INTERNATIONAL JOURNAL OF BIOLOGY AND BIOMEDICAL ENGINEERING
DOI: 10.46300/91011.2022.16.15 Volume 16, 2022

# Peculiarities of inheriting coat colour shades in the breed "Golden Retriever"

Alexander Alexandrovich Ermakov,
Moscow Aviation Institute (National Research University),
4 Volokolamskoe shosse, 125993, Moscow
Russian Federation
aleral@mail.ru

Received: June 14, 2021. Revised: December 1, 2021. Accepted: December 17, 2021. Published: January 3, 2022.

**Abstract—The Article describes the peculiarities of colour shades in the Golden Retriever breed. The separate attention was given to nowadays existence of different standards of coat colour in this breed across the world, and these standards admit a broad variety of different shades in golden colour. Herewith it has been established that the white pigmentation colour of golden retrievers is unallowable in any of "Kennel Club" standards valid for today. The special focus was put to the genotype of breed, which presupposes the existence of double recessive gene (*e/e*) that predetermines creamy shade of coat colour. It was discovered that exactly the gene *MC1R,* its autosomal-recessive inheritance, plays the leading role in defining the coat colour of dogs and in the exterior of the breed. It was assumed how and why this genotype is widespread in population, at which extent sub-populations are distinguished (American and English-European ones), and also there was the option offered related to breeding the pure line on the basis of knowledge about karyotype of dogs, that were obtained in a course of predicative screening of E-locus.**

**Keywords—Golden Retriever, coat colour, breed standard, e/e genotype, MCIR.**

## I. Introduction

Golden Retriever — is a breed of relatively big hunting dogs having the coat of medium length, of characteristic golden shade, which are also good companions and «family» dogs due to the kind complaisant character of breed. The Golden Retriever breed has its origins in Scotland in 1868, when the sportsmen started using dogs in hunting. Breed was developed from British tweed water spaniel (now extinct breed) by way of crossing with Newfoundland, and also the blood of Irish Setters and Bloodhounds was transfused. For the first time ever the Golden Retrievers were registered in British «Kennel Club» in 1903. They were finally acknowledged as a breed 1913, in 1954 - recognized by World's canine organization [1].

The breed standard is the following: this is a dog of harmonious, relatively big constitution, with brawny limbs and roundish paw pads. Head is of size proportional with reference to the body, has a widening in the area of parietal lobe and well-defined transition from forehead to foreface. Nose is black, eyes are of dark brown colour (hues are acceptable), and eyelid edges are dark. Eyes are wide-set, ears are located approximately at the level of eyes: of medium size, hanging. Tail is long, never curled, and continues the natural line of spine. Height at the withers for male dogs is 56-61cm, for female dogs - 51-56 cm, weight of male dogs varies from 26 to 41.5 kg, weight of female dogs - from 25 to 37 kg, respectively [2].

In terms of behaviour, Golden Retrievers are friendly, open-minded dogs that are usually not aggressive. Studies have shown that the lack of pronounced aggressiveness in Golden Retrievers is genetically determined. Therefore, dogs of this breed are often got in families with children, and are also used as guide dogs and in rescue operations. Eye-pleasing colour and friendly muzzle makes such a dog attractive even for people who are afraid of large dogs [3].

## II. Coat of Golden retriever

Coat is with thick undercoat, straight or slightly wavy, of golden shades. To this date, the colours of golden retriever include: creamy, dark golden, light golden, red and, of course, classic golden (see Fig. 1). In official standards of Golden retriever breed it's mentioned that this dog should always have one-coloured coat, white coat colour is considered to be the fault of the breed. Although «featherings» (the

longer coat on limbs and tail) can be of shade lighter than the coat colour of the rest of the body [4].

Different shades of golden retriever's coat colour are conditioned solely by breed genotype. Same as for other breeds, different coat colour of these dogs depends on pigmentation, which defines tint and shade of coat colour.

Fig. 1 Admissible shades of golden retriever's coat colour [4].

Colour of a dog's coat depends on the gene *PMEL*, controlling production of melanin under the name *Pheomelanin* [5, 6]. This is pigment of red colour with golden and yellow shade as a default. *Pheomelanin* creates coat colour shades from dark red to orange, creamy, golden, yellow and yellow-brown. Range of genes (*Slc7a11, ASIP, a-MSH*) controls the level of pheomelanin expression and proliferative rate of melanocytes, which makes the colour stronger or weaker [7, 8].

The pathway of regulation of the *Pheomelanin* and *Eumelanin* genes through the interaction of the *Agouti* genes and *Melanocortin-1 receptor (Mc1r)* gene looks especially interesting. Studies have shown that in many mammals, the interaction between these two genes acts as a switch: either dark color (*eumelanin*), or light or tan/red (*pheomelanin*). This is considered the main way of regulating the color of laboratory mice and rats, for example. Moreover, in dogs, apparently, this type of regulation is somewhat different from events in other mammals. The gene *Agouti* in dogs is 98% identical to the homologue of such a gene in the fox and is located in a conserved region of the chromosome. However, its expression has practically no effect on the level of expression of *pheomelanin* genes. The only exception is black German Shepherds, in which the inheritance of color is associated with a polymorphism of the color gene [9, 10].

Accordingly, according to modern data, in the absence of significant polymorphisms in the breed, the color of the dog completely depends on the level of expression of the *PMEL* gene. However, in the presence of polymorphisms, the color of a dog can be influenced by the standard for mammalian regulation pathway through the *Agouti* gene.

Golden Retrievers can be found around the globe, but its appearance can vary depending on the country. In spite of mutual English origin, for today, the differences in coat colour of Golden retriever breed in American and European continents have formed and they are connected with the fact that in USA and Canada the breed was developing and develops according to the standards of American «Kennel Club», but in Europe — according to the standards of «Kennel Club» in Great Britain [2, 4].

Subject of genetics related to dog's coat colour is very difficult, and unfortunately, not fully researched yet. Currently, from scientific and practical points of view, the questions connected with genetic diversity of golden retrievers draw the significant attention, because all purebred dogs of this breed have double recessive gene (*e/e*), which doesn't allow for black colour (its pigment) of the coat, but at the same time, the genotype (*e/e*) predetermines the existence of creamy shade in coat colour instead of traditional yellow-gold, which was the most wide-spread in the past.

The start of research related to dog DNA was made in Broad Institute in Cambridge (USA) by means of dog's genome sequencing. In 2004 the scientific employees of this institute published the result of the whole genome sequence (approximately 2.5 billion base pairs) of the Boxer female [11]. Later on, the following authors dedicated their works to carrying out a molecular genetic analysis of dogs belonging to different breeds: Adams J R, Leonard J A, Waits L P, Johnston E, Halverson J, Marshall K, Rosenfeld D, McKenna S, Shap T, Edwards J Basically, the genetic researches were conducted with Microsatellite DNA loci, and in our country also: these are the works of Grafodatsky A S, Rajabli, S I, Semenova S K, Illarionova N A, Vasilyev V A, Shubkina A V, Rysakov A P and others.

Although, the questions of selection, scientific researches, experimental and exploratory work, in frames of which the preferred genotypes are created and educed, including those, connected with dog's coat colour and representing the starting material for improvement of existing breeds and development of new ones, remain open and require further profound research.

Therefore, taking into account the above said, the aim of the article is to explore the peculiarities of coat colour shades in the breed of Golden Retriever from the point of view of existing American and European standards of this breed and with respect to its genotype.

Thus, at present time, the American «Kennel Club» allows registering only for golden colours in the following standard coat colours: dark-golden (registration code 080), golden (registration code 093) and light golden (registration code 119).

The indicated colours are also set in the standard of Great Britain «Kennel Club», although, in 1936 the creamy colour was added to English standard. After a few years of selection these lighter samples got the name «English cream golden retrievers» [12].


Astarta degenia velebitika hayek


Astarta anturium sherzerianum album

Nowadays, these samples of light colour are called «English golden retrievers», «European golden retrievers», «light-golden retrievers», and even «white golden» or «platinum blond golden». Although some golden colours can seem very light, they are not white in fact: basically the white colour does not genetically occur in this breed. «White gold» same way is not admitted both by British and American clubs of kennels: any quantity of fair hairs is considered to be the mistake and breed fault [12].

It's known that a dog's chromosome has at least ten regions where the pair of genes, which can influence the colour of coat, occurs. Some of genes control the colour itself (black, brown, yellow), while the other genes influence the distribution of colour, which results in occurrence of light dots (like Dobermann), dark faces (like Mastiff and German shepherd), tiger colour (like Boxer), or stains (like Beagle). Besides, the genes from certain regions of chromosomes interact with genes from other regions, more often in accordance with pleiotropic mechanism, more rarely — through epistatic interaction, which in the end leads to absolutely unexpected result, in comparison with the case when genes would be in isolation and wouldn't interact in any ways [15].

Of course, it is also worth mentioning the epigenetic regulation of the expression level of individual genes in dogs. Modern research argues that epigenetic regulation is developed in all mammals and plays an important, if not the main role in the regulation of the level of expression of individual genes. In particular, it is already known that epigenetically in dogs the work of certain steroidogenic factors, as well as the aging process, is regulated [13, 14]. However, in this work, aspects of epigenetics will not be considered specifically. Firstly, at present there are no detailed, precise works covering the issue of epigenetic regulation of the color of dogs, especially golden retrievers. Second, until the end of all the ways of "classical" regulation of the color genes through intergenic interactions and transcription factors have not been fully investigated - it is premature to talk about epigenetics.

However, because this article deals with peculiarities of coat colour exclusively of golden retrievers — the research gets simplified to some extent, because there are only 3 genes, affecting the coat colour, distinguished in this breed. The rest 7 regions are constant, that's why retrievers don't have dark masks, dots, marks and stains nowhere on the body. So, we will tell just about 3 genes.

As it was mentioned earlier, for the dogs of Golden retriever breed, the high level of gene expression in locus E is observed, which leads to dilution or paleness of phaeomelanin pigmentation, caused by this karyotype, and ensures golden colour of coat. As a result of using STR-markers, which are very polymorphous and have high ability of defining genetic differences between species and breeds, in sampling of 691 golden retrievers it was found that the creamy coat colour, corresponding to autosomal-recessive type of inheritance, occurs in dogs with *e/e MC1R* genotype. Although, it's of particular interest to note that in wild type this genotype does not cause the occurrence of creamy colour, that's why the assumption that some more genes exist, which as a result of interaction with *e/e*, lead to emerge of creamy shade instead of yellow or red colour, was put forward [16].

Fig. 2 shows the interaction of genes in the breed of Golden retriever in four loci, characteristic for the genome of this bread, from the point of view of their role in coat colour. If the circle is filled with colour, this means that the dog's coat colour was defined at

this point. If the circle remains white — this means that the information about the additional gene is required.

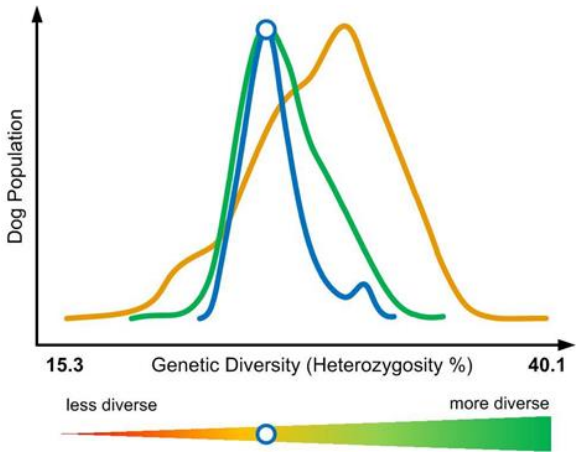


Fig. 3 Chart of genetic diversity in Golden retriever breed [17]. All statistics and graphic illustrations for them, presented in the figures, are taken from specific articles dedicated to Golden Retrievers. Graphic illustrations are cited without changes, since the author of the article does not have the original data.

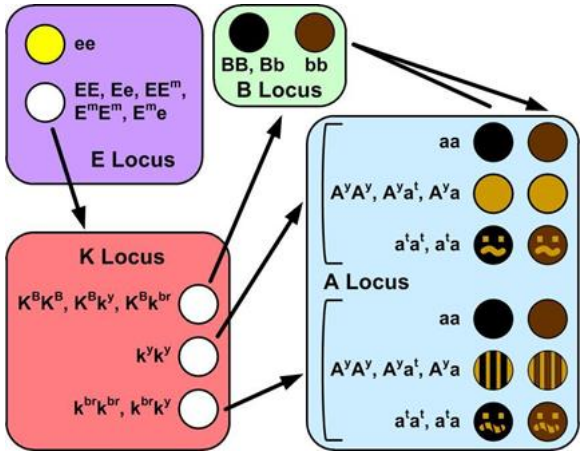


Fig. 2 Influence of loci A, B, E and K in defining coat colour of Golden retriever [16]. A graphical diagram illustrating the interaction of genes, cited by Bauer et al. Perhaps this scheme is subject to clarification or change, but the author did not set his task as an applied study of the interaction of loci, and therefore did not modify it in any way.

The genotype *e/e MC1R,* which is under consideration, has at least eight variations affecting dog's appearance: *E, Em, eh, ed* and *e, e2, e3*. There are totally 36 possible combinations of these alleles, although some of them are theoretically predicted, because till now it has not yet been witnessed that any of dog breeds could have all eight variants.

It's worth to note that E-locus is dominating among all other loci of golden retriever coat colour. Moreover, it controls the production of eumelanin (black pigment) in melanocytes: dominant form of *MC1R* gene (*E*) ensures normal production of eumelanin in melanocytes.

Although, the variant of DNA in gene *MC1R* (*e*) stops the production of black pigment, and saves only the production of pheomelanin (yellow/red pigment) in melanocytes. Given that, the gene *e* is autosomal-recessive, the both alleles of this variant of *MC1R (e/e)* should be present in order to get solid «yellow/red» coat colour. The dog, which possesses two alleles *E* (*E/E*), theoretically won't have yellow-red coat and won't be able to pass yellow-red coat to its generation. The dog which has one allele *E* and one allele *e* (*E/e*), won't have yellow-red coat, but can transfer the gene of yellow/red coat colour *e* (or gene of black coat colour *E*), segregation in its generation will happen according to the Mendel's First Law as 1:2:1 (*EE/Ee/Ee/ee*) in genotype and 3:1 in phenotype (black colour prevails). The dog, which has both alleles *e* (*e/e*), will get a yellow-red coat and can only pass the gene of yellow/red coat colour to its generation [5,7,12,16].

The more detailed genetic studies of dogs, referred to Golden retriever breed, testify that the median of their genetic diversity presently amounts to 25,4% (marked with blue-white dot on Fig. 3). This means that dogs under consideration inherited different forms of alleles of coat colour genes from mother and father approximately in 25,4% of cases. In general, in frames of breed the level of diversity varies in the range of 22,6-31,6%, i.e. coat colour gene *MC1R* is inherited according to Non-Mendelian inheritance and thereon, the percentage of heterozygotes (E/e) doesn't exceed 31,6% (while theoretical expectation is 50%). It can be assumed that homozygote according to recessive (e/e) is prioritized inherited coat colour, which is also indirectly confirmed by its phenotype expression (standard of Golden Retriever breed). It's remarkable that the median for all tested dogs of different breeds amounts to 28,8% (orange line on Fig. 3). This means that, in general, the recessive type of inheriting this trait occurs among dogs more often, which can be explained both by predominance of pure lines among breedy dogs (exactly such dogs were used in experiment), and by lack of selection pressure on coat colour: really and truly, in conditions of urban territories the dark coat actually doesn't allow any competitive advantages in comparison with lighter coat, therewith, among many breeds in exterior, light shades of coat are more highly valued [1, 2, 17].

It's important to note that the chart of diversity demonstrates two separate peaks that comply with two sub-populations of golden retrievers: American and English (European). This helps to make the conclusion that two different lines of breeding golden retrievers lead to the fact that nowadays the breed is clearly divided in two sub-populations, which, by being genetically uniform in its area, to a certain

degree differ from each other, and coat colour is a clear expression of this diversity.

## III. Discussion

In order to be able to draw a definite conclusion regarding the heritability of coat color in golden retrievers, it is necessary to first summarize a number of data.

First, as shown in the literature, the color itself of golden retrievers can vary very widely. Of course, when we say "Golden Retriever", we primarily mean a golden-colored dog. However, all sorts of shades and half-tones of color lead to the fact that as such a "golden" color is defined rather as "from deep golden dark tones to platinum, almost white." Since all such shades are acceptable according to international standards, it is necessary to recognize the possibility of genetic diversity of the parent individuals by color. Then, no matter how much the dog handlers would like it, it is impossible to introduce the concept of "ideal genotype" by color for a given breed.

Secondly, it is necessary to understand that color is inherited as a combination of the work of several genes. Since each gene in the body of mammals is normally represented by two alleles, respectively, the number of possible combinations of alleles of several genes is extremely large. Suppose that all color genes work for us "by a direct mechanism", that is, directly the product of the expression of a particular gene (that is, a protein) interacts with the expression product of another gene and gives a certain color. Of course, as we saw with the example of the interaction of loci of *MCIR*, such a scheme is very simplified and gives the most general idea of the process of inheriting the color of the breed. But even so, with only two genes working with three variants (recessive homozygote, dominant homozygote, and heterozygote), we can have nine variants of the dog's genotype. Since in our case there are at least two genes (*PMEL* and *MC1R*), the work of at least one of them is controlled by three more genes (*Slc7a11, ASIP, a-MSH*), and at the same time, the other gene may have four different loci (A, B, K, E) – in the end, this gives us 1728 ((4^3)*(3^3)) variants of interaction between the alleles of the genes that control pheomelanin: itself gene pheomelanin, its receptors and loci in the gene of this receptor. It is clear that there are the most common genotypes, however, analysis of the frequency distribution of pheomelanin receptor (*MC1R*) loci showed that they are not normally distributed in the population, that is, large-scale screening is needed to build an accurate genetic map of the Golden Retriever population.

With all this, we, in principle, do not take into account the possibility of intergenic interaction (for example, pleiotropy or epistasis), do not take into account the possibility of the existence of other regulatory genes (in addition to those mentioned by us), and also fundamentally ignore epigenetic regulation. So it's a long way to go to a complete map of the Golden Retriever population.

Third, it is necessary to take into account the factor of evolution. On the one hand, differences in subpopulations of American and European Golden Retrievers clearly show how fast evolutionary processes proceed in relatively isolated groups with relatively rapid generational change. The breed standards of many dogs have changed and continue to change, so variations in color, as well as the preferences of owners on different sides of the ocean, can lead to the emergence of virtually two breeds - the European and American Golden Retrievers.

At the same time, one should not forget that «Kennel Clubs» appeared a little over a hundred years ago, and before that many breeds were bred "handicraft", simply by crossing the dogs with the most pleasant exterior. That is why phylogenetic studies show a "jumble" in terms of the genetics of most breeds: often there are several breeds in the past behind a particular breed in the present [19, 20]. There are cases when a breed from the past is now lost or its appearance has changed significantly. Actually, the Golden Retrievers have the blood of three existing breeds and one that has been lost. All this "blurs" the pattern of color inheritance: in relatively different breeds, the same genes from a common ancestor can be responsible for color, and, conversely, in related breeds, color can be determined in completely different ways. This also significantly complicates the issue of identifying the "ideal genotype" of the Golden Retriever color and entering it into the catalog.

And finally, we must remember about such a thing as "a matter of taste". As numerous studies show, when choosing a dog, most people are guided not so much by its working qualities as by its exterior [21]. Moreover, many people are not confused by the visible defects of the dog or hereditary diseases [22]. The popularity of a breed depends on the fashion for a particular dog, on its properties as a companion dog and simply on which country you are in. Actually, this is clearly demonstrated by the differences in shades of Golden Retrievers in America (USA) and Europe (European Union): residents of different countries consider different shades of color to be "ideal", more often buying dogs of a particular color. All this not only constantly "artificially mixes" the population of one breed, but also leads to the emergence of subpopulations by country and even sub-subpopulations by regions of one country. In such a situation, any theoretical prediction of the genetic structure of a population turns out to be incorrect in practice.

Of course, it is possible to identify the main genes responsible for the inheritance of the color, recognized as the standard by various Kennel clubs, which was done in the conclusion. But we must understand that after a few years, the ratio of individuals in the population and the penetrance of color genes may be completely different simply because a white Golden Retriever starred in an advertisement for dog food.

## IV. Conclusion

To sum up the conducted research, it's possible to draw the following conclusions. Firstly, nowadays in «Kennel Clubs» of different countries there is a broad range of dog coat colours that is accepted in the frames of standard for Golden retriever breed. The most diverse coat colours in the segment «gold of different shades» are set in the standard of Great Britain «Kennel Club». While in the USA the predominant colour of a dog's body, which is either too pale or dark, is considered to be unwanted. Secondly, dominance of one or another shade is connected with proportion of pheomelanin and eumelanin, which is defined by E-locus and genes *PMEL* and *MC1R*. Thirdly, the key role in formation of coat colour in Golden retriever breed is played by autosomal-recessive inheritance of eumelanin pigment in genotype *e/e*, which predominates nowadays in both sub-populations of breed: American and English (European). In connection to this in the process of breeding dogs belonging to such breed, it's necessary to make predictive DNA testing for availability of allele *e* and karyotype *e/e*, and also to study the other loci, which will help to limit the possibility of occurring modified phenotype in generation, and, probably, will lead to breeding of «absolutely» pure line of Golden retriever breed with genotype *e/e*.

## References

[1] https://www.thekennelclub.org.uk/search/breeds-a-to-z/breeds/gundog/retriever-golden/.
[2] http://www.fci.be/en/nomenclature/GOLDEN-RETRIEVER-111.html.
[3] Liinamo, Anna-Elisa, et al. "Genetic variation in aggression-related traits in Golden Retriever dogs." Applied Animal Behaviour Science 104.1-2 (2007): 95-106.
[4] Wendy Andrews, "Golden Retriever. The Breed Standard Illustrated".
[5] E. Voslarova, et al., "Coat Color of Shelter Dogs and Its Role in Dog Adoption", Society and animals: social scientific studies of the human experience of other animals, vol. 27, no. 1, pp. 25-35, 2019.
[6] Available: https://vertebrate.genenames.org/data/gene-symbol-report/#!/vgnc_id/VGNC:44730.
[7] S. Chintala, et al., "Slc7a11 gene controls production of pheomelanin pigment and proliferation of cultured cells" Proceedings of the National Academy of Sciences, vol. 102, no. 31, pp 10964-10969, 2005.
[8] T. Kondo and V. J. Hearing, "Update on the regulation of mammalian melanocyte function and skin pigmentation", Expert review of dermatology, vol. 6, no. 1, pp 97-108, 2011.
[9] Kerns, Julie A., et al. "Characterization of the dog Agouti gene and a nonagouti mutation in German Shepherd Dogs." Mammalian Genome 15.10 (2004): 798-808.
[10] Boyko, Adam R., et al. "A simple genetic architecture underlies morphological variation in dogs." PLoS biology 8.8 (2010): e1000451.
[11] T. Casci, "Association studies: Dog genes mapped at a SNP" Nature reviews. Genetics, vol. 8, no. 11, pp 824-825, 2007.
[12] http://www.fci.be/Nomenclature/Standards/111g08-en.pdf.
[13] Sekiya, Asato, et al. "Dog Steroidogenic Factor-1: Molecular cloning and analysis of epigenetic regulation." Journal of Veterinary Medical Science (2020): 20-0050.
[14] Thompson, Michael J. "An epigenetic aging clock for dogs and wolves." Aging (Albany NY) 9.3 (2017): 1055.
[15] Jinny Johnson, Golden retriever. London: Franklin Watts, 2013, p. 24.
[16] A. Bauer, et al., "A novel MLPH variant in dogs with coat color dilution", Animal genetics: immunogenetics, biochemical genetics, molecular genetics, vol. 49, no. 1, pp, 94-97, 2018.
[17] T. E. Perez, et al., "Relationship between the melanocortin-1 receptor (MC1R) variant R306ter and physiological responses to mechanical or thermal stimuli in Labrador Retriever dogs", Veterinary anaesthesia and analgesia, vol. 44, no. 2, pp. 370-374, 2017.
[18] Farm RKF «ASTARTA», Available: www.astartakennel.ru.
[19] Vilà, Carles, Jesus E. Maldonado, and Robert K. Wayne. "Phylogenetic relationships, evolution, and genetic diversity of the domestic dog." Journal of Heredity 90.1 (1999): 71-77.
[20] Pollinger, John P., et al. "Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication." Nature 464.7290 (2010): 898-902.
[21] Ghirlanda, Stefano, et al. "Fashion vs. function in cultural evolution: The case of dog breed popularity." PLoS One 8.9 (2013): e74770.
[22] Sandøe, Peter, et al. "Why do people buy dogs with potential welfare problems related to extreme conformation and inherited disease? A representative study of Danish owners of four small dog breeds." PloS one 12.2 (2017): e0172091.