browser is recommended for viewing the graphics.

INTRODUCTION

Breeding horses for color patterns, without regard to genetic problems, conformation, and disposition, can result in undesirable traits pervading the breed. Morgan horses are not a "color" breed, nevertheless the emergence of the Rainbow Morgan Horse Association[1], which highlights "colorful" Morgans, is a testimonial to the recent popularity of certain color patterns in the Morgan breed. One of these desirable color patterns is flaxen manes and/or tails. The purpose of this research project is to produce useful flaxen breeding statistics for Morgan breeders, and to prepare a data set which may be useful for subsequent molecular research to find the DNA marker(s) for the flaxen trait in the horse genome.

It is not known whether the flaxen trait in Morgan horses is controlled by a single or by multiple genes, or by a body color modifier gene so that the color of the body hairs are lightened when distributed to the mane and/or tail. It is not known whether the trait is recessive, however visual expression of the flaxen trait in pedigrees of flaxen horses suggests that it is. The study described here has been designed to confirm the probable inherited expression of the flaxen trait in the Morgan horse breed.

DNA markers for the flaxen trait are not yet identified. However through pedigree analysis of horses in our data set, likely expression of the flaxen trait can be predicted. For example, a particular bay stallion mated with a particular black mare has consistently produced flaxen offspring, as is shown in an illustration herein.

MATERIALS AND METHODS

This study's data collection and organization relied heavily upon the collaborative nature of Yahoo Flickr Photo Albums (http://www.flickr.com), Google Spreadsheets (http://docs.google.com), and Internet access to data sets on personal websites (such as http://www.thesokolows.com). While the first 5 years of photo collection and organization relied primarily on emails, email photo attachments, color printers, website browsing, and internal spreadsheets, by the 6th year, photos were relatively easily uploaded onto the Internet and the spreadsheets were updated with almost-daily online collaborative communications. Without the ease of viewing and editing the same documents online by several collaborators, it is doubtful that this study would have been completed. The methods were developed one step at a time, objectively and clearly documented with typed instructions distributed either online or to internal staff so that computer input and output flowed easily and reliably. An outline of the research methods is summarized here.

1. Obtain a sample of digital photos of horses meeting the study population criteria. These photos were of registered Morgan horses with chestnut body color, some of which have flaxen manes and/or tails. The photos were uploaded into a Flickr album, organized in sets by owner at the time the photos were obtained.
2. Create a spreadsheet to record observations and upload the spreadsheet to Google Docs for collaboration on the Internet.

1 http://www.**rainbowmorganhorse**assoc.com

3. Select a subset of photos having varying degrees of flaxen color trait, including non-flaxen, for method validation testing.
4. Two observers (one being principal investigator Sokolow and the other being horse owner Hanley) recorded their rating of horse body color, mane color, and tail color in categories of light, medium, dark, and non-flaxen, and light, medium, and dark chestnut body color. Comments on complications or variations were also recorded.
5. As a pilot study, two other observers viewed the validation sample and measured the brightness of color at 3 representative points in each of the horses' mane, tail, and body. This measurement was done using the "B" (brightness) value in the "HSB" tool[2] of Adobe Photoshop version 5.0LE. The spreadsheet computed the average of the 3 values for each body part. The difference between the tail average value and body average value for each horse was computed as a measure of the degree of lightness of tail color relative to body color. The mane was similarly compared with body. The average of these mane and tail relative lightness measures was used as the measure of "flaxenness" of the horse.
6. Statistical tests were done to determine the variation between observers within the objective (computerized) method. Also, a replication of observations by one observer using additional photos of each of 4 horses were made and analyzed to determine between-photo variation. After this analysis and further replications with more data points from each observer, per horse, it was decided that 10 points per body part (mane, body, tail) per horse would be measured and that the average of those 10 points would be used without further averaging across body parts, so that within-horse variations could also be studied. To eliminate between-observer variation and bias, only one observer would make all of the photographic measurements.
7. A computer program ("Pedigree Draw") was written to display pedigrees of each horse supplemented with offspring and siblings of the proband, to produce a comprehensive mating node graph, and to summarize flaxen and non-flaxen mating outcomes. In addition, a program ("Color Capture") was written to allow the rapid capturing of the color measurements from multiple points on the photos of the horses. The programs can be modified to produce output which is in a format required by other existing computer programs for genetic analysis. The output of these programs was uploaded to the study's folder on a web site for easy viewing by the collaborators on the project.
8. Lists of "Interesting Horses", both flaxen and non-flaxen were created. The criteria for including a particular horse is these lists were based on both ancestry and progeny, as illustrated in the computer-generated pedigrees.
9. Data sources for pedigrees were American Morgan Horse Association (AMHA) online registry and www.allbreedpedigree.com online services.
10. Occasional meetings at UC Davis VGL provided focus for further data needed.

2 "HSB" is a scheme for representing colors using numerical values of hue, saturation, and brightness, sometimes called HSV, where V is "value". The "B" brightness or "V" value was used in this study. It ranges from zero for black to 100 for pure white, and all integer values between for varying degrees of lightness and darkness.

In an effort to create a clean data set showing Morgan horses (many of which came from Lambert Morgans, the Quietude prefix), accomplishments and findings to date are described below. All spreadsheets were maintained online.

Our objectives were to produce a clean data set from which DNA analysis may be done, and to perform statistical and Mendelian analyses which may narrow the possible genotypes consistent with the phenotypes observed. The pedigrees revealed three non-flaxen horses that produced only nonflaxen offspring from their matings with a flaxen mate based upon at least 4 offspring each, which was presumptive of likely homozygous non-flaxen genotype in these two horses. Their offspring were therefore likely heterozygous. The pedigrees also included eleven non-flaxen horses having only nonflaxen parents and grandparents. These "interesting" horses may be good candidates for DNA analysis. Our preliminary findings were based on simple classification as flaxen if the mane or tail of the horse showed primarily lighter hairs than what is the primary body color of the horse. Methodology was developed to quantify the degree of the trait photometrically for further analysis as a continuous trait, possibly multidimensional. Our custom software programs produced graphical color-coded hyper-linked pedigrees, mating node graphs, and extensive reports. They could be rapidly modified to extract other data for reports or to pass data on to other software for further analysis.

The pedigrees, mating node graphs, legends, and reports are viewable online at http://www.thesokolows.com/colorresearch/pedigrees . The Mozilla Firefox 2 web browser is recommended for viewing the graphics. A sample of a Pedigree Analysis is illustrated in this article.

INSTRUMENTATION DEVELOPED

1. A master data spreadsheet describing all Morgan horses in the study, including ancestors, showing each horse's characteristics such as their flaxen classification tags and URL hyperlinks to their photos on the Internet (if available).
2. A computer-generated mating node diagram of the Morgans in the master spreadsheet..
3. A statistical analysis of the reliability of an objective computer-based method of identifying a quantification of flaxen in the photos.
4. A collaborative Lambert Morgans photo album accessible online.
5. A spreadsheet showing Lambert Morgans mating pairs (sire X dam) with their flaxen/non-flaxen progeny ratio.
6. A computer-generated pedigree for each horse, showing proband, ancestors, full siblings, and progeny of matings, including each horse's flaxen/non-flaxen color code, cross-linked to the pedigrees of all horses in the proband's pedigree, and links to photos (when available)..
7. A collaborative spreadsheet available at Google Docs showing descriptions and flaxen tags of Lambert Morgans.
8. A list of "interesting" horses which includes very flaxen, very non-flaxen, and horses which are non-flaxen but probably carry flaxen based upon patterns in their pedigrees.
9. A description of computer programs written and used for this research.
10. Computer-generated reports showing various summaries of the spreadsheet data, such as the offspring of each (sire X dam) pair listed with their flaxen classification tags,

RESULTS

1. All flaxen X flaxen matings have produced flaxen progeny: there were 68 flaxen-sire X flaxen-dam pairs mated and 121 flaxen offspring from these matings.
2. Three horses would be interesting for DNA study because they are non-flaxen horses that are likely homozygous non-flaxen based upon their being offspring of non-flaxen X non-flaxen, and their production of only non-flaxen offspring (4 each) from matings with a (presumed homozygous) flaxen mate.
3. Also interesting for DNA study are 13 likely heterozygous non-flaxen horses based upon their being offspring of presumed homozygous non-flaxen mated with presumed homozygous flaxen parent, and 11 non-flaxen horses having only non-flaxen parents and grandparents.
4. We have 33 flaxen horses having only flaxen parents and grandparents.
5. We have documented a bay stallion mated with a black mare which consistently have produced flaxen offspring (see Illustration showing EF Hot Wheels).
6. Photometric data obtained from the Color Capture program suggest by casual observation of the data that the manes and forelocks of the horses tend to have a lighter color than the tail of the same horse. In some horses the difference is greater than in others. This caudal-rostral differential in expression of the phenotype within each horse brings to mind the phenomenon of morphogenic gradients, which may be an aspect for further study.[3] The data also highlights the importance of establishing an operational definition of the flaxen trait since the trait is not so simple as the presence or absence of color (such as in albinism), but rather it is expressed as a continuous variable ranging from barely flaxen in the mane and non-flaxen in the tail to very boldly flaxen in both the mane and the tail.

DISCUSSION

Limitations of the data:

- Ideally, the photographs should be dated so the age of the horse at the time of the photo could be determined, but almost none of the photographs could be dated by the photo providers (generally the owner or breeder). Photo date is not included in the database.
- Color photography is known to have variations due to lighting conditions, age degradation of pigments in non-digital photographs (film and paper prints), film or camera characteristics, and so on. Older horses in the pedigrees only had monochrome photographs if they had a photo at all. Since our photometric software separated the "lightness" value of the HSV (HSB) color space from the hue and saturation of the color, all of our analysis was done on the monochrome (gray-scale) dimension, so these older photos were included in the metrics. The bias that this variable (film characteristics) may have introduced has not been studied here.
- Some of the horse photos had to be excluded from color measurement because they did not adequately show the mane or tail. Ideally, photos should be taken from a standardized position and lighting. That was not possible in this data set since it was a retrospective study.

3 See "The 'Re-discovery' of Morphogenic Fields" in chapter 3 section 3.3 at http://8e.devbio.com/index.php .

Possible Future directions:

- Use the color measurements to investigate correlation between intensity of the flaxen trait expression and the underlying genotypes.
- Consider additional statistical modeling methods, such as cluster analysis, Bayesian statistics, and Monte Carlo methods, for extracting inheritance patterns and testing various genotype models for consistency with observed phenotypes.
- Consider the impact of inbreeding, since Susan Hanley's horses are pasture-bred and do have instances of inbreeding withing recent generations.
- Enlisting collaborators who can identify the genotypes from the DNA samples.

The team of collaborators includes:

- Sonya Sokolow, Ph.D., the principal driving force behind the project.
- Stanley Sokolow, D.D.S., her husband, whom Sonya classifies as a lifelong “geek” and who is the Chief Technology Officer (CTO) of the team.
- Susanne Sokolow, D.V.M., Ph.D., who has done the statistical analyses and provided guidance from her research and publishing experience.
- Susan Hanley, owner of Lambert Morgans (Quietude Morgans), who has owned and bred Morgans for decades and who has provided and continues to provide us with invaluable information on her past and present horses.
- Steve Giberti, a friend of Susan Hanley and owner of two Quietude Morgans, who provided spreadsheets of Susan Hanley's herd of horses.

ILLUSTRATION (EF HOT WHEELS)

NON-FLAXEN PARENTS MAY PRODUCE FLAXEN CHESTNUT OFFSPRING: The two flaxen chestnut Morgan offspring above are among the progeny of the bay sire and black dam. Neither parent show the flaxen trait, yet they produced three very flaxen chestnut offspring, which are this mare's only offspring. With other mares, the stallion produced 9 other chestnuts, but none were flaxen. It is known that the flaxen trait is expressed only in horses with red (chestnut) body hair pigment, not black or bay body colors, but the trait may be hidden within the sire's and dam's genetic code and can be passed on to offspring.

Seasons Fleetson 167636

Seasons Fine N Dandy 176461

SIRE

EF Hot Wheels 124892

DAM

Season's Midnight Majoret 0140369

ILLUSTRATION (PEDIGREE ANALYSIS)

AN EXAMPLE OF AN ENHANCED PEDIGREE. The "proband" is the horse which is the subject or root of the pedigree. The oval boxes within the pedigree represent females; the rectangular boxes represent males. Yellow indicates flaxen horses; orange indicates non-flaxen horses. Notice that in this particular pedigree, flaxen horses (yellow) mated with non-flaxen horses (orange) produce both flaxen and non-flaxen offspring, while flaxen horses mated with flaxen horses always produce flaxen offspring, and non-flaxen horses mated with non-flaxen horses always produce non-flaxen offspring. This appears to be consistently true for all chestnut matings among our entire sample of Morgan horses.