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Heterozygote Advantage: The Effect of Artificial Selection in Livestock and Pets

Philip W. Hedrick

From the School of Life Sciences, Arizona State University, Tempe, AZ 85287.

Address correspondence to Philip W. Hedrick at the address above, or e-mail: philip.hedrick@asu.edu.

Abstract

There are a number of mutants in livestock and pets that have a heterozygote advantage because of artificial selection for these mutants in heterozygotes and strong detrimental effects from natural selection in homozygotes. In livestock, these mutants include ones that influence milk yield in dairy cattle, fecundity in sheep, litter size in pigs, muscling in beef cattle, color in horses, lean meat content in pigs, and comb morphology in chickens. In pets, these mutants include ones that influence tail length in cats and hairlessness, muscling, color, or ridgeback hair in dogs. A large variety of mutants are responsible, including small or large deletions or insertions and single base-pair nonsynonymous changes. Many of the mutants cause loss of function for the genes involved, a change that results in the pleiotropic effects of a desired phenotype in heterozygotes and low fitness or an undesirable phenotype in mutant homozygotes. I examine how selection changes the frequency of these mutants and provide an approach to estimate the amount of artificial selection that is necessary to maintain these mutants at the high frequencies often observed. The amount of artificial selection ranges from low selection favoring heterozygotes for double muscling in whippet dogs to very strong selection favoring the "flash" (part white, part solid) heterozygote in boxer dogs and the rose comb in chickens. In several examples (rose comb in Wyandotte chickens and the hair ridge in Rhodesian ridgeback dogs), there is actually stronger selection for the mutant than against it, making the frequency of the mutant greater than 50%.

Subject areas: *Molecular adaptation and selection, Genomics and gene mapping*

Key words: *heterozygote advantage, lethal, mutant, overdominance, pleiotropy, selection*

A detailed search for genetic variation maintained by heterozygote advantage (also called overdominance) in animals by Hedrick (2012) found mainly the well-known examples, such as sickle cell anemia in humans, major histocompatibility complex in vertebrates, and complementary sex determination (*csd*) in hymenoptera. Overall, there were only a few new instances of heterozygote advantage selection. Even genomic surveys (Asthana et al. 2005; Bubb et al. 2006; Andrés et al. 2009; Leffler et al. 2013; DeGiorgio et al. 2014)) have only found a few potential examples of heterozygote advantage selection. The one major exception found by Hedrick (2012) was a number of examples of heterozygote advantage in livestock and pets for mutants that are advantageous as heterozygotes because they are artificially selected for but have detrimental effects, often quite severe, when homozygous.

These examples might be generally described as ones of balancing selection, a term that includes heterozygote advantage, negative frequency-dependent selection, selection varying in time, varying in space, between the sexes, in different life stages, or between different fitness components (Hedrick 2012). Perhaps useful here is the concept of marginal heterozygote advantage (Wallace 1968), an umbrella definition for these different types of selection that result in some overall heterozygote advantage. For example, for these mutants in livestock and pets, the balance of natural selection (or artificial selection in some cases) against mutant homozygotes and artificial human selection favoring heterozygotes over wild-type homozygotes can result in a marginal heterozygote advantage.

Many of the traits that are discussed here, favored in heterozygotes for breeding in livestock or pets, are phenotypes that might be detrimental in heterozygotes if only natural selection acted. In other words, artificial selection can give an advantage to a phenotype, and the genotype that determines it, that is desirable for production in livestock or some phenotypic characteristic in pets that would generally be selected against in a wild population. As examples in livestock (discussed further below), very high milk production in dairy cattle or extreme musculature in beef cattle might be

agriculturally desirable but these traits would probably have negative pleiotropic effects and consequently overall detrimental impacts on heterozygous fitness in a wild population. As examples in pets, lack of a tail as in Manx cats or hairlessness as in Mexican hairless dogs are artificially selected for but presumably would be selected against in heterozygotes in a wild population. In addition, many of these variants have even stronger detrimental effects as homozygotes and in some instances result in embryonic lethality or are greatly selected against by humans because of their undesirable phenotypic effects. In a number of cases, the variants are loss-of-function mutants caused by major genetic changes (insertions, deletions, and so on), which have an impact on some trait that is selected for artificially in heterozygotes, while in homozygotes, the loss-of-function results in detrimental fitness effects or undesirable phenotypes.

These mutants can be considered pleiotropic because they influence both some favored phenotypic trait as heterozygotes and aspects of fitness detrimentally as homozygotes. The most extreme artificial selection effect would be if only heterozygotes are selected over wild-type homozygotes. The most extreme natural selection effect would be to cause zero fitness in mutant homozygotes because of low survival, mating, and/or reproduction. Here when artificial selection or another fitness component besides survival results in zero fitness, we will indicate this using quotes as "lethal." Typically such recessive lethals would generally be at a very low frequency in a population because this strong selection reduces their frequency and mutation only introduces them back at a very low rate. However, if heterozygotes for such a recessive lethal exhibit some phenotypic trait that is artificially selected for more than the wild-type homozygote, overall then heterozygotes would have a selective advantage.

Below I will first discuss 12 examples of heterozygote advantage mutants in livestock and pets. Then I will give some population genetic models that can be used to understand and predict the frequencies of these mutants in populations. Finally, I will apply these models to specific examples to illustrate how these models can help understand the dynamics and maintenance of these mutants and determine the extent of artificial selection.

Examples

The 12 examples that I discuss here are well-documented examples of heterozygote advantage in livestock and pets. In nearly all these cases, the genes involved, or the genetic location of the genes involved, and the type of mutant causing the phenotypic change have been identified. For details of the approaches used to identify the genes and the variants associated with particular phenotypic traits, please examine the references cited below for the 12 examples. Further, in nearly all cases, a reasonable explanation for the phenotypic trait and the advantage in heterozygotes and the phenotypic trait and fitness disadvantage in homozygotes fitness has been documented. As more genes important for production traits in livestock and artificially selected phenotypic variation in pets are identified, the number of genes with heterozygote advantage because of the combination of artificial and natural selection is likely to grow. For example, some other potential examples that have not been as thoroughly examined as the 12 discussed here are complex vertebral malformation in Holstein cattle (Qin et al. 2010), bulldog dwarfism in Dexter cattle (Cavanagh et al. 2007), lethal white foal syndrome in horses (Santschi et al. 1998), polledness in goats (Pailhoux et al. 2001), and a number of mutants found in livestock and dog breeds (Reissman and Ludwig 2013; Kadri et al. 2014).

Livestock

Milk Yield (Dairy Cattle)

Kadri et al. (2014) found that a 660-kb deletion when homozygous resulted in recessive embryonic lethality in Nordic Red cattle, possibly from the loss of gene *RNASEH2B* within the deletion (Table 1). They also found that the deletion had substantial positive effects on milk yield and milk composition in heterozygotes although it is not known which gene associated with the deletion causes these effects. The deletion is present as a heterozygote in 13%, 23%, and 32% of the Danish, Swedish, and Finnish Red cattle sampled, respectively. Kadri et al. (2014) suggested that this embryonic lethal, and others with similar characteristics, might account for much of the reduction in dairy cattle fertility observed in recent years.

Fecundity (Sheep)

Davis (2005) and Gemmell and Slate (2006) discussed five different mutants in several breeds of sheep (Romney, Belclare, and Cambridge) that exhibited heterozygote advantage. These mutants increased ovulation rate and fecundity in heterozygotes, but homozygous mutants had impaired oocyte development and maturation, resulting in undeveloped ovaries and female infertility. The mutants were at the related *BMP15* (X-linked) and *GDF9* (autosomal) genes, both of which code for proteins that are members of the transforming growth factor β superfamily. The effects of the mutants appear sex-limited to females, and there are no known impacts to males for any of these mutants.

The frequencies of these mutants appear quite high in some breeds (Hanrahan et al. 2004). In a recent survey, Mullen and Hanrahan (2014) found that in 181 Belclare sheep, 32% were heterozygous and 1.7% were homozygous for a *GDF9* mutant ($Fec G^{H}$) that increased ovulation and litter size in heterozygotes. Mullen et al. (2013) surveyed other breeds of sheep to determine the source of these mutants and concluded that they came from the Lleyn sheep breed and the High Fertility Line developed in the 1960s. In 812 Cambridge sheep born in 1987 and later, the frequency of another mutant at *GDF9* ($Fec G^{H}$) was even higher with 44.0% heterozygotes and 8.3% homozygous mutant (Hanrahan JP, personal communication)

Litter Size (Pigs)

In the Finnish Yorkshire pig breed, some infertile boars were identified in the 1980s with immotile short-tail sperm, and

Table 1 Examples of seven mutants in livestock where the heterozygote has a selective advantage (the phenotypic difference is given) and the mutant homozygote is selected against (the detrimental trait is indicated). See the text for references for these mutants and further discussion

Species (breed)	Trait	Gene	Type of mutant	Heterozygote	Homozygote
Cattle (Nordic Red)	Milk yield	*RNASEH2B*[a]	660-kb deletion	High milk yield	Embryonic lethal
Sheep (Romney, Cambridge, Belclare)	Fecundity	*BMP15, GDF9*	Most 1-bp change[b]	Increased female fecundity	Female infertility
Pig (Finnish Yorkshire)	Litter size	*SPEF2*	9-kb insertion	Higher female litter size	Male infertility
Chicken (Wyandotte)	Rose comb	*MNR2*	7.4-Mb inversion	Rose comb	Male infertility
Cattle (Belgian Blue)	Crooked tail	*MRC2*	2-bp deletion	High muscle	Crooked tail
Horse (Appaloosa, Knabstrupper)	Leopard phenotype	*TRPM1*	1378-bp insertion	Leopard complex spotting	Congenital night blindness
Pig (Pietrain, Landrace)	Halothane sensitivity	*RYR1*	1-bp change	High lean meat content	Porcine stress syndrome

[a]*RNASEH2B* is thought to be a good candidate for the gene that causes embryonic mortality in homozygotes in Nordic Red cattle, but the gene or genes that causes higher milk production in heterozygotes might be a different and has not been identified.
[b]For references for 12 different mutations, see Mullen and Hanrahan (2014).

this male infertility was associated with an insertion in an intron in gene *SPEF2* (Sironen et al. 2012). The mutant was traced to a single boar in 1982, and by 2001, the frequency of heterozygotes for this mutant had increased to 36%. Because it was thought that an association of this mutant with production or reproduction traits might be responsible for a selective advantage, Sironen et al. (2012) examined a number of reproductive traits. They found that heterozygous females had a significantly higher litter size in the first parity (0.51 piglets higher) than females not carrying the insertion. By instituting gene-assisted selection using a diagnostic test developed before the mutant was fully described, the heterozygote frequency was reduced very quickly to 0.06 by 2008.

Rose Comb (Chickens)

In chickens, the comb (a fleshy crest on the top of the head of gallinaceous birds) has an altered morphology from a mutant called rose comb (Figure 1A) compared with the wild-type single comb. Rose combs are found in both heterozygotes and mutant homozygotes in Wyandotte chickens and many other breeds. This phenotype is generally due to a 7.4-Mb inversion that changes the position of the homeodomain protein gene *MNR2* (Imsland et al. 2012). In this mutant, disruption of gene *CCDC108* at one of breakpoints of the inversion causes poor sperm motility in homozygotes and essentially male infertility in homozygotes when in competition with heterozygous males (Imsland et al. 2012) while females homozygous for the mutant have normal fertility. For example, Crawford (1965) found that when equal numbers of sperm from homozygous mutants and heterozygotes were pooled and were used to inseminate hens, all chicks were sired by the sperm from heterozygotes. The balance between artificial selection for heterozygotes with rose-comb and natural selection against male homozygotes resulted in 15.5% single comb (wild-type) homozygotes in a sample of 4298 Wyandotte chicks (Wehrhahn and Crawford 1965).

Crooked Tail (Beef Cattle)

In Belgian Blue beef cattle, a two-base pair, loss-of-function mutation at the mannose receptor *MCR2* gene increases muscle mass in heterozygotes and causes skeletal and muscular malformations, called the crooked-tail syndrome (Figure 1B) in homozygotes (Fasquelle et al. 2009). Although the crooked-tail phenotype is not lethal, some severe cases have been euthanized and the rest have retarded growth and poor carcass quality. In a survey of 1899 healthy Belgian Blue cattle, 24.7% were heterozygous for the mutant and none were homozygous (Fasquelle et al. 2009).

Examination of pedigree and molecular data discovered that a bull named Précieux, born in 1980, was a carrier of the mutant and that his extensive utilization as a sire greatly increased the frequency of the mutant in the 1980s and resulted in a selective sweep. Using computer simulations, Fasquelle et al. (2009) suggested that during this period heterozygotes for this mutant were twice as likely to be selected as their normal siblings. Since 2008, selection against carriers and/or avoiding at-risk matings has largely eliminated this mutant from the breed. However, Sartelet et al. (2012) subsequently identified a different point mutant in the *MCR2* gene that also caused crooked-tail syndrome in Belgian Blue beef cattle. Because it had a frequency of only 0.3% heterozygotes in 3188 animals tested, it appears to be a new, but similar, crooked-tail mutant.

Leopard Complex Spotting (Horse)

Bellone et al. (2013) found that a 1378-bp insertion into gene *TRPM1* resulted in leopard complex spotting in heterozygotes in several horse breeds, including Appaloosa and Knabstrupper (Figure 1C). In homozygotes, there are varying amounts of white and few, to no, leopard spots but there is congenital stationary night blindness. The cave paintings of wild spotted horses (close to the leopard phenotype in modern horses) at Pech-Merle, France date back about 25 000 years (Pruvost et al. 2011). In a survey of ancient western European samples, 4 out of 10 horses were heterozygous for a single nucleotide polymorphism used to detect this mutant allele (allele frequency of 0.2; Pruvost et al. 2011) and Bellone et al. (2013) confirmed that three ancient samples had the insertion-causing mutation. In other words, this variant appears to have been in substantial frequency in ancient

Figure 1. Photos of (**A**) a rose comb heterozygote in chickens (Imsland et al. 2012), (**B**) a crooked tail homozygote in Belgian Blue cattle (A. Sartelet and C. Charlier), (**C**) a leopard complex spotting heterozygote in horses (R. Bellone), (**D**) a tailless heterozygote Manx cat (Creative Commons), (**E**) a hairless heterozygote Mexican hairless dog (Creative Commons), (**F**) a homozygote bully whippet dog (D. Mosher, H. Parker, and E. Ostrander), (**G**) a flash heterozygote boxer dog (N. Salmon Hillbertz), and (**H**) a homozygote Rhodesian ridgeback dog (N. Salmon Hillbertz).

horses even though as a homozygote it was presumed to have caused night blindness. Pruvost et al. (2011) suggested that the leopard phenotype in heterozygotes might have advantageously provided camouflage in the snowy Pleistocene. However, because the frequency of the leopard phenotype is substantial in a number of modern breeds, it appears that artificial selection for the phenotype is now important.

Porcine Stress Syndrome (Pig)

During handling, pigs with porcine stress syndrome demonstrate various behavioral symptoms, display discoloration of the skin and muscle rigidity, and can die without intervention. Homozygosity for a single-nucleotide mutation in the skeletal muscle receptor *RYR1* is responsible for this syndrome (Fujii et al. 1991). Before DNA testing was available, affected pigs were detected by challenging them with the general anesthesia halothane gas and homozygotes exhibited muscle rigidity, skin discoloration, and limb tremors. An association between this *RYR1* mutation and higher lean meat content has been documented (see references in Salmi et al. 2010).

It has been suggested that heterozygotes for this mutant might have been selected because they had leaner meat or had larger musculature due to an increased incidence of muscle contractions that burn fat and stimulate muscle growth. A meta-analysis of studies showed statistically significant effects on meat quality (carcass leanness) in heterozygotes (Salmi et al. 2010). A survey by O'Brien et al. (1993) showed large variation in the frequency of the mutant over breeds with the highest allele frequency in Pietrain (51.7% heterozygotes, 44.8% homozygotes, and an allele frequency of 0.707 in 58 pigs) and Landrace (33.2% heterozygotes, 2.1% homozygotes, and an allele frequency of 0.187 in 1962 pigs). After the mutant was identified molecularly, selection was able to eliminate the mutant from these breeds.

Pets

Manx (Cats)

Buckingham et al. (2013) documented that multiple different mutants cause frameshifts and premature truncation of the transcription factor Brachyury, encoded by the gene *T* that causes taillessness or a short-tail phenotype in cats. Taillessness, or a short-tail phenotype, is the most distinguishing characteristic of the Manx breed (Figure 1D) and generally normally tailed cats, even though they might have two heterozygous Manx parents, are not considered of the Manx breed. Buckingham et al. (2013) found that all cats with this phenotype were heterozygous for mutants at the *T* gene. They

found no tailless or short-tailed cats homozygous for the *T* mutants, consistent with the findings in other species that lack of Brachyury results in early embryonic lethality. Deforest and Basrur (1979) found that litters from crosses between Manx cats are often smaller than typical litters, consistent with embryonic lethality of homozygotes. In a summary of many crosses between Manx cats, Robinson (1993) observed 440 (66.8%) Manx cats and 219 (33.2%) tailed cats, very close to the 2:1 ratio expected if there were lethality of homozygotes for the Manx allele. Todd (1979) suggested that the high frequency of Manx cats (32.6%) on the Isle of Man (Table 2), where the most common mutation was thought to have originated, probably resulted from human preference for Manx cats.

Hairlessness (Dogs)

A seven-base-pair duplication in the gene *FOX13* when heterozygous results in hairlessness (Figure 1E) and abnormal teeth in three breeds of dogs, Mexican hairless, Peruvian hairless, and Chinese crested (Drögemüller et al. 2008). The protein from the *FOX13* gene is thought to be part of downstream target of the ectodysplasin signaling pathway. Dogs homozygous for this mutant die during embryogenesis, and as a result, when two Mexican hairless dogs are crossed, about 66.7% are hairless and 33.3% are haired (Kimura et al. 1993). However, the survival of these hairless dogs was only 31.3% compared with 80.0% for their haired littermates although survival of hairless dogs increased significantly when given extra heat (Kimura et al. 1993). Interestingly, Mexican hairless dogs were considered sacred by the Aztecs and statues of hairless dogs date back 3700 years, suggesting that the mutant is quite old and has been maintained by selective breeding.

Double Muscling (Dogs)

A two-base-pair deletion in the *MSTN* gene in whippets causes a premature truncation of myostatin, which is a negative regulator of muscle mass, and consequently results in an increase in the number of muscle fibers produced (Mosher et al. 2007). Homozygotes are known as "bully" whippets because of their well-developed musculature (Figure 1F). However, because bully whippets do not conform to breed standards, they are often euthanized. Heterozygotes for the deletion are more muscled than normal whippets and are successful racers (Mosher et al. 2007). As a consequence, artificial selection for racing performance appears to have caused an increase in the frequency of the deletion. In a sample of 146 whippets, which were both racers and nonracers, Mosher et al. (2007) found that 13.7% were heterozygous and 1.4% were homozygous for the mutant.

White Color (Dogs)

White color occurs in boxers and bull terriers that are homozygous for an apparent regulatory variant at the gene *MITF*, an important developmental gene related to pigmentary and auditory diseases in humans and mice (Karlsson et al. 2007). Both homozygous white boxers and white bull terriers have an incidence of deafness of around 10%. Heterozygous boxers for this mutant have a part white, part solid phenotype called "flash" (Figure 1G), which is often favored by breeders, and do not have any increased risk of deafness. Because of the increased incidence of deafness and the unpopularity of white boxers, homozygous white boxers are often put down or are not used for breeding, resulting in heterozygote advantage (Barsh 2007). In a large cohort of 2629 boxer puppies, 17.9% were white and were euthanized (Nielen et al. 1998). Assuming that these were all homozygous for the regulatory variant, it is estimated that 48.8% were heterozygotes, or flash phenotypes, in this population.

Ridgeback (Dogs)

A 133-kb duplication involving three fibroblast growth factor (*FGF*) genes (*FGF3, FGF4,* and *FGF19*) when heterozygous (and homozygous) causes a dorsal hair ridge (hair running in the opposite direction of the rest of the coat) on the back (Figure 1H) in the hunting and guard Rhodesian ridgeback and Thai ridgeback breeds (Salmon Hillbertz et al. 2007). Dogs homozygous for the duplication have a high risk of dermoid sinus, which resembles a neural tube defect in humans. Dermoid sinus results in negative health effects and dogs with it are often euthanized. In a sample of 32 ridged Rhodesian ridgebacks, Salmon Hillbertz et al. (2007) found that 56.2% were heterozygous for the duplication and 43.8% were homozygous. In the same sample, only 11.1% of the dogs heterozygous for the duplication had dermoid sinus while 71.4% of the homozygotes had dermoid sinus. Neither ridgeless dogs nor dogs with dermoid sinus are allowed to breed as Rhodesian ridgeback dogs, but because homozygotes for the duplication do not always have a dermoid sinus, selection is not complete against homozygotes.

Table 2 Examples of five mutants in pets where the heterozygote has a selective advantage (the phenotypic difference is given) and the mutant homozygote is selected against (the detrimental trait is indicated). See the text for references for these mutants and further discussion

Species (breed)	Trait	Gene	Type of mutant	Heterozygote	Homozygote
Cat (Manx)	Taillessness	*T*	1-bp deletion	Short or no tail	Lethal
Dog (Mexican hairless)	Hairless	*FOX13*	7-bp duplication	Hairless	Lethal
Dog (whippet)	Muscle	*MSTN*	2-bp deletion	More muscle, fast racer	Double muscle
Dog (boxer)	White spotting	*MITF*	Regulatory, from insertion	White spotting pattern	White and increased risk of deafness
Dog (ridgeback)	Hair ridge	3 *FGF* genes	133-kb duplication	Dorsal hair ridge	High risk of dermoid sinus

Salmon Hillbertz et al. (2007) found that 80% of the ridged dogs without dermoid sinus were heterozygous, which suggests that about 16% [(0.8)(0.8)(0.25) = 0.16] of the progeny from crosses between these ridged dogs would be ridgeless, wild-type homozygotes. However, only about 5%–6% of newborn dogs are ridgeless, suggesting that either there is underreporting of the undesirable ridgeless phenotype or that homozygotes without dermoid sinus are over-represented in the breeding population.

Model

Let us assume that allele *A* is the wild-type (or normal) allele in the population and genotype *AA* is the wild-type (or normal) homozygote. First, assume that a mutant occurs at low frequency and that it is effectively lethal as a homozygote *aa*, that is, it does not have any surviving offspring because of the negative effects on survival, mating, and/or reproduction. As a result, the relative fitness of genotype *aa* is 0 ($s_2 = 1$ below) as in the first row of Table 3. If the heterozygote *Aa* is phenotypically different from the ancestral wild-type homozygote *AA* because it has a higher value for a production trait in a livestock breed or has a phenotype thought important for a pet breed, then the heterozygote *Aa* can have a selective advantage s_h due to human or artificial selection as shown in the second row of Table 3.

The notation here uses a lowercase letter for the mutant because it is generally recessive for its fitness effect. However, the mutants here are dominant for some phenotypic trait expressed in the heterozygotes, a fact that is not conveyed by using a lowercase letter to indicate a recessive mutant. For example, this phenotypic dominance is traditionally symbolized by using *M* for the Manx cat mutant and *m* for the wild-type allele and *R* for rose-comb chicken mutant and *r* for the wild-type allele.

For mathematical convenience, the relative fitnesses in the second row of Table 3 can be standardized so that the heterozygote *Aa* has the highest relative fitness of 1. These relative fitnesses are given in the third row of Table 3 where

$$s_1 = \frac{s_h}{1+s_h} \tag{1a}$$

When the fitness of the heterozygote is standardized to be 1, then the amount of selection against the wild-type heterozygote *AA* is s_1. Further, s_h can be given as a function of the difference in fitness between the wild-type homozygote and the heterozygote as

$$s_h = \frac{s_1}{1-s_1} \tag{1b}$$

Second, assume that the mutant homozygote *aa* has a nonlethal detrimental effect so that its fitness is $1 - s_2$ and the artificially selected heterozygote *Aa* still has a selective advantage s_h. The relative fitnesses, again assuming that the standardized relative fitness of *Aa* is equal to 1, can be given as in the bottom half (b) of Table 3, where

$$s_2' = \frac{s_h + s_2}{1+s_h} \tag{1c}$$

Let us assume that selection occurs in two stages a12.5s it appears to in many of the examples we will discuss. Here, p and q are the frequencies of alleles *A* and *a*, and *P*, *H*12.5, and *Q* are the frequencies of genotypes *AA*, *Aa*, and *aa*, respectively, First, natural selection against genotype *aa* occurs so that the genotype frequencies after this selection become

$$P' = \frac{p^2}{\bar{w}}$$

$$H' = \frac{2pq}{\bar{w}} \tag{2a}$$

$$Q' = \frac{q^2(1-s_2')}{\bar{w}}$$

where

$$\bar{w} = 1 - s_2' q^2$$

In general, the frequency of heterozygotes that are reported in the examples we will discuss are after natural selection but before artificial selection, or similar to H' here.

Next after artificial selection, the genotype frequencies become

Table 3 The relative fitnesses of the wild-type homozygote *AA*, the heterozygote *Aa*, and the mutant homozygote *aa* when (a) genotype *aa* is a recessive lethal and (b) genotype *aa* is a recessive detrimental

	Fitness		
Genotype	*AA*	*Aa*	*aa*
(a) Recessive lethal	1	1	0
Lethal with heterozygous advantage	1	$1 + s_h$	0
Standardized fitnesses for lethal with heterozygote advantage	$1 - s_1$	1	0
(b) Recessive detrimental	1	1	$1 - s_2$
Detrimental with heterozygous advantage	1	$1 + s_h$	$1 - s_2$
Standardized fitnesses for detrimental with heterozygote advantage	$1 - s_1$	1	$1 - s_2'$

s_1 is the selective disadvantage of the wild-type *AA* homozygotes compared with the heterozygote, s_h is the selective advantage of the heterozygote resulting from artificial or human selection, and s_2 is the selective disadvantage of mutant homozygote *aa* ($s_2 = 1$ for a recessive lethal).

$$P'' = \frac{P'(1-s_1)}{\bar{w}'}$$

$$H'' = \frac{H'}{\bar{w}'} \quad (2b)$$

$$Q'' = \frac{Q'}{\bar{w}'}$$

where

$$\bar{w}' = 1 - s_1 P'$$

The expected change in the frequency of mutant *a* is

$$\Delta q = \frac{pq(s_1 p - s_2' q)}{1 - s_1 p^2 - s_2' q^2} \quad (3a)$$

and the expected equilibrium frequency of mutant *a* is

$$q_e = \frac{s_1}{s_1 + s_2'} \quad (3b)$$

(e.g., Hedrick 2011). In both of these equations, when there is a lethal, $s_2 = s_2' = 1$. The expected equilibrium can also be given as

$$q_e = \frac{s_h}{2s_h + s_2} \quad (3c)$$

In some cases, there is selection against genotype *aa* in only one sex because of no reproductive success in that sex. Let us assume that genotype *aa* is not reproductive in males so that there are only the six mating types given in Table 4 (a) (these results are the same when there is no reproductive success in *aa* females instead of males). The frequencies of the three genotypes in the progeny are then

$$P' = \frac{p^2}{\bar{w}}$$

$$H' = \frac{p(Q+H)}{\bar{w}} \quad (4a)$$

$$Q' = \frac{qH}{2\bar{w}}$$

where

$$\bar{w} = p(p + Q + H) + qH/2$$

Then, after artificial selection favoring the heterozygote, the genotype frequencies become

$$P'' = \frac{P'(1-s_1)}{\bar{w}'}$$

$$H'' = \frac{H'}{\bar{w}'} \quad (4b)$$

$$Q'' = \frac{Q'}{\bar{w}'}$$

where

$$\bar{w}' = 1 - s_1 P'$$

A more extreme situation occurs when no wild-type individuals are allowed to mate, that is, complete selection against genotype *AA* ($s_1 = 1$) as in the rose-comb example in chickens discussed below. In this situation, again assuming that *aa* males are not successful in mating, there are only two possible mating types, *Aa* × *Aa* and *Aa* males × *aa* females (Table 4 (b)) and the progeny frequencies after natural selection are

$$P' = H/4$$

$$H' = 1/2 \quad (5a)$$

$$Q' = (1+Q)/4$$

Then, after artificial selection, they become

Table 4 (a) The six possible matings when *aa* males are not successful reproductively and (b) the two possible matings when only *Aa* males can mate and *AA* animals are not allowed to mate and the proportion of different progeny types produced

	Mating			Progeny		
	Male	Female	Frequency	*AA*	*Aa*	*aa*
(a)	*AA*	*AA*	P^2	P^2	—	—
	AA	*Aa*	*PH*	*PH*/2	*PH*/2	—
	AA	*Aa*	*PQ*	—	*PQ*	—
	Aa	*AA*	*PH*	*PH*/2	*PH*/2	—
	Aa	*Aa*	H^2	$H^2/4$	$H^2/2$	$H^2/4$
	Aa	*Aa*	*QH*	—	*QH*/2	*QH*/2
				$p^2/\bar{w}$	$p(Q+H)/\bar{w}$	$qH/2\bar{w}$
(b)	*Aa*	*Aa*	H^2	$H^2/4$	$H^2/2$	$H^2/4$
	Aa	*Aa*	*QH*	—	*QH*/2	*QH*/2
				H/4	½	(1 + *Q*)/4

P, *H*, and *Q* are the frequencies of genotypes *AA*, *Aa*, and *aa*, respectively, and $\bar{w}$ is the mean fitness.

$$P'' = 0$$

$$H'' = \frac{2}{3+Q'} \qquad (5b)$$

$$Q'' = \frac{1+Q'}{3+Q'}$$

Using this last equation for Q'' and substituting into it the expression for Q' from equation (5a), then

$$Q'' = \frac{1+\frac{1}{4}(1+Q)}{3+\frac{1}{4}(1+Q)} \qquad (6a)$$

At equilibrium, $Q'' = Q = Q_e$ and this expression becomes

$$Q_e^2 + 12Q_e - 5 = 0 \qquad (6b)$$

Solving this equation, the positive root is $Q_e = 0.403$ and by subtraction, $H_e = 0.597$. Therefore, the expected frequency of AA homozygotes in the progeny is $P' = H_e/4 = 0.149$.

Results

General

Let us begin by examining the expected increase in allele frequency for a new mutant with heterozygote advantage. The selection levels in these examples that are known are quite strong so that the expectation is that the increase in mutant frequency from a low level would be fast. To illustrate, Figure 2 gives the increase from an initial frequency of the new mutant of 0.01 (1%) for different levels of selection.

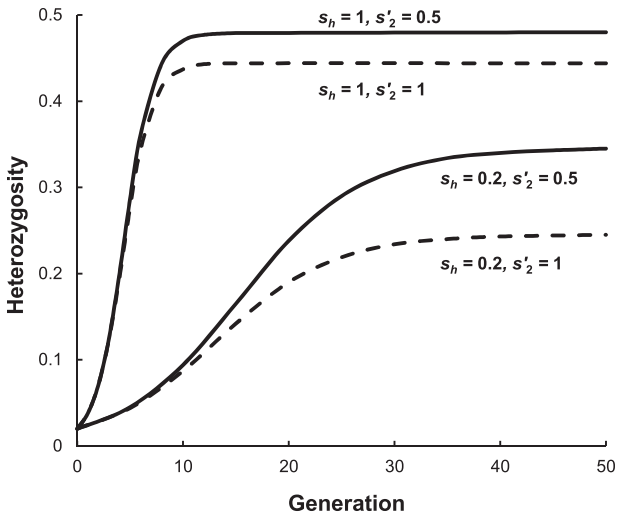

Figure 2. The expected increase in heterozygosity when a new mutant *a* is introduced at a frequency of 0.01 for different levels of artificial selection s_h for the heterozygote and natural selection s'_2 against the mutant *aa* homozygote.

When artificial selection is large so that $s_h = 1$, or heterozygotes for the mutant are twice as likely to be selected as wild-type homozygotes, then the frequency of heterozygotes increases very quickly and reaches very close to its equilibrium frequency in only 10 generations. Depending upon the generation length in a particular species, this could be as low as 10 years when the generation length is only 1 year as when young pets or livestock are bred.

Interestingly, this fast rate of increase is somewhat independent on the level of natural selection against the mutant homozygote because the increase from a low frequency is mainly determined by the difference in the fitness of the wild-type homozygote and the heterozygote. When the level of artificial selection is lower as when $s_h = 0.2$, then the increase is slower and it takes around 30 generations to reach a heterozygosity close to the equilibrium frequency (Figure 2).

The most extreme artificial selection and fastest increase in the frequency of a mutant would be if an individual with the mutant was identified and then used as the sole breeding individual for his or her sex, say the sole sire. In this case, if one out of 50 individuals was a heterozygous for a mutant, then the frequency of mutant allele *a* would initially be 0.01, as in the examples in Figure 2. If that heterozygous (*Aa*) individual was selected to be the sole sire and mated to females that were homozygous AA for the wild-type allele, then in their progeny, the frequency of heterozygotes would be 0.5 and the frequency of the mutant allele 0.25. Then if only heterozygous progeny were selected as parents in the next generation, in their progeny before selection, the mutant allele frequency would be 0.5, the maximum frequency expected if the homozygous mutant were lethal. Obviously, there is the potential if most heterozygotes can be correctly identified and given mating preference, to increase the mutant allele in a very few generations.

The equilibrium heterozygosity is a function of both artificial selection for the heterozygote (against the wild-type AA homozygote) and natural selection against the mutant *aa* homozygote. Figure 3 gives the equilibrium heterozygosity as a function of the level of artificial selection s_h for two levels of selection against the mutant homozygote where it is lethal, $s'_2 = 1$, and where it has half the fitness of the standardized heterozygote, $s'_2 = 0.5$. First, the equilibrium heterozygosity is higher when s'_2 is lower because this results in a more even amount of selection against the two homozygotes for the standardized fitnesses. Second, the four solid circles in Figure 3 indicate the equilibria for the four selection combinations in Figure 2. For example, for $s_h = 0.2$ and a lethal with $s'_2 = 1$, the equilibrium heterozygosity is 0.25, indicated by the lower leftmost solid circle. Finally, the level of heterozygosity for this solid circle demonstrates that it does not take very much artificial selection, only $s_h = 0.2$, to result in the substantial equilibrium heterozygosity frequency of 0.25.

Examples

The amount of artificial selection for these examples was estimated in the following manner assuming that the mutants are at, or near, their equilibrium frequency. First, a level of

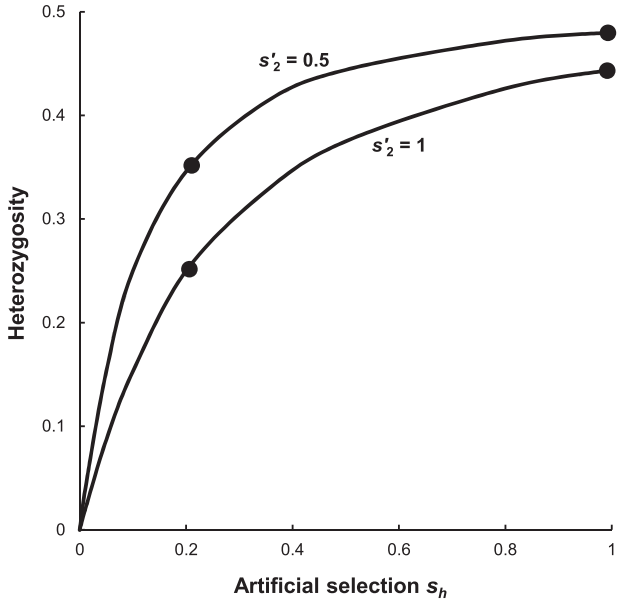

Figure 3. The equilibrium heterozygosity for different levels of artificial selection s_h for mutant heterozygotes and natural selection s'_2 against mutant *aa* homozygotes. The closed circles indicate the equilibria for the four combinations of artificial and natural selection given in Figure 2.

selection s'_2 against the mutant homozygote was assumed for a particular example as given in Table 4. Then, the amount of selection against the wild-type homozygote s_1 was assumed to determine the equilibrium allele frequency from equation (3b) and the predicted value of H' from equation (2a). The value of s_1 was varied until the heterozygosity level predicted from equation (2a) was equal to that observed in the particular example. The amount of artificial selection for the heterozygote s_h was then estimated from estimated value of s_1 using equation (1b).

Lethal

Of the examples of mutants in livestock and pets, there are three that are lethal as mutant homozygotes in both sexes; milk yield in Nordic red cattle, taillessness in Manx cats, and hairlessness in Mexican hairless dogs (Table 4). First, the lethal mutant increasing milk yield had heterozygosity frequencies of 13%, 23%, and 32% in samples of Danish, Swedish, and Finnish Red cattle. Therefore, for these frequencies, the levels of s_h necessary for these frequencies are 0.080, 0.175, and 0.307, respectively, in the three breeds. For example, in Finnish Red cattle, the heterozygote would be 1.307 times as likely to be selected as the wild-type homozygote. Each generation, 25% of the offspring from heterozygous by heterozygous matings would be lethal. Estimating that the frequency of heterozygotes after both natural and artificial selection is $H'' = 0.381$, then given random mating $(0.381)^2/4 = 0.036$ (3.6%) of the conceptions would be lost in this breed.

Second, the Manx cats (heterozygotes) have a frequency of 32.9% on the Isle of Man, and using the same approach as above, the estimate of artificial selection s_h is 0.324. However, because some of the matings in the population are not controlled (are probably close to random), the level of artificial selection is actually higher in the rest of the population. Third, both the breed populations of Manx cats and the breed population of Mexican hairless dogs are captive and presumably all (or nearly all) the matings are controlled. In other words, essentially s_h is very large and s_1 approaches 1 so that H'' approaches 1 as well. As a result, nearly one-fourth of the progeny die as mutant homozygotes. Of the surviving individuals, 33.3% are tailed or haired and 66.7% are tailless or hairless each generation. In these two breeds, the mutant is maintained essentially as a balanced lethal, complete natural selection against the mutant homozygote *aa* and complete artificial selection against the wild-type *AA*.

"Lethal" in One Sex

For three of the examples, there is no reproduction for homozygotes in one sex and the other sex appears to have normal reproduction. For the fecundity mutants in sheep, females are not reproductive when mutant homozygotes and mutant homozygote males appear to have normal reproduction. For the litter size mutant in pigs and rose comb in chickens, males are not reproductive when mutant homozygotes and mutant homozygote females appear to have normal reproduction. In these cases, I used two different approaches to estimate the amount of artificial selection and the expected frequencies of the genotypes. First, the average fitness over the two sexes can be used for the mutant homozygotes, that is, the average of 0 fitness in one sex and normal fitness of 1 gives an average fitness of 0.5 for the mutant homozygotes. Second, for these examples, I also either iterated Equation (4) for the sheep fecundity and pig litter size mutants or for rose comb in chickens, I used the solution to equation (6b).

A survey of the litter size mutant in Finnish Yorkshire pigs estimated that the frequency of heterozygotes was 36%. Therefore, using the approach with $s'_2 = 0.5$, an estimate of the amount of artificial selection was $s_h = 0.182$ and 5.5% of the births would be homozygous for the mutant (Table 5). The more exact approach (indicated by 1.0, 0.0 in the s'_2 column) was very similar with $s_h = 0.185$ and again 5.5% of the births mutant homozygotes.

Similarly, the heterozygosity for the sheep fecundity mutant in the Belclare breed was estimated to be 32%, resulting in an estimate of artificial selection for the heterozygotes of $s_h = 0.143$ and that 4.0% of the births would be homozygous for the mutant. The more exact approach was again very similar with $s_h = 0.148$ and 3.9% of the births mutant homozygotes. In this sample, 1.7% of the sheep were observed to be mutant homozygotes (Mullen and Hanrahan 2014), slightly less than these expectations. The even higher frequency of heterozygotes (44.0%) for a different mutant in the Cambridge sheep breed (above and Hanrahan JP, personal communication) results in an even higher estimate of $s_h = 0.321$ using the less exact approach. In addition, 10.7% of the births would be predicted to be homozygous mutants compared with the 8.3% observed. One consideration that might influence the

Table 5 The 12 heterozygote advantage mutants in livestock and pets organized as to their fitness in mutant homozygotes as lethal, "lethal" in one sex, near "lethal," and less selection

Mutant	Trait	Population	H'	s_2'	s_1	s_h	Q
Lethal	Milk yield (cattle)	Danish	0.13	1.0	0.075	0.080	0.005
		Swedish	0.23	1.0	0.149	0.175	0.017
		Finnish	0.32	1.0	0.235	0.307	0.036
	Manx (cat)	Isle of Man	0.329	1.0	0.245	0.324	0.039
		Breed	0.667	1.0	1.0	∞	0.25
	Hairless (dog)	Breed	0.667	1.0	1.0	∞	0.25
"Lethal" in one sex	Fecundity (sheep)	Belclare	0.320[a]	0.5	0.125	0.143	0.040
			0.320[a]	0.0, 1.0	0.129	0.148	0.039
	Litter size (pig)	Finnish Yorkshire	0.36[a]	0.5	0.154	0.182	0.055
			0.36[a]	1.0, 0.0	0.156	0.185	0.055
	Rose comb (chicken)	Wyandotte	0.667[b]	0.5	1.0	∞	0.444
			0.597[b]	1.0, 0.0	1.0	∞	0.351
Near "lethal"	Crooked tail (cattle)	Belgian Blue	0.247[a]	1.0	0.169	0.203	0.021
			0.247[a]	0.5	0.084	0.092	0.021
	Double muscling (dog)	Whippet	0.137[a]	1.0	0.080	0.087	0.006
			0.137[a]	0.5	0.040	0.042	0.006
	White color (dog)	Boxer	0.594[c]	1.0	0.733	2.704	0.179
			0.536[c]	0.5	0.367	0.580	0.179
Less selection	Ridgeback (dog)	Breed	0.556[b]	0.2	1.0	∞	0.444
	Leopard spotting (horse)	Ancient	0.40	0.5	0.177	0.215	0.068
			0.40	0.2	0.074	0.078	0.073
	Porcine stress syndrome (pig)	Pietrain	0.517	0.2	0.3	0.429	0.360
		Landrace	0.332	0.5	0.128	0.147	0.042

The parentheses around "lethal" indicate that the lowered fitness is not due to survival but to either artificial selection or other fitness components. H' is the observed frequency of heterozygotes after natural selection (except where noted), s_2' is the selective disadvantage of *aa* mutant homozygotes (when two values are given, the first is for males and the second for females), s_1 is the estimated selective disadvantage for AA wild-type homozygotes, s_h is the estimated selective advantage of Aa heterozygotes, and Q is the estimated frequency of *aa* mutant homozygotes before natural selection.

[a]Because these mutants do not influence survival in homozygotes, the frequency of heterozygotes here is before natural selection has occurred, not afterwards.

[b]For rose comb chickens, there is complete selection against single comb chickens, so this heterozygosity is that in rose comb chickens. For ridgeback dogs, there is complete selection against ridgeless dogs, so this heterozygosity is that in ridged dogs.

[c]It was assumed that 0.179 was the equilibrium frequency of white boxer homozygotes before selection and the equilibrium mutant frequency was $(0.179)^{1/2}$. Assuming the s_2' values given, then the s_1 values necessary to maintain this equilibrium were calculated.

frequency of these genotypes in these breeds is that there are other mutants in this breed that have similar effects and appear additive over loci (Hanrahan et al. 2004).

Gemmell and Slate (2006) estimated the amount of selection favoring heterozygotes for these mutants based on ovulation rates and litter size and found that the advantage was 0.328 for *BMP15* (X-linked) mutants and 0.368 for *GDF9* (autosomal) mutants. Standardizing the fitnesses for the *GDF9* mutant so that the heterozygote has a fitness of 1, and averaging over both sexes, the relative fitnesses are 0.866, 1, and 0.5 for genotypes AA, Aa, and *aa*. With these fitnesses, the equilibrium frequency of heterozygotes is 0.333, $s_h = 0.155$, and the frequency of mutant homozygotes is 0.045, not very different from that estimated above with a different approach. For the X-linked data for the *BMP15* mutant, the relative fitnesses are 0.868, 1, and 0.5 and the equilibrium frequency of female heterozygotes is 0.331, $s_h = 0.152$, and the frequency of female mutant homozygotes is 0.044.

For rose comb in chickens, the two approaches gave somewhat different results. The approach using $s_2' = 0.5$ gave the frequency of the wild-type homozygotes as 11.1% and mutant homozygotes as 44.4% before selection while the exact approach from expression (6b) gave the frequency of wild-type homozygotes as 14.9% and mutant homozygotes as 35.1% before selection. This latter estimate of 14.9% homozygotes is very close the 15.5% observed by Wehrhahn and Crawford (1965) and suggests that the more exact approach is preferable. Presumably the combination of strong artificial selection against wild-type AA homozygotes and the different amounts of selection in the two sexes can result in the less exact approach giving a significantly different estimate. Using a different theoretical approach, Wehrhahn and Crawford (1965) found a similar equilibrium frequency of wild-type AA homozygotes in chicks of 14.6%, given complete artificial selection against wild-type AA homozygotes and no reproduction from *aa* males.

Using the exact approach, the frequency of heterozygotes in chicks before artificial selection is 50% and the frequency of *aa* homozygotes is 35.1%, so that the equilibrium frequency of mutant *a* before artificial selection is 60.1%. Because in this case there is stronger selection against the wild-type homozygotes (none are allowed to breed) than the mutant homozygotes (only males do not successfully reproduce), the mutant allele at equilibrium is at a higher frequency than the wild-type allele. This is not as extreme natural

selection as the balanced lethal type selection against the Manx and hairless mutants where all mutant homozygotes *aa* were lethal because here there is selection against only the males. As I will discuss below, the strong artificial selection for ridged dogs (against homozygote ridgeless dogs) along with less natural selection against *aa* homozygotes results in a similar high frequency of the ridged mutant.

Near "Lethal"

For three of the examples (crooked tail in Belgian Blue beef cattle, double muscling in whippet dogs, and white color in boxer dogs), because of the extreme phenotype of the homozygote or its pleiotropic effect, the homozygous mutant animals that survive are often not used for breeding. For the Belgian Blue cattle and whippet mutant, I give two different values for s_2' in Table 4 because if all mutant homozygotes are identified and not allowed to breed, then homozygotes are lethal and $s_2' = 1$, or if half are identified and not allowed to breed, then s_2' is about 0.5, the exact value depending upon the size of s_h. For the mutant in Belgian Blue cattle, the amount of artificial selection favoring heterozygotes (s_h) is 0.169 and 0.084 when the mutant is lethal in homozygotes or $s_2' = 0.5$, respectively. For the mutant in whippets, the amount of selection favoring heterozygotes (s_h) is 0.080 and 0.040 when the mutant is lethal in homozygotes or $s_2' = 0.5$, respectively. It is possible that in some whippet lineages strongly selected for racing, both the amount of selection estimated and the frequency of the mutant might be higher.

For the mutant that results in white boxers, a different approach was used to calculate the amount of selection. In the population from the Netherlands examined (Nielen et al. 1998), the frequency of white mutant homozygotes was 17.9%. Assuming that this is the equilibrium frequency and two different values of selection against white homozygotes, $s_2' = 1$ or 0.5, then the amount of selection favoring the flash heterozygotes is quite high at $s_h = 2.704$ and 0.580. The high expected frequency of flash heterozygotes reflects this high selection.

Less Selection

In three examples, there is less selection against the mutant homozygotes; ridgeback in dogs, leopard complex spotting in horses, and porcine stress syndrome in pigs. First, for the ridgeback mutant, like the tailed progeny from Manx cats, the haired progeny from Mexican hairless dogs, and the single comb chickens in Wyandottes and other breeds, wild-type ridgeless dogs from Rhodesian ridgeback dogs are not used for breeding. However, selection is not as strong against mutant homozygotes because some homozygotes do not have dermoid sinus which is selected against. If $s_2' = 0.2$ and $s_1 = 1$ (no dogs without the mutant are ridged), then the expected frequency of heterozygotes is 55.6% and that of mutant homozygotes is 44.4%, not very different from the observed values of 56.2% for heterozygotes and 43.8% for homozygotes (Salmon Hillbertz et al. 2007).

In the ancient sample of horses, 40% were heterozygous for the leopard spotting mutant. Assuming that, as in contemporary horses, homozygotes for this mutant have night blindness, then homozygous horses would be selected against. If we assume that $s_2' = 0.5$ or 0.2, then the amount of selection favoring the heterozygote is $s_h = 0.215$ and 0.078, respectively. In this case, it is assumed that this is natural selection favoring the heterozygote with leopard spotting but in contemporary breeds, such as Appaloosa and Knabstrupper, artificial selection favors this color mutant.

The porcine stress syndrome mutant was found in extremely high frequency as both heterozygotes and homozygotes in the Pietrain pig breed, suggesting that the phenotype in heterozygotes was strongly artificially selected and that the negative effect in some homozygotes was not very large. For example, if we assume that $s_2' = 0.2$, then if $s_h = 0.429$ (note that this is larger selection favoring the heterozygote than against the mutant homozygote), the frequency of homozygotes is 36%. Although this value is lower than the frequency of homozygotes observed (0.448), it still indicates the type and magnitude of selection necessary to have these genotype frequencies. In the Landrace pig sample, the frequency of heterozygotes is lower and selection values of $s_2' = 0.5$ and $s_h = 0.147$ are consistent with it. For this breed, 4.2% mutant homozygotes are predicted while the observed frequency is 2.1%.

Discussion

There are a number of mutants in livestock and pets that have been maintained by heterozygote advantage adding significantly to the number of polymorphisms maintained by heterozygote advantage in populations. Here I have given details of the 12 best documented examples, seven of them in livestock and five in pets (see other potential examples in Santschi et al. 1998; Pailhoux et al. 2001; Cavanagh et al. 2007; Qin et al. 2010; Reissman and Ludwig 2013; Kadri et al. 2014). These mutants with a heterozygote advantage constitute a great diversity of mutation types (Tables 1 and 2) with some causing phenotypic and fitness effects because of small changes, either nonsynonymous substitutions in coding regions or small insertions or deletions that cause reading-frame changes. Other mutants are caused by large duplications or deletions that either duplicate or eliminate whole genes or linked groups of genes. Overall, most of the mutants appear to cause loss-of-function changes that result in lack of production or significantly altered function of an important protein.

Embryonic lethals or mutants with strong detrimental pleiotropic effects segregate in low frequency in humans and other mammal populations. However, in livestock and pets because of strong selection favoring them as heterozygotes, some of these mutants are segregating at much higher frequencies within breeds. Some mutants in high frequency in livestock or pets might have this high frequency within breeds because of chance effects due to small founder numbers or other chance effects during the formation of the breed. In addition, chance effects might be important in many breeds because of contemporary small effective population size

and/or inbreeding. For example, Leroy et al. (2013) estimated the effective population size in 60 dog breeds, 40 sheep breeds, 20 cattle breeds, and 20 horse breeds from contemporary pedigree data in French populations and found that the effective population sizes in many breeds are quite small.

In some cases, it might be difficult to differentiate between mutants that have been increased by chance and those at high frequency because of heterozygote advantage, as discussed here, because of the lack of detailed data on the impact of the mutant in heterozygotes. However, in general mutants that are in high frequency because of chance effects would probably be completely recessive, are seen primarily as homozygotes because of recent, close inbreeding, and do not have phenotypic or fitness effects in heterozygotes.

There are several examples here in which it appears that there is as strong, or stronger, artificial selection for the mutant heterozygote than there is natural selection against the mutant homozygote. In two cases, the Manx mutant in cats and the hairless mutant in dogs, equally strong complete selection against the two homozygotes has effectively resulted in a balanced lethal. For the rose-comb mutant in chickens and the ridged mutant in dogs, there is actually stronger selection against the wild-type homozygote than the mutant homozygote, resulting in the frequency of the mutant being greater than 0.5. Similarly, although the levels of selection do not appear to have been as strong, for the porcine stress syndrome mutant in pigs, it appears that in the past there was stronger artificial selection for the mutant in heterozygotes than against mutant homozygote, resulting in the frequency of the porcine stress mutant greater than 0.5 in some breeds.

Overall, these mutants are generally maintained by a balance of artificial selection favoring heterozygotes and natural (and sometimes artificial) selection against mutant and/or wild-type homozygotes. From the models I have discussed, only a relative small artificial selective advantage in heterozygotes, say 10%, can result in a substantial frequency of heterozygotes, even though there is strong selection against homozygous mutants. For example, if the mutant homozygote is lethal and there is a 10% advantage to heterozygotes, then 15.4% of the population would be expected to be heterozygous at equilibrium.

When the level of selection is estimated in the way used above, it is assumed that the mutant was at or near equilibrium frequency. Because the amount of selection is often large for these mutants, it might not take very long to reach the equilibrium frequency, making the use of the equilibrium value reasonable. However, if the heterozygosity has not yet reached the equilibrium value, then the amount of artificial selection favoring the mutant might actually be larger than was estimated here.

The general impact of a mutant in livestock production can potentially be examined from the benefit in heterozygotes, the frequency of heterozygotes times the increase in the desired phenotype over the wild-type homozygote, minus the cost in homozygotes, the frequency of the mutant homozygote times the loss in phenotypic value compared to the wild-type homozygote. For example, if there were a 10% increase in the desired phenotype in heterozygotes and 0.3 heterozygote frequency and lethality in mutant homozygotes with a frequency of 0.03, then the

$$\text{benefit} - \text{cost} = (0.10)(0.3) - (1.0)(0.03) = 0$$

and the benefits and costs are the same. If there were was no net benefit, then selecting for the presence of the mutant in livestock or pets would be of questionable value unless other factors played an important role for its maintenance.

For phenotypes that have been artificially selected for in livestock and pets, it would be beneficial to identify mutants that cause the same phenotypic effect but do not have a detrimental pleiotropic effect on fitness or other phenotypes. For example, mutants at other loci result in high ovulation rate in sheep but do not have negative fitness impacts (Davis 2005), other short-tail variants, such as in bobcats and lynx, are not lethal as homozygotes like the Manx mutant in cats (Buckingham et al. 2013), and another mutant results in rose comb in chickens that does not have infertility effects in homozygous mutant males (Imsland et al. 2012). That is, some recessive mutants might have desirable traits for livestock and pets in heterozygotes but have not too negative pleiotropic effects on some component of fitness or phenotype. In these cases, it would be possible to have the mutant fixed in the breed or population, but this would not lower fitness due to the negative pleiotropic effects very much. In addition to not having negative pleiotropic effects, these variants would be true breeding and would not produce progeny by segregation that do not have the desired phenotype.

Both livestock and pets have provided examples of fast evolutionary change and selective sweeps for some genes that have major effects on traits favored by artificial selection. However, the high frequencies of these mutants presumably would not be maintained without artificial selection because of their strong negative pleiotropy effects, and once they are no longer favored, their decrease might occur very quickly. For example in dogs, strong phenotypic selection has resulted extreme dwarfism, miniaturization, gigantism, and hairlessness. Selection for appearance has been so extreme that in Cavalier King Charles spaniels their brains are too large for the size of the skull and in Boston terriers their heads are so large that 92% of them must be born by Cesarean section (Williams 2010). Needless to say, these and other phenotypes and genotypes produced by extreme artificial selection would have very low viability and fitness without extensive human intervention and could be quickly eliminated if artificial selection did not favor them.

When there was a molecular detection approach for mutants in heterozygotes and the detrimental impacts were known for the litter size mutant in pigs, the porcine stress syndrome in pigs, the crooked tail in cattle, and bulldog dwarfism in Dexter cattle (Cavanagh et al. 2007), selection against these mutants resulted in a very fast reduction in the frequency or near elimination of these mutants. For example, if all heterozygotes can be identified and breeding of heterozygotes can be eliminated, then the mutant could be eliminated in one generation.

On the other hand, if the mutant provided some important value, then when the impact is sex-limited, as for the fecundity mutants in sheep and the litter size mutant in pigs, then the following approach could be used. For the fecundity mutants in sheep that have no known impact in males, homozygous male mutants *aa* could be mated to homozygous females *AA*. All the female progeny would have the desired *Aa* genotype with high fecundity. For the litter size mutant in pigs, homozygous female mutants *aa* could be mated to *AA* male to obtain all female *Aa* progeny with higher litter size. Of course to continue this breeding scheme over generations, source populations for the *AA* and *aa* genotypes would need to be maintained, making this breeding scheme more difficult to continue and potentially not giving enough benefit for the costs of implementing it.

Another potential example of heterozygote advantage is that for the black coat color phenotype in wolves and dogs. Anderson et al. (2009) concluded that the same dominant allele at a defensin gene in 30 breeds of dogs that resulted in black coat color also caused black coat color in wild populations of wolves. Coulson et al. (2011) estimated fitnesses in wolves from Yellowstone National Park and found that black heterozygote wolves had the highest fitness, gray homozygote wild-type wolves had somewhat lower fitness, and black mutant homozygotes had much lower fitness (see also Hedrick et al. 2014), similar to the examples given here. However, some dog breeds are true breeding for this same black allele and do not appear to have a lowered fitness as estimated in the black homozygous wolves in Yellowstone. Assuming that the fitnesses estimates are accurate in Yellowstone wolves, then perhaps something in the natural wolf environment causes lower fitness in black homozygotes in wolves but not in dogs, such as some stress or disease, or there is some type of epistasis such that black homozygotes in the wolf genetic background have a lower fitness than the same black homozygote genotype in the dog genetic background.

Hedrick (2012) and Hedrick et al. (2014) discussed the somewhat counterintuitive findings possible when there is both asymmetric heterozygote advantage and a small population. However, those impacts are likely mostly in naturally breeding populations and are generally unlikely in closed populations of breeds of livestock and pets where matings are often carefully controlled. For example, as I have discussed here, a single artificially selected individual might quickly change the frequency of a new mutant with a desired phenotype. In other words, the high potential selective effects on these mutants, even when rare, appear to generally outweigh the chance changes due to small population size.

Overall, including these mutants in livestock and pets, the number of polymorphisms maintained by heterozygote advantage are many more than the almost always cited case of sickle cell anemia. Of course, for these mutants, human artificial selection plays an essential role in their maintenance by heterozygote advantage and without it presumably the polymorphism would be lost. However, these examples might be important for identifying other genetic variants maintained by heterozygote advantage. For example, given very strong selection from an environmental or other change, a new mutant that has an advantage as a heterozygote might increase in frequency. However, if it had a lowered fitness as a homozygote, it would be maintained as a polymorphism due to its overall heterozygote advantage much like the variants discussed here.

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Corresponding Editor: Fred Allendorf