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The insights of smallholder farmers on special attributes of the genetically robust mule

TAKELE TAYE DESTA[1,♥], HAIMANOT TEKLEMARIAM[2], TEWODROS MULUGETA[1]

[1]Department of Biology, College of Natural and Computational Science, Kotebe University of Education. Addis Ababa, Ethiopia.
Tel./fax. +251-11-660-0921, ♥email: takele_taye@yahoo.com

[2]Department of Forest Protection, Ethiopian Environment and Forest Research Institute. Addis Ababa, Ethiopia

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Abstract. *Desta TT, Teklemariam H, Mulugeta T. 2022. The insight of smallholder farmers on special attributes of the genetically robust mule. Biodiversitas 23: 3561-3566.* A mule (*Equus asinus* X *Equus caballus*) is the offspring of a jack, a mature male donkey, and a mare, a mature female horse. Mule is the artifact of distant hybridization, and interspecific breeding has endowed mule with appreciable hardiness. In the history of humans, mule breeding represents a novel undying, and successful traditional biotechnology. This qualitative study reports the advantages and disadvantages of using mules as working and riding equid and insights of smallholder farmers on mule's behavior and robustness. For most of the socio-economically important traits reported mule surpasses both the parental species, donkey and horse. This superiority is most likely associated with the genetic robustness of mules aroused from interspecific hybridization. It was reported that a mare mule is more intelligent, and quite the opposite, it is more aggressive than its male counterpart. Presumably, X-inactivation in distant hybrid animals like mules possessing heterologous X-chromosomes is suppressed; consequently, some of the X-chromosome genes might be escaped the inactivation process which makes the mare mule produce more of the protein products and surpass its male counterpart. The general sterility of mare mule may have also made it to invest virtually none in the costly task, nurturing of offspring. Mule's sterility and aggression make some of the community members develop negative insights. Regardless of this, the mule is highly appreciated for the various services it delivers to the rural community.

Keywords: Distant hybridization, enhanced performance, genetic robustness, insight, mule

INTRODUCTION

The equid mule (*Equus asinus* X *Equus caballus*) is most likely an invention of traditional biotechnology and carries the legacy of successful breeding practices that have been accomplished in the agricultural history of humans. It has been hypothesized that the distant crossbreeding of donkeys and horses to produce mules might have rooted its bequest in 5000 BP in Anatolia which represents most of present-day Turkey (Yilmaz and Wilson 2012). It might have been then mule production was acknowledged as plausible breeding practice and dispersed across the agriculturally important world. Nowadays, there are about 8.5 million mules (FAOSTAT 2020) across the world delivering various socio-economic and ecological services. Interestingly, regardless of the general sterility of mules, the demanding interspecific breeding has remained important practice due to the robust phenogenetic characteristics of the mule and the indispensable services it is delivering to the rural community. The mule is serving as a working, riding, and companion animal (McLean et al. 2019; Câmara et al. 2020), and involves in landscape management - grazing by mule modifies the sward structure and composition of the flora. Mule can serve as a model animal to study the genomic landscape of distant hybridization.

The genome-wide hybrid vigor that the mule has amassed from its distant hybrid origin, makes it resilient to various environmental challenges and surpasses companion domestic equids in most of the agriculturally important traits, even under difficult working and living conditions (McLean et al. 2019). Heterosis may take different forms such as dominance, overdominance, and pseudo-overdominance (Timberlake 2013). Heterosis through its favorable effect makes the bearer exhibit enhanced performance at least above the average of the parents. Heterosis has been exploited undesigned or in a deliberate fashion for millennia to enhance the productivity and resilience of the agricultural system. Due to the distant hybrid nature of the mule karyotype, the genetic robustness it carries may go beyond the realm of the usual hybrid vigor. This genetic complexity makes the definition and interpretation of the genetic robustness of the mule a demanding task. Nevertheless, this distant crossbreeding has made the mule virtually sterile attributable to the incompatibility in chromosomal morphology and number - the virtual absence of chromosomal homology for successful meiotic pairing (Benirschke and Ryder 1985). This sterility forces smallholder farmers to keep separate breeding stock of donkey jack and horse mare to produce a mule, which is a costly and demanding venture.

There is a scarcity of indisputable evidence on how and when the distant crossbreeding technique for the production of the mule was dispersed across the globe and adopted in different parts of the world. Smallholder farmers could have been engaged in the distant crossbreeding program of a mule for time immemorial to make the best use of mules in the subsistence farming system. For

example, the mule has been commonly found in rural Ethiopia and it forms the integral part of mixed crop-livestock agriculture. Ethiopia possesses the fourth-largest population of mules (340k) in the world following Mexico, China (mainland), and Brazil (FAOSTAT 2020). In some regions of Ethiopia possessing a well-trained saddle mule is a symbol of high prestige. However, this thought has gradually dwindled following the expansion of rural road and motorized transport and the growing incitement on the use of motorized transport which is perceived as a turning point of civilization. Regardless of this, the mule remains an important component of the rural economy which is largely dependent on animal power to execute agricultural and socio-economic activities.

Like companion equids, the expansion of motorized transport and agricultural mechanization in the rural landscape has threatened the mere survival of equids as working and riding animals. Regardless of this, equids are vital working animals in rural Ethiopia. Mountainous Ethiopia contains a large mass of gorges and prominent hills which are difficult to access through motorized transport due to the huge investment needed to build roads on such terrains. The importance of mules in the life of smallholder farmers is determined by eliciting their insights on the relative importance of mules in the subsistence farming system. This study reports the thoughts and practical insights of farmers on the importance of mules in their daily life, the use-values and special attributes of a mule, and highlights on stud breeding of mules.

MATERIALS AND METHODS

The study site

The study site is the Chole district, and it is found in the Arsi zone, Oromia National Regional State, south-eastern Ethiopia. The sampled villages are located at an elevation of ~2700 meters above sea level. Chole district covers a total area of ~766 square kilometers. The daily temperature ranges from 15 to 25°C making it exhibit a temperate climate. The mean annual rainfall is 1000mm. Subsistence farmers make their livings and earnings from mixed crop-livestock agriculture.

Methodologies

This cross-sectional qualitative study involved 6 key informants (model farmers) face-to-face in-depth individual interviews (Figure 1) and 3 focus group discussions (Figure 2) and accidental informal talks. The key informants were selected in consultation with the staff of the district's Office of Agriculture and verbal consent was obtained from all discussants ahead of the conversation. The key informants are belonged to 3 kebeles (a sort of counties) out of 6 high lying and main belts of traditional equine (horse, donkey, and mule) breeding sites (18 kebeles make the study district).

Information gathering and interpretation

The information collected from key informants and focus group discussions include the advantages and disadvantages of keeping a mule as working and riding animal and snapshots of stud breeding of mule and special attributes of a mule. The information is presented in a narrative form which is then prudently interpreted and discussed.

RESULTS AND DISCUSSION

Results

The reported production system of mules in smallholder farmers' settings is highlighted in the following sections. Mule can be used to trash harvested crops (Figure 3). It has been discovered that the use of equines to trash harvested crops has gained importance in recent years due to their fast movement and ease of management, although oxen place heavier pressure while trashing. Mule is a sociable animal and can build a good relationship with companion equids (Figure 4).

Figure 1. One of the key informants and the knowledgeable person was individually interviewed by the lead author

Figure 2. Knowledgeable farmers participated in focus group discussions dealing with general issues of the mule production system

Figure 3. Farmers use mules and horses to trash manually harvested crops

Figure 4. Mules build a good relationship with horses in their everyday life

Figure 5. A saddled mule waiting for its owner to carry him back home

Attributes of mule versus horse and donkey

The mule is a hardy equid that usually surpasses the distinct parental species, i.e., horse and donkey in its performance. For example, a mule carries a heavier load than a horse and donkey. Mule has appreciable stamina; consequently, it works longer compared to horse and donkey. Accordingly, the mule travels long distances without showing a sign of exhaustion. Mule also travels up the hill faster than horse and donkey while packed and during riding. Mule is surefooted and is excellent at trekking on rocky pavements and rough escarpments. Vis-à-vis trekking speed, the mule is intermediate between horse and donkey on flat areas. In flat landscapes, the horse is the fastest and the donkey is the sluggish one.

Like a donkey, the mule is resilient to environmental challenges such as disease and scarcity of feed. Mule adapts to a wide range of environmental conditions. Consequently, the hardy mule is sold at a higher price compared to the horse and donkey. This hardiness could have aroused because the mule is the product of two discrete species of equids and this might have enabled it to combine the best qualities of the genetically diverged species. However, the mule often kicks and bites attendants, which requires care while handling. Concerning resilience to disease, the mule is better than the horse but is usually surpassed by the donkey and at best it is resilient to disease as much as a donkey. For example, a mule could contract the disease in humid regions but this could not usually happen to a donkey. Mule lives longer than both horse and donkey. Mule can live up to 60 years, that is the life expectancy of a significant proportion of the people. Farmers believe that nature confers the mule with desirable attributes which make it possess appreciable endurance.

Comparative performance of female and male mules

The riding performance of mules usually depends on the degree and type of training, not sex. However, mare mules are often shined in riding so long as they are adequately trained. Moreover, the mare mule is more intelligent than john mule; subsequently, the mare mule is highly preferred for riding (Figure 5). The mare mule also has an excellent side gait which makes it produce smooth moving. Moreover, the mare mule treks faster than john mule. From a manageableness perspective, a gelded john mule is safer than a mare mule for riding. Mare mule is more aggressive than its male counterpart especially when it is in heat. The mare mule is shy away hastily while facing strange things. However, both sexes are good at carrying loads. Nevertheless, a john mule is better than mare mule at carrying heavier loads. The john mule is masculine which might have enabled it to carry a heavier load.

The selection criteria of stud jack and breeding mare

Black roan (locally called *tirign*, i.e., □□□ in Amharic) mare is preferred, and the mare should have to have large body frame. The mare has to have an attractive look and a stretched-out face. The jack has to have a long-drawn-out face, should be large, it should stand high in the front, and should have an attractive look. Black jack is highly preferred because it can produce the much-needed black roan mule. The black roan mule is believed to be hardy, resilient to drought, and draws a higher price.

The trend of mule production

The number of mules and companion equids has been reduced following the scarcity of grazing land and feed. The scarcity of feed is triggered by the encroachment of crop farming to feed the ever-increasing human population and the encroachment of eucalyptus plantations into

grazing lands. The rarely encountered stud breeding of mule has reduced and most of which had been operated a couple of decades ago have abandoned this business. The expansion of motorized transport and road infrastructure, and the growing incitement toward modernization, i.e., the use of motorized transport have reduced the breeding practice and utility of equids.

Variation between mare carrying horse and mule pregnancies

The belly of a pregnant mare is carried downward in the case of a mule-carrying pregnancy; however, it spreads out when carrying a horse pregnancy. A mare carrying a mule pregnancy usually loses its body condition. However, the mare does not show noticeable variation in behavior whether carrying mule or horse pregnancies. Farmers provide more care for mares that carry mule pregnancies because they so often encounter abortion. Accordingly, the mare carrying mule pregnancy is isolated from the stallion at the first month of conception to maintain the pregnancy. For example, when the jack or a john mule mates with the mare carrying mule pregnancy she urinates and could abort the fetus. If the mare carries mule pregnancy the foaling occurs at 11 months whereas if it carries horse pregnancy foaling requires 12 months. Farmers prefer experienced mares with several parities to carry the physiologically demanding mule pregnancy. The mare provides similar care regardless of the type of foal it nurses, i.e., either mule or horse foal. There is a proverb that says horses do not produce mules but humans. This is to show that the mule is the product of a human-mediated breeding system.

Mating management

Mare mule can be sired by john mule and stallion but less often by a jack. This indicates that, regardless of the sterility of mule, it shows noticeable libido. A john mule is gelded when it is sexually matured to make it sturdy, and manageable, and to lessen the loss of energy associated with libido.

Perception of the community on the behavior of mule

Mule is mannerless. If a mule is well-fed and properly managed it reproaches with aggression involving kicks and bites. Thus, farmers believed that rather than giving much leisure time and care to the mule, it is better to stuff it with a lot of workloads. When people came across a woman with an attractive physique, they usually say that this woman looks like a mare mule that kills its owner (in Amharic, □□□□ □□□□□ □□□ □□□□□□). This proverb testifies that when a mule is well-managed and kept in excellent condition like a good-looking woman, rather than acknowledging its owner, the mule kills its owner. A sterile woman is also perceived as a mule. People do not allow a bride to ride on a mule on her wedding day because they believed that if they did that the bride will become sterile. Mule does not like care and respect if so, it boasts swiftly. Mule is the product of a distant hybrid, and a distant hybrid is practically aggressive. Some people equate the mule to the devil because she often does wrong stuff in response to good offerings. However, most of the community members do not have a negative impression of a mule.

For the root cause of mule sterility, farmers shared their insights. It is believed that the mule is cursed by God as she tried to kick Him and God made it sterile as a fine against its wrongdoing. Because the mule is obtained from discrete species and this might have made it sterile.

Managing the stud jack

Starting from an early age, the jack is penned with a horse to acquaint itself with horses. The stud jack is well-fed with grains such as whole barely to keep it in good condition. The stud jack is not allowed to intermingle with donkeys. During mating, one of the front legs and the corresponding hind leg of the mare are tied together and the mare is drugged down into a pit excavated for this purpose to fit the height of a shorter jack. Sometimes mating is assisted by a human. To agitate the jack for mating an adequate amount of whole grain (barley) is provided and when the mare is in heat and sexually excited the jack is allowed to mount. The owner of the mare brings with him/her 10kg of barley grain as a service charge for the stud breeding and, in some places, a service charge of 1.5 birrs (the then 1 Ethiopian Birr was exchanged for 2.07 US$) was collected during the Derg regime (before ~40 years).

Fortunately, one of the key informants used to be a stud breeder of a mule. He reported that he owned a jack, and he confined, well-fed, penned the jack with horses, and did not allow the jack to mix with donkeys but to have a sexual disposition towards horses. On account of this, the jack bites when it came across donkeys. When the jack erupted 2 teeth (~3 years of age) he owned it and when the jack erupted 4 teeth (four years old) it was found to be well trained and matured enough to provide the stud breeding service.

Discussions

Distant hybridization as in the case of mule involves the crossing of unlike but closely related species (Liu et al. 2022); subsequently, this breeding practice combines distant genomes and biological characteristics in the progeny (Liu et al. 2020). Distant hybridization may also combine the best qualities of the distinct parental species which makes the distant hybrid progeny genetically robust. For example, the mule is known for its stamina in mountain trekking, a valuable resource for ecotourism in rugged topographies (Canisso et al. 2019). In line with what has been reported by the farmers, the mule is believed to be intelligent equid, although this needs to be proved through extensive studies (Haines and Goliszek 2019).

Like other distant hybrids, the mule has lost its reproductive fitness due to chromosomal incompatibility in parental gametes (Johnson 2008); therefore, its mere survival as a distant hybrid solely depends on the artificially induced distant hybridization or perhaps in very rare cases accidental mating of jack and mare. Since mule inherits heterologous genomes (heterokaryons) it might be privileged from the additive effect of coding and noncoding (regulatory) regions and species-specific epistatic effects

besides the usual hybrid vigor. Although in the haploid state, the mule may contain more genes than a donkey or a horse does due to the presence of species-specific genes or at least due to the possession of several species-specific allelic variants. Results show that the mule possesses several traits expressed in the over-dominance pattern. This superiority might be aroused from significant variation in the genomic landscape of the distinct parental species.

Aggression is associated with high genetic polymorphism (Manuck et al. 1999; Butovskaya et al. 2013) and can be used as an adaptative mechanism to maximize survival and reproductive fitness (Xu et al. 2018). Sexual dimorphism in aggression is a common phenomenon; for example, in mammalian species, males are often more aggressive than their female counterparts (for humans, see, for example, Butovskaya et al. 2013). This disparity could arise from the impact of sex hormones that brought about masculine behavior and due to sexual selection. In males, testosterone triggers aggression, and males as common guardians of their species have been naturally selected to retain a significant level of aggression. Nevertheless, in mules, mare mules are reportedly more aggressive than john mules. X-inactivation is performed by the cellular mechanism as a means of dosage compensation. Consequently, usually, one of the X-chromosomes is randomly inactivated in mammalian females. However, in mare mules, where the heterologous genome (X-chromosome) is possessed X-inactivation might not be stable, and reactivation of silenced genes (Graves 1982) might have made the mare mules produce more proteins and display elaborated adaptive behaviors such as aggression, intelligence, fast and advanced gait, and explorative behavior. Because mare mules are virtually sterile, they do not engage in demanding tasks such as nurturing young and carrying pregnancies and showing regular oestrus. Aggression in a male can be controlled by gelding.

Strangely, the donkey shows a wide range of gestation length, 11 to 14.5 months, but this range is narrower in the horse which is 11 to 12 months. Consistent with the report of the respondents, the short gestation length in mares carrying mule pregnancies compared to horses and donkeys was reported in the work of Boakari et al. (2019). Carrying heterologous pregnancy may create some challenges to the uterine environment (Boetam and Zarco 2012) hence this might be circumvented by earlier foaling as an adaptive mechanism. However, both donkey and horse can gestate for 11 months equivalent to the reported gestation length for mare-carrying mule pregnancies.

Selection criteria for breeding donkeys and horses reported in this finding focus on appearance traits, including coat color. For example, such dark colors as in the case of black roan could help mules to adapt to the prolonged exposure to solar radiation of the tropical environment. Dark coat colors are also served as camouflage against predators' attack.

Selection criteria for breeding donkeys and horses reported in this study focus on appearance traits, which is in line with the extensive review made by Klecel and Martyniuk (2021) on ancient breeding and management of horses in Greece, including coat color, facial profile, and head shape (Maśko et al. 2022). For example, dark colors such as black roan could make mules adapt to the prolonged exposure to solar radiation of the tropical environment, which is reflected by shade-seeking behavior of mules (Haddy et al. 2020). Dark coat colors also serve as camouflage mechanisms against predator's attack. Coat color is important trait in the adaptation history of equines (Zhou et al. 2020).

In conclusion, this study reports novel results on insights of smallholder farmers on mule performance, behavioral ecology, and breeding system. The outcomes of this study elicit research questions to work further on the phenogenetic mechanisms underlying the robustness of mules. The findings from this study however need to be supported by extensive research and experimental studies to enhance and ratify the prevailing knowledge of farmers on behavioral ecology and the production system of a mule.

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REFERENCES

Benirschke K, Ryder OA. 1985. Genetic aspects of equids with particular reference to their hybrids. Equine Vet J 17 (S3): 1-10. DOI: 10.1111/j.2042-3306.1985.tb04583.x.

Boeta M, Zarco L. 2012. Luteogenic and luteotropic effects of eCG during pregnancy in the mare. Anim Reprod Sci 130 (1-2): 57-62. DOI: 10.1016/j.anireprosci.2012.01.001.

Boakari YL, Alonso MA, Riccio AV, Fernandes CB. 2019. Are mule pregnancies really longer than equine pregnancies? Comparison between mule and equine pregnancies. Reprod Domest Anim 54 (6): 823-827. DOI: 10.1111/rda.13423.

Butovskaya ML, Vasilyev VA, Lazebny OE, Suchodolskaya EM, Shibalev DV, Kulikov AM, Karelin DV, Burkova VN, Mabulla A, Ryskov AP. 2013. Aggression and polymorphisms in AR, DAT1, DRD2 and COMT genes in Datoga pastoralists of Tanzania. Sci Rep 3: 3148. DOI: 10.1038/srep03148.

Câmara RJ, Bueno BL, Resende CF, Balasuriya UB, Sakamoto SM, Reis JK. 2020. Viral diseases that affect donkeys and mules. Animals 10 (12): 2203. DOI: 10.3390/ani10122203.

Canisso IF, Panzani D, Miró J, Ellerbrock RE. 2019. Key aspects of donkey and mule reproduction. Vet Clin Vet Clin North Am Equine Pract 35 (3): 607-642. DOI: 10.1016/j.cveq.2019.08.014.

FAO S. FAOSTAT database. 2020. Food Agric Organ UN Rome Italy. 2020. Available: http://www.fao.org/faostat/en/

Graves JA. 1982. 5-azacytidine-induced re-expression of alleles on the inactive X chromosome in a hybrid mouse cell line. Exp Cell Res 141 (1): 99-105. DOI: 10.1016/0014-4827(82)90072-6.

Haddy E, Burden F, Proops L. 2020. Shelter seeking behaviour of healthy donkeys and mules in a hot climate. Appl Anim Behav Sci 222: 104898. DOI: 10.1016/j.applanim.2019.104898.

Haines A, Goliszek J. 2019. Donkey and mule behaviour for the veterinary team. UK-Vet Equine 3 (1): 27-32. DOI: 10.12968/ukve.2019.3.1.27.

Johnson N. 2008. Hybrid incompatibility and speciation. Nat Educ 1 (1): 20. https://www.nature.com/scitable/topicpage/hybrid-incompatibility-and-speciation-820/

Klecel W, Martyniuk E. 2021. From the Eurasian steppes to the Roman circuses: A review of early development of horse breeding and management. Animals 11 (7): 1859. DOI: 10.3390/ani11071859.

Liu Q, Liu J, Yuan L, Li L, Tao M, Zhang C, Qin Q, Chen B, Ma M, Tang C, Liu S. 2020. The establishment of the fertile fish lineages derived from distant hybridization by overcoming the reproductive barriers. Reproduction 159 (6): R237-R249. DOI: DOI: 10.1530/REP-19-0576.

Liu S, Wang S, Liu Q, Wu C, Zhou Y, Tao M, Zhang C, Qin Q, Luo K. 2022. The Research Advances in Animal Distant Hybridization and Polyploid Organisms. Fish Distant Hybridization. Springer, Singapore. DOI: 10.1007/978-981-16-5067-3_1.

Manuck SB, Flory JD, Ferrell RE, Dent KM, Mann JJ, Muldoon MF. 1999. Aggression and anger-related traits associated with a polymorphism of the tryptophan hydroxylase gene. Biol Psychiatry 45 (5) :603-614. DOI: 10.1016/s0006-3223(98)00375-8.

Maśko M, Wierzbicka M, Zdrojkowski Ł, Jasiński T, Sikorska U, Pawliński B, Domino M. 2022. Comparison of donkey, pony, and horse dorsal profiles and head shapes using geometric morphometrics. Animals 12 (7): 931. DOI: 10.3390/ani12070931.

McLean A, Varnum A, Ali A, Heleski C, Navas González FJ. 2019. Comparing and contrasting knowledge on mules and hinnies as a tool to comprehend their behavior and improve their welfare. Animals 9 (8): 488. DOI: 10.3390/ani9080488.

Timberlake WE. 2013. Heterosis. Brenner's Encyclopaedia of Genetics. Academic Press, San Diego.

Xu XM, Chi QS, Cao J, Zhao ZJ. 2018. The effect of aggression I: The increases of metabolic cost and mobilization of fat reserves in male striped hamsters. Horm Behav 98: 55-62. DOI: 10.1016/j.yhbeh.2017.12.015.

Yilmaz O, Wilson RT. 2012. The domestic livestock resources of Turkey: Status, use and some physical characteristics of mules. J Equine Sci 23 (4): 47-52. DOI: 10.1294/jes.23.47.

Zhou Z, Fan Y, Wang G, Lai Z, Gao Y, Wu F, Lei C, Dang R. 2020. Detection of selection signatures underlying production and adaptive traits based on whole-genome sequencing of six donkey populations. Animals 10 (10): 1823. DOI: 10.3390/ani10101823.