Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Medical Genetics Unit

Philosophiae Doctor (PhD)
Thesis 2023:1

Noise reactivity and fear of fireworks in dogs: a study of genetic and phenotypic variables

Lydangst og frykt for fyrverkeri hos hund: en studie av genetiske og fenotypiske variabler

Karin Westereng Handegård

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Ås 2022

Thesis number: 2023:1

ISSN: 1894-6402

ISBN: 978-82-575-2020-5

"Stotting is jumping upward with all four legs simultaneously.
My advice: do not die until you've seen a large black poodle stotting in the snow."
-Douglas Adams

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Acknowledgments

First of all, I am very thankful for all the expert guidance and support provided by my supervisors, Prof. Frode Lingaas and Dr. Linn Mari Storengen. A special thank you to my main supervisor Frode, who has shown great patience in the face of a lengthy doctoral work. Thank you also to my colleagues and fellow PhD-students at the Medical Genetics unit for all the wonderful lunch conversations and all the cakes we have eaten. Thank you, Ingrid and Mariella, for taking the time to help a struggling student, your feedback was very helpful. Also, a massive thank you to Dina, for saving me through the last intense months of writing, with both professional input and mental support – you have been absolutely invaluable.

I am forever grateful to all my friends for not giving up on me! Thank you, Ida, for supporting my interest in canine genetics and for contributing with important input about dogs in general and poodles in particular. Thank you, Tove, for allowing me to share unlimited pictures of my children without judgment. And to my dear Miriam, my rock through studies and work life, thank you for being my friend and for sharing my love for animals with little hats!

I want to thank my siblings for not caring about either genetics or dogs and making it possible to maintain a social life without work-related topics. Thanks for having cooler jobs than me so that my boys have someone to look up to. I love you all to pieces! Thank you, dad, for all the lousy jokes, for always having a positive attitude, and for having that unshakable belief that I would be able to do this, even when I have felt insufficient and stupid.

Mamma. You are my best friend, my closest confidante, and the first person I call when life is difficult, or I need advice. You always know what to say or do, and you always have an answer. I know you have carried so many worries and sorrows on my behalf, had sleepless nights, and defended me and my choices many times. You are the kindest, warmest, and most loving person I know, my number one hero, and a person I strive to live up to. The existence of this study is as much your merit as mine. I would never have made it without you, and I cannot thank you enough.

Last but not least, a thousand loving thoughts to my two most precious ones, Syver and Vebjørn, who give the warmest hugs and hold my hand every day. I need those moments more than you can imagine. You keep my feet firmly planted on the ground and have given me an entirely new perspective on life and what is really important. You remind me every day of the importance of playfulness, how fun it is to jump in puddles, and that the best thing about cooking is tasting the ingredients. Being your mom is the greatest thing I've ever experienced, and I'm infinitely proud of the life the three of us share every day. I love you to the moon and back!

This study is dedicated to Luna, my faithful furry companion of more than a decade. She herself was never a fearful dog; on the contrary, she was confident, calm, and reliable, and she was the best friend I have ever had. Thank you, my darling Luna, for miles of walks in the woods, for late-night writing company, for making sure I took breaks, and for reminding me daily when it was time for lunch. Even a long dog life is brutally short in the eyes of a human, but you will live forever in my heart. I miss you.

Ås, October 2022

Karin Westereng Handegård

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1 Abbreviations and definitions

AKC	The American Kennel Club
C-BARQ	Canine Behavioral Assessment and Research Questionnaire
CCD	Canine compulsive disorder
CFA	Notation of canine chromosomes - Canis Familiaris
FCI	*Fédération Cynoloquique Internationale,* the largest international federation of kennel clubs
GCTA	A software tool for Genome-wide Complex Trait Analysis
GWAS	Genome-wide association study
HR	Heart rate
HRV	Heart rate variability
ISCWT	Irish soft-coated wheaten terrier
LD	Linkage disequilibrium
MDS	Multi-dimensional scaling
NGS	Next generation sequencing
NKC	The Norwegian Kennel Club / Norsk Kennel Klub
NPC	The Norwegian Poodle Club / Norsk Puddelklubb
NSPA	The Norwegian Society for Protection of Animals / Dyrebeskyttelsen
OR	Odds ratio
QQ-plot	Quantile quantile plot
SNP	Single nucleotide polymorphism
SP	Standard poodle
TGS	Third generation sequencing
WES	Whole exome sequencing
WGS	Whole genome sequencing

2 List of papers

Paper I

Noise reactivity in standard poodles and Irish soft-coated wheaten terriers

Karin Westereng Handegård, Linn Mari Storengen, Frode Lingaas

Journal of Veterinary Behavior 2020 vol. 36 pp. 4-12

Paper II

Genetic parameters for noise reactivity in standard poodles

Karin Westereng Handegård, Per Madsen, Linn Mari Storengen, Frode Lingaas

Journal of Veterinary Behavior 2021 vol.45 pp. 33-36

Paper III

Genomic analysis of firework fear and noise reactivity in standard poodles

Karin Westereng Handegård, Linn Mari Storengen, Dina Jørgensen, Frode Lingaas

Submitted manuscript

3 Abstract

Noise reactivity is one of the most common behavioral problems in dogs. Even though various studies find significant differences in prevalence between breeds, and some also find gender differences, noise reactivity seems to occur in all types of dogs. Dogs may react to everyday noises such as clattering from kitchen utensils or vacuum cleaners, roaring traffic, and construction work, but also weather phenomena such as thunder and wind, or the noise of fireworks and gunshots. Owners often state that it is the sharp, sudden noises that dogs react to, and fear of fireworks, in particular, is a well-known and frequently discussed topic among dog owners. Fear of loud noises and fireworks are probably influenced both by complex genetics and environmental factors.

This thesis deals with the genetic background of fear of fireworks and noise reactivity in dogs, with a particular focus on the standard poodle breed. A series of surveys have been carried out where dog owners have classified the level of noise reactivity in their dogs. Two questionnaires were aimed at owners of standard poodles or Irish soft-coated wheaten terriers, while the others were aimed at only owners of standard poodles. One epidemiological study, one pedigree-based heritability study, and one heritability study based on genetic association have been carried out.

The surveys show that 49.4% of standard poodles and 53.6% of Irish soft-coated wheaten terriers display weak to extremely strong fear of fireworks and/or loud noises. It was also found that the dogs with a stronger degree of fear showed more and stronger fear-related behaviors. The two breeds also express a somewhat different frequency of fear-related behaviors when they experience a noise-related fearful situation. Repeated evaluations of many dogs show that owners are consistent in their assessment of the noise reactivity of their dogs and that there is a correlation between noise reactivity and the number of displayed fear behaviors.

It was found that Irish soft-coated wheaten terriers have a slightly higher prevalence of noise reactivity and firework fear than standard poodles, and that older dogs are more fearful than younger dogs. Most dogs that show signs of noise reactivity display symptoms before the age of 4, but the degree of fear can worsen later in the dog's life. The study also shows that the most fearful dogs are more nervous in general, and that dogs that are the only dog in the household are more fearful than dogs that are accompanied by another dog.

Estimated heritabilities in the pedigree-based study are 0.09-0.16 for fear of fireworks and 0.13-0.16 for fear of loud noise, with a high positive correlation between the two traits. The heritability estimates in this study indicate that fear of fireworks and loud noises has significant genetic components, even if the pedigree-based heritability estimates are relatively low.

DNA samples from 400 standard poodles were analyzed to estimate genomic heritabilities and detect SNPs that may be associated with fear of fireworks or noise reactivity. Genomic heritabilities were calculated to be 0.28 for fear of fireworks and 0.15 for noise reactivity, with a strong genetic correlation between the two traits. Genome-wide association identified one region on chromosome 17, which shows a possible association, and this region harbors genes that may be of interest. More extensive studies with higher sample sizes will be needed to verify the study.

This study found that there is a significant genetic component to fear of fireworks and loud noises and that these two complex genetic traits are strongly correlated. This study did not identify specific genes or gene variants that play a significant role in the development of a dog's fear of fireworks and loud noises. The estimated heritability of both fear of fireworks and fear of loud noises indicate that it should be possible to reduce the prevalence of these traits using selective breeding.

4 Norsk sammendrag

Ett av de vanligste atferdsproblemene hos hund er frykt for lyd. Ulike undersøkelser finner store raseforskjeller, og noen også kjønnsforskjeller, men frykt for lyd synes å forekomme hos alle typer hunder. Hunder kan reagere på hverdagslyder som gryteskrammel og støvsugere, trafikk og anleggsarbeider, men også værfenomen som torden og vind, eller lyden av fyrverkeri og skudd. Eiere opplyser oftest at det er de skarpe, plutselige lydene hundene reagerer mest på, og spesielt frykt for fyrverkeri er et velkjent og hyppig diskutert tema blant hundeeiere. Frykt for fyrverkeri og lyd er trolig komplekse genetiske egenskaper som i stor grad er påvirket av ulike miljøfaktorer.

Denne avhandlingen ser nærmere på de genetiske årsakene til frykt for fyrverkeri og høy lyd hos hund, med et spesielt fokus på rasen storpuddel. Det er gjennomført en serie spørreundersøkelser der hundeeiere har klassifisert hundene sine når det gjelder graden av frykt for ulike høye lyder. To av undersøkelsene er rettet mot eiere av storpuddel og Irish softcoated wheaten terriere, mens de øvrige kun er rettet mot eiere av storpuddel. Det er gjennomført ett epidemiologisk studie, ett arvegradsstudie basert på stamtavle, og et genomisk assosiasjonsstudium der målet var å beregne genomisk arvegrader og forsøke å identifisere kromosomale regioner assosiert med frykt for høye lyder.

Undersøkelsene viser at 49,4% av storpudler og 53,6% av wheaten terriere viser svak til ekstremt sterk frykt for fyrverkeri og/eller høy lyd. Det ble også funnet at hundene med sterkere grad av frykt viser flere og tydeligere fryktrelaterte atferder, og at de to rasene uttrykker noe forskjellig oppførsel når de er redde. Studiet som inkluderer repeterte besvarelser fra samme eier/hund over tid, viser at undersøkelser basert på web-baserte spørreskjema gir en høy reproduserbarhet ved at eiere er konsekvente i sin vurdering av hvor redd hunden er over tid. Undersøkelsene viser at det er tydelig sammenheng mellom hvor redd eieren opplever hunden og hvor mange tegn på frykt den viser.

Det ble funnet at wheaten terriere har noe hyppigere forekomst av lydangst enn storpudler, og at eldre hunder er reddere enn yngre hunder. De fleste hunder som vil vise tegn på frykt i løpet av livet gjør det senest ved 4-års alder, men frykten kan bli sterkere senere i hundenes liv. Undersøkelsen viser også at de mest redde hundene oftere er nervøse generelt, og at hunder som er den eneste hunden i husholdningen oftere er redde enn hunder som lever sammen med en annen hund.

Estimerte arvegrader i det stamtavlebaserte studiet er 0,09-0,16 for frykt for fyrverkeri og 0,13-0,16 for frykt for høy lyd, med høy positiv korrelasjon mellom de to egenskapene. Arvegradene i dette studiet viser at frykt for fyrverkeri og høye lyder har klare genetiske komponenter, men at variasjonen mellom ulike hunder også i stor grad skyldes miljøfaktorer.

DNA-prøver fra 400 storpudler ble sammenlignet for å estimere genomiske arvegrader, og påvise SNPer som kan være assosiert med frykt for fyrverkeri eller høy lyd. Genomisk arvegrad ble beregnet til å være 0,28 for frykt for fyrverkeri og 0,15 for frykt for høye lyder, med sterk genetisk korrelasjon mellom de to egenskapene. De genomiske assosiasjonsstudiene identifiserte ett område på kromosom 17 med en mulig assosiasjon til de to egenskapene. I denne regionen ligger det noen gener som kan ha en mulig betydning for egenskapene. Større studier med flere hunder er nødvendig for å verifisere disse funnene og for å finne genvarianter som kan være assosiert med økt risiko for lydfølsomhet.

Resultatene fra dette studiet finner at det er en genetisk komponent til frykt for fyrverkeri og høye lyder, og at disse to egenskapene er tett knyttet til hverandre. Det er sett på forekomst og arvbarhet av både frykt for fyrverkeri og frykt for høye lyder, og identifisert ett område på genomet som kan være knyttet til lydsensitivitet hos storpuddel. I studiet har vi ikke klart å identifiser spesifikke gener med en sikker sammenheng med frykt for fyrverkeri og lyd hos hund, samtidig som det viser at det er mulig av å selektere bevisst på ikke-redde hunder for en mindre redd rase over tid.

5 Synopsis

5.1 Introduction

5.1.1 Origin and genetics of the domestic dog

Domestication of the dog

The dog (*Canis lupus familiaris*) was the first animal to be domesticated by humans (Vilà et al., 1997, Freedman et al., 2014). Archeologic and genetic studies suggest that dogs probably evolved from domesticated grey wolves at least 15,000 years ago, possibly in Europe or Asia (Thalmann et al., 2013, Wang et al., 2016). Fossils of wolf bones have been found at the site of early human settlements several hundred thousand years old, proving that wolves and humans have reigned in the same areas for a very long time (Clutton-Brock, 1995). It is believed that the domestication of dogs is a result of this co-existence which at some point became beneficiary to both species due to shared food sources and living spaces (Clutton-Brock, 1995, Lindblad-Toh et al., 2005).

Exactly when and where dogs originated is still debated, and even recent studies on ancient dog- and wolf-DNA have not given any clear evidence as to where the first dogs emerged (vonHoldt et al., 2010, Bergstrom et al., 2020). It has also been hypothesized that dogs were domesticated more than once at different places in the world simultaneously (Vilà et al., 1997, Botigue et al., 2017, Bergström et al., 2022). In the thousands of years that have followed the initial domestication, dogs have been bred to meet the needs of humans for hunting, herding, guarding, and companionship. This has resulted in the development of a wide range of unique breeds, with complex behavior traits and massive variations in physical features such as size, head shapes, and coat-texture (Ostrander et al., 2000, Lindblad-Toh et al., 2005, Boyko, 2011). Phylogenetic analysis suggests that more than 20 clades of breeds, representing clearly defined breed types, existed long before the formation of breed clubs and pedigree registrations, some possibly thousands of years ago (figure 1) (Parker et al., 2017). Modern breeds as we know them today were however officially formed as late as the 19th and 20th centuries.

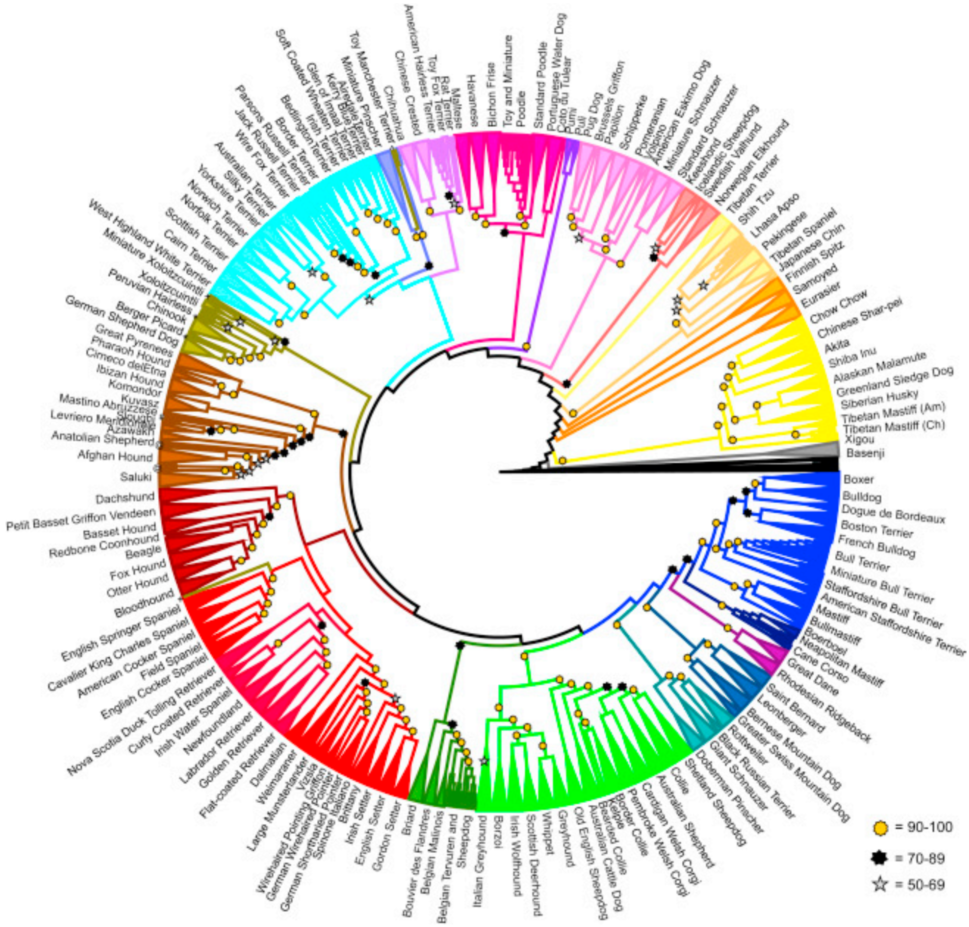

Figure 1: Cladogram of 161 domestic dog breeds, showing their phylogenetic relationship. 150 of those breeds can be divided into 23 clades of 2–18 breeds each (Parker et al., 2017).

Population bottlenecks

A genetic bottleneck describes the loss of genetic variation that occurs after a severe restriction in population size, followed by a great rise in the number of individuals. Only a few individuals (founders) remain or are selected to breed and pass their genes on to their offspring. The founder effect, with relatively few parents giving rise to the next generations, causes longer linkage disequilibrium patterns (LD). Such bottlenecks can be caused by natural disasters, war, diseases, and restrictive inbreeding. Also, genetic diversity may be lost, and higher levels of homozygosity are usually seen (Lindblad-Toh et al., 2005, Karlsson and Lindblad-Toh, 2008).

In purebred dogs, two main population bottlenecks are most likely associated with the loss of genetic diversity (figure 2). The first presumably occurred during domestication when dogs started to differ phenotypically from wolves several thousand years ago (Lindblad-Toh et al., 2005, Bergstrom et al., 2020). The second is thought to have occurred during breed foundation in the late 1800s and early 1900s. The American Kennel Club (AKC) was founded in 1884, and the Fédération Cynoloquique Internationale (FCI) was founded in 1911, which marks the beginning of the official registrations of pedigree dogs. Nowadays, more than 400 different recognized pedigree dog breeds are known worldwide, each defined by closed breeding populations with a unique pattern of size, morphology, and behavior. The variation in dogs' size and conformation is greater than that of any other domestic animal (figure 3). Many breeds have derived from the same few founders, separated into different breeds based on what physical or behavioral properties the breeders sought to promote (Ostrander et al., 2000). In that process, only a limited number of individuals, often closely related and with the desired qualities, were selected for breeding. Once a breed was established, the "stud book" was typically closed, and all dogs had ancestors of the same breed. This way, breeds emerged as closed breeding populations that received no new genetic variation (Shearin and Ostrander, 2010). The use of a limited number of popular sires, line breeding, phenotypic trait breeding, and the promotion of the breed barrier all contribute to loss of genetic variation in dogs (Leroy, 2011). The extensive use of popular sires during breed formation can be observed in the canine genome through comparisons of mitochondrial DNA (maternal inheritance) and Y chromosome (paternal inheritance) diversity, where male contributors are far fewer than female contributors (Sundqvist et al., 2006). Studies have also found that LD in dog breeds can vary from 0.5 Mb to >3 Mb, up to one hundred times more extensive than in humans (<100 kb) (Sutter et al., 2004, Lindblad-Toh et al., 2005).

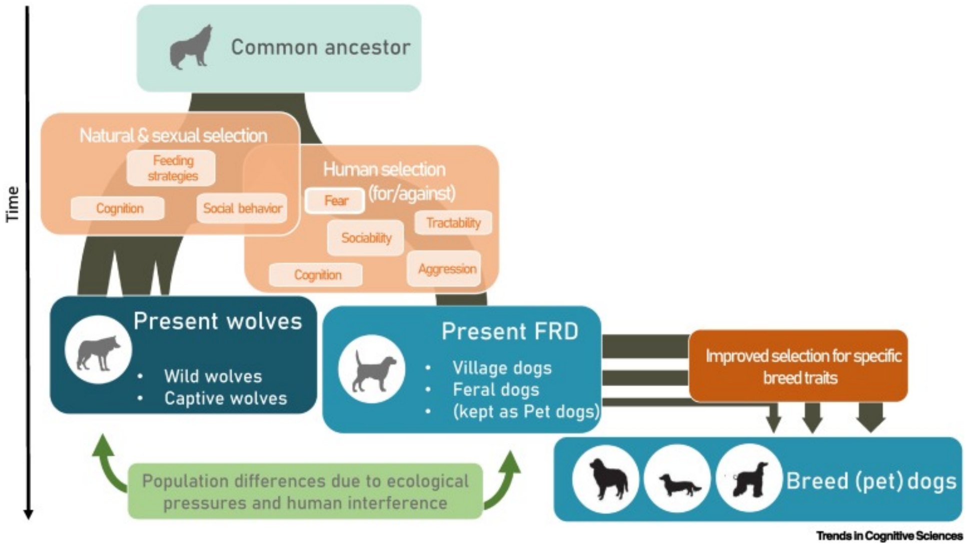

Figure 2: A summary of the wolf domestication process and the selective pressures thought to have been active. The narrowing of the arrows represents genetic bottlenecks. FRD=Free-ranging dogs. Reused with permission from Elsevier.

The most common recent bottlenecks are the result of deliberate breeding. An example can be found in the standard poodle population, with what is known as the Wycliffe bottleneck in the 50s and 60s. A small group of only five line-bred, American black standard poodles from kennel Wycliffe produced large numbers of top-winning offspring, which were later exported and bred all over the USA and Europe. In an article from The New York Times in 1968, it is described how the poodle (all size variants) went from being a rare breed in 1950 to become the number one dog in the country from 1960 to 1968, covering 30% of the total breed registrations, and that inbreeding for the desired traits were common practice (Rice, 1968). Today the five mentioned Wycliffe founders can be traced in almost every standard poodle pedigree (Armstrong, 1997, Pedersen et al., 2015).

High levels of relatedness among members of a population leads to the accumulation of specific alleles, which increase the probability of offspring receiving identical alleles from both parents (homozygosity). High homozygosity increases, in turn, the risk of disease if some of the accumulated alleles are causative of recessive disease traits. Old and newer bottlenecks have reduced genetic variation within dog breeds and caused various accumulation of risk alleles, thus increased the frequency of different recessive diseases within different breeds (Ostrander and Wayne, 2005, Farrell et al., 2015, Donner et al., 2018). Today, many inherited disorders in dogs are found within just one or a few breeds (Ostrander and Wayne, 2005).

Figure 3: Examples of morphological variation between dog breeds. From top left: Dogue de Bordeaux, Borzoi, Russian toy terrier. Middle: Siberian husky, Sharpei, Barbet. Bottom: Mexican hairless dog, Tibetan mastiff, Skye terrier. Images used under license from Shutterstock.com

Animal welfare issues and ways forward

Selective breeding in the past century has led to an increase in homozygosity and continuously smaller gene pools within many breeds. An important question is how these changes impact animal welfare. The notion "animal welfare" has many different interpretations, focusing on negative or positive welfare, physical health, and/or emotional well-being, some measuring a specific moment in time, while others measure welfare over the animal's whole lifespan (Harfeld, 2016). The public attitude towards animal welfare has changed with the increase in living standards around the world. In later years, there has been increased pressure from both the media and the public for Kennel clubs to take active measures to avoid the breeding of unhealthy dogs. This debate intensified after the publication of the BBC-documentary *Pedigree Dogs Exposed* in 2008, which looks critically at the health and welfare issues with pedigree dogs in Great Britain.

Many breed standards, and thus judges on dog shows, may focus on extreme physical attributes like short muzzles, short legs, bulging eyes and round skulls, allowing for the continuous breeding of such physical traits. Breathing difficulties, heart disease, the inability to naturally mate and give birth, allergies, excessive skinfolds, among many others, are frequently mentioned features of some purebred dog breeds, correlated to unhealthy breeding for extreme aesthetics (Bovenkerk and Nijland, 2017). At the same time, selective breeding on a small number of founders has led to reduced genetic variation and increased homozygosity, resulting in an increased frequency of clinical disease due to recessive variants within some breeds. Approximately 400 disorders that are caused, or suspected to be caused, by genetic mechanisms, have been identified in pedigree and mixed-breed dogs (Summers et al., 2010, Farrell et al., 2015, Donner et al., 2018). Many of these disorders are strongly reduced in frequency or removed from many breeds due to genetic testing. Mixed-breed dogs are more likely to be carriers of a recessive disease, while purebreds are more likely to be genetically affected by a recessive disease since they have a higher degree of homozygosity (Donner et al., 2018).

The *Five Freedoms-approach,* initially formed by the UK Farm Animal Welfare Council in 1979, states that an animal has good welfare when it is free from hunger and thirst, free from discomfort, pain, injury, or disease, free to express normal behaviors, and free from fear and distress (The National Archives, 1979). Fear, phobias, and anxiety are thus regarded as negative emotions that may have a significant effect on the welfare of dogs (Sherman and Mills, 2008, Bovenkerk and Nijland, 2017). In selected breeds, nearly 50% of dog owners report that their dogs demonstrate signs of fear when exposed to loud and sudden noises (Blackwell et al., 2013), making it one of the most extensive welfare issues for companion dogs. Behavioral problems can disrupt the bond between dog and owner to such an extent that the owner feels unable to keep the dog. Studies find that a least one behavioral reason was recorded for 10-50% of relinquished dogs (Salman et al., 2000, Marston et al., 2004, Kwan and Bain, 2013, Salonen et al., 2020) and that the majority of these dogs are younger than three years old (Kwan and Bain, 2013). Two studies based on veterinary records, one from England (2018) and one from Australia (2021), both found that undesired behavior was the predominant cause of mortality for dogs aged three years or younger (Boyd et al., 2018, Yu et al., 2021). One study found that anxiety disorders and phobias were the most common presenting complaint, second only to aggression, of dogs referred to a behavior clinic (Bamberger and Houpt, 2006).

While selective breeding for unique physical traits has led to a high frequency of extreme morphologies associated with disease in some breeds, the way forward is not simple. Several strategies will have to be put into action, including screening programs, genetic testing, genomic selection combined with strict health requirements for potential breeding dogs, and, if necessary, cross-breeding with other breeds, in order to significantly reduce the number of inherited disorders and improving the overall health in pedigree dogs (Farrell et al., 2015). Screening for hip- and elbow dysplasia, patellar luxation and brachycephalic obstructive airway syndrome are among the strategies implemented in many breeds (Nilsson et al., 2018, Hedhammar, 2020). Many breed clubs also encourage behavior testing (Svartberg and Forkman, 2002). Given that important traits are recorded at population level with validated registration methods, estimated breeding values or genomic selection may be helpful tools to decrease the prevalence of inherited disorders. Genomic selection use estimated association between several single nucleotide polymorphisms (SNPs) in LD with the phenotype, to give a better estimate of the animal's true breeding value (Stock and Reents, 2013). Breeding values may also be estimated for animals without known phenotype if they are genotyped and have a sufficient number of phenotyped relatives typed for the SNPs. Such strategies do, however, increase costs for the breeders regarding genetic testing and sometimes complicated logistics and routines for DNA-typing and evaluation. Genomic selection has the advantage that it corrects for environmental influences (Sánchez-Molano et al., 2014).

The rapid advances in canine genetics and the development of new diagnostic DNA-tests have given breeders valuable tools to breed healthier dogs. Several laboratories now offer commercial genetic testing for several hereditary diseases and disorders in dogs, and several kennel clubs encourage breeders to use relevant tests for the various breeds. Examples of recommended genetic tests in poodles include progressive retinal atrophy, von Willebrand´s disease and neonatal encephalopathy, all traits with known causative variants. As more variants and traits are genetically mapped, the number of available tests will probably increase, and in the future it might also be possible to test for different problem behaviors (Zapata et al., 2022). Used correctly and ordered from reliable laboratories, such tests can help lower the incidence of suffering in the different dog breeds (Shaffer et al., 2019). However, such tests must be used with care, and require that the owner is knowledgeable and well-informed, as there is currently no regulation of the home DNA-testing market (Moses et al., 2018, Shaffer et al., 2019).

The dog in the modern society

There are more than 70 million pet dogs in the EU (European Union) today (Statista, 2021a), and estimated >480 thousand in Norway (Statista, 2021b). In most households, dogs are considered family members, and the relationship between human and dog has been compared to that of mother and child (Laurent-Simpson, 2017). The strong relationship humans have with their dogs has many physical and psychological benefits, as owning a dog is associated with increased physical activity, lower blood pressure, lower heart rate, and lower cortisol levels (Anderson et al., 1992, Lentino et al., 2012, Christian et al., 2018). Dogs also provide companionship, decrease feelings of loneliness and isolation, and have a positive effect on mental health (O'haire et al., 2015, Hoffman et al., 2018, Hughes et al., 2020). During the COVID-19 pandemic, the number of pet owners increased significantly, as it has been found that owning a dog during the pandemic protects against loneliness (Bussolari et al., 2021, Oliva and Johnston, 2021).

The working dog also stands strong and does not appear superfluous even as new technological advances are constantly being made. Even when modern technology can help us with an increasing number of tasks, the dog's position as co-worker, assistant and protector is very strong in the western society. Assistance dogs (guide dogs, hearing dogs, service dogs, e.g.) are trained to increase a person's functional self-sufficiency level. For instance, a guide dog is of great value to the user, not only improving travel performance and independence, but also providing a positive effect on travel habits, social interaction, self-esteem, and mental health (Allen and Blascovich, 1996, Lloyd et al., 2008, Sanders et al., 2015, Brady et al., 2018).

Not only do dogs fulfill their given tasks, but they also often outperform technologies designed with the same purpose. Law enforcement uses dogs to aid in locating and controlling suspects, for detection of illegal substances, and to find traces of blood at a crime scene. Similarly, the military use dogs to search for people or explosives, and for security, while search-and-rescue dogs can locate missing or dead people in the wilderness or buried in snow or ruins much faster than humans alone, saving many lives every year. Border control has detection dogs that can sniff out money, drugs, and explosives. Dogs are even being trained to detect different types of cancers or infections at early stages (Browne et al., 2006, Moser and McCulloch, 2010).

The dog as a model organism

The dog is regarded as a good model organism for medical and genetic research because it has such a wide morphological variety, including in the prevalence of diseases and personality traits. Many of these diseases and personality traits are very similar to those of humans in etiology, development, diagnosis, and treatment (Sutter and Ostrander, 2004, Karlsson and Lindblad-Toh, 2008, Boyko, 2011). In clinical studies laboratory mice and other rodents are usually the preferred animal models; however, they have great limitations in studies of more complex human diseases. Dogs are physiologically more similar to humans than mice are, as they are bigger in size, live longer, have a comparable heart size, and have bigger brains where signs of disease are much easier to detect than in the much smaller mice (Ostrander et al., 2000). Dogs are usually not recommended as laboratory animals for obvious animal welfare reasons. The potential as models for human disease is still great if information/records from daily life, including records about clinical disease, is collected. The complete canine genome has been sequenced, which revealed that the human genome differs less from the canine genome than from the mouse genome (Lindblad-Toh et al., 2005), making dogs especially well-suited for genetic studies, as it is possible to align more of the human genomic sequence to the canine genome. Humans and dogs suffer from a range of similar diseases, such as diabetes, epilepsy, metabolic diseases, autoimmune diseases, cancers, arthritis, and eye diseases (Tsai et al., 2007, Shaffer, 2019), and several hundred inherited traits in dogs are considered potential models for human traits (Online Mendelian Inheritance in Animals). Recently, companion dogs have been suggested as good models for studies on human aging (Hoffman et al., 2018, Wang et al., 2020), and as a potential model for rare human disorders (Hytönen and Lohi, 2016). Notably, dogs also display a range of behaviors that could be comparable with human psychiatric disorders, making them excellent candidates for behavior genetic studies (Overall, 2000).

For many human diseases and psychiatric disorders, a known or suspected gene variant is linked to the disease or disorder (Claussnitzer et al., 2020), and those genes are interesting candidates for studying similar diseases or behaviors in dogs. Such candidate gene approaches can significantly reduce the time it takes to detect mutation in an orthologous canine gene in small materials. However, due to the reduction in costs of genome sequencing, it is usually feasible and very cost-effective to combine a candidate gene strategy with genome sequencing to identify novel canine variants (Cadieu and Ostrander, 2007, Grall et al., 2012). Identifying genetic causes of rare disorders in humans is sometimes challenging, as it is often hard to find enough affected families. With the selective breeding of dogs, high frequencies of undesired alleles have emerged within breeds, resulting in "breed-specific" diseases (Sutter and Ostrander, 2004, Donner et al., 2016, Donner et al., 2018). High prevalence of a disease within a breed is evidence of heritable components of that disease and makes it easier to conduct case-control studies (Karlsson and Lindblad-Toh, 2008). It is also easier to narrow down the search for causal variants in dogs with smaller sample sizes than in humans due to the long LD in dogs (Ostrander and Wayne, 2005, Karlsson et al., 2007).

Companion and working dogs have adapted to an environment where they live their whole lives with the same environmental influences as their human companions. This may contribute to making some of the environmental effects of different traits more comparable between dogs and humans (Ostrander et al., 2000). Dogs are often treated like family members and will receive complicated medical attention for both common and complex diseases, many of which have human analogs, thus medical research benefits both species. Still, dogs have a significantly lower life expectancy, and diseases typically manifest at an earlier age and progress faster than in humans (Hansen and Khanna, 2004). This significantly shortens the duration of clinical trials, both in the observation of pathogenesis and testing of treatments. Due to the relative similarity in body size (of a medium/large sized dog), it is also sometimes possible to compare equipment and dosages used for dogs with that used for humans (Pearson, 2006).

5.1.2 The canine genome

The domestic dog (*Canis lupus familiaris*) belongs to the genus Canis, which also includes other dog-like carnivores like wolves (*Canis lupus*), coyotes (*Canis latrans*), and jackals (*Canis aureus*). Genetically all these species are very similar, with 78 chromosomes (38 autosomal and two sex chromosomes) and the potential to interbreed (Wayne and Ostrander, 1999). The canine genome is smaller than the human genome and contains approximately 2.5 billion DNA base pairs. Because of restrictive breeding practices, there has been an increase in genetic drift, resulting in a loss of genetic diversity and high homogeneity within breeds, and unique phenotypical variation between breeds (Ostrander and Wayne, 2005).

The first draft of the dog genome sequence was published in July 2004 by the Canine Genome Sequencing Project, a Broad institute team led by Kerstin Lindblad-Toh (Lindblad-Toh et al., 2005). This first high-quality 7.5x coverage sequence was sequenced from a female boxer named Tasha, which was then compared with an earlier 1.5x coverage sequence from a male standard poodle (Kirkness et al., 2003), shotgun sequence data of nine other diverse dog breeds, four grey wolves and one coyote. The result was a dense SNP map, with more than 2.5 million evenly distributed SNPs. Later, an improved canine genome assembly, CanFam3.1, was published in 2014 (Hoeppner et al. (2014). In 2015 a group of researchers sent an open invitation to the Canine Genomics Community, asking colleagues to unite in a globally coordinated effort in canine genome sequencing, with a vision of conducting whole genome sequencing of 10.000 dogs over a 5-year period. This project is called the Dog10K and aims to produce an integrated reference genome with the caliber of the human genome (Ostrander et al., 2019, Wang et al., 2019).

With new technology available, more recent assemblies have been published. In February 2021, Wang et al. (2021) published a new canine reference genome assembly GSD_1.0 (CanFam4), and shortly after, in June 2021, Jagannathan et al. (2021) and the Dog10K group published the assembly Dog10K_Boxer_Tasha_1.0. While the GSD-assembly is based on a de novo assembly from a female German shepherd, the Dog10K is based on the same boxer as the first canine reference assembly. Both assemblies are created using long-read technologies, increase the sequence contiguity by 55-100fold, and close >23,000 gaps of the CanFam3.1 reference assembly. The improved continuity and completeness of the canine reference genomes will be valuable resources for both canine and comparative genetics research, including in the field of low pass genotyping and imputation for trait mapping (Jagannathan et al., 2021, Wang et al., 2021).

5.1.3 Canine behavior and fearfulness

Normal behavior

Normal dog behaviors are usual, expected, or typical behaviors displayed by dogs in any given dog population (definition of “normal” from the Oxford English Dictionary, 2022). The age, breed, and previous experiences influence how a normal, healthy dog behaves. Nonetheless, most dogs are naturally lively, social, and friendly, they like playing with toys, people, and other dogs, love to walk, run, and sniff around their neighborhood, and show affection to “their pack” – they behave like dogs (Overall, 2013). Even so, the purpose with which the dog breed was initially created will affect how normal a behavior trait is in that breed. What is sometimes misinterpreted as undesirable behavior is simply a dog being a dog in a manner the owner disapproves of. Undesirable behaviors may be a function of the owner or environment and can be entirely normal for the dog, even behaviors that the owner does not like (Overall, 2005). Examples may be digging in the grass bed, chasing after the neighborhood kids while they ride their bikes, or barking at stray cats outside the windows. Some behaviors fall inside or just outside the expected range of normal behavior, which can occur in contexts or frequencies that make it worth questioning the environment or owner handling/training methods (Kwan and Bain, 2013, Pirrone et al., 2015). Some behaviors like very rough play, chewing on clothes/shoes/other objects, and herding of people/pets may be normal, and still inappropriate or harmful to other animals and humans, and can therefore be more challenging to manage (Landsberg and Denenberg, 2014). Other examples of such behaviors are urine marking, predation/hunting, eating/rolling in feces, and mounting. However, many behavioral challenges can be handled through changes in the dogs' environment and adequate training and socialization, especially in young dogs (Blackwell et al., 2008, González-Martínez et al., 2019).

Pathological Behavior

Truly pathological canine behaviors may potentially be harmful to the dog itself or to people and animals the dog encounters and can be a source of reduced quality of life for the dog. Abnormal behaviors may have developed due to insufficient/negative socialization or training, traumatic experiences, medical conditions, as a result of genetic susceptibility, old age, or a combination of several factors (Landsberg and Denenberg, 2014). Also, in many cases where dogs display abnormal behavior, the behavior may be a sign of physical pain or disease (Hedhammar and Hultin-Jäderlund, 2007). A wide range of infectious diseases, malnourishment, hormonal changes, congenital or acquired conditions affecting the brain, and so on, may cause behavioral changes in dogs (Hedhammar and Hultin-Jäderlund, 2007). However, abnormal behavior occurs frequently in otherwise healthy dogs. Different behavioral disorders are often comorbid, meaning the dog displays more than one problematic behavior, which may make them challenging to diagnose or tell apart. Fearfulness and aggression, or noise reactivity and separation anxiety, are examples of such traits (Tiira et al., 2016, Dinwoodie et al., 2019, Salonen et al., 2020). Treatment of behavioral disorders generally requires both alterations in the dogs´ environment and the owner's handling of the dog, often combined with behavior-altering prescription drugs or pheromones. This section briefly deliberates some of the most frequently occurring pathological behaviors.

Fear and anxiety

Fear is a basic natural response in a potentially dangerous situation or in exposure to a potentially dangerous stimulus and is necessary for survival (McFarland, 1981, Erhardt and Spoormaker, 2013, Ressler, 2020). When the fear response is excessive and out of context, it may, however, be problematic and could be pathological (Overall, 2013). Anxiety may be described as "a psychological, physiological, and behavioral state induced in animals and humans by a threat to well-being or survival, either actual or potential" (Steimer, 2002). It is characterized by autonomic and neuroendocrine activation, increased alertness, and heightened arousal, with a change in behavior from ongoing behaviors to flight/other defensive behaviors, for instance, aggression. The impact of fearfulness and anxiety disorders in dogs can be huge, both for the individual dog and the animals and humans it interacts with (Rugbjerg et al., 2003, Shore, 2005, Cannas et al., 2018), and may have a profound impact the life expectancy of the dog (Scarlett et al., 1999, Dreschel, 2010).

Fear and anxiety-related behavior issues are also a problem in working dogs. It has been found that behavioral problems account for more than 80% of the discharged adult military dogs younger than five years old (Evans et al., 2007). Moreover, fear-related behavior is one of the most common reasons for dogs being released from guide dog training programs or withdrawn after successful guide dog training (Goddard and Beilharz, 1985, Serpell and Hsu, 2001, Batt et al., 2008, Caron-Lormier et al., 2016). One study found that guide dogs being "passed back" from the original training class because of behavioral issues were much less likely to eventually graduate as guide dogs (Ennik et al., 2006). Selective breeding for dogs with the appropriate behavioral traits is crucial, as the cost of breeding and training a puppy to become a working dog is high (Chen et al., 2021).

Fearfulness and anxiety disorders make up a large proportion of behavioral problems in both working and companion dogs, and noise reactivity constitutes a large part of these problems (Blackwell et al., 2013, Storengen and Lingaas, 2015, Tiira et al., 2016, Dinwoodie et al., 2019, Salonen et al., 2020). The etiology of fear and anxiety is, however, still poorly understood. Behaviors and different forms of fearfulness are considered complex traits, as they show a wide specter of varying phenotypes, and are likely affected by a combination of genes, environmental factors, life experiences, and training. The prevalence of fear in dogs varies between breeds, although present to some extent in all breeds/mixed breeds. Some breeds have a higher risk of developing anxiety, phobias, or other forms of fear behavior (Storengen et al., 2014, Overall et al., 2016). Comorbidity has been found between different fear behaviors (Tiira et al., 2016), and several studies have found that many behavioral traits, including different types of fear, have moderate to high heritability (Goddard and Beilharz, 1985, Persson et al., 2015, Ilska et al., 2017). Despite many studies in both dogs, humans and many other species, the information about the genetic architecture of phobias, anxieties, and fearful behavior is very limited (Hohoff, 2009, Ogata, 2016).

Aggression

In the United States, aggression is the most common referral behavior concern, accounting for more than 70% of caseloads of referral practices (Bamberger and Houpt, 2006, Landsberg and Denenberg, 2014). At least five million people in the United States are referred to hospitals yearly to treat dog bites, making dog-human aggression a significant public health problem (Landsberg and Denenberg, 2014). Aggressive behavior can be motivated by various factors, and it is known that aggression can be caused by fear and insecurity (Asp et al., 2015, Tiira et al., 2016, Salonen et al., 2020, Salonen et al., 2022). Apart from predation, the most common form of aggression in dogs is distance-increasing behavior. Such behavior is displayed if the dog is actively trying to distance itself from the fear-inducing stimulus and will be recognized as typical aggressive behavior shown by, for instance, barred teeth, flattened ears, growling, barking, and lunges forwards (Landsberg and Denenberg, 2014).

Noise reactivity

Many companion dogs are met every day by a range of loud noises and general commotion, and fear of loud and sudden noises is a significant problem for a worrying number of them. Studies have shown that up to 30% of dogs show a strong or very strong fear of loud noises, and as many as 50% may show some signs of noise reactivity (Blackwell et al., 2013, Storengen and Lingaas, 2015, Tiira et al., 2016, Riemer, 2019, Salonen et al., 2020). Several studies point to noise reactivity as one of the most common behavior problems for pet dogs, and very often associated with other behavioral issues like separation anxiety, aggression, and general fearfulness (Overall et al., 2001, Tiira et al., 2016, Salonen et al., 2020).

The dogs may react to everyday noises such as the rattling of kitchen utensils or vacuum cleaners, traffic noises and construction work, but also weather phenomena such as rain, thunder and wind, or very sharp and sudden noises like the sound of fireworks or gunshots. The most common fear reactions are reported to happen when the dogs are exposed to sudden and loud noises like fireworks, gunshots, thunder, and roaring traffic noises (Iimura, 2006, Sherman and Mills, 2008, Storengen and Lingaas, 2015, Salonen et al., 2020). Commonly displayed behaviors with noise reactivity include trembling, attention seeking, hiding, vocalization, panting, drooling, restlessness, destructiveness, indoor defecation/urination, self-mutilation, and refusal of food/water (Overall et al., 2001, Mills, 2005, Tiira et al., 2016). Many owners dedicate much time to preparing their dog before possibly fear-inducing happenings, and the use of behavior-altering prescription drugs have increased in later years (Riemer, 2020). Noise reactivity should therefore be considered an important welfare issue, causing both agony for the dog and frustration and worry for the owner (Sherman and Mills, 2008, Overall et al., 2019, Salonen et al., 2020).

Noise reactivity is, like other behaviors and personality traits, considered to be a complex trait. It shows a wide specter of varying phenotypes, and is likely affected by a combination of genes, environmental factors, experiences, and training (Blackwell et al., 2013). Some dogs are fearful of a range of different noises, while others are fearful of just one or two very specific noises. Some studies find that factors such as gender, age, castration status, early experiences and owner's gender are important factors (Blackwell et al., 2013, Tiira and Lohi, 2015, Cannas et al., 2018, Salonen et al., 2022). Fear of noise seems to occur in all types of purebred and mixed-breed dogs; however, studies do find significant differences between breeds/mixed breeds, which suggest a relevant genetic component to noise reactivity (Morrow et al., 2015, Storengen and Lingaas, 2015, Overall et al., 2016).

Separation anxiety

Another frequently occurring behavior problem is separation anxiety, for which different studies find a prevalence of 14-20% of all dogs (Sherman and Mills, 2008, Martinez et al., 2011, Tiira et al., 2016). Separation anxiety in dogs is commonly recognized as behaviors that the dog displays when left alone or separated from the caretaker(s) (Overall et al., 2001). The behaviors may include destructiveness, indoor urination and defecation, vocalization, restlessness, and a range of other signs of fear and distress (Overall et al., 2001, Sherman and Mills, 2008, Palestrini et al., 2010). Several studies have found comorbidity between separation anxiety and noise reactivity (Overall et al., 2001, Martinez et al., 2011, Storengen et al., 2014). Separation anxiety can be very disruptive to the dog-owner bond, especially if the dog displays destructive behavior or indoor soiling (Sherman and Mills, 2008).

Canine compulsive-disorder

Canine compulsive-disorder (CCD) is often considered to be the canine equivalent of obsessive-compulsive disorder (OCD) in humans. CCD is a collective term for normal behaviors performed at an abnormal frequency and in an extreme and often repetitive way for no apparent reason (Luescher et al., 1991). Different studies have found a high prevalence of compulsive behavior in dogs (Dinwoodie et al., 2019, Salonen et al., 2020). The behavior may be very difficult to stop and cause much frustration and severe medical problems. CCD behaviors include flank sucking, spinning, tail chasing, fly snapping and self-mutilation by excessive licking and biting on different parts of the body. It is thought that CCD may have sporadic and heritable forms, as it often manifests around the time of social maturity, and appears not to be related to lack of training or other social effects (Overall and Dunham, 2002). Comorbidity with noise reactivity and separation anxiety has been found (Tiira et al., 2012, Salonen et al., 2020). Progress has been made in the genetic research on CCD in dogs: Dodman et al. (2010) successfully identified a significant association between CCD and one gene on chromosome 7 in a study on Doberman pinchers, and later another study by Tang et al. (2014) confirmed this gene and identified three additional candidate genes that overlap human neuropsychiatric loci.

5.1.4 Behavior genetics

The etiology of personality traits and psychiatric disorders has been discussed since the times of the ancient Greek philosophers, and yet it is still mainly unknown. A wide range of hypotheses have been proposed, but due to its complexity and strong environmental effects, scientists are still far from a complete understanding of what personality traits are and why some animals, including humans, develop personality disorders (Corniquel et al., 2019, Dinwoodie et al., 2019, Fariba et al., 2022). Because the dog is such a good model organism, many behavior studies have been conducted as genetic studies on dogs. Studies have found that breed has a significant impact on many everyday behavior traits, such as trainability, playfulness, and fearfulness (Asp et al., 2015). At the same time, there are big within-breed differences in behavior traits (Mehrkam and Wynne, 2014). This knowledge is important in understanding behavioral genetics, as the differences both between breeds and within breeds indicate that genetic differences also influence behavior.

5.1.4.1 Identifying the phenotypes

Phenotypes are the traits of an individual that can be observed and are the results of a combination of genotype and environmental effects. Phenotyping behavior traits is often very challenging, as there are no simple diagnostic tools to use or any specific physical traits to observe. Instead, it has to rely on the observation of varied and often non-specific behaviors that are open to interpretation by the observer (van Rooy et al., 2014). These observed behaviors are symptoms of the underlying trait one wishes to study and may vary in frequency and intensity both between individual dogs and within one specific dog, making results inconsistent and difficult to reproduce. Also, a behavior phenotype may change throughout a dog's life, depending on its age and how its environment changes (Overall et al., 2006). Earlier experiences will, for instance, affect the displayed behavior, and some studies exclude puppies in behavior studies because they are still developing their cognitive phenotypes (Watowich et al., 2020). Most behavior conditions will, however, manifest between one and two years of age as the dog reaches social maturity (Overall et al., 2006).

Studies on fear and anxiety have been conducted in various species, including laboratory animals, as well as domestic and wild animals, yet no consistent definition of fearfulness as a behavior trait exists (Mobbs et al., 2019). Likewise, there is a lack of unified terminology and standardized well validated recording schemes in the field of behavioral assessment in dogs, making it difficult to compare data between studies (Overall, 2013). This also makes epidemiological studies of both genetic background and treatment of behavior problems very challenging. For instance, different literature uses terminology like "noise aversion", "noise sensitivity", "noise phobia" and "fear response to noise", while they very often loosely describe the same phenomenon of dogs showing abnormally strong reactions to specific noises (Stephens-Lewis et al., 2022). How much and what sort of reaction the dogs show will vary a lot between studies, and whether a dog is included or not as a "fearful" dog in case/control studies will depend on the study design and diagnostic methods/inclusion criteria. Behavior traits are regarded as complex traits where the environmental influence is strong yet hard to measure.

Recording reliable, reproducible phenotypes is essential in genetic analysis, and one of the main challenges with behavior genetic studies is, therefore, the definition of phenotypes (van Rooy et al., 2014). Developing reliable ways of phenotyping behaviors in dogs has been challenging due to its complexity. There are four primary methodologies that can be used to map diverse canine behaviors: Battery testing, owner-directed survey, expert breed assessment, and observational studies, in which battery testing has been most commonly used (Jones and Gosling, 2005, Spady and Ostrander, 2008).

Battery testing is frequently used in the assessment of working dogs, for example police dogs, guide dogs, and military dogs (Spady and Ostrander, 2008). The test exposes the dogs to several different, often novel situations/stimuli, for instance contact with strangers, play tests, and several potential fear- and aggression-evoking stimuli, including sudden sharp noises (gunshot) (Svartberg and Forkman, 2002). Many different versions of this type of test are in use, adapted to different breeds, ages, and purposes, e.g., the "Dog Mentality Assessment", the "Behavior and Personality Assessment", and the "Public Access Test". Special tests have been adapted to puppies, like the Puppy Profiling assessment, to select the best qualified puppies for future training (Asher et al., 2013). The results of such tests may be used to select breeding animals or potential working dogs or assess the heritability of different personality traits (Wilsson and Sundgren, 1997, Ruefenacht et al., 2002, Lazarowski et al., 2021). Many breed associations, including the Norwegian Poodle Club (NPC), also include battery testing as part of their recommended assessment of breeding animals.

Another common approach is owner-based surveys, where owners or caregivers evaluate their dogs. This is an effective method for collecting data on many dogs simultaneously, as the questionnaires may be published online and, therefore, easily accessible to many dog owners simultaneously. Different versions of questionnaires have been developed, the most frequently used to date is the Canine Behavioral Assessment & Research Questionnaire (C-BARQ), developed and validated by Hsu and Serpell in 2001-2003 (Serpell and Hsu, 2001, Hsu and Serpell, 2003). The C-BARQ is a standardized behavioral evaluation tool for dog owners, handlers, and professionals, designed to measure the prevalence and severity of behavioral problems in dogs, firstly for working and assistance dogs and later for pet dogs. However, it is now also used to assess canine personality. Several studies have found that data collected with C-BARQ or other similar tests can be used in genetic studies and as screening tools for breeding purposes (Svartberg and Forkman, 2002, Svartberg, 2005, van den Berg et al., 2006, Serpell and Duffy, 2014, Zapata et al., 2016, Bray et al., 2019).

The biggest weakness in owner-based behavior assessments is that peoples' personal conception of fear/other behaviors may vary, and especially when owner-based questionnaires are the basis for phenotyping, there is a risk of misclassifications that must be taken into account (Hsu and Serpell, 2003, Stephens-Lewis et al., 2022). Studies have found that humans varying experiences with dogs will affect the way they observe the dog's emotional state, and their perception of the dogs' fearfulness will vary accordingly. Thus there may be a difference between new inexperienced dog owners and owners who have had dogs for a long time (Kujala et al., 2012, Wan et al., 2012). Another study found that owners interpret their dogs' emotions well, regardless of the dog-owner relationship, however, the dog-owner relationship did affect the dogs' emotional reactions (Somppi et al., 2022). Studies have also shown that humans are better skilled at recognizing positive than negative emotions in dogs (Dalla Costa et al., 2014, Hawkins et al., 2022), and that humans' belief in animal emotions affects how they perceive the dog's behavior (Hawkins et al., 2022). One study found that members of the same household may give significantly different answers about their dogs on behavioral questionnaires (Jakuba et al., 2013), while others find that owner-based questionnaire surveys can show excellent correlation with data from behavior tests, especially for the traits related to noise reactivity (Tiira and Lohi, 2014, Flint et al., 2018). Either way, information about the validity of web-based surveys is limited (Jones and Gosling, 2005). Owner-based surveys are generally considered very good for collecting data on breed-specific variation, as the large number of participants makes up for differences related to personal opinions. They are, however, not so good for collecting data on individual dogs for that same reason. Nonetheless, it might be possible to overcome some of the issues with owner-based surveys by collecting data on severity, intensity, and type of behavior in any given situation, thereby assessing the dogs' specific behaviors rather than asking the owner's impressions of the behavior (Overall et al., 2006).

Expert breed assessment and observational studies are not as frequently used. Expert rating of breeds is performed by veterinarians, dog show judges, or other qualified personnel. By ranking or rating/describing breeds as a whole, cluster analysis or factor analysis can be performed to make comparisons between different breeds or within breeds of different populations, for instance, based on behavior or gender (Bradshaw and Goodwin, 1999, Hart and Hart, 2005, Takeuchi and Mori, 2006). Observational studies are much like battery testing, while performed in a more natural environment, where the dogs' behavior is assessed by an observer from a distance and/or via videotape. Observational studies are typically performed when selecting guide/service dogs (Murphy, 1995).

Noise reactivity and firework fear might be affected by factors related to the sense of hearing. Noise-reactive dogs have been suggested to suffer from physical pain in the ears and/or a change in auditory response (Scheifele et al., 2016, Tiira et al., 2016). Likewise, studies on fearfulness in dogs point out its association with candidate genes related to hearing (Sarviaho et al., 2019). Musculoskeletal pain has also been suggested as an important factor in noise reactivity-related behavior (Lopes Fagundes et al., 2018). These hypotheses regarding pain as a factor of noise reactivity will require further investigation before any conclusions can be made. A thorough clinical health check by a veterinarian is advised in any dog that displays abnormal behavior, including excessive fearfulness (Hedhammar and Hultin-Jäderlund, 2007).

Recently, physiological measurements have been implemented as part of the phenotyping assessment, including measuring heart rate (HR) and heart rate variability (HRV). Such parameters can be used as a way of detecting alterations between activation of the sympathetic and parasympathetic nervous systems, thus detecting emotional changes in the dog (Palestrini et al., 2005, Katayama et al., 2016). Fearful dogs have a higher HR when exposed to a fear-inducing stimulus (Hydbring-Sandberg et al., 2004, Franzini de Souza et al., 2018). Reduced HRV has been associated with anxiety and aggression-related behaviors in dogs and humans but also positive anticipation (Zupan et al., 2016, Wormald et al., 2017). HRV-registration is non-invasive and does not hurt the dog or interfere with the results. The correlative data gathering of physiological changes and observed behaviors may give a more accurate assessment of the dogs' emotional state when exposed to a possible fear-inducing or stressful stimuli (Bidoli et al., 2022).

5.1.4.2 Heritability

Heritability estimation is a quantitative approach that usually calculates how much of the variation in a trait is due to additive genetic variation (Visscher et al., 2008). Narrow-sense heritability (h^2) is usually estimated in related individuals, in humans typically through twin studies. The heritability ranges from 0 to 1, where h^2 close to 0 indicates that most/all of the variability in a trait within that breed is due to environmental factors, while a h^2 close to 1 indicates that most/all of the variability in a trait is due to genetic variation (Visscher et al., 2008, Tenesa and Haley, 2013).

Human heritability studies have found high heritabilities for several personality traits, as well as mental disorders such as major depressive disorder, specific phobias, and social anxiety (Beatty et al., 2002, Van Houtem et al., 2013, Guffanti et al., 2016, Moreno et al., 2016, Sanchez-Roige et al., 2018). In dogs, several behavior traits have been found to have high heritability (Table 1), e.g., retrieving, social behavior, courage, and cooperation (Saetre et al., 2006, Houpt, 2007, Evans et al., 2015, Persson et al., 2015, Gnanadesikan et al., 2020). Heritabilities have also been estimated to be high for different types of fearfulness in dogs (Goddard and Beilharz, 1985, Arvelius et al., 2014, Persson et al., 2015, Ilska et al., 2017); however, there is still limited knowledge about the genetic factors that affect canine phobias, anxieties and other fear-related behaviors (Hohoff, 2009, Ogata, 2016). Heritability estimates are useful in breeding programs to measure how efficient selective breeding may be for the desired/undesired trait in a particular population. It is also important information when considering the most efficient selection method; With high heritabilities reasonable genetic progress can be expected by mass selection, while low heritabilities indicate that estimated breeding values may be needed to secure genetic progress.

The "missing heritability" problem

The term "missing heritability" describes how single genetic variations are unable to explain much of the estimated heritability in different disorders, diseases, or behavior traits (Manolio et al., 2009, Slatkin, 2009, Eichler et al., 2010). When the human genome was first sequenced (Venter et al., 2001), the expectations were that one would soon have mapped genes and genetic variants that would explain much or most of the genetic contribution to many traits and diseases. Traditional heritability studies had estimated high heritabilities for many complex traits and diseases, and while candidate genes were initially discovered, with the more extensive use of genome-wide association studies (GWAS), most of these candidate genes turned out to be false positives (Hewitt, 2012). Traits like height and intelligence had heritability estimates around 0.8, yet those genetic variants that have been identified explain only a small bit of the variation. In a large study on schizophrenia by Ripke et al. (2014), SNPs explained only about 3% of the heritability, whereas the heritability of schizophrenia in population studies has been estimated at 64% (h^2=0.64) (Lichtenstein et al., 2009).

The missing heritability raises many questions about the nature of complex phenotypes, traditional methods of genetic studies, the accuracy of phenotyping, sample sizing, and the role of epigenetics (Maher, 2008, Manolio et al., 2009, Zuk et al., 2012, Nishino et al., 2018). Many of the traits with high heritability are hypothesized to be highly polygenic, meaning that they are caused by the additive effects of a large number of variants. For example, approximately 700 common variants have been identified in association with height (Marouli et al., 2017). When a trait is highly polygenic, very large sample sizes will be necessary to generate genome-wide significant results (figure 4) (Lee et al., 2011, Chaufan and Joseph, 2013, Nishino et al., 2018). Other theories include the possibility that traits are caused by rare variants with large effects, but that these variants are not tagged by common SNPs on genotyping arrays, or that pedigree-based h^2 is overestimated due to shared environmental effects (Manolio et al., 2009, Yang et al., 2017).

Traditionally, estimates of h^2 are based on closely related individuals. However, with SNP-based heritability estimation (genomic heritability - h_g^2) through Genome-wide complex trait analysis (GCTA) the variance is explained by all SNPs in a dataset, for instance, a GWAS or whole genome sequencing, which can be performed on unrelated individuals. Calculations of h_g^2 can therefore give an estimate of how much of the total variance can be attributed to common variants. It has been demonstrated that for many traits h_g^2 is high, even when the GWAS significance is missing. This indicates that there may be a large number of common variants with effect sizes too small to pass the GWAS significant threshold (Yang et al., 2017).

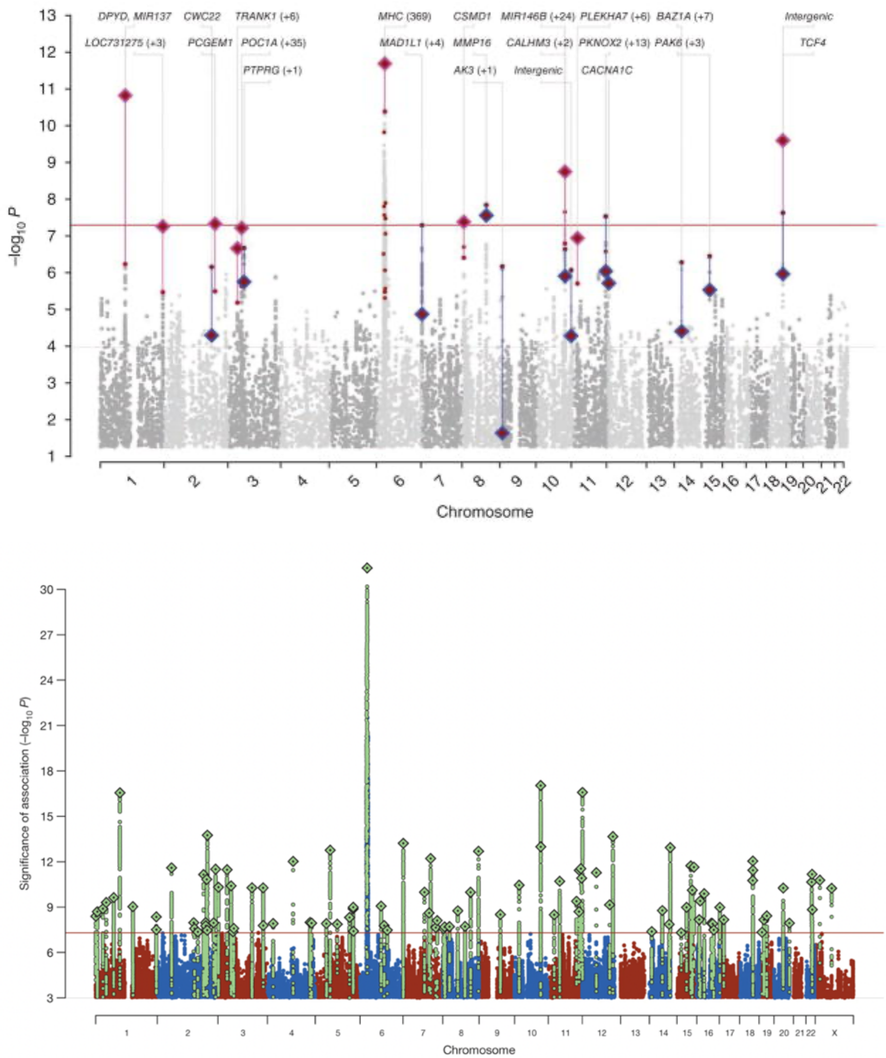

Figure 4: Two Manhattan plots by the Schizophrenia Working Group of the Psychiatric Genomics Consortium. The first study (top, 2011) had 9.394 cases and 12.462 controls. The second study (bottom, 2014) had 36.989 cases and 113.075 controls. This is an example of how a more extensive sample size may increase power and precision in the estimation of the effect of highly polygenic traits (Ripke et al., 2011, Ripke et al., 2014). Reused with permission from Nature.

Table 1: Heritabilities (narrow) for a selection of personality and behavior traits in dogs.
ECS=English cocker spaniel, GR=Golden retriever, GSD=German shepherd dog, LR=Labrador retriever, FCR=Flat coated retriever, BSD=Belgian shepherd dog.

Trait	**Heritability (h^2)**	**Breed**	**Reference**
Aggression (dominant)	0.20	ECS	(Pérez-Guisado et al., 2006)
Aggression (to humans)	0.90	GR	(Liinamo et al., 2007)
Aggressiveness	0.13	GSD	(Strandberg et al., 2005)
Communication skills	0.39	Many	(Gnanadesikan et al., 2020)
Cooperation	0.24/0.17	LR/GSD	(van der Waaij et al., 2008)
Cooperation	0.09-0.21	Gun hunting dogs	(Brenøe et al., 2002)
Cooperation	0.13	Gun hunting dogs	(Schmutz and Schmutz, 1998)
Cooperation	0.12	FCR	(Lindberg et al., 2004)
Courage	0.28/0.26	LR/GSD	(Wilsson and Sundgren, 1997)
Courage	0.13/0.19	LR/GSD	(van der Waaij et al., 2008)
Curiosity/fearlessness	0.23	GSD	(Strandberg et al., 2005)
Fear	0.46	Guide dogs (LR)	(Goddard and Beilharz, 1983)
Greeting behavior	0.09/0.05	LR/GSD	(Saetre et al., 2006)
Guarding	0.14	BSD	(Courreau and Langlois, 2005)
Gun shyness	0.56/0.21	LR/GSD	(van der Waaij et al., 2008)
Gunfire reaction	0.23	GSD	(Ruefenacht et al., 2002)
Gunfire reaction	0.19	GSD	(Meyer et al., 2012)
Gunshot fear	0.37	FCR	(Lindberg et al., 2004)
Human-directed social interaction	0.23	Lab. beagle	(Persson et al., 2015)
Independence	0.06-0.21	Gun hunting dogs	(Brenøe et al., 2002)
Inhibitory control	0.70	Many	(Gnanadesikan et al., 2020)
Memory	0.17	Many	(Gnanadesikan et al., 2020)
Non-social fear	0.27	GR	(Schiefelbein, 2012)
Obedience	0.13	BSD	(Courreau and Langlois, 2005)
Physical reasoning	0.21	Many	(Gnanadesikan et al., 2020)
Self confidence	0.18	GSD	(Ruefenacht et al., 2002)
Self confidence	0.20	GSD	(Meyer et al., 2012)
Temperament	0.18	LR/GSD	(van der Waaij et al., 2008)
Temperament	0.10/0.15	LR/GSD	(Wilsson and Sundgren, 1997)
Temperament	0.51	GSD	(Mackenzie et al., 1985)
Temperament	0.10	GSD	(Meyer et al., 2012)
Temperament	0.17	GSD	(Ruefenacht et al., 2002)

5.1.5 Genetic mapping

There are many methods for mapping the association between genes and various physical and mental characteristics. Some traits have simple Mendelian inheritance, where the causative variant can be recessive and only expressed if both parents are carriers (homozygosity), or dominant and expressed by all individuals carrying at least one copy of that allele (heterozygosity). Other characteristics, as behavioral traits are assumed to be, are complex and likely caused by both many different genes (polygenetic) and by many different external influences (environmental factors). In this section, some of the most common methods for genetic mapping are presented. Some of them, such as linkage studies and candidate gene studies were more common before the sequencing of whole genomes. Genome-wide association studies have been widely used in the last decade but are now being rapidly replaced by more modern methods. The development in gene technology is progressing fast, and new and improved methods are being developed continuously.

Candidate gene approach

A candidate gene study investigates the association between a trait and the genetic variation within a pre-specified gene. A gene may be a target for further investigation because of its chromosomal position (e.g., because of its central position in an identified chromosomal region resulting from a genetic association study) or known activity, for example due to a gene association with similar traits in other species. In behavior studies, candidates are typically genes involved in neurotransmitter regulation, or genes associated with similar behavior traits in humans or other animal species (Arata et al., 2008, Våge et al., 2010, Kis et al., 2014, Bellamy et al., 2018, Luo et al., 2018). As mentioned earlier, a candidate gene study by Sarviaho et al. (2019) found an association between fearfulness and genes related to hearing. Candidate gene studies are usually performed as case-control studies and are relatively low-cost and straightforward to conduct. The candidate gene approach only studies genes that influence the phenotype in question, and therefore does not identify novel variants (Zhu and Zhao, 2007).

Linkage studies

Linkage studies are well suited, first of all, for following phenotypes (e.g., diseases) segregating in high-risk families, searching for genetic markers with known chromosomal positions co-inherited with the phenotype of interest. If big families are available, it is possible to locate a disease-causing gene (Dawn Teare and Barrett, 2005), but linkage studies are less used today due to the development of efficient mapping strategies based on association studies.

Genome-wide association studies

Complex traits, including many quantitative traits and diseases, are often the result of both multiple environmental factors and polygenetic factors, and identifying those genetic factors have been very difficult by traditional candidate gene and linkage studies. GWAS has revolutionized the way we search for genotypes behind both mendelian and complex phenotypes with high speed and accuracy (Hardy and Singleton, 2009, Horwitz et al., 2019).

A GWA study is an observational study of genetic variation across the genome, designed to find genetic variation in SNPs associated with a particular phenotype. A GWAS is often conducted by comparing the DNA of two groups of unrelated individuals from the same population with opposing phenotypes (cases and controls) of the condition of interest. The comparisons look at how the frequency of different SNP variants differs between those two groups. The result is typically presented as a Manhattan plot, with the genomic coordinates on the x-axis sorted by chromosome numbers, and the association p-value for each SNP on the y-axis. The p-values are presented on a negative logarithmic scale, as the smallest p-values represent the highest association (figure 4). To account for the challenge of multiple testing when a large number of SNPs are used and compared at once, the significance threshold of GWAS has traditionally been set by applying Bonferroni correction adjusting for the number of tests, usually the number of SNPs tested, to reduce the chance of getting false positive results. By applying Bonferroni correction, the significance level of each SNP is divided by the total number of tested SNPs. Thus, the alpha value is adjusted from α=0.05 to α =0.05/nSNP. The Bonferroni correction assumes that each association test on a GWAS data set is independent, which is generally untrue due to LD among the GWAS markers. As an example, a GWAS using 500k SNPs would have a recommended significance level of a SNP association set at 1e-7. In GWAS, the Bonferroni correction often gives a too conservative p-value threshold. If the significance level is too stringent it will result in a loss of power and a large number of false negative results, especially where there are highly polygenic traits where each associated SNP accounts for a very small fraction of the genetic effect (Luciano et al., 2018). With more extensive use of mixed linear model and GCTA for GWAS, it has been argued that other methods of estimating the significance threshold should be considered (Kaler and Purcell, 2019).

Since GWAS is based on SNPs evenly spaced over the genome, the method mostly picks up an indirect association, meaning it is used to locate SNPs that are in LD of the causal genetic variants. Once an associated SNP is located, fine mapping needs to be conducted to look for candidate genes or other causal variants that are in LD with said SNP on the genome (Schaid et al., 2018). Due to the long LD in dogs, fine mapping may be challenging.

Genome-wide association studies have been widely used in the past two decades to identify genomic regions (on the human genome) that harbor genetic causal variants of complex traits like personality or behavior traits and psychiatric disorders (Jones et al., 2008, Ripke et al., 2013, Machiela and Chanock, 2014). Because of their domestication and breeding history, domestic dogs show extreme phenotypic variation between breeds, with low within-breed genetic diversity and a high degree of genetic homozygosity (Sutter et al., 2004, Lindblad-Toh et al., 2005). Some of these factors have contributed to the increased prevalence of specific recessive diseases in some breeds, many of which mimic human analogs. For this reason, dogs are a very good models for genetic studies, as it is estimated that it will be possible to find an association between phenotypes and SNPs with a much smaller sample size than in human studies (Machiela and Chanock, 2014, Hayward et al., 2016), even with a relatively sparse SNP array (Karlsson et al., 2007). When considering the significance threshold of dog GWAS it has been argued that the Bonferroni correction is far too conservative. This can be corrected through a genome-wide significance threshold based on an average LD in dogs being approximately 1Mb, and that the genome consists of roughly 2400 haplotype blocks (Lindblad-Toh et al., 2005, Karlsson et al., 2013).

GWAS have successfully identified SNPs and candidate genes for several complex traits in dogs (Karlsson et al., 2013, Hayward et al., 2016, Agler et al., 2019, Das et al., 2019), including behavior traits/disorders like obsessive-compulsive disorder, sociability and fearfulness, among many others (Dodman et al., 2010, Tang et al., 2014, Persson et al., 2016, Zapata et al., 2016, Sarviaho et al., 2019, Sarviaho et al., 2020, Shan et al., 2021). Nonetheless, when a high number of minor genetic effects influence a trait, as is predicted with behavioral characteristics, large datasets are probably necessary to find genomic relationships or to make genomic predictions, even in dogs (Hayward et al., 2016). Up until recently, the densest SNP array for the dog genome was the 230K Illumina HD Canine SNP-Array, which is the same density used for the GWAS in paper III. A canine HD genotyping array that covers over 700.000 SNPs and insertions/deletions is now available by Applied Biosystems™ Axiom™. The density increment in more recent array technologies may improve the statistical power in studies across breeds/mixed breeds, and may help identify other novel loci (Hayward et al., 2019). It will hopefully be possible to impute data from 230K or lower densities onto >700K data, which will be cost-effective and increase GWAS power (Quick et al., 2020).

Whole genome / whole exome sequencing

Whole genome sequencing (WGS) is the process of sequencing the entire genomic DNA, including non-coding sequences, by using modern sequencing techniques based on high throughput next-generation sequencing (NGS). WGS makes it possible to investigate both intronic regions, untranslated regions, microRNAs, promoters, enhancers/repressor genes and ultra-conserved regions. WGS has successfully identified novel variants associated with specific diseases like congenital stationary night blindness, hereditary footpad hyperkeratosis, and cerebellar ataxia in dogs (Sayyab et al., 2016, Das et al., 2019, Jenkins et al., 2020, Bellamy et al., 2022).

Whole exome sequencing (WES) is a technique for sequencing all the protein-coding regions in the genome (exome). WES aims to identify genetic variants that alter protein sequences, but at a much lower cost than WGS. Both WGS and WES intend to identify disease-causing variants directly, as they are more comprehensive and detect more types of genetic variation than GWAS. Canine WES enrichment kits are now available and may become an important tool in future canine genetic studies (Broeckx et al., 2014, Broeckx et al., 2017), as the reduced costs of WES and the increased focus on intergenic regions in genomic studies may indicate that WES will be the preferred method in the future.

One of the major challenges with WGS is the massive amounts of data output, which have been difficult to manage, store and analyze with available bioinformatic tools. Combined with the advances in computer technology, the challenges of WGS will likely diminish over time, increasing the availability and potential of finding new genetic variants for many different traits and diseases (Zhang et al., 2011, Stranneheim and Wedell, 2016, Adams and Eng, 2018).

Third generation sequencing – introduction of longer reads

GWAS and traditional NGS are "short-read" sequencing technologies, which read smaller fragments of the genome that must be reassembled when sequencing longer stretches of DNA. Because of this, they are not well suited for sequencing large copy-number variations, and large duplicated regions. Third generation sequencing (TGS) has been presented in recent years as an alternative "long-read" technology that can generate sequences >10 kb directly from native DNA (Check Hayden, 2009). These long-read technologies can overcome some of the short-read issues, such as genome-wide repeats and structural variant detection. One of the limitations of TGS compared to GWAS/NGS methods is the accuracy of the reads. However, this is improving day by day, particularly with advancements in software analysis (Hu et al., 2021).

Low pass sequencing and imputation

Low-pass whole genome sequencing (LP-WGS) is a cost-effective, high-throughput, DNA-sequencing technology now being used as an alternative to genotyping arrays (Buckley et al., 2022). With LP-WGS the genome is sequenced with an average coverage equal to or lower than 1x (Li et al., 2021). The genotypes can then be imputed to a reference panel that has been genotyped at much higher coverage, such as a canine reference genome assembly (Friedenberg and Meurs, 2016, Buckley et al., 2022). This allows for the assessment of more variants than those on the micro-arrays (Friedenberg and Meurs, 2016). Imputation also facilitates meta-analysis of datasets genotyped on different arrays (Lou et al., 2021). Individual genotypes cannot reliably be considered at low coverage; however, that is usually not that interesting for population-level studies, where one instead wishes to look at the overall population characteristics like allele frequencies and LD- patterns. Studies have suggested that LP-WGS, combined with increasing sample sizes, maximizes the information gained about the population, increases the power of GWAS and decreases measurement error of polygenic risk scores (Hayward et al., 2019, Li et al., 2021, Lou et al., 2021).

Epigenetics

Epigenetics is the study of phenotypical changes produced by alterations in gene activity or gene expression that does not involve changes in the DNA sequence. Examples of epigenetic mechanisms are DNA methylation and histone modification, along with a range of alterations of other proteins and non-coding RNA. DNA methylation adds a methyl group onto a region of the DNA, affecting how or if that DNA sequence is transcribed. Variation in DNA-methylation has been shown to affect several phenotypes, including social behavior (Cimarelli et al., 2017) and domestication and dog-breed differences (Sundman et al., 2020).

Normal growth and mental development are regulated by epigenetic mechanisms as mediators of the responses to environmental factors. It is hypothesized that epigenetics has an important role in the development of psychiatric disorders and has been linked to several human behaviors and mental illnesses (Nestler et al., 2016, Palumbo et al., 2018, Chmielewska et al., 2019). The hope is that epigenetic changes in selected genes may become diagnostic tools able to identify individuals with a high risk of mental disorders, with the possibility of more precise diagnosis and treatment (Chmielewska et al., 2019).

5.2 Hypothesis and aims of the thesis

The study's main hypothesis was that fear of fireworks and loud noises in dogs has a strong genetic component and that some of this variation may be identified through genetic studies.

The aims of the study were:

- To better understand the relevance and prevalence of fear of fireworks and other loud noises in some Norwegian companion dog breeds.
- To identify the relationship between fear of fireworks/loud noises and variables like gender and age, and environmental factors such as the presence of other dogs in the same household.
- To estimate the pedigree-based and genomic heritability of firework fear and noise reactivity in standard poodles.
- To identify genomic regions that might be associated with fear of fireworks/loud noises in standard poodles.

5.3 Materials and Methods

5.3.1 Dogs

Data on dogs included in papers I, II and III was collected through questionnaires based on owner participation. Previous data collection had indicated that the standard poodle (SP) and Irish soft-coated wheaten terrier (ISCWT) breeds included many individuals with fear of fireworks and loud noises, and they were chosen for further study for that reason (paper I). The SP is a more numerous dog breed than ISCWT in Norway and was, for practical reasons, chosen as the breed for continued data collection. Pedigree data (paper II) was collected through the NKC database. DNA samples (paper III) from standard poodles (n=400) were collected through dog shows, home visits, and by owners sending buccal swabs by mail. All samples were collected for research purposes, with the owners' consent. An overview of the dogs included in different papers is given in table 2.

Table 2: Number of included dogs in the three papers. SP=Standard poodle, ISCWT=Irish soft-coated wheaten terrier.

Paper	Breed	Number of included dogs
I	SP	672
I	ISCWT	474
II	SP	1148
III	SP	400

The total number of SPs with at least one entry in any one of the questionnaires is 1206.

5.3.2 Questionnaires

Behavior trait data was collected from web- and telephone-based questionnaires (Table 3). The surveys were performed in collaboration with the Norwegian Kennel Club (NKC) and the Norwegian poodle club (NPC). In the telephone questionnaire (2017), owners of either SP or ISCWT with a valid telephone number in the NKC registry were called; the telephone questionnaire was standardized and presented the same way for all participants (paper I). The participants of the telephone questionnaire were also encouraged to answer a follow-up online questionnaire (2017), which was more extensive than the one carried out over the phone. A second and third online questionnaires (2020/2022) were published with the aim of reaching owners who had not previously participated. This was done to collect more data and DNA from fearful and non-fearful SPs for heritability studies (paper II) and GWAS (paper III). Only dogs with a registration number in NKC are included in paper II. Owners of SPs were encouraged to answer the questionnaires in the NPC member magazine, on social media (Facebook), and on flyers handed out at NKC/NPC dog shows. Relevant data from a previous questionnaire (2014) about SPs were also included (paper I, II, III). All questionnaires included: (i) Contact information to the owner: name, e-mail address, (ii) general information about the dog: name, age, gender, and registration number, (iii) information about the dogs' fear of fireworks and fear of loud noises on a 1-5 Likert scale where 1 was "shows no fear" and 5 was "very fearful", inspired by other surveys often used for this purpose (Hsu and Serpell, 2003, Overall et al., 2006). The online follow-up questionnaire (2017) also included more in-depth questions about displayed behaviors in fearful situations, other stimuli the dog might be fearful of, and other dogs in the household (paper I). The use of questionnaires has been proven effective and reliable in this type of study (Tiira and Lohi, 2014, Flint et al., 2018).

Table 3: Overview of questionnaires and in which papers they are included. SP=Standard poodle, ISCWT=Irish soft-coated wheaten terrier

Questionnaire	Year	Type	Breed	Paper
Health- and behavior study	2014	Online	SP	I, II, III
Noise reactivity/Firework fear	2017	Telephone	SP	I, II, III
Noise reactivity/Firework fear	2017	Telephone	ISCWT	I
Follow-up online survey	2017	Online	SP	I, II, III
Follow-up online survey	2017	Online	ISCWT	I
Supplementary study I	2020	Online	SP	II, III
Supplementary study II	2022	Online	SP	III

5.3.3 Statistical and genomic analyses

In paper I, statistical analysis was performed using JMP® Pro 14.0.0 software (SAS Institute Inc., Cary, NC, 1989-2019) and Microsoft excel 2016. Generalized Linear Model (GLM) calculations were performed with noise/firework fear as dependent variables and breed, age, gender, and interviewer as independent variables. To study the changes in repeated measures, a Pearson's correlation analysis and a multivariate analysis of variance were run on the scores for fear of fireworks in the telephone survey and the follow-up survey. Correlations between fear of fireworks, fear of noise, and general signs of fear were calculated using Spearman's Rho correlation analysis. Odds ratios (OR) were estimated for comparing different age groups using a clinical research calculator (medcalc.org), as well as for fearfulness in other situations and the presence of other dogs in the household. The α value was set at 0.05 for all statistical tests.

The heritability analysis in paper II was estimated using a bi-variate animal model. Three different definitions of fixed effects were used to account for possible environmental factors related to change over time: year of birth, year of birth grouped in 5-year intervals, and without the year of birth. Variance components were estimated with REML (Restricted Maximum likelihood) (Patterson and Thompson, 1971) using the average information algorithm (AIREML) (Jensen et al., 1997) as implemented in the DMU package (Madsen and Jensen, 2013).

The GWAS and SNP heritability calculations in paper III were performed using **GCTA** v.1.93.2 (Yang et al., 2011). The SNP heritability was calculated using a genetic relationship matrix (GRM) based on the autosomal SNPs in the dataset. The GRM was included in a bivariate genomic restricted maximum likelihood (GREML) analysis using the model, $y = \mu + g + e$, where y is a vector of one of the two phenotypes, μ is the mean term (fixed effect), g is the random genetic effect, and e is the residual error. The GWAS were performed using a mixed linear model where noise reactivity and fear of fireworks were used as dependent categorical variables. A relationship matrix was included as a random effect to correct for relationship and population structure. The model was tested with potential covariates like sex, coat color and questionnaire. Manhattan plots and QQ-plots were created using the QQ-man package in **R** (Turner, 2014).

5.3.4 DNA sampling and extraction

In paper III, DNA samples were collected using DNA Genotec™ Performagene PG-100 buccal swabs or EDTA (Ethylenediaminetetraacetic acid) blood collected by a veterinarian. DNA from buccal swabs was extracted with Performagene 0.5mL purification protocol using the PG-100 kit. DNA from blood samples was extracted using the Omega Bio-tek - E.Z.N.A® Blood DNA Mini Kit following the manufacturer's instructions. The DNA samples were genotyped with the 230K Illumina HD Canine SNP-Array.

Each participating dog was given a unique ID number, which follows the dog in questionaries, datasets, and DNA sampling / genotyping. The DNA samples were marked with their respective ID numbers and stored in plastic Eppendorf tubes at -20°C.

5.4 Synopsis of papers

5.4.1 Paper I

Noise reactivity in standard poodles and Irish soft-coated wheaten terriers

A total of 1146 owners of standard poodles (N = 672) and Irish soft-coated wheaten terriers (N = 474) volunteered to participate in a telephone survey in which they answered questions about the dog's fear of loud noises and fireworks. 45% of the owners also participated in a web-based follow-up survey. Relevant data from an earlier survey on standard poodles was also included. It was found that age and breed significantly contribute to fear of loud noises and fireworks, and that fear of noise and fear of fireworks is strongly correlated (Cor. 0.77 for ISCWT and 0.73 for SP). We found that ISCWTs are more fearful than poodles, and that in both breeds older dogs are more fearful than younger dogs. We found that most dogs that show some signs of fear do so at the latest at the age of 4, but that the fear may be stronger later in the dogs' life. The surveys show that up to 53.6% of ISCWTs and 49.4% of SPs display some degree of fear of noise/fireworks. A weak correlation was found (0.22-0.33) between fear of noise/fireworks and fear of all other stimuli in both breeds. It was shown that the dogs with a stronger degree of fear display more and clearer fear-related behaviors. The two breeds also express different behaviors when they are fearful. For example, ISCWTs have been found to vocalize almost three times as much as SP. It was found that the most fearful dogs are more often nervous in general, and that dogs that are the only dog in the household are more often fearful than dogs that are accompanied by another dog. We concluded that web-based surveys are a reliable tool for documenting fear and behavior in dogs.

5.4.2 Paper II

Genetic parameters for noise reactivity in standard poodles

Data was collected from four owner-based surveys, one by telephone and three online. Owners were encouraged to include all their dogs if they had more than one.

The owners classified the dogs' fear of fireworks and loud noises from 1 to 5 on a Likert scale. The dogs had to be registered with a registration number and pedigree in the NKC to be included in the study. Eventually, 1148 standard poodles were included in a pedigree-based heritability study. A pedigree file for all dogs related to the included dogs was extracted from NKC's database, and the filtered file included 3457 dogs. Heritability was calculated to be between 0.091 and 0.16 for fireworks and 0.125 and 0.159 for loud noise, depending on fixed effects in the model, with a high correlation between the two traits. The possibility of development over time and the four different data sources (questionnaires) were considered. The results indicate that fear of fireworks and loud noise is hereditary, and that systematic breeding can, over time, contribute to a reduction in the prevalence of reactivity to loud noises.

5.4.3 Paper III

Genomic analysis of firework fear and noise reactivity in standard poodles

DNA samples from 400 standard poodles were collected using data from 5 owner-based surveys. The owners classified the dogs' fear of fireworks and loud noises from 1 to 5 on a Likert scale, where dogs with a score of 1 were included as controls, and dogs with scores 4 or 5 were included as cases. Genomic heritability for the two traits was moderate to low (fear of firework=0.28 and noise reactivity=0.15). A genome-wide association study of fear of fireworks and noise reactivity was conducted and identified one area on CFA17 with a suggestive association with firework fear and noise reactivity. The identified region harbors genes that have previously been associated with different psychiatric traits with anxiety components in humans.

5.5 Discussion

Fearfulness, anxiety, and other behavioral traits are complex traits and challenging to describe and understand thoroughly. Behavioral traits have a complex genetic background and are influenced by a number of environmental effects, and possibly also important epigenetic effects. Precise phenotyping of behavior traits is invaluable for detecting genetic effects influencing the traits. Fearfulness in dogs is common, difficult to manage for the owners, and has a profound impact on the welfare of the affected dogs. This section will discuss the aims, methodology, results, and impacts of this thesis, and put those into a broader perspective.

5.5.1 Data collection

During the planning and design of the surveys, emphasis was placed on primarily gathering information from owners about the dogs' degree of fear of fireworks and loud noises. It was chosen to use a 1-5 scale to measure the degree of fearfulness, in accordance with the C-BARQ and similar behavioral survey designs (Hsu and Serpell, 2003, Overall et al., 2006) (paper I-III). The follow-up study was particularly designed with inspiration from surveys designed by Overall et al. (2006), to look more closely at the behaviors displayed by the dogs during exposure to fireworks/loud noises. It was prioritized to collect information about dogs that were likely to be alive, for the possibility of sampling DNA (paper III), and at the same, time old enough to have experienced fireworks, preferably on several occasions. When the telephone survey was carried out in 2017, therefore, owners of dogs born in 2006 or later (<12 years) were contacted, the owners of the oldest dogs first. For the purpose of pedigree heritability estimation (paper II), it was also relevant that the majority of the dogs had a NKC valid registration number. All owners of SPs born in the period 2006-2019 (N=4192) who were registered with an e-mail or telephone number at NKC have been attempted contacted at least once through one or both means.

Both extensive and short questionnaires were designed for this study, and different approaches for recruiting owners were made. In general, the questionnaires should both collect good quality data, ideally with detailed information about possible influencing or confounding factors, and at the same time aim at a high quantity of submitted answers. Extensive questionnaires are more time consuming to answer, which makes it more difficult to recruit owners to participate. The telephone survey produced the highest number of responses and was the least time-consuming for the owners to answer, as it took approximately five minutes. However, it was very time-consuming to conduct, and there was a limited amount of information that could be collected during that five-minute phone call. In the online questionnaire sent as a follow-up to the telephone survey, in-depth questions were included about the frequency of various behaviors and other parameters of interest, such as the age of onset, other dogs in the household, and reactions to other types of noises. About half of all those who said they would answer the follow-up questionnaire did so, even though they received multiple reminders by email. It was equally challenging to recruit owners for the subsequent online questionnaires (2020, 2022). Many attempts at reaching out to SP owners were made, both personally by emailing them directly or by reaching out to SP owners in general, for example, through the breed club magazine ("Puddelposten"), different Facebook pages and other social media posts, as well as flyers handed out at dog shows.

The total number of SPs with at least one entry in any one of the questionnaires is 1206. This is approximately 29% of the total registered SPs during the time period from 2006-2019, in which the majority of the included poodles were born (N=4192). A limited number of the included SPs were born before or after that time period.

5.5.2 Dog breeds

SPs and ISCWTs are found to have a high frequency of firework and noise fear, while the ethology differs some between them. The SPs are described as clingier with the owner and have a very high frequency of shivering, while the ISCWTs are considerably more prone to vocalization. The survey did not ask about the frequency of vocalization in non-fearful situations, making it difficult to know if the difference is only measurable in fearful situations, or is a more general behavior difference between the breeds. In hindsight, this and many other interesting behaviors could have been included in the questionnaires. Nevertheless, no single behavior is seen exclusively in one of the breeds, which is in accordance with the findings of a new study by Morrill et al. (2022), which challenges the stereotypes that behavior is breed-dependent and that although breed may explain some of the variances in behavior traits, it is not a good predictor of individual dog behavior. They too found that no behavior is exclusive to any breed, which gives evidence to support that selection in dogs has focused strongly on aesthetics rather than behavior. Other studies have pointed out the same tendency, that the level of within-breed behavioral variation is very similar to the between-breeds variation (Svartberg, 2006, Mehrkam and Wynne, 2014). This study does not alter that conception, and the presence or lack of firework fear or noise reactivity would not be a good tool to differentiate between SP and ISCWT.

The origin of the poodle is thought to have been as a waterfowl retriever in Germany or France. Dogs of poodle type have been described in arts and popular literature as mostly a companion dog since at least the 17th century, and since the first pedigree registrations, poodles have been mostly known as a lapdog. The Irish soft-coated wheaten terrier was probably developed from other terrier breeds in Ireland in the early 20th century and was mainly used for hunting small animals and as guard dogs. Today, the ISCWT is also best known as a companion dog. It is interesting to note that both breeds have been used for hunting, in which gun shyness is an obvious disadvantage. It seems plausible that during the original selection for working abilities, dogs with a high degree of noise reactivity were not selected as breeding animals. Still, the frequency of noise reactivity is, maybe surprisingly, high in both breeds.

The ISCWT got officially recognized as a breed in 1937, and the SP was pedigree registered by the English kennel club in 1874. Therefore, there has been no cross-breeding of the two breeds in at least 30+ generations of dogs, if we assume a generation length of 4-5 years (Leroy et al., 2009). Both breeds were included in a large phylogenetic study from 2017, which finds that they belong to two separate and not conjoining clades (Parker et al., 2017). Due to their different origins, one might assume that the two breeds could exhibit different behavior traits, including frequency and exhibition of fearfulness. This appears not to be true in the case of firework fear and noise reactivity, as the two breeds share very similar behaviors and frequencies of both those traits (paper I). It would be interesting to compare one or both breeds to a set of other breeds from the same or different clades with higher or lower frequencies of firework and noise fear as a means of looking at the degree of fixation of noise-related fearfulness in dogs in general.

5.5.3 Phenotyping

A common denominator, particularly in papers II and III, is the challenges related to the lack of precision in phenotyping. The data in this study was collected using owner-based behavior assessments through questionnaires (papers I-III), which was considered the most effective method for collecting data on a large number of dogs for the study. Battery testing or an observation study design could possibly have given more accurate phenotypes but would have been far more time-consuming and would have severely restricted the number of available participants, thus given a much smaller sample size.

There are many possible pitfalls in owner-based data collection, where the biggest weakness probably is the risk that the owners' personal conception of fear behavior influences their scoring of the dogs. For example, a recent (preprint) study questions the use of the term "reactivity" in dogs, as the understanding of the word varies a lot, as well as the classification of different displayed behaviors (Stephens-Lewis et al., 2022). In this study, the dog owners seem able to detect fear reactions regardless of what behaviors the dogs display, as there is a consistency between fear score and number of displayed behaviors in exposure to a fear-inducing stimulus (fireworks/noise), even if the type of displayed behavior varies (paper I) (figure 5). The more fearful dogs are also prone to showing signs of fear before and after firework exposure, indicating that they have a sense of expectation of what is to come/may continue, possibly caused by subtle changes in the environment, such as owners preparing themselves.

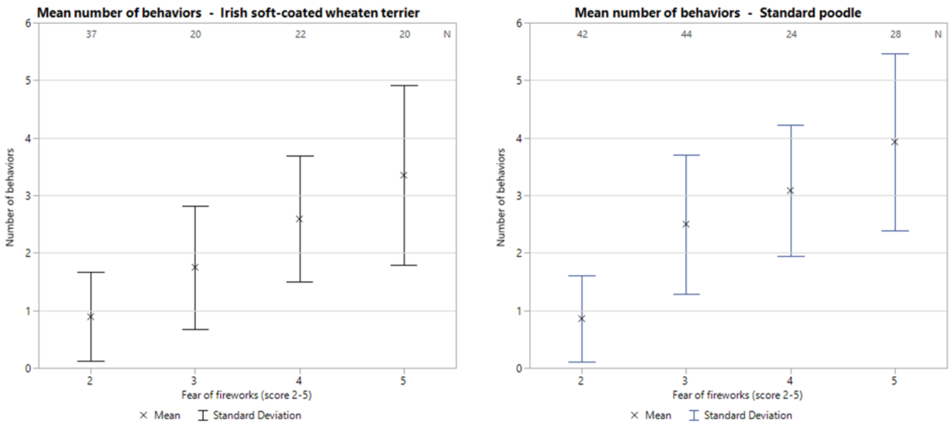

Figure 5: Number of fear-related behaviors shown by dogs with scores 2-5 for firework fear in ISCWT (left) and SPs (right). The figure is from paper I.

We found consistency in scores in those cases where dogs are scored more than once. Other studies have presented various results in consistency in behavior scoring within the same household. There was an especially high correlation between scores in the telephone survey and the follow-up survey (paper I), which was expected since these two surveys were close in time. Most of the deviating scores were on those dogs with intermediate scores of 2 (some reaction to fireworks/noise) or 3 (medium reaction), indicating that these less fearful dogs are the most difficult for the owners to classify. This is also the reason for the decision to exclude dogs with scores 2 or 3 from the genomic heritability/GWAS in paper III. Other studies have included all reactive dogs regardless of score >1 with success (Sarviaho et al., 2019, Salonen et al., 2020, Sarviaho et al., 2020). However, by including only the most severe cases, it is less likely to misclassify a non-fearful dog as fearful. For the control group (score 1) it is possible that some dogs with very subtle signs of fear might be included, which would be difficult to discover with this type of study design. However, many owners have participated more than once; thus, several dogs have more than one score, and only dogs with a consistent score of 1 are included as controls. It is worth noting that dogs with a score of 1 in any of the surveys are very rarely reclassified in any of the others, and thus this problem should be minimal and without great effect on the results.

In the questionnaires used in this thesis and papers I-III, the term “noise reactivity” is meant as a description of dogs with abnormally strong reactions to all loud and sudden noises. The questions in the surveys did not differentiate between types/sources of loud noises (traffic, thunder, gunshots, honking cars, and many more); they are all grouped together in the “noise reactivity” trait. It should be noted that thunder is a relatively rare occurrence in Norway and that the severity of thunder in Norway may not be comparable with the thunderstorms experienced in many other countries. The noise reactive phenotype may be more challenging to measure than fear of fireworks, as fear of fireworks is a much more predictable, precise, and specific behavior to observe. In Norway, it is prohibited to use private fireworks at any time except during an 8-hour window (18:00-02.00) on New Year’s Eve, and dog owners are, therefore, rarely surprised by unexpected fireworks. In paper II the difference in heritability between fear of fireworks and noise reactivity is small, while the genomic heritability is higher for fear of fireworks than noise reactivity in paper III. Whether these findings are related to noise reactivity as a more unprecise phenotype to register than fear of fireworks is more of a speculation at this point and requires further investigation.

Both pedigree heritability (paper II) and genomic heritability (paper III) find that fear of fireworks and noise reactivity are genetically highly correlated, with correlations that do not differ significantly from 1.0. In paper I, the correlation between scores of firework fear and noise reactivity is 0.73 in SPs. One of the questions raised is whether fear of fireworks and fear of other sudden loud noises are two different traits, or different expressions of the same trait. When treated as two traits, there is an obvious overlap between them, and in paper III, all dogs that are described as very or extremely fearful of loud noises (cases) will always be very fearful of fireworks as well. Interestingly, only about half of all dogs included as cases for the fear of fireworks trait are also classified as cases in the noise reactivity trait, and a few firework-fearful dogs are even included as noise reactivity controls - not fearful of loud noises (figure 6). This would mean that many of the firework fear cases may react to the noise regardless of its origin, while the rest react exclusively or more severely to fireworks. This suggests that they are two different traits, though very hard to distinguish, as that also would make it entirely plausible that some dogs are affected by both traits. It is, for instance, possible that some of the dogs with firework fear react to other things than noise, such as the smell of gunpowder, the flashing lights, or changes in the home environment/owner behavior during the New Year's celebrations. With raised attention to the problem of firework fear in dogs, it is also possible that owners expect and take notice of firework fear more easily than general noise reactivity. In that case, the scores on firework fear could be overestimated, and/or the noise reactivity scores possibly underestimated.

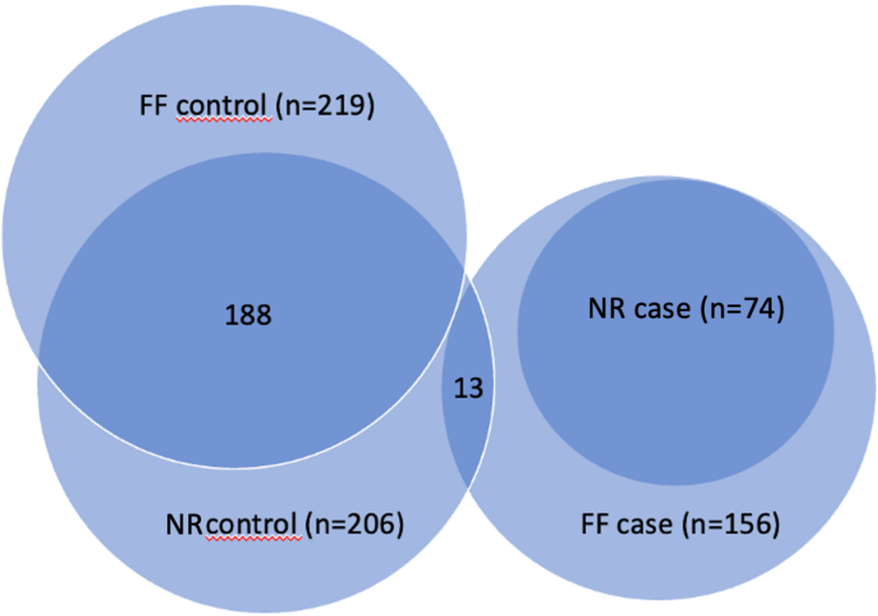

Figure 6: All dogs included as noise reactivity cases (NR) are always included as fireworks fear cases (FF), but only half of all dogs included as FF cases are also classified as NR cases. A small number of dogs are included as FF cases and NR controls. This illustrates that they are overlapping traits with some variation. Figure from paper III.

In this study, older dogs are found to be more fearful than younger dogs (paper I). From 2-3 years of age, most behavior traits seem to have developed and can be reliably recorded (Asher et al., 2013, Overall et al., 2016), and most noise-reactive dogs seem to show some signs of fearfulness by this age. In paper I, the average age of onset was found to be <3.9 years for both firework fear and noise reactivity in SPs, which is in accordance with previous reports. All the surveys in the study (papers I-III) primarily targeted owners of older dogs, preferably those older than three years. However, owners of younger dogs were allowed to reply to the surveys. About 12% of the included dogs were younger than three years old, and ~6% were younger than two years old, at the time the behavior score was recorded. Some of those younger dogs might have been included as controls in the genetic analysis and start to show signs of fearfulness later in life. Age at the time of sampling/scoring could, in retrospect, have been added as an inclusion criterion without significantly lowering the sample size, as it involves a very limited number of dogs. Because it is such a small number, however, it should not have a major effect on the overall results.

This study finds that dogs who are the only dog in the household are more fearful than dogs living with other dogs, which is in accordance with a recent study by Hakanen et al. (2020). It seems that one fearful dog in the household does not negatively affect the other dogs, which it might be natural to think it could. On the contrary, it might seem as though a non-fearful dog does have a positive impact on its housemates. As environmental factors seem to have a large impact on the development of noise reactivity, it would also have been interesting to look at similarities and differences between homes with one and homes with multiple dogs. Is the presence of another dog enough to make a dog less fearful, or are there other factors in multiple dog homes that have a significant impact, like location (urban/rural)? Could the presence of other animals in general have the same effect, and does the breed/genetic relationship of the other dogs have an influence? Early life experiences and socialization, amount of daily exercise, and whether or not the dogs primary caretaker is present during exposure to fireworks/loud noises are also factors that have been found to have a significant impact on the degree of fearfulness (Tiira and Lohi, 2015, Stellato et al., 2020). Only the follow-up questionnaire (2017) gave explicit information about selected environmental factors related to fearfulness in this study (paper I), for instance, the number of dogs in the household. A more thorough study of environmental factors related to noise reactivity would be needed to shed light on these and many other questions.

5.5.4 Heritability studies

In paper II, the estimated heritabilities in the pedigree-based study are 0.09-0.16 for fear of fireworks and 0.13-0.16 for fear of loud noise, while the genomic heritabilities in paper III were calculated to be 0.28 for fear of fireworks and 0.15 for noise reactivity. The heritabilities in both paper II and paper III are close to what other studies previously have found for gunfire reaction (0.19-0.23) and non-social fear (0.27) in other breeds (Ruefenacht et al., 2002, Meyer et al., 2012, Schiefelbein, 2012). The results from both studies suggest that much of the phenotypic variation can be explained by environmental factors, meaning that mass selection on the least fearful dogs would probably lead to a relatively slow selection response. Over time, however, the effect should be measurable, and the frequency of noise reactivity should drop.

The pedigree heritability estimate is lower than the genomic heritability. It is likely that higher pedigree heritabilities (paper II) could be obtained by including other effects like age of onset in the model or with improved phenotypical classifications by including records of additional, associated phenotypes (Overall, 2013), or other traits like duration of the symptoms before and after exposure to loud noise or fireworks. The NKC offers an official mentality assessment test for dogs ("*Mentalbeskrivelse hund/MH*"), which includes testing of noise reactivity and gun shyness. The NPC encourages members to participate and have their dogs tested, and all tested dogs must have a NKC valid registration number. As of 2022, a relatively small number of standard poodles (N=53) have records from these tests, but this could also provide a useful source of phenotypes in the future.

5.5.5 Genome-wide association studies

It was hypothesized that it could be possible to identify genetic associations to firework fear or noise reactivity in SPs. There were few studies published on genes associated with canine fearfulness, and GWAS had become very available and affordable. The results showed (like in many human studies) that it was very challenging to identify strongly associated regions/loci. This might be due to a combination of unprecise phenotyping, difficulties in the collection of large enough sample sizes, and the complexity of the studied traits themselves. The GWAS performed in paper III identified a region of interest on CFA17, and further investigation using a bigger sample size will be needed to verify if the genes in this region can indeed be associated with firework fear and noise reactivity.

The MDS plot indicated that there was a structure in the dataset with at least two subpopulations. When coat color was added to the plot, there was an indication that the subpopulations were associated with solid color variants (figure 7). The silver standard poodle, in particular, stands out, and even if the NPC allows the breeding of silver/black and silver/white, this is rarely done.

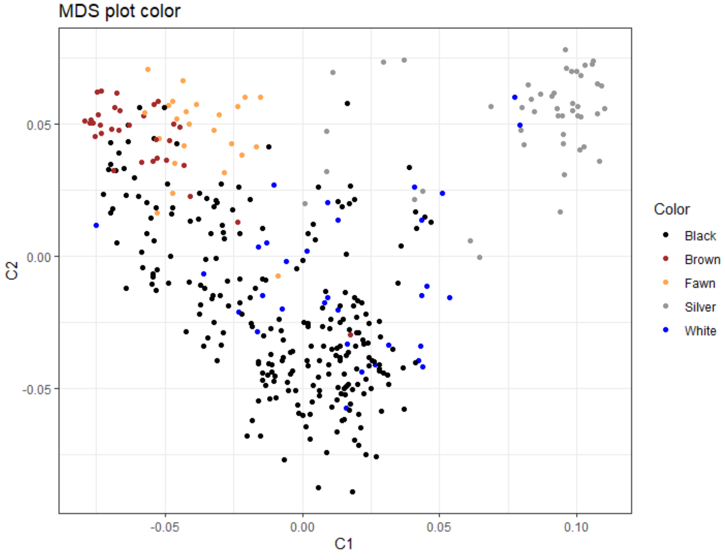

Figure 7: MDS plot, which illustrates that clustering in the standard poodle population correlates with coat color. The figure is from the supplementary in paper III.

5.5.6 Animal welfare

There has been an increased focus on animal welfare in the last years, including the adverse effects of breeding that may have a negative influence on animal health and welfare. In 2017, the EU presented a platform of animal welfare, and in 2020 it was supplemented with a series of guidelines for pet animal breeding.

The current Norwegian Animal Welfare Act was adopted in 2009 and replaced the previous Animal Protection Act. The animal welfare act 2009, section 25, regulates the breeding of animals, including dogs, and requires that the animals are bred with good function and health (Animal Welfare Act, 2009). This study shows that up to 50% of all dogs in the included breeds show some degree of fear of fireworks or loud noises (paper I), and other studies of the prevalence of noise reactivity and firework fear show similar numbers (Blackwell et al., 2013, Tiira et al., 2016, Salonen et al., 2020). It is difficult to claim that dogs with some degree of noise reactivity, in general, have reduced animal welfare. However, with the findings in papers II and III suggesting that firework fear and noise reactivity have a significant hereditary component, it should be considered to recommend that dogs seriously affected by noise reactivity should not be used for breeding.

5.5.7 Implications of the study and future prospects

The use of fireworks is challenging and affects the lives of many dogs and owners negatively (Riemer, 2019). This study finds that a significant proportion of family dogs experience strong noise reactivity to fireworks (paper I). It is likely that dogs strongly affected by fireworks may also have a higher noise reactivity in general. The knowledge provided by this study documents a negative effect on animal welfare in situations where fireworks are being used and should support attempts to prohibit the use of fireworks.

In this study, it is found that fear of loud noises and fireworks is influenced by genetic factors in Norwegian standard poodles, and that a proportion of the variation within the breed can be explained by genetic differences (paper II, paper III). Estimation of breeding value based on owner records would make it possible to reduce the prevalence and degree of noise reactivity and contribute to better animal welfare in the future.

The region on CFA17 that is identified in paper III requires further investigation. The most important is probably an increase in sample size to verify the present results and look for novel associated regions that may improve knowledge of the genetic etiology of noise reactivity.

There is still much more to learn about noise reactivity in dogs, and further epidemiological and genetic studies are warranted to address this issue. Lastly, the importance of international collaboration needs to be highlighted, as data sharing will be essential for increasing sample sizes to be able to identify genomic regions associated with noise reactivity.

5.6 Conclusions

Fear of fireworks and noise reactivity in dogs are genetically complex traits influenced by many environmental factors. This thesis aimed to contribute new knowledge about firework fear and noise reactivity in dogs. The study has given evidence that both firework fear and fear of other loud noises have a high prevalence in Irish soft-coated wheaten terriers and standard poodles in Norway. As many as half of the dogs of the two breeds show some degree of fear of fireworks and noise reactivity. Strong fear of fireworks and noise reactivity is, therefore, likely to have a significant negative impact on the welfare of a large number of dogs. When exposed to fear-inducing stimuli, fearful dogs experience a high degree of stress and emotional discomfort, which may last for several hours. There may be a need for comprehensive adjustments in the home environment and possibly psychopharmaceutic drugs. In the most severe cases, noise reactivity may disrupt the dog-owner dynamic to such an extent that it can lead to the relinquishment or euthanasia of the dog. Fear of noises may also negatively affect the working dog's ability to perform as expected. A reduction in the prevalence of fearful dogs would benefit animal welfare.

This study finds that there is a significant genetic component to fear of fireworks and loud noises in standard poodles, and that these two traits are closely associated. The study also finds that there is a good agreement between the owner's assessment of the severity of the dog's fear and the number of behaviors it exhibits when exposed to fireworks or loud noises. Owner surveys are, therefore, considered a good and efficient method for collecting data on many dogs at the same time, and thus a good tool in genetic studies of firework fear and noise reactivity. By using owner-based surveys, it will be possible to develop methods to select the best breeding animals to contribute to a reduced prevalence and severity of noise reactivity in the future.

This study has not uncovered specific genes that are associated with fear of fireworks or noise anxiety. An interesting region on chromosome 17 showed a tentative association to firework fear and noise reactivity, but further studies with increased sample size would be needed to verify the findings. The region could be the subject of further investigations since it contains genes previously associated with human psychiatric disorders, including the Catenin Alpha-2 gene. The Catenin Alpha-2 gene has been associated with canine compulsive disorder, which also is believed to have an anxiety component.

The genetic background for firework fear and noise reactivity in dogs is far from fully understood, and further research is needed to increase our knowledge about causative genetic variants for both within- and across-breed variations of the two traits. This thesis provides new and interesting knowledge about the prevalence of firework fear and noise reactivity in Norwegian standard poodles. That knowledge can be useful to breeders, veterinarians, and dog owners alike, as it is in common interest to breed and keep fearless, happy dogs.

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7 Enclosed papers I-III

I

Journal of Veterinary Behavior 36 (2020) 4–12

Contents lists available at ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Canine Research

Noise reactivity in standard poodles and Irish soft-coated wheaten terriers

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ARTICLE INFO

Article history:
Received 14 August 2019
Received in revised form
30 December 2019
Accepted 23 January 2020
Available online 27 February 2020

Keywords:
dog
fear
noise reactivity
fear of fireworks
survey
behavior

ABSTRACT

The purpose of this study was to estimate the frequency of noise reactivity in two dog breeds, standard poodles and Irish soft-coated wheaten terriers, and investigate how fear of noises is influenced by sex and age and fear in other situations. Owners were initially contacted by telephone and later answered a follow-up Web-based survey. In this study, both breeds have a high frequency of noise reactivity to both loud noises and fireworks, the soft-coated wheaten terrier more so than the standard poodle. There was a positive correlation between noise reactivity and age. The frequency of fear-related behaviors displayed when exposed to fireworks/loud noises is higher in the most fearful individuals. An association is found between fear in everyday situations and noise reactivity. Dogs in households with other dogs have a lower frequency of fear of fireworks. The validity of the survey, interviewer effect, and differences between the different dog owners' assessment are considered. The study finds excellent test-retest reliability, showing that Web-based surveys may be a reliable and cost-efficient tool to study noise reactivity and identify dogs to collect DNA samples for genetic studies.

Introduction

Fearfulness and anxiety disorders are among the main challenges for both family dogs and working dogs and make up a large proportion of behavioral problems in dogs (Blackwell et al., 2013; Storengen and Lingaas, 2015; Tiira et al., 2016). Fear is a natural response to a potentially dangerous stimuli/situation and is necessary for survival (McFarland, 1981). Excessive fear responses out of context are however problematic and may be pathological (Overall, 2013). Anxiety is an uncomfortable emotional state that has substantial negative affect on animal welfare (Rugbjerg et al., 2003), as well as the dog-owner relationship (Shore, 2005; Udell et al., 2010), and may affect the life expectancy of the dog (Scarlett et al., 1999; Dreschel, 2010). Anxiety and fear in dogs can have different causes and expressions, and for many dogs, fear of loud and sudden noises is a particular problem. A study by the same research group showed that up to 30% of dogs of some breeds show a strong or extreme fear of fireworks (Storengen and Lingaas, 2015).

Canine behavior can be evaluated through observation in everyday situations, standardized behavior testing, or owner/handler questionnaire surveys (Overall, 2013). The increase in popularity and availability of behavioral tests has provided a new source of data for behavior studies. The participation in formal behavior tests may be time-consuming and costly, require a test-area/field, and present logistical challenges for many owners. The assessment of canine behavior is therefore still mainly performed in working or service dogs for breeding or training purposes, and thus this type of data from the companion dog is often limited. In addition, some important traits, such as fear of fireworks, are difficult to fully measure in a test-environment (Hsu and Serpell, 2003). Questionnaire surveys may be an attractive alternative for registration of some behavior traits. One advantage of such studies is that they are cheap, can reach a large number of respondents, and provide additional information about the dogs' environment, past experiences, and reaction patterns in specific situations, as well as information on the dogs' physical health (Svartberg et al., 2005; Tiira and Lohi, 2014). Data from owner-based questionnaire surveys can show excellent correlation with data from behavior tests, especially for the traits related to noise reactivity (Tiira and Lohi, 2014; Flint et al., 2018), but information about the validity of Web-based surveys is limited.

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https://doi.org/10.1016/j.jveb.2020.01.002
1558-7878/

The etiology of excessive fear is poorly understood. The occurrence of fear in dogs varies between breeds, with some breeds showing higher risk of developing anxiety, phobias, or various forms of fear behavior (Storengen et al., 2014; Overall et al., 2016). Comorbidity has been found between several different fear behaviors (Tiira et al., 2016), and a number of studies have shown that most behavioral traits, including different types of fear, have high heritability (Goddard and Beilharz, 1985; Persson et al., 2015; Ilska et al., 2017). In spite of many studies in human beings and several other species, there is limited information about the genetic architecture of phobias, anxieties, and fearful behavior (Hohoff, 2009; Ogata, 2016).

The domestic dog is an excellent model animal for genetic studies because of their large breed diversity and variety of behaviors and physical appearance both between and within breeds (Lindblad-Toh et al., 2005; Wayne and Ostrander, 2007). The dog shows several of the same illnesses and behaviors as humans do, and it is expected that mapping of the genetic component in fear and anxiety in dogs also may shed light on similar human disorders (Sargan, 2004; Persson et al., 2015). A recent genome-wide association study of German shepherds found loci significantly associated to noise reactivity and fear of strangers, with regions that overlap previously described human neuropsychiatric loci (Sarviaho et al., 2019). Mapping of fearfulness requires efficient identification of large "genetic" case-control groups (Spady and Ostrander, 2008; Overall et al., 2014).

Health surveys performed on a number of different dog breeds have indicated that some breeds show a significantly higher frequency of noise reactivity (Storengen and Lingaas, 2015). In the present study, two of these breeds, standard poodles (SPs) and Irish soft-coated wheaten terriers (ISCWT), were selected because of high prevalence and examined more thoroughly for fear of fireworks/loud noises and the influence of sex and age.

This study includes results from a telephone interview with owners of the two breeds, a follow-up Web-based survey, and also a comparison with an earlier Web-based health and behavior survey where many of the same owners with the same dogs had responded to the same questions, giving the possibility to compare within-owner repeatability. The study estimates the validity of these owner-based behavior questionnaires and identifies dogs that are mildly, very, or extremely fearful of noises from firework, and dogs not showing fear, in two separate breeds. It also identifies some fear-related behaviors that provide additional information about the dogs' severity of fearfulness.

Materials and methods

Telephone survey

A telephone survey was conducted by calling owners of either an SP or ISCWT registered with a pedigree in the Norwegian Kennel Club. To ensure that the dogs had experienced firework noises, only owners of dogs older than 2 years were called (the oldest first). Owners of 672 poodles and 474 wheaten terriers (N = 1146) dogs participated in the telephone survey. The average age was 8.2 years for SP and 6.5 years for ISCWT.

The telephone interview was very brief, with just 4 questions about the dogs' behavior. Each owner was asked to indicate their dog's fear of fireworks and (their dog's) fear of loud noises, including thunder and gun shots, on a 1 to 5 Likert scale, where 1 is "shows no fear" and 5 is "very fearful". The owner was also asked if the dog's fear of noise had changed through its life (yes/no). Finally, the owners were asked at what frequency the dog show any "other fear-related behaviors," including any fear-related behavior to other stimuli than noise, on a 1 to 5 Likert scale where 1 is "never" and 5 is "very often".

Seven students were responsible for performing the telephone interviews, with the number of owners interviewed by each student varying from N = 34 to N = 337. The effect of the interviewer on the owners' response was considered.

Web-based survey

All participants of the telephone survey were invited to participate in a more extensive Web-based survey. The Web-based survey was sent by e-mail 1-2 weeks after the telephone survey was completed. The questions about fear of fireworks and fear of loud noises were repeated with the same 1 to 5 Likert scale, as in the telephone survey. Questions about age, sex, age of onset (if fearful of noises), and registration number were added. Owners also choose from a list of up to 11 behaviors (Table 3) that their dog displays when exposed to fireworks/loud noises. Questions about time of onset and duration of fear-related behaviors before/after fireworks were answered from a scroll down menu:

"How long before midnight does the dog show signs of distress?"

1) More than 12 hours before, 2) a few hours before, 3) From the first fireworks, and 4) Unsure.

"How long after midnight does the dog show signs of distress?"

1) Nothing after the last fireworks, 2) A few hours, 3) The whole night, 4) Unsure.

This was performed to be able to evaluate how strongly the dogs were influenced. Questions about fear in several other situations, reactions to household machines, and grooming were also included (Table 4). Owners of 513 dogs (SP = 310/ISCWT = 203) participated in the follow-up Web-based survey.

Health and behavior survey

Two years before this study, another health and behavior survey (HB survey) was performed in SPs. The survey was performed in collaboration with the breed club and included the identical questions about noise reactivity included in the present telephone survey and Web-based survey. Many SPs are included in both surveys.

Case/control

For the purpose of illustrating an effect of age, the dogs were classified as case/control and the odds ratio (OR) was estimated between age groups. Cases were defined as dogs rated 4 or 5 on fear of fireworks (strong or very strong fear) and controls defined as dogs rated as 1 (not fearful) in the telephone survey. The same definition is set for all the OR calculations. In the descriptive evaluations, dogs were considered not fearful if they had a score of 1 or fearful (slightly-very) with scores 2-5, if not stated otherwise.

Statistical analysis

Statistical analyses were performed using the JMP® Pro 14.0.0 software (SAS Institute Inc., Cary, NC, 1989-2019) and Microsoft excel 2016. Generalized linear model (GLM) calculations were performed using the results from the telephone survey, with fear of firework/fear of noise as dependent variables and breed, age, sex, and interviewer as independent variables. The effect of age was also illustrated using OR where the risk in increasing age groups were compared with the risk in the group of dogs younger than 5 years. A Pearson's correlation analysis and a multivariate analysis of variance were run on the scores for fear of fireworks in the telephone survey and the Web-based survey to study the change in these repeated measures. The correlation between fear of firework, fear of noise, and general signs of fear was calculated using Spearman's Rho correlation analysis. The α value was set *a priori* at 0.05 for all statistical tests.

Results

The results of the GLM analysis showed that for both fear of fireworks and fear of other loud noises, age and breed are both significant contributors ($P < 0.05$), while sex and interviewer do not have a significant effect.

Firework fear score distribution

From the telephone survey, a total of N = 1146 dogs were included (SP, N = 672; ISCWT, N = 474). Of these, there were 560 dogs not fearful of fireworks (score 1) and 586 dogs (fearful of fireworks) that were rated with scores 2-5. Five hundred and thirteen (44.8%) of those who responded to the telephone interview participated in the follow-up Web-based survey (SP, N = 310; ISCWT, N = 203; Total N = 513). Of those, 266 were classified as not fearful and 237 as fearful dogs (Table 1). The frequency of nonfearful dogs was about the same in the telephone study as in the Web-based study; however, the frequency of the highest firework/noise fear scores (4/5) was lower for both breeds in the Web-based study than that in the telephone study. The distribution of the different firework/noise fear scores is statistically significantly different between the two breeds, with a higher frequency of fear in the ISCWT ($P \leq 0.05$).

From the telephone survey, a significant positive correlation was found between fear of fireworks and fear of loud noises and also between fear of fireworks/noise and frequency of "other fear-related behaviors" for both breeds (Table 2). The correlation between fear of fireworks and fear of loud noises is 0.77 (ISCWT) and 0.73 (SP), indicating a strong positive monotonic relationship. The correlation between fear of firework/loud noises and frequency of fear of any other stimuli is weak (0.22-0.33) for both correlations in both breeds.

Table 2
Spearman's Rho correlation between fear of fireworks, fear of loud noises, and frequency of fear of any other stimuli, in Irish soft-coated wheaten terriers (ISCWT) and standard poodles (SP)

Breed	Trait	N	Spearman's Rho	*P* value
ISCWT	Fireworks × loud noises	468	0.77	<0.001
	Firework × other fear	463	0.22	<0.001
	Loud noises × other fear	462	0.24	<0.001
SP	Fireworks × loud noises	660	0.73	<0.001
	Firework × other fear	629	0.28	<0.001
	Loud noises × other fear	625	0.33	<0.001

Frequencies of behaviors

In the Web-based survey, owners of fearful dogs (score 2-5) subclassified their dogs according to the presence of 11 different fear-related behaviors (present = 1; not present = 0), (Table 3). Ninety-nine ISCWTs and 138 SPs were included. Most of the behaviors were observed between 13 and 54 times, but salivation, indoor urinating/defecating, and destructive behavior were seldom observed. The lowest number of observed behaviors was 0, and the highest number of observed behaviors was 7. The more fearful dogs display, on average, a higher number of fearful behaviors than the less fearful dogs. The least fearful dogs (score 2) display on average 0.9 fear-related behaviors for both breeds (Table 3; ISCWT: 33/37; SP: 36/42). In ISCWTs (Figure 1A), the least fearful dogs show from 0 to 3 fear-related behaviors, while the most fearful dogs (score 5) show between 1 and 7 behaviors, with an average of 3.4. In SPs (Figure 1B), the least fearful dogs range from 0 to 2 behaviors, while the most fearful dogs show between 1 and 7 behaviors (average 3.9). The Tukey-Kramer HSD *P* values show significant difference ($P<0.05$) between scores 2-5, 2-4, and 3-5 (ISCWT) and between scores 2-5, 2-4, 2-3, and 3-5 (SP), proving that very fearful dogs show a higher number of fear-related behaviors than less fearful dogs (Figure 1).

There are some differences in frequencies of the different fear behaviors in the two breeds (Table 3). SPs have a 1.4-2.4 times higher frequency than ISCWTs of behaviors such as being clingy with the owner, hiding, refusing to go outdoors, shivering, and refusing food and water, while ISCWTs are reported to vocalize a lot more than SP, with a 2.9 times higher frequency.

Severity and duration of fear-related behaviors vary between the most fearful (score 4 or 5, ISCWT, N = 42; SP, N = 52) and the least fearful (score 2, ISCWT, N = 37; SP, N = 42). Twenty-six percent (N = 11) (ISCWT) and 42% (N = 22) (SP) of the owners of the most fearful dogs state that it is impossible to calm or comfort the dog to any degree during exposure to firework, while 0% of the owners of the least fearful dogs state the same. Owners of only 2% (N = 1) (ISCWT) and 4% (N = 2) (SP) state that the most fearful dogs can be calmed down completely, the same numbers being 68% (N = 25) (ISCWT) and 61% (N = 27) (SP) for the least fearful dogs. Five percent (N = 2) (ISCWT) and 13% (N = 7) (SP) of the most fearful dogs show signs of distress several hours before a firework event, while 3% (N = 1) (ISCWT) and 0% (SP) of the least fearful dogs show the same. In addition, 17% (N = 7) (ISCWT) and 21% (N = 11) (SP) of the most fearful dogs show signs of distress the whole night or the day after, while 0% of the least fearful dogs show any sign of distress after the firework event.

ORs were calculated for fear of fireworks in groups of dogs showing fearful reactions to some other situations. The results

Table 1
Frequencies of fear scores for fireworks and loud noises (scores 1, not fearful; to 5, very fearful) from the telephone survey and the web-based survey

Items			Total	1, Not fearful	2	3	4	5, Very fearful	N/A
Fireworks	ISCWT	Telephone	474	220 (46.4)	80 (16.9)	42 (8.9)	62 (13.1)	67 (14.1)	3 (0.6)
		Web	203	102 (50.2)	37 (18.2)	20 (9.9)	22 (10.8)	20 (9.9)	2 (1.0)
	SP	Telephone	672	340 (50.6)	102 (15.2)	66 (9.8)	63 (9.4)	93 (13.8)	8 (1.2)
		Web	310	164 (52.9)	42 (13.5)	44 (14.2)	24 (7.7)	28 (9.0)	8 (2.6)
Noise	ISCWT	Telephone	474	230 (48.6)	86 (18.1)	60 (12.7)	45 (9.5)	48 (10.1)	5 (1.1)
		Web	203	99 (48.8)	54 (26.6)	29 (14.3)	12 (5.9)	7 (3.4)	2 (1.0)
	SP	Telephone	672	380 (56.5)	102 (15.2)	73 (10.9)	58 (8.6)	47 (7.0)	12 (1.8)
		Web	310	170 (54.8)	78 (25.2)	40 (12.9)	11 (3.5)	10 (3.2)	1 (0.3)

SP, standard poodle; ISCWT, Irish soft-coated wheaten terrier.
Percentage values are given within brackets.

Table 3
Frequencies of 11 fear-related behaviors observed when dogs are exposed to fireworks, at different firework fear scores (2, mild fear to 5, very fearful) in standard poodles (SP, N = 138) and Irish soft-coated wheaten terriers (ISCWT, N = 99)

Fear-related behavior	Fear score	2	3	4	5	Total %
ISCWT						
Shivering		1 (2)	4 (20)	14 (63)	14 (70)	33
Pacing		11 (29)	11 (55)	9 (40)	11 (55)	42
Hiding		2 (5)	4 (20)	8 (36)	7 (35)	21
Refuse food/water		–	–	5 (22)	8 (40)	13
Fussy/clingy with owner		6 (16)	1 (5)	5 (22)	8 (40)	20
Refuse to go outdoors		4 (10)	1 (5)	5 (22)	6 (30)	16
Vocalizing		9 (24)	12 (60)	8 (36)	8 (40)	37
Salivation		–	1 (5)	3 (13)	3 (15)	7
Urinating indoors		–	1 (5)	–	2 (10)	3
Defecating indoors		–	–	–	–	0
Destroys objects		–	–	–	–	0
Total behaviors (N)		33	35	57	67	
Dogs with score (N)		37	20	22	20	
SP						
Shivering		2 (4)	28 (63)	18 (75)	26 (92)	54
Pacing		14 (33)	27 (61)	11 (45)	14 (50)	48
Hiding		4 (9)	10 (22)	13 (54)	16 (57)	31
Refuse food/water		–	12 (27)	12 (50)	19 (67)	31
Fussy/clingy with owner		11 (26)	12 (27)	8 (33)	8 (28)	28
Refuse to go outdoors		2 (4)	14 (31)	7 (29)	13 (46)	26
Vocalizing		3 (7)	6 (13)	2 (8)	7 (25)	13
Salivation		–	–	2 (8)	6 (21)	6
Urinating indoors		–	–	–	1 (3)	1
Defecating indoors		–	1 (2)	–	–	1
Destroys objects		–	–	1 (4)	–	1
Total behaviors (N)		36	110	74	110	
Dogs with score (N)		42	44	24	28	

Brackets show percent of affected dogs for the behavior within the related firework fear score.

show that dogs that are fearful to some other situations in general are more reactive/fearful to fireworks. The results also show that dogs who live in single-dog households are more fearful of fireworks than dogs who live with other dogs. It also shows that dogs fearful of fireworks have a higher frequency of fear of household machines such as vacuum cleaners (Table 4).

Effect of interviewer

Distribution of scores between the interviewers (N = 7) had a high (87.4%, N = 1002) agreement (Figure 2). GLM calculations find no significant *P* values for any of the interviewers in either breed ($P > 0.05$). Interviewer number 7 is excluded from the figure because of too low number of interviews for any of the breeds but was

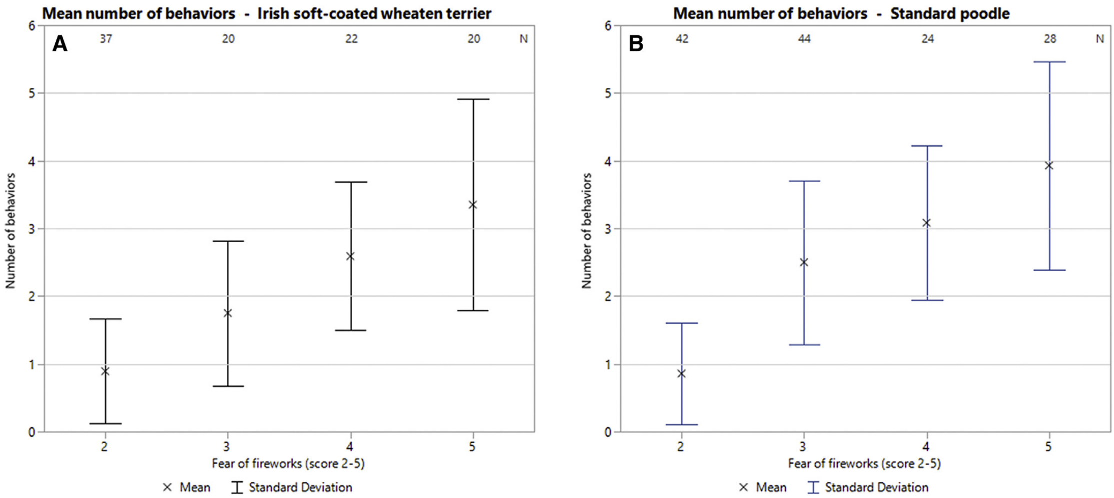

Figure 1. Number of fear-related behaviors shown by dogs with scores 2-5 for firework fear in A) Irish soft-coated wheaten terriers (ISCWTs) and B) standard poodles (SPs). The means and standard deviation error bars are shown, and error bars are constructed using 1 standard deviation from the mean. Both breeds show 0-7 behaviors.

Table 4
Odds ratio (OR) for fear of fireworks (score 4 or 5) in different situations

Fear/trait	OR	*P* value	z	95% Confidence interval
Often nervous/anxious	7.8	0.0001	4.0	2.85-21.77
Only dog in family	2.1	0.004	2.9	1.27-3.50
Fear of household machines	3.2	0.014	2.5	1.27-8.11
Fear of other dogs	2.3	0.13	1.5	0.78-6.94
Fear of grooming	1.1	0.80	0.3	0.50-2.44

included in the GLM. When answering the Web-based survey, 13% (N = 65) of owners give a lower score than that they gave in the telephone survey, while only 3.6% (N = 18) give a higher score. There is no significant difference between the 7 different students who performed the interviews. The owner rarely lower the score by more than one value (Table 5).

For both ISCWT and SP, the correlation between the answers given in the telephone survey and the Web-based survey is good (ISCWT: R = 0.95, $P < 0.001$, $R^2 = 0.9$, N = 200, SP: R = 0.95, $R^2 = 0.91$, N = 297). The overall means drop from 2.2 (telephone) to 2.1 (Web-based) in both breeds. This indicates that if the owner changes the answer from the first survey to the next, he or she moderates the response by giving a lower score. This is consistent with the findings in Table 5.

Ninety-two percent (ISCWT, N = 187) and 83% (SP, N = 247) of the deviating scores were classified 2 or 3 in the telephone survey and/or the Web-based survey, indicating that these values may be difficult to differentiate. This tendency is also seen in Table 6, which shows the change of firework fear score in SPs from the health and behavior survey (2014) to the Web-based survey (2016).

Effect of sex

Five hundred seventy-six female and 570 male dogs were included (SP female = 357, male = 315, ISCWT female = 219, male = 255). Fifty percent (N = 128) (ISCWT) and 54% (N = 170) (SP) of males show no fear of noise, while 45% (N = 98) (ISCWT) and 49% (N = 175) (SP) of the female dogs show no signs of fear of noise (not significant) (Figure 3). The effect of sex on noise reactivity was also tested with case/control groups (case score 4/5, strong/very strong fear; control 1, not fearful), also not significant.

Effect of age

In the telephone survey, the older dogs tend to be more fearful than the younger dogs. Furthermore, 14% (SP, N = 94) and 15.2% (ISCWT, N = 72) of the owners state that their dogs are more fearful now than they were earlier in life.

Table 5
Distribution of deviating scores between the telephone survey and the Web-based survey for dogs included in both surveys (N = 499), by the different interviewers (N = 7)

Interviewer	−2	−1	0	+1	+2
No. 1	1	10	61	0	0
No. 2	2	19	105	5	0
No. 3	1	7	72	2	0
No. 4	2	13	109	5	0
No. 5	0	2	34	2	0
No. 6	0	5	23	1	0
No. 7	0	3	12	3	0
Total N = 499	6	59	416	18	0
Total %	1.2	11.8	83.4	3.6	0.0

The Web-based survey shows that 43.5% (N = 138) of SPs and 47.8% (N = 99) of ISCWTs show some degree of fear of fireworks (2-5, mild-severe fear). For SPs, 61% (N = 84) were not fearful the first year, while 42% (N = 42) of the ISCWTs were not fearful the first year. For both breeds, the mean age of onset is 3.9 years if they have become fearful later than the first year of life (>1 year at first sign of fear), while the median age of onset is 3 years for SPs and 2.5 years for ISCWTs, and a variation from 1 to 11 years (SP) and 1 to 9 years (ISCWT). Among the owners, 24.6% (N = 34) (SP) and 25.3% (N = 25) (ISCWT) state that the dog has become increasingly more fearful with older age. Only 30% (N = 42) (SP) and 24% (N = 24) (ISCWT) of the owners believe the fear started after a specific situation with loud noises.

The increase in risk of fear was also illustrated comparing the risk (OR) of fearful dogs in increasing age groups to dogs younger than 5 years (Table 6).

Health and behavior (HB) survey in SP (2014)

The health survey included 454 SPs, where 242 were also included in the telephone survey two years later, where 223 had answered the questions about behavior. One hundred and fifty-two dogs participated in all three surveys.

Two hundred and twenty-three dogs from the behavior survey two years prior were included in the telephone survey. Sixty-six percent (N = 148) of owners did not change their score from the HB survey to the telephone survey, with the outlying scores (1, not fearful; 5, very fearful) being most consistent. The rest of the dogs (N = 75) show no clear trend, either of increasing or decreasing

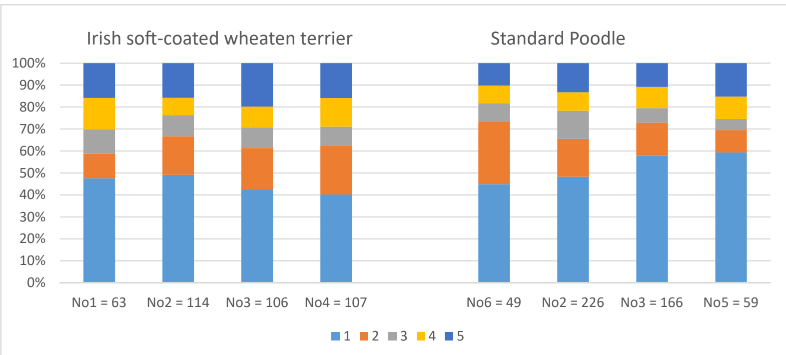

Figure 2. Scores 1-5 distributed by the four students with the highest number of interviewed owners (N = 34-337 [SP: student 2, 3, 5, and 6; ISCWT: student 1, 2, 3, and 4]).

Table 6
Odds ratio case (score 4, strong fear or score; 5, very strong fear) and control (score 1, no fear) at different ages for standard poodles (SP) and Irish soft-coated wheaten terriers (ISCWT), with age <5 as the comparative group

Age (years)	Odds ratio ISCWT	Odds ratio SP
<5		
5-6	1.3	1.2
7-8	1.3	2.4
9-10	1.9	3.2

ISCWT ($P = 0.07$; CI, 1.0-3.8), SP ($P < 0.001$; CI, 1.65-6.32).

scores (Table 7), but most of the variation can be observed in the group with scores 2 and 3, indicating that these scores might be difficult to define/separate.

Discussion

The frequency of fear of fireworks/loud noises is slightly higher in the ISCWT, with a small difference in the frequency of scores 2-5. The difference is statistically significant ($P \leq 0.05$). Approximately half of the responding owners of both breeds report that the dog shows some sign of fear of fireworks, which is consistent with findings in other studies by the same research group (Storengen and Lingaas, 2015). For both breeds, there is a significant correlation between fear of fireworks and fear of loud noises (e.g., thunder or gun shots), which is consistent with findings in other studies (Overall et al., 2001; Tiira et al., 2016; Dinwoodie et al., 2019). Differences in frequencies of fears and phobias between breeds are documented by several studies (Mahut, 1958; Plutchik, 1971; Martinez et al., 2011; Blackwell et al., 2013; Mehrkam and Wynne, 2014; Morrow et al., 2015). Even if there may be differences in inclusion criteria or classification of phenotypes between studies, these differences between breeds is an indicator that behavior might have a genetic component and that risk allele variants frequencies may vary between different breeds' gene pools. From an animal welfare perspective, it is worrying that 20%-30% of the dogs show a strong or very strong fear of fireworks and that half the dog population may be experiencing some level of discomfort when exposed to loud noises.

Both breeds show the same tendency of older dogs being more afraid of fireworks/loud noises than younger dogs (Figure 4).

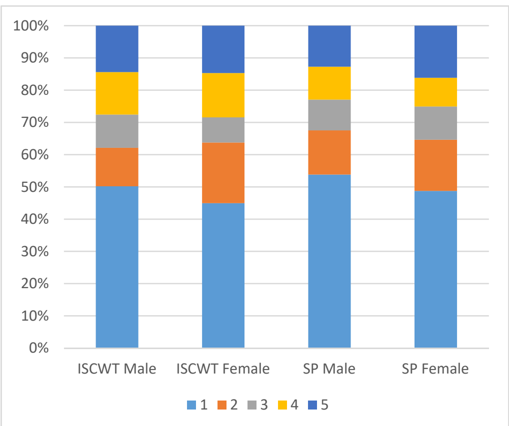

Figure 3. Distribution of firework fear score frequencies based on sex for standard poodles (SPs) and Irish soft-coated wheaten terriers (ISCWTs). Pearson's correlation $P = 0.3$ indicates no significant difference between the sex of either breed.

Table 7
Distribution of deviating firework fear scores between the older behavior (HB) survey (2014) and the telephone survey (2016) for standard poodles included in both surveys (N = 223)

Score in HB survey	N	−2	−1	0	1	2	3
1	111	–	–	91	15	4	1
2	27	–	14	8	4	0	1
3	33	4	9	12	7	1	–
4	17	1	2	10	4	–	–
5	35	3	5	27	–	–	–
Over all	223	8	30	148	30	5	2
Score in HB survey	**%**	**−2**	**−1**	**0**	**1**	**2**	**3**
1		–	–	82	14	4	1
2		–	52	30	15	0	4
3		12	27	36	21	3	–
4		6	12	59	24	–	–
5		9	14	77	–	–	–
Over all		4	13	66	13	2	1

Negative numbers indicate that the score is lower on the telephone survey than it was in the health and behavior survey, while positive numbers indicate that the score is higher than before.

Among owners, 24.6% (SP, N = 34) and 25.3% (ISCWT, N = 24) say their dogs have become more fearful with time, which is consistent with findings in other studies (Blackwell et al., 2013; Dale et al., 2010). In cases where the dog was not fearful the first year, but later became fearful, the mean age of onset is 3.9 years in both breeds, with median age of onset being 2.5 (ISCWT) and 3 (SP) years and a variation from 1 to 11. This is consistent with what others have found (Tiira et al., 2016). Age of onset for different types of phobias varies a lot between different studies, from 1 year old to 6.5 years (Bamberger and Houpt, 2006; Doring et al., 2009; Hakosalo et al., 2014). There is a general agreement that frequency of noise reactivity increase with older age, while the reason for this is unknown. Past experiences, owner-related factors, and auditory function have all been suggested (Goddard and Beilharz, 1985; Blackwell et al., 2013; Tiira and Lohi, 2015; Scheifele et al., 2016). In this study, only 30% (SP, N = 42) and 24% (ISCWT, N = 24) of the fearful dogs are reported to have had a frightening experience with loud noises/fireworks that may have triggered the fear. Negative experience with noise may possibly affect the distribution of fear of fireworks. For 62.3% (SP, N = 86) and 66.6% (ISCWT, N = 66) of the fearful dogs in this study, there is however no indication that there has been any negative experience before the debut of the fear. It is worth noting that the average age of the included dogs is relatively high in this study (SP, 8.2 years; ISCWT, 6.5 years). The high occurrence of signs of fearfulness to fireworks should therefore also be evaluated in this context because the frequency might be lower if large number of younger dogs had been included.

There is a marked difference in the percentage of dogs that are afraid from the first year/first exposure in the two breeds, as fearful ISCWTs have a 50% higher frequency of being fearful as puppies than SPs. Other studies have found breed differences in onset of fear reactions in puppies (Morrow et al., 2015). For a proportion of the fearful dogs (40% SP, N = 56, and 60% ISCWT, N = 59), the fear of noise seems to be innate or developed very early, as the owners claim they were fearful at the first exposure to fireworks, before the age of 1. For the remainder of the fearful dogs, the fear of noise has developed some time after they were one year old. It may seem that the great majority of the fearful dogs have shown signs of noise reactivity by the age of 4, even though the fear may worsen even after that. For studies comparing fearful dogs with fearless dogs, such as genetic studies, it is important to take the effect of age into account in the inclusion criteria for fearless dogs to reduce misclassification.

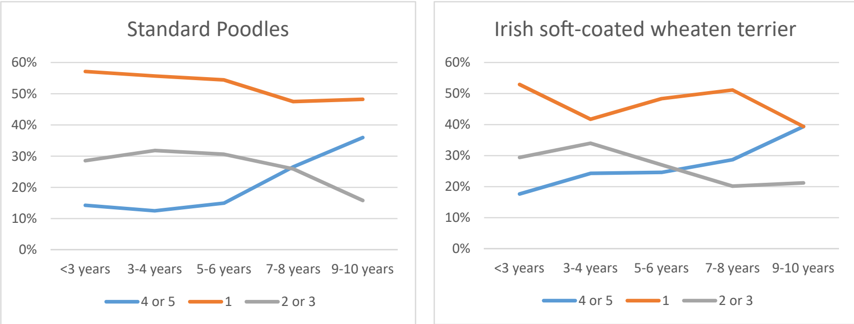

Figure 4. Percent of dogs with firework fear scores 4 or 5 (case), 1 (control), and 2 or 3 for firework fear, at five age groups (1 to 10 years), for standard poodles and Irish soft-coated wheaten terriers. The figure highlights the tendency that the higher firework fear scores are more frequently observed in the older dogs.

Although the degree of fear and frequency of additional recorded behaviors in the fearful dogs are surprisingly similar between the two breeds, there are some noticeable differences. The SPs show a higher degree of need for proximity to the owner/little independence, while the ISCWTs vocalize more. For both breeds, shivering is according to owners the most frequently observed single behavior in the fearful dogs (highest in SP), together with restlessness/pacing behaviors. Results/numbers shown in Table 3 also indicate that some behaviors might be related to a higher grade of fear (e.g., clinginess, salivation, refusal to go out, shivering, and refusal of food/water) and have a higher frequency in the dogs with higher scores. Defecation/urination indoors and destructive behavior are very rare, even in the most fearful dogs. Shivering is also seen in other fear-related situations such as thunder storms and visits to the veterinary clinic (Doring et al., 2009; Blackwell et al., 2013; Tiira et al., 2016). These dogs are primarily companion dogs, and one can expect that they live in rather similar environments. Several other studies find similar breed differences with fear/phobias and with aggression (Mahut, 1958; Hsu and Sun, 2010; Martinez et al., 2011; Mehrkam and Wynne, 2014). These differences may possibly be explained by genetic differences, and other studies have found heritability ranging from 0.23 to 0.56 for gun shyness and noise fear (Ruefenacht et al., 2002; van der Waaij et al., 2008; Ilska et al., 2017). As it is unlikely that these traits are selected for, the high frequency could be a result of random genetic drift or that they may have been in linkage disequilibrium with other preferred traits in major breeding dogs when the breed was founded. It is also suggested that breed-typical behavior is also a result of more recent indirect selection (Svartberg, 2006).

In this study, a high number of fear-related behaviors seem to correlate directly with a high firework fear score (Figure 1), which is consistent with other studies (Blackwell et al., 2013). The owner consistently rates dogs that show many fear-related behaviors higher on firework fear scores. The recording of several additional behaviors in the fearful dogs, as well as duration of fear-related behaviors before and after the exposure, was informative and helped understanding the degree of discomfort of affected dogs. OR calculations report that dogs showing general fearfulness in everyday situations also have an increased risk to be more fearful of fireworks/noise. There is an association between “general nervousness” and reaction to fireworks but also a tendency that dogs that react to the noise of household machines are more fearful to fireworks. These findings are consistent with the results of the telephone survey, where a significant correlation is found between fear of fireworks and how often the dogs show signs of fear in other situations. This study also shows that dogs who are the only dog in the household have a higher risk of firework fear, indicating that the existence or lack of firework fear/noise reactivity might have a social/interdog component. One study found that dogs in single-dog households react more pronounced when exposed to thunderstorms (Dreschel and Granger, 2005), and another found that dogs in single-dog households are more fearful (Tiira and Lohi, 2015), supporting our findings. In studies on separation anxiety, no effect/support of a second dog could be seen (Flannigan and Dodman, 2001; Palestrini et al., 2010). These relationships require further studies with a higher number of individuals but are interesting supplements in the understanding of canine fear behavior and noise reactivity.

Although the frequency of fear in female dogs is higher than that in males in both breeds, the difference was not significant. Previous studies have found that female ISCWTs are more fearful of firework noises than males (Storengen and Lingaas, 2015). Other studies have also found that females show more fear behaviors than males (Tiira et al., 2016; Wells and Hepper, 2000) and that females have a higher frequency of phobias and demand for owner attention (Bamberger and Houpt, 2006; Bradshaw et al., 1996), while another study found that male dogs are more fearful of gunshot noises (Blackwell et al., 2013). In studies of separation anxiety, some studies found an effect of sex (McGreevy and Masters, 2008; Storengen et al., 2014), while others did not (Wright and Nesselrote, 1987; Flannigan and Dodman, 2001). The lack of significance in this study may be influenced by the high mean age, if sex has a greater impact on younger dogs, or possibly reflects that the effect of sex is small. The effect of sex may also be small in this study because of a generally high frequency of noise reactivity in the selected breeds and selected dogs. Data collection variables, definitions of fearfulness, and geographic/population differences may contribute to variation in reported results. The correlation between the responses from the telephone survey and the Web-based questionnaire shows that owners have a consistent opinion about their dogs’ fear of noises/fireworks and that the answer is not significantly influenced by these two methods of data collection. These are promising results and consistent with similar studies (Tiira and Lohi, 2014; Wiener and Haskell, 2016). A few owners give a different score for fear of firework or noise fear score in the Web-based survey compared to the telephone survey. In general, they reported a slightly lower score, mainly by reducing the number of 4/5 scores in the Web-based survey. It therefore looks like Web-based questionnaires are time- and cost-efficient alternatives to collect reliable information about noise fear from the field. The distribution of fearful and nonfearful dogs is approximately the same in the two surveys, and no evidence has been found that owners of fearful or fearless dogs are more or less represented in either survey. It may be necessary to assess how to reach the owners with Web-based questionnaires, and how to motivate them to participate.

Conclusions

This study shows that dog owners are consistent in their assessment of dogs' fears and that the phenotypes they provide are likely to be correct. ISCWTs are slightly more fearful than SPs, the two breeds differ in their reaction to fireworks, and the number of behaviors presented indicates the severity of noise reactivity. The most fearful dogs are more likely to be anxious before and after expected exposure to fireworks and are often difficult for the owner to calm down. OR calculations show that dogs with fear of fireworks are more insecure and more fearful of everyday situations and household noises than fearless dogs. Dogs who are the only dog in the household are more fearful than dogs living with other dogs.

The study also shows that older dogs tend to be more fearful of fireworks and noises than younger dogs, with an age of onset of 3.9 years for dogs who are not fearful during their first year.

Acknowledgments

The project is partly funded by the Norwegian research council; project number NFR-244591.

Conflict of interest

The authors declare no conflict of interest.

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Journal of Veterinary Behavior 45 (2021) 33–36

Contents lists available at ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Genetic parameters for noise reactivity in standard poodles

Check for updates

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ARTICLE INFO

Article history:
Received 31 August 2020
Revised 27 May 2021
Accepted 12 June 2021
Available online 19 June 2021

Keywords:
Noise reactivity
heritability
dog
firework fear
behavior
genetics

ABSTRACT

The aim of this study was to increase knowledge on the genetic background of noise reactivity in dogs, with a study on heritability based on owner-collected data on standard poodles' degree of fear of sudden loud noises and fireworks. Previous studies have shown significant breed differences, and we expected to find significant heritability for noise reactivity. 1148 standard poodles are included in the study. The estimated heritabilities are between 0.09 and 0.16. Reaction to sudden loud noises and fireworks are genetically highly correlated ($r_g > 0.95$).

Introduction

Behavior and behavior disorders have a large effect on both dogs' and dog owners' everyday lives, and anxiety-related disorders such as separation anxiety and phobias may have profound welfare implications (Rugbjerg et al., 2003). Studies find that deviant or problematic behavior in dogs may result in a shorter life expectancy (Scarlett et al., 1999; Dreschel 2010) and unwanted behaviors such as aggression, destructive behavior and indoor urination and defecation ("loss of learned behavior") (Overall et al., 2001; Handegård et al., 2020). These behaviors can ultimately lead to a damaged dog-owner relationship (Shore 2005; Udell et al., 2010).

Additionally, dogs with noise reactivity are not suitable as working dogs. Fear and anxiety are one of the most common reasons for withdrawal of guide dogs from training programs (Goddard & Beilharz 1984; Goddard & Beilharz 1985; Serpell & Hsu 2001; Batt et al., 2008; Caron-Lormier et al., 2016).

Canine temperament and behavior, including fear and phobias, are influenced by both genetic and environmental factors (Overall, 2013). Behavior and behavioral challenges vary within and between breeds. While various breeds have been chosen for specific behaviors, the genetic factors that influence behavior are mostly unknown.

Using twin studies, human behavioral research has found heritabilities between 0.30 and 0.70 for personality traits, aggressiveness, and disorders like major depression disorder and social anxiety (Plomin et al., 1994; Beatty et al., 2002; Guffanti et al., 2016; Moreno et al., 2016). Several behavior traits have been found to have high heritability in dogs: retrieving, social behavior, courage and cooperation (Saetre et al., 2006; Houpt 2007; Evans et al., 2015; Persson et al., 2015; Gnanadesikan et al., 2020). High heritabilities have been found for different types of fear in dogs (Goddard & Beilharz 1985; Arvelius et al., 2014; Persson et al., 2015; Ilska et al., 2017), but there is limited information about the genetic factors that affect phobias, anxieties and fearful behavior (Hohoff 2009; Ogata 2016). The possibility to reduce the incidence and severity of anxiety through selection depends on the possibility to record phenotypic information from a number of dogs and estimate the heritability based on data. The collection of phenotypic information and the calculation of heritabilities in standard poodles is the focus of this study.

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https://doi.org/10.1016/j.jveb.2021.06.005

Table 1
Summary statistics and distribution on the 4 data sources (DS). The average score is based on a Likert scale 1-5 where 1 is not fearful and 5 is very fearful.

Trait	Number	Average	Dispersion	Number of observations per DS			
				1	2	3	4
Fireworks	1,148	2.042	1.379	361	288	248	251
Noise	1,148	1.801	1.134				

A previous study showed that up to 50% of standard poodles show some degree of reactivity towards fireworks / loud noises (Handegård et al., 2020). In the current study we collect data from 1148 standard poodles, and present heritability estimates for noise reactivity and firework fear, as well as the genetic correlation between the 2. The purpose is to evaluate if heritability based on owner-based records is sufficient to achieve reasonable genetic progress through breeding.

Materials and methods

Behavioral data

Data on behavior traits was collected from web- and telephone-based questionnaires, in collaboration with the Norwegian poodle club (NPC) and the Norwegian Kennel Club (NKC).

A total of 4 surveys were performed over the course of 5 years to collect data from which we could identify phenotypes involving anxiety. The first (2014) was a behavior study which included questions about fear of fireworks and noise reactivity. The second and third (2017) were performed as a telephone survey with a follow-up online questionnaire repeating the questions about fear of firework and noise reactivity. The fourth was an online questionnaire (2020) with the same questions about fear of firework / loud noises, sent to owners who had not participated in either of the earlier studies. Some dogs were included in more than 1 study. If a dog had more than 1 observation only the score from the newest online questionnaire was used. The 4 data collections are referred to as “Data Source” 1 to 4. (1: Questionnaire 2020, 2: Q2017, 3: Q2014, 4: Telephone 2017).

In all 4 studies the dog owners were asked to indicate reactivity to 2 traits (1) their dogs’ fear of fireworks and (2) their dogs’ general reactivity to loud noises on a 1 to 5 Likert scale where 1 is "shows no fear" and 5 is "very fearful". Firework fear and noise reactivity are thus handled as 2 different phenotypes as defined in a previous paper (Handegård et al., 2020). In the web-based study owners added information on up to 11 behaviors typically associated with noise reactivity (Overall, 2013) that their dog displays when exposed to fireworks or loud noises. The number of observed behaviors showed a high correlation to the Likert classification where dogs with score 2-5 showed on average 2.4 of these behaviors (Handegård et al., 2020) with shivering, pacing, refusing food/water, seeking the owner and hiding most often reported. None of the dogs with score 1 had any remarks. In this paper any dog with score 2 or higher is therefore included as fearful (case) while dogs with a score of 1 is considered not fearful (control). Fear of thunder / gun shots are included in noise reactivity, thunderstorms are however rarely occurring in Norway, and no dogs are included exclusively for fear of thunderstorms/gun shots.

To be sure that all dogs had experienced fireworks we primarily asked for information about older dogs. Eighty-eight percent of the dogs were >3 years old (36 months). One study of 3 breeds found that noise reactivity/ phobia occurred by 20 months for most dogs, as the dog reaches social maturity (Overall et al., 2016). Recent studies have suggested that some dogs may worsen throughout life (Salonen et al., 2020).

Table 2
Fixed effects included and number of levels for the models used (NA indicates that this effect is not in the model).

Model	Sex	Number of Data sources	Y of birth	Time of birth; 5 y-group
M1	2	4	30	NA
M2	2	4	NA	NA
M3	2	4	NA	5

Only dogs with a pedigree registered in the NKC (N = 1,148) were included and information was collected through 4 surveys from 2014 to 2020. If a dog had more than 1 observation across the studies, only the score from the most recent online questionnaire was selected. The 1148 dogs were born between 1983 and 2019. A pedigree file for all dogs related to the 1148 was dogs extracted from NKC’s database and included,3457 dogs.

Summary statistics and number of observations from the 4 data sources are shown in Table 1.

Statistical analysis

Data was analyzed using the following bi-variate animal model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Za} + \mathbf{e}$$

where **y** represents the vector of phenotypes (Firework and Sound); β is a vector of fixed effects, **a** is a vector of additive genetic effects following $N(0, A \otimes G_0)$, where A is the numerator relationship matrix, and G_0 is the additive genetic co-variance matrix; **X** and **Z** are design matrices relating phenotypes to fixed effects and random animal effects, respectively; and e is a vector of residuals following $N(0, I \otimes R_0)$, where R_0 is the residual co-variance matrix.

In order to account for possible environmental factors over the timespan, 3 different definitions of fixed effects were used (see Table 2):

Variance components were estimated with REML Patterson & Thompson (1971) using the average information algorithm (AIREML) Jensen et.al (1997) as implemented in the DMU package (Madsen & Jensen, 2013).

Model validation

Models validation was conducted by regression of EBV’s from the full data on EBV’s from a reduced data set. Two different definitions of the reduced data set were used: either the data for the dogs born in 2019 was deleted (Val1) or that for dogs born 2018 and 2019 was deleted. (Val2). The EBV’s for dogs with deleted observations in Val1 and Val2 will have EBV’s based on parent average, while the EBV’s from the full data also include own information. The expectation for the regression of EBV’s from full data on EBV’s from reduced data is 1.0. Deviation from the expectation indicated that the model does not describe the genetic trend correctly.

Table 3
Estimated co-variance components, correlations (in bold) and heritabilities from the 3 different models (SE of estimate in brackets).

Model	Trait	Genetic co-variance		Residual co-variance		Heritability
		Fireworks	Noise	Fireworks	Noise	
M1	Fireworks	0.166 (0.083)	0.153 (0.070)	1.659 (0.100)	1.012 (0.077)	0.091 (0.045)
	Noise	0.953 (0.075)	0.156 (0.069)	0.753 (0.018)	1.091 (0.074)	0.125 (0.054)
M2	Fireworks	0.303 (0.103)	0.241 (0.089)	1.590 (0.107)	0.977 (0.081)	0.160 (0.052)
	Noise	0.970 (0.045)	0.204 (0.074)	0.7474 (0.020)	1.077 (0.075)	0.159 (0.055)
M3	Fireworks	0.205 (0.091)	0.181 (0.075)	1.658 (0.103)	1.021 (0.080)	0.110 (0.048)
	Noise	0.961 (0.060)	0.173 (0.072)	0.755 (0.018)	1.102 (0.075)	0.135 (0.054)

Table 4
Validation of models 1, 2, and 3.

Model	Trait	Val 1 (n = 47)		Val 2 (n = 84)	
		Intercept	b	Intercept	b
M1	Fireworks	-0.011 (0.011)	0.976 (0.144)	-0.005 (0.008)	1.051 (0.102)
	Noise	-0.013 (0.010)	0.923 (0.157)	-0.006 (0.008)	1.042 (0.109)
M2	Fireworks	-0.132 (0.031)	0.981 (0.239)	-0.101 (0.015)	1.172 (0.119)
	Noise	-0.106 (0.024)	0.968 (0.240)	-0.082 (0.012)	1.171 (0.124)
M3	Fireworks	-0.075 (0.017)	0.986 (0.202)	-0.056 (0.010)	1.186 (0.111)
	Noise	-0.067 (0.015)	0.934 (0.203)	-0.050 (0.009)	1.158 (0.116)

Results

Table 3 shows the estimated co-variance components and derived correlations and heritabilities. Correlation is high between the 2 traits (0.75-0.97). The heritability varies somewhat between the models (fireworks 0.091-0.160, noise 0.125-0.159). The heritability and correlations are highest in model 2 without fixed effects.

Due to the relatively small dataset, the estimated parameters have quite large standard errors.

Table 4 shows results from model validation. Again, due to the small dataset, the standard errors for the regression of EBV's from full data on EBV's from reduced data are large, and none of the regression coefficients differs significantly from 1.0.

Discussion

The 2 traits "Firework" and "Noise" are genetically highly correlated, with correlations that do not significantly differ from 1.0. The estimated heritabilities are low to medium. If they correctly represent the relative genetic contribution of the traits, a relatively slow selection response might be expected based on mass selection. The estimates are lower than we had expected due to the reported variation in frequency between different dog breeds, which indicates a relatively strong additive genetic component.

This study is based on observations from owner-based questionnaire surveys. Such surveys have previously shown good correlation with data from behavior tests (Tiira & Lohi 2014; Flint et al., 2018), especially with traits such as fear of firework noise, which is difficult to measure in a field-test environment (Hsu & Serpell 2003). Even if the correlation between repeated observations is high, it is possible that higher (realized) heritabilities/faster selection response could be obtained by including other effects like age of onset in the model. Of the 1148 dogs included in this study, 1,023 dogs were born in 2005 or later. In the same period (2005-2019) 4270 standard poodles were registered in the NKC, so this study includes approximately 24% of those dogs.

Correct classification, in the sense of recording the genetic part of the trait, of noise reactive dogs is challenging, as a number of environmental factors may wholly or partially influence the development and classification of the traits. Owners also have different experience in evaluating their dogs' behavior, and there might be a difference in interpretation between breeders and ordinary dog owners. The use of a semi-quantitative Likert scale should take into account part of the continuous variation of the trait, but it should be recognized that it is difficult for the owners to indicate a "correct" classification, especially between 2 to 4.

A previous study on the same material shows that the latest age of onset is <3.9 years for both firework fear and noise reactivity in standard poodles. In the current study all surveys are performed as cohort studies where we initially have addressed owners of grown-up dogs (3 years and older). The majority of records are therefore from dogs above 3 years of age. At this age dogs tend to have developed many behaviors and can be reliably recorded (Asher et al., 2013; Overall et al., 2016), and most dogs at risk of noise reactivity show signs of the phenotype. However, since we also allowed owners of younger dogs to reply, a low percentage (N = 141, 12%) are younger than 3 years. 30% of the dogs below 3 years show some degree of noise reactivity, versus 47% of the dogs aged 3 and above, which means 20 to 25 dogs (≈2%) could have been wrongly classified as not fearful. Because of the small number of younger dogs where a fear diagnosis might have been missed due to the low age, we believe these dogs do not have a significant influence on the results.

It is interesting to see the difference between the model with and without the year of birth in the model even if the study is covering a relatively short time interval. Since all surveys are done as cohorts, covering dogs from many of the "time periods", we did not expect year of birth to have strong effect. During the past years there has however been some systematic differences in the type of noise that challenge the dogs. In 2008 new governmental rules in the sale of fireworks were established to reduce the frequency of human accidents, prohibiting sky rockets and bottle rockets while allowing battery missiles. This has also changed the type and intensity of noise from fireworks, as battery missiles usually do not contain the same whistle effects as bottle rockets. To account for potential differences between data source/surveys, the data source was included in one of the explored statistical models and was the major effect that influenced the estimated heritability. Even if the records are collected from dogs born in a relatively short time period (15 years), and there may have been systematic changes in environmental effects, there may also have been a change in the parent-population between dogs born in the beginning of the period versus those born in the end of the period. If this is the case, the inclusion of time effects (and data source) could overestimate the effect of cohorts and underestimate the heritability.

In some dogs the fear of fireworks is present from early puppyhood, while others start to show signs of fear at a later age, possibly affected by negative experiences in the dog's life. It is also difficult to determine whether it is the noise or other factors like the smell of smoke and/or flashing lights that the dog is fearful of. It has been suggested that noise reactive dogs might have a change in auditory response (Scheifele et al., 2016), and that dogs fearful of noises could be feeling pain when exposed to loud noises (Tiira et al., 2016). Studies from other species have also found a link between hearing loss and noise reactivity (Jastreboff & Jastreboff 2015; Manohar et al., 2017), in contradiction to a human twin study which found significant heritability of noise reactivity even when hearing impairment was corrected for (Heinonen-Guzejev et al., 2005). In this study, no data were collected on auditory dysfunction / hearing loss.

It is likely that the realized heritabilities are higher than those indicated by our estimates. But to obtain a better recording of the underlying genetics of the phenotypes, higher heritabilities and a better selection response, it would be important to record phenotypes with higher precision. This could be obtained by a better description of each score, asking for age at recording, or ask owners to classify their dogs at specific age point, for *example 5 to 6* years. The recording of additional phenotypes known to be associated with noise reactivity (Overall, 2013) may facilitate the classification of phenotypes, especially of dogs with mild symptoms. Other traits like duration of the symptoms might also be included.

Conclusions

Low to medium sized heritabilities were estimated for noise reactivity and fear of fireworks in standard poodles based on owners' records. There is a significant difference between the lowest and highest estimate in the different models. The results show that it should be possible to obtain a reduction in the prevalence of reactivity to loud noises based on owner's registrations by systematic breeding. The results also indicate that optimization of models by inclusion of other environmental effects could improve heritabilities and support a faster breeding response.

Conflict of interest

The authors declare that they have no conflict of interest.

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III

Genomic analysis of firework fear and noise reactivity in standard poodles

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Plain English summary

The prevalence of fear of fireworks and loud noises is very high in many dog breeds and can be a serious problem for both the dogs and the owners. A genetic study of fear of fireworks and fear of other loud noises was conducted on standard poodles. The study was based on online surveys, where the dog owners scored their dogs degree of fearfulness from 1 (not fearful) to 5 (extremely fearful). We estimated genomic heritabilities to 28% for fear of fireworks and 15% for noise reactivity. We also identified a region on chromosome 17, with a possible association with the two traits. This genomic region contains genes of interest in human anxiety-related disorders. Genomic studies in dog breeds where the individuals with and without fear-associated phenotypes can be clearly distinguished, based on owner records, may provide opportunities for implementation of methods for genomic selection for those fear-associated traits. This can become a tool for breeders in their selection of breeding animals, which ultimately will contribute to better animal welfare.

Abstract

Background: Fear of firework noises and other loud, sudden noises (noise reactivity) is a significant problem for many dogs and may have a negative effect on both welfare and, in severe cases, the life expectancy of dogs. A wide range of behavior traits, including fear-related behaviors, have high heritability estimates in dogs. The aim of this study was to estimate genomic heritability for fear of fireworks and loud noises in dogs.

Results: A genomic heritability estimate was performed based on genome-wide SNPs from standard poodles with records of fear of firework and noise reactivity. The study was based on questionnaires filled in by owners, who also volunteered to return a cheek swab from their dog for DNA analyses. SNP-based heritability was estimated to be 0.28 for firework fear and 0.15 for noise reactivity. We also identified an interesting region on chromosome 17 that was weakly associated with both traits.

Conclusions: We have estimated low to medium genomic heritabilities for fear of fireworks and noise reactivity in standard poodles. We have also identified an interesting region on chromosome 17, which harbors genes that have been shown to be involved in different psychiatric traits with anxiety components in humans. The region was associated with both traits; however, the association was weak and need further verification from other studies.

Keywords: Noise reactivity, GWAS, dog, fear of fireworks, behavior, behavioral genetics, genome heritability, SNP-based heritability

Background

The pet dog is challenged by a range of loud noises and general commotion, and fear of loud and sudden noises is a significant problem for many dogs. Studies have shown that up to 30% of dogs of some breeds, including the standard poodle, show a strong or extreme fear of loud noises and/or fireworks (1-6).

Fearfulness and anxiety disorders constitute a large proportion of behavioral problems in both family dogs and working dogs, and noise reactivity is a large part of these problems (1-3, 5). Noise reactivity is a complex trait with a wide specter of phenotypes, and is likely affected by both environmental factors, as well as heritage. The etiology of fear and anxiety is poorly understood. While fear is a natural response to a potentially dangerous stimuli or situation, and is necessary for survival (7-11), excessive fear responses out of context are problematic, and may be pathological (12). Anxiety and fear can also have a strong negative effect on animal welfare (13), as well as the relationship between the owner and the dog (14, 15). In severe cases, anxiety and fear may impact the life expectancy of the dog (16, 17).

Studies from human behavior research have estimated heritabilities for many personality and behavior traits to be between 20 and 70% (18-20). These traits include aggressiveness, and disorders like social anxiety and major depression disorder (21-24). Identifying specific genes associated with behavior traits has, however, proven to be difficult. Several behavior traits, including a range of fear phenotypes, have been shown to have high heritability also in dogs. For example, Goddard and Beilharz found heritability estimates of 0.46 for fearfulness in guide dogs, and Ruefenacht et al. found heritability estimated to 0.23 for reaction to gunfire, similar results have been reported by other researchers (25-28). The relatively high heritability of behavior traits in dogs is also supported by the variation in prevalence of different behaviors between breeds (29, 30). Despite the many studies in both humans and several other species, there is limited information about the genetic architecture of phobias, anxieties, and fearful behavior (12, 31, 32).

Many single genes found to be associated with behavior traits, including traits with high heritabilities, explain only a small proportion of the variation in the traits, giving rise to the term "missing heritability" (33-35). The reason for the limited success of identifying associated genes with large effects is thought to be that the traits emerge as a result of complex genetic interactions, and the traits may be inconsistently diagnosed, biased, or highly polygenic caused by a large number of genes with small or moderate additive effect (36-40). Epigenetics might also add to the complexity of these traits and contribute to camouflage single gene effects (41, 42). Later, hundreds of genome-wide association studies (GWAS) have provided new information about genetic associations to disorders like general anxiety, major depressive disorder, schizophrenia, autism spectrum disorders and bipolar disorder and others, indicating that most behavior traits have a complex genetic background where many different loci may be involved (43-47). GWAS in dogs has successfully identified candidate markers and genes for several behavior traits (48-52) including fearfulness (50, 53, 54).

The evolutionary bottlenecks and breeding for specific traits have caused purebred dogs to have lower genetic diversity with longer linkage disequilibrium (LD) compared to humans (55). Studies have found that LD in some dog breeds is up to a hundred times more extensive than in humans (55-57). The accumulation of risk alleles associated with specific behaviors, as well as the limited genetic heterogeneity within breeds, makes the dog a good model for identifying associated loci for complex traits, even with a limited sample size.

The aim of this study was to estimate the genomic (SNP-based) heritability of fear of fireworks and loud noises in standard poodles and search for potential genomic regions associated with these traits.

Results

Heritability estimates

The genomic heritability estimates were 0.28 (SE 0.10) for firework fear and 0.16 (SE 0.10) for noise reactivity, with a high genetic correlation between the two traits, 0.99 (SE 0.05).

Genome-wide association study

Mixed linear models for the phenotypes firework fear and noise reactivity each identified suggestive association ($p<2x10^{-05}$) to a region on chromosome 17 (CFA17). For firework fear the top SNP (BICF2P1194351) was found in position 44.487.783 ($p=6.065x10^{-06}$) and the top SNP for noise reactivity (BICF2P966078) in position 44.409.723 (Table 1). Both top SNPs are within the same intron of the Catenin alpha 2 gene (CTNNA2), and in <1Mb range of two other genes; Leucine rich repeat transmembrane neuronal 1 (LRRTM1) and Regenerating islet-derived protein III-alpha (REG3A). SNPs on chromosomes 7 and 15 also reach near-suggestive p-values (Figure 1). All genomic positions are given according to the assembly GSD_1.0 (CanFam4) (58).

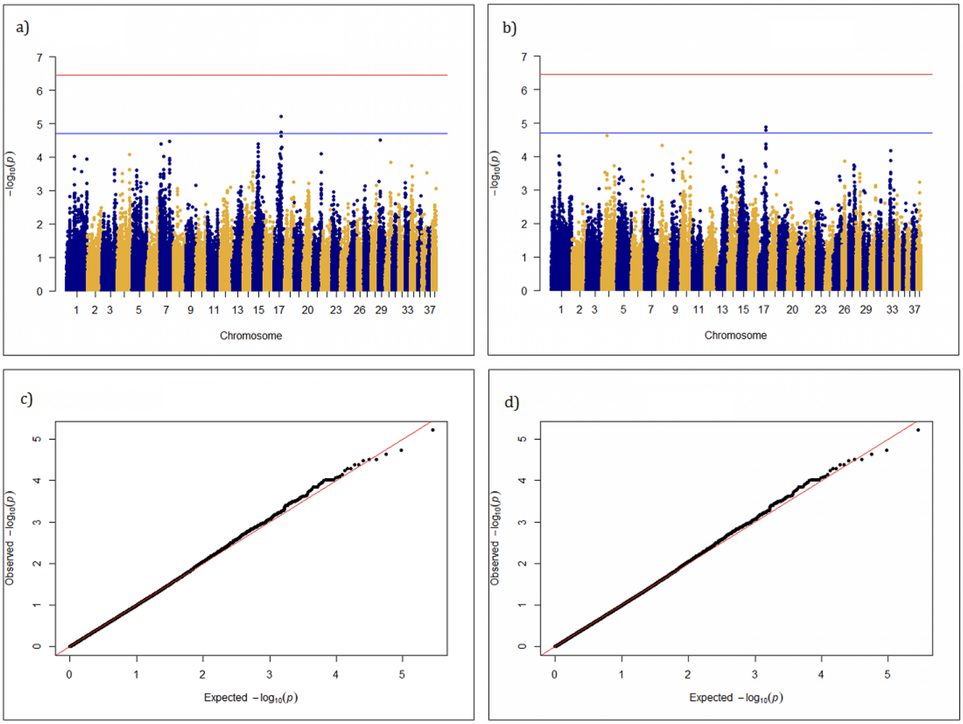

Figure 1: Manhattan plots for a) firework fear and b) noise reactivity. A suggestive line (blue) was placed at p=2x10-^{05}, based on Bonferroni adjustment using the number of independent haplotype blocks (Karlsson et al., 2013). The more conservative GWAS significance line (red) was placed at p=3.54x10^{-07} using the total number of tested SNPs (141.174 SNPs after QC). Quantile-quantile plots for c) firework fear and d) noise reactivity. Lambda was calculated at 1.0 for both traits.

Table 1: SNP-positions top SNPs and genes in the nearby region

Chromosome SNP position	Top SNP ID and p-value	Gene	Association summary	Distance from top SNP
chr17:44.487.783	BICF2P1194351 $p = 6.065 \times 10^{-06}$	*CTNNA2*	Canine: Canine compulsive disorder Human: Excitement seeking, ADHD, Schizophrenia, Bipolar disorder	0.00Mb
		LRRTM1	Mice: claustrophobic-like behavior in knock-out-mice Human: Schizophrenia	0.21Mb
		REG3A	Human: Gastrointestinal cancers, (no reported association to behavior-traits)	0.81Mb

The Multidimensional scaling (MDS)-plot show an even spread of cases and controls for both firework fear and noise reactivity (Figure 2) and for male and female between the clusters (supplementary figure 1). Clustering in the population can be partly explained by subpopulations with different solid colors, but cases and controls are evenly distributed in both subpopulations (supplementary figure 2).

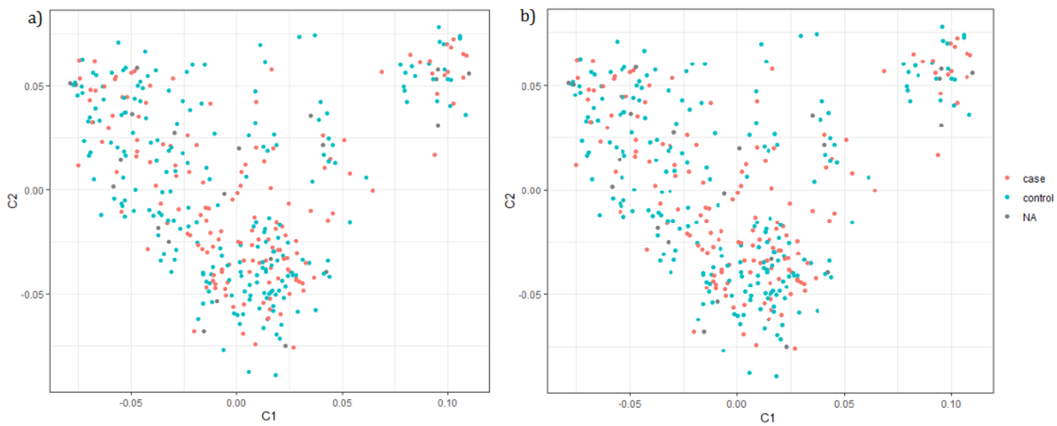

Figure 2: Multidimensional scaling plot for a) firework fear and b) noise reactivity. NA=dogs with score 2 or 3.

Discussion

Our estimates showed medium to low genomic heritabilities for fear of firework and noise reactivity. In a previous study, pedigree based heritability for noise reactivity and fear of fireworks was estimated to be 0.09 (noise reactivity) and 0.16 (fear of fireworks), with high genetic correlation between the two traits ($r_g > 0.95$) (59). It has been argued that GCTA may overestimate the genomic heritability (60), but both the pedigree-based and genomic heritabilities are based on partly different and relatively small materials.

Our study also supports the challenge of identifying genomic regions associated with behavior traits also in dogs, even if the genetic heterogeneity is lower than in humans and it should be easier to identify genes for complex traits in small materials compared to human. The region on CFA17 with a suggestive association to fear of fireworks and noise reactivity is just above the suggestive threshold with much "background noise", as visualized on the Manhattan plots (Figure 1). It is however notable that the two traits in question were both associated with the same region, and this region contains several genes with a potential effect on the studied traits. The results are not significant according to strict Bonferroni thresholds. Studies have argued that the traditional GWAS significance levels, using the number of tested SNPs, are too conservative in canine GWAS studies due to long LD and closely linked SNPs (57, 61). Karlsson, Sigurdsson (61) suggest that p-values < $2x10^{-05}$ (depending on breed) may be an alternative to correct for the number of haplotype-blocks. The top SNPs passed the significance threshold when we used less strict criteria based on average LD of 1Mb, 2400 blocks and p-values < $2x10^{-05}$. The study is likely to be underpowered, and an increase in sample size would be preferable.

One of the genes in the candidate region, CTNNA2 (Catenin alpha 2), has been thought to be involved in bipolar disorder, a disease with a component of anxiety. The CTNNA2 gene encodes a catenin protein which is associated with several human psychiatric disorders with components of anxiety, including bipolar disorder (62) and schizophrenia (63). The CTNNA2 gene is proposed as a candidate gene by Tang et al. (2014) in canine compulsive disorder in Doberman pinchers, a condition closely linked to anxiety (48, 64).

LRRTM1 (Leucine-rich repeat transmembrane neuronal 1) is a small protein coding gene with only 1568 base pairs. LRRTM1 is a nested gene within the bounds of the CTNNA2 gene. The LRRTM1 gene has previously been related to claustrophobia-like behavior in LRRTM1-deficient mice (65), and schizophrenia in humans (66-68).

Classification of noise reactive dogs is challenging, as a number of environmental factors influence the development and expression of the traits. The owners' abilities to perform an "objective" and correct classification will vary, which may be a challenge as each dog has a different owner (69, 70). Fear of fireworks might be present from early puppyhood but could also be a result of previous experiences in the dog's life, like reactions to the smell of smoke, flashing lights, and other environmental factors. It has also been suggested that some noise reactive dogs are suffering from physical pain in the ears or changes in auditory response (3, 71). In general, however, owner-based questionnaires are considered an acceptable method for collecting behavioral phenotypes (72-75).

Fear of fireworks and noise reactivity may be biologically similar traits, and the two traits are shown to have a high correlation in the same breed (76). Still, we believe that they are two different traits that largely overlap, rather than different expressions of the same trait. All the dogs that are described as very or extremely fearful of loud noises (cases) are also found to be very or extremely fearful of fireworks. Only about half of all dogs included as cases for the fear of fireworks trait are also classified as cases in the noise reactivity trait (Figure 3). It, therefore, seems that many of the firework cases could be reacting to the noise regardless of its origin, while the rest react exclusively or more severely to fireworks. This could be because some of the firework fear is caused by other stimuli, like flashing lights or the smell of gunpowder, and suggest that fear of noise and fear of fireworks at least to some extents are two different traits, which are very hard to distinguish. That would also make it likely that some dogs are affected by both traits, which complicates the phenotyping further.

Phenotypes were collected from owner-based questionaries, where the dogs were scored from 1 (not fearful) to 5 (extremely fearful). To increase the likelihood of identifying associated loci, we have aimed at keeping a maximum contrast between observed phenotype in cases and controls. Accordingly, dogs with a score of 2 or 3 were not included in the study (n=111). Several recent studies (2, 53, 54) have included all dogs with scores >1 as cases. One advantage of such classification is a significant increase in the number of dogs analyzed, but the disadvantage is a smaller contrast between cases and controls where owners' ability to correctly classify the dogs' behavior may be a challenge. By including all dogs with scores >1 it is possible that the statistical power could be increased somewhat.

One study by Zapata (2016) found an associated region of non-social fear on CFA18 in a study on 11 different breeds (50). Another study by Sarviaho (2019) found an association of noise reactivity in an area on chromosome 20 in German shepherd dogs (53). Our study does not replicate those results in standard poodles.

Conclusion

Using material from standard poodles and owner-based records, we have estimated medium and low genomic heritabilities for fear of fireworks and noise reactivity, respectively. The significant medium genomic heritability for fear of fireworks may provide helpful information for genomic selection of breeding animals in future breeding programs, which may in turn reduce the prevalence of these behavior traits. We have also identified an interesting region on CFA17, which harbors genes that have been shown to be involved in several psychiatric traits with anxiety components in humans. As in human studies, it has been challenging to identify strongly associated loci, and further research is needed to validate the suggested associated region.

Materials and methods

Questionnaires

Data on behavior traits in Norwegian standard poodles were collected from web- and telephone-based questionnaires. The surveys were performed in collaboration with the Norwegian poodle club (NPC) and the Norwegian Kennel Club (NKC).

The first survey (2014) had a set of questions regarding behavior and fearfulness, including fear of fireworks and noise reactivity. The second and third surveys (2017) were performed as an initial telephone survey followed by an online survey repeating the questions about fear of firework and noise reactivity. Finally, a fourth and fifth online questionnaire (2020 and 2022) with the same questions about fear of firework and loud noises was sent to owners that had not participated in the earlier studies. In all five surveys, the dog owners were asked to indicate 1) their dogs' fear of fireworks and 2) their dogs' reactivity to loud noises, including thunder and gun shots, on a 1 to 5 Likert scale, where 1 was "shows no fear" and 5 was "very fearful".

Included dogs

Dogs with a score of 1 (no fear) were included as controls, and dogs with a score of 4 or 5 (fearful, very fearful) were included as cases. Some dogs were included in more than one study. If the dog had more than one observation, the score from the newest online questionnaire was selected. An overview of distribution of the selected cases and controls is given in figure 3.

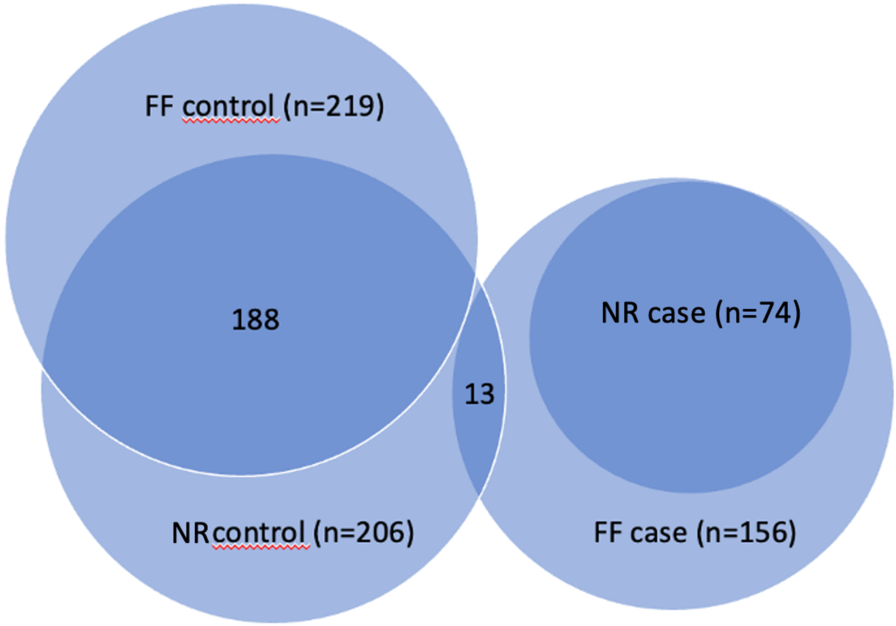

Figure 3: Distribution of cases (score 4+5) and controls (score 1) for noise reactivity (NR) and firework fear (FF) in the 400 standard poodles included in the GWAS.

DNA samples were collected using DNA Genotec™ Performagene PG-100 buccal swabs or EDTA blood collected by a veterinarian. All materials were gathered in accordance with the Norwegian National Committee for Research Ethics in Science and Technology's (NENT) guidelines for research ethics in science and technology (2007). Extraction of DNA was done in accordance with Performagene 0.5mL purification protocol using the PG-100 kit (buccal swabs) or Omega Bio-tek - E.Z.N.A® Blood DNA Mini Kit (blood). A total of 378 dogs were genotyped with the 230K Illumina HD Canine SNP-Array.

Quality control

A genotyping quality control (QC) was performed. Markers with a minor allele frequency threshold (MAF) less than 0.05 and a call rate <95% were excluded from the analyses, as well as markers failing the Hardy- Weinberg equilibrium exact test with a level of $< 10^{-6}$ in controls and $< 10^{-10}$ in the cases. Samples with a genotyping rate below 95% and a heterozygosity rate above three standard deviations from the mean were removed. In addition, a control for duplicates and a gender check to identify potential sample mix-ups were performed. After quality control, 145725 SNPs remained for the noise reactivity dataset and 145723 SNPs in the firework datasets. Three hundred eighty-five dogs remained in both datasets, including 150 cases and 212 controls for the firework phenotype and 72 cases and 200 controls for the noise reactivity phenotype (categorical trait).

Heritability estimates

The SNP based heritability was calculated in GCTA. A genetic relationship matrix (GRM) was calculated based on all the autosomal SNPs in the dataset. The GRM was included in a bivariate genomic restricted maximum likelihood (GREML) analysis to estimate the variance explained by the autosomal markers in the dataset using the model,

$y = \mu + g + e$, where y is a vector of one of the two phenotypes (fear of fireworks and noise reactivity), μ is the mean term (fixed effect), g is the random genetic effect, and e is the residual error (77)

Genome wide association analyses

The association analyses were performed using a mixed linear model in GCTA (77), including 145,097 autosomal markers where noise reactivity and fear of fireworks were used as dependent categorical variables, and a relationship matrix was included as a random effect to correct for relationship and population structure. Initial model-testing showed limited effects of potential covariates like sex, coat color and questionnaire. Manhattan plots and QQ-plots were created using R with the QQ-man package (78)

Declarations

Ethics approval and consent to participate

All samples were collected as cheek swabs taken by the owner and with the owners' consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

The project was funded by the Norwegian University of Life Sciences (Veterinary faculty)

Authors' contributions

K.W., L.M.S. and F.L. designed the study and the questionnaires. K.W. collected data through telephone surveys. K.W. and F.L. collected data from online surveys. K.W. performed the DNA extraction. K.W. and D.J. performed the GWAS analysis. D.J. estimated SNP heritability. K.W. wrote the manuscript with support from L.M.S, D.J and F.L. All authors read and approved the final manuscript

Acknowledgements

Thank you to the Norwegian Kennel Club and the Norwegian Poodle Club for their support in data collection.

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ISBN: 978-82-575-2020-5
ISSN: 1894-6402

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