Linköping Studies in Science and Technology
Dissertation No. 1989

Dog behaviour

Intricate picture of genetics, epigenetics,
and human-dog relations

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and human-dog relations

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Haru hunni bunni hunn?

Till mormor,
vad tokigt det kan bli

Abstract

Dogs, *Canis familiaris*, share the lives of humans all over the world. That dogs, and the behaviour of dogs, are of interest to many is therefore no surprise. In this thesis, the main aim has been to identify factors that affect dogs' behaviours.

The dog, *Canis familiaris*, is our first domesticated animal. Since domestication, various types of dogs have developed through adaptation to an environment shared with humans and through our selective breeding, resulting in a unique variation in morphology and behaviour. Although there is an individual variation in the behaviour of dogs, there is also a difference between breeds. Moreover, selection during the last decades has split some breeds into divergent types. Labrador and golden retrievers are divided into a common type, for show and companionship, and a field type, for hunting. By comparing the breed types, we can study the effects of recent selection. In **Paper I**, we investigate differences in general behavioural traits between Labrador and golden retriever and between common and field type within the two breeds by using results from the standardized behaviour test Dog Mentality Assessment. There were differences between breeds and types for all behavioural traits. However, there was also an interaction between breed and type. Thus, a common/field-type Labrador does not behave like a common/field-type golden retriever. Even though they have been selected for similar traits, the selection has affected the general behavioural traits differently in the two breeds.

In **paper II**, we were interested in dogs' human-directed social skills. Dogs have a high social competence when it comes to humans. Two experiments commonly used to study these skills are the problem-solving test, where dogs' human-directed behaviours when faced with a problem are measured, and the pointing test, where dogs are tested on how well they understand human gestures. We compared the social skills of German shepherds and Labrador retrievers, and of common- and field-type Labradors. Labradors were more successful in the pointing test and German shepherds stayed closer to their owners during the problem solving. Among Labrador types, the field type had more human eye contact than the common type. Importantly, when comparing the two experiments, we found no positive correlations between the problem-solving test and the pointing test, suggesting that the two tests measure different aspects of human-directed social behaviour in dogs.

A previous study has identified two suggestive genetic regions for human-directed social behaviours during the problem-solving test in beagles. In **paper III**, we show that these SNPs are also associated to social behaviours in Labrador and golden retrievers. Moreover, the Labrador breed types differed significantly in allele frequencies. This indicates that the two SNPs have been affected by recent selection and may have a part in the differences in sociability between common and field type.

The behaviour of dogs cannot simply be explained by genetics, there is also an environmental component. In **paper IV**, we study which factors that affect long-term stress in dogs. Long-term cortisol can be measured by hair samples. We found a clear synchronization in hair cortisol concentrations between dogs and their owners. Neither dogs' activity levels nor their behavioural traits affected the cortisol, however, the personality of the owners did. Therefore, we suggest that dogs mirror the stress level of their owners.

The mediator between genes and the environment is epigenetics, and one epigenetic factor is DNA methylation. In **paper V**, we compared methylation patterns of wolves and dogs as well as dog breeds. Between both wolves and dogs and among dogs there were substantial differences in methylated DNA regions, suggesting that DNA methylation is likely to contribute to the vast variation among canines. We hypothesize that epigenetic factors have been important during domestication and in breed formation.

In this thesis, I cover several aspects on how dogs' behaviours can be affected, and paint an intricate picture on how genetics, epigenetics, and human-dog relations forms dog behaviour.

Populärvetenskaplig sammanfattning

Varför gör hunden som den gör? Det är en fråga som kan få ett mycket långt svar. I den här avhandlingen har jag undersökt vad som påverkar beteendet hos våra hundar. Jag har undersökt skillnader i beteende mellan raser och mellan rastyper, tittat närmare på den genetiska grunden till beteenden och vad för miljöaspekter som påverkar våra hundar. Jag har också studerat skillnader i epigenetiska faktorer mellan varg och hund och mellan hundraser.

Hunden är den första arten som domesticerades och detta skedde för mer än 15 000 år sedan. Under domesticeringsprocessen och in i nutid har vi människor valt ut de individer som har de önskvärda egenskaper som vi uppskattar och avlat vidare på dessa (selektion). Det har gett upphov till ett antal olika hundtyper och under de tvåhundra senaste åren har många hundraser med stängda stamböcker uppkommit. Inom vissa raser har aveln under de senaste årtiondena dessutom gett upphov till typer inom raserna. Det kan vi till exempel se hos de två retrieverraserna labrador och golden. Det finns en typ som har avlats för utställning och sällskap, den vanliga typen, och en som avlats för sina jaktegenskaper, jakttypen.

I **artikel I** visar vi att det finns skillnader i de beteendeegenskaper som testas i ett standardiserat beteendetest mellan labrador och golden och mellan den vanliga typen och jakttypen. Skillnaderna är dock inte desamma inom de två raserna mellan typerna. Jaktlabradoren beter sig alltså inte som en jaktgolden och en labrador av vanlig typ beter sig inte som en golden av vanlig typ. Detta till trots att de selekterats för motsvarande egenskaper under de senaste årtiondena. I **artikel II** jämförs hundraserna schäfer och labrador (och labradortyperna) i hur väl de kommunicerar med människan. Hundar har en exceptionell förmåga att kommunicera med oss. De tar till exempel hjälp av oss när de stöter på problem och kan läsa av vårt kroppsspråk för att hitta godis. Men det finns också en variation mellan hundar. Vi fann att labradorer söker mer ögonkontakt under problemlösningen medan schäfrar är mer passiva och spenderar mer tid nära sin ägare. Under pektestet presterade båda raserna bättre än slumpen men labradorerna var snäppet bättre på att följa pekgesterna. Vi undersökte också om det finns något samband mellan att söka människans kontakt under problemlösningen och förmågan att läsa av människans gester vid pektestet. Det finns det inte utan hundarnas sociala förmåga är mångfacetterad.

Både gener och miljö spelar in när hundars beteende formas. De beteenden som hundar visar upp under problemlösningen har en hög arvbarhet och det har hittats

två områden i DNA:t hos beaglar som verkar påverka dessa beteenden. I **artikel 3** visar vi att dessa områden även påverkar beteendet hos labrador och golden. Vi visar också en skillnad mellan labradorertyperna i vilka genvarianter de har och vi vet därför att aveln har påverkat dessa varianter.

I **artikel 4** intresserade vi oss istället för vilka miljöaspekter som påverkar hundarnas beteende. Fokus var hundarnas stressnivåer över tid, vilka kan mätas via kortisol som lagrats in i håret. Vi visar att hundar speglar sin ägares stressnivå och en ägare med höga nivåer av stresshormon har hundar med höga nivåer av stresshormon. Det visade sig också att ägarens personlighetsdrag har en inverkan på hundarnas stressnivåer.

I **artikel 5** har vi studerat epigenetiska skillnader mellan olika hundgrupper. Epigenetik handlar om hur genernas aktivitet regleras, till exempel utifrån miljö, utan att DNA-sekvensen förändras. En epigenetisk faktor är DNA-metylering. Vi har jämfört de generella metyleringsprofilerna hos varg och hund samt olika hundraser och vi kan konstatera att det finns stora skillnader. Epigenetiska faktorer kan därmed ha en bidragande orsak till den stora variation som finns inom arten hund.

Dessa fem artiklar visar alltså att våra hundars beteende påverkas av selektion, gener och den miljö de lever i (dvs. ägaren de delar livet med), samt att epigenetiska faktorer nog har en inverkan på den stora variationen.

Papers included in this thesis

Papers will be referred to by their Roman numerals.

Paper I

Ann-Sofie Sundman, Martin Johnsson, Dominic Wright, and Per Jensen, 2016. Similar recent selection criteria associated with different behavioural effects in two dog breeds. *Genes, Brain and Behavior*, 15(8), pp.750-756.
<http://doi.org/10.1111/gbb.12317>

Paper II

Ann-Sofie Sundman, Mia E. Persson, Anna Grozelier, Lise-Lotte Halldén, Per Jensen, and Lina S.V. Roth, 2018. Understanding of human referential gestures is not correlated to human-directed social behaviour in Labrador retrievers and German shepherd dogs. *Applied Animal Behaviour Science*, 201, pp.46-53.
<http://doi.org/10.1016/j.applanim.2017.12.017>

Paper III

Mia E. Persson*, Ann-Sofie Sundman*, Lise-Lotte Halldén, Agaia J. Trottier, and Per Jensen, 2018. Sociality genes are associated with human-directed social behaviour in golden and Labrador retriever dogs. *PeerJ*, 6, p.e5889.
<http://doi.org/10.7717/peerj.5889>
*Equal contribution

Paper IV

Ann-Sofie Sundman, Enya Van Poucke, Ann-Charlotte Svensson-Holm, Åshild Faresjö, Elvar Theodorsson, Per Jensen, and Lina S.V. Roth, 2019. Long-term stress levels are synchronized in dogs and their owners. *Submitted manuscript*.

Paper V

Ann-Sofie Sundman, Fábio Pértile, Carlos Guerrero-Bosagna, Luiz Lehmann Coutinho, Elena Jazin, and Per Jensen, 2019. DNA methylation in canine brains is related to domestication and dog-breed formation. *Manuscript*.

Contribution

Paper I: Conceived and designed the experiment, gathered data, analysed the data, authored the paper; in collaboration with co-authors

Papers II-V: Conceived and designed the experiments, performed the experiments, analysed the data, authored the paper; in collaboration with co-authors

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1 Why dogs?

The dog, *Canis familiaris*, was the first species to be domesticated. Today, dogs are a natural part of human society and can be found wherever in the world there are humans. Both behaviourally and morphologically, dogs show an impressive variation and is one of the most variable species in the world. There are many breeds of dogs, with forms and behaviours to suit people of different lifestyles and for a variety of tasks. They are highly appreciated as a companion pet and for their hunting, herding and guarding abilities, just to mention but a few of their abilities. They also have an important role within police, military, and search and rescue, and are invaluable to many as service dogs.

Being the popular and commonly found animal that they are, together with their importance as a model animal, dogs have a high research value. There is an interest to learn more about the behaviour of dogs, and this is of importance to both pet owners, dog trainers and researchers, and, of course, to the dogs themselves from a welfare perspective.

The dog did not start to interest ethologists until rather recently. Focus in the beginning was instead on the behavioural ecology of wild animals. During the first decades of the 20th century, those that used dogs in research were psychologists who were interested in the process of learning. In the middle of the 20th century, one of the first, and to date one of the most extensive, studies on dog behaviour started, namely Scott and Fuller's 20-year-long project. They studied behavioural patterns, breed differences, developmental stages, and genetics of behaviour, an important ground work for researchers (1). From this point on, studies on dog behaviour became increasingly popular. Especially during the last 20 years, studies on, for example, dogs' personality, social cognition, and behavioural genetics have increased greatly (e.g. 2, 3, 4).

1.1 General aim of this thesis

The behaviour of dogs varies both on a group level and on an individual level. In this thesis, my aim has been to study which factors affect and contribute to behavioural variation in dogs. I have investigated the behavioural differences between breeds to understand the selection of behaviour, the genetic effects on dogs' behaviour, and the owners' effects on dog behaviour. I have also studied epigenetic differences between wolf and dogs, as well as differences across dog breeds.

2 History of the dog

The dog has been formed through domestication, morphologically as well as behaviourally. To understand dogs' behaviour, the variation within the dog, and dogs' importance to humans, we need to start from the beginning.

2.1 Domestication

Several species have been domesticated, but the dog was the first by several thousand years. Domestication is the process where a population of animals, or plants, through genetic changes is adapted to life with humans. Studying domestication processes will also increase our knowledge about evolutionary processes.

While Darwin (1868) expressed about the dogs' ancestor(s) that "[w]e shall probably never be able to ascertain their origin with certainty" (5), today we do know that the grey wolf, and only the wolf, is the ancestor of the dog (6-8). The questions of when, where, why, and how the dog was domesticated, however, remain subjects of scientific debate.

2.1.1 When?

Due to several bottlenecks and admixtures between dogs and wolves and between dog lineages, it is not an easy task to find neither time point nor geographical location for domestication. According to archaeological findings, the domesticated dog existed 15,000 years ago. Skeletal remains from this time are clearly distinguishable from those of wolves (9). Early molecular work suggests a divergence between wolves and dogs long before that, over 100,000 years ago (8). However, more recent studies are in congruence with archaeological data and suggest domestication to have taken place closer to 15,000 years ago (10-13), although between 20,000-40,000 years has also been indicated (14, 15).

2.1.2 Where?

Several geographical locations for the origin of dogs have been pointed out. Basically, these locations are based on where the highest genetic diversity can be found, which is an indication of closeness to the origin. However, the results vary according to method, which wolves and dogs that are included, and whether DNA

from ancient samples are taken into account. Places of origin that have been suggested are Southeast Asia (10, 11, 16), the Middle East (17), and Europe (14, 18). Most studies suggest a single domestication event, whereas Frantz, *et al.* (19) hypothesized that dogs were domesticated twice: once in Southeast Asia and once in Europe.

As the techniques for using ancient DNA are increasingly more robust, and dense genotyping increasingly faster and less expensive, new discoveries to the when and where of the dog's origin are likely to occur.

2.1.3 Why and how?

As to the why and how, it is generally accepted that the dog's ancestors entered domestication through a commensal route that started by them taking advantage of human wastes. Wolves are opportunistic scavengers and it is believed that less fearful wolves were drawn to the refuse of the human encampments, hereby finding a new niche where they habituated to humans (20, 21). Thus, at least initially, the domestication process of wolves into dogs was not deliberate or conceived by human hunters and gatherers, but based on natural selection, a process called the self-domestication hypothesis (22). It is hypothesized, however, that that humans in the beginning inadvertently selected the most adjustable wolves by dispelling or dispatching wolves that did not conform to human societal rules (21, 23). The Russian geneticist Dmitry Belyaev even wrote that "[i]t is obvious that selection for behavior has been unconsciously carried out by man since the earliest stages of animal domestication" based on the fact that contact with humans as well as obeying them and reproducing in their care are prerequisites for domestication (24).

At some point the commensal relationship between the two species developed into a mutual relationship where both parties benefited from the association and, eventually, intentional breeding for desired traits (20). Dogs, or rather proto-dogs, got food and protection from their human partners who in return probably used the dogs for, among other things, guarding, support during scavenging and hunting, and for food and pelts. What is believed to be one of the earliest documentation of dogs are cave paintings in Saudi Arabia, dated to 9,000-10,000 years ago, depicting humans and dogs hunting together (25). Interestingly, already at the beginning of our partnership, dogs seem to have been important to humans, shown for example by the findings of burial sites where dogs were buried and site in Israel dated to 11,000-12,000 years ago where an elderly person was buried together with a puppy (26).

2.1.4 The domestic phenotype

The early archaeological records find that dogs' skulls and teeth show a general reduction in size, and especially a shortening and widening of the muzzle and snout compared to wolves (27). These characteristics are in concurrence with what is called 'the domestic phenotype'. Indeed, compared to their wild ancestor, domesticated animals have several traits in common. Morphological differences include changes in body size, body proportions (e.g. brachycephalia and chondrodystrophia), brain size, and coat colour alteration, specifically an increase of white colouration. Domesticated animals also show altered physiological traits, they for example differ in endocrine responses and reproductive cycles. More specifically, domestic animals become sexually mature earlier, have a shorter generation time, and have larger litters. Of course, there is also a difference in behaviour between wild and domestic counterparts, where the latter show a decrease in wariness of humans but also a lower general fear, lower reactivity, and an increased sociability (20, 27-29).

These effects have also been found in experimental settings. In a famous experiment by geneticist Dmitry Belyaev, farmed silver foxes were bred for tameness (24). Even though tameness was the only trait that was selected for, in few generations the selected foxes showed other characteristics not found in the control population. These changes included coat depigmentation (white marks), floppy ears, curled tails, and alterations in reproductive pattern. Further into the selection the foxes showed even more changes, for example reduction in both body size and relative brain size, and altered body proportions (24, 30). Similar selection experiments in other species have also shown changes in concurrence with the domestic phenotype, for example in chickens (29) and rats (31).

Hence, even though the initial part of the domestication process was not directed by focused artificial selection, solely a reduced fear of humans would predict phenotypic changes. This happens through a correlated selection response. Even if selection only targets few genes, these may lead to a cascade of effects through genetic mechanisms such as pleiotropy, epistasis, and linkage (29). Belyaev (24) suggested that the domestication effects may be due to changes in gene regulation rather than genetic structure, and a recent hypothesis suggests that the domestic phenotype is caused by deficiencies of neural crest cells during embryogenesis (32). It is also reasonable that epigenetic mechanisms have had an important part during animal domestication. These can occur in direct response to environmental changes by modifying gene expression without changing the genome (29).

2.2 Breed formation

Since domestication, various types of dogs have developed through adaptation to an environment shared with humans and through selective breeding. This has resulted in a unique within-species variation in morphology and behaviour between breeds. Today, the international kennel organization Fédération Cynologique Internationale (FCI) recognizes 346 breeds (www.fci.be), but there are likely more than 400 breeds around the world.

Early depictions and archaeological evidence suggest that morphologically different dogs have existed for a long time. An excavation in Sværdborg, Denmark, of an 8000-year-old site identified three differently sized dog types, and remains from ancient Egypt, 4000 years ago, show that dogs similar to mastiffs and greyhounds existed at that time (9, 33). Interestingly though, the breeding practise of today, with stringent selective breeding, closed stud books, and breed standards with focus on form rather than function, is a quite recent invention that has its origin in the 19th century Victorian era (years 1837-1901) (34). Genetic comparisons of breeds have failed to find unique mitochondrial DNA (maternal inheritance) and Y chromosome haplotypes (paternal inheritance) within breeds, indicating that breeds have not been isolated for a long period of time (33). In a study on the origin of breeds, Parker, *et al.* (35) estimated, based on haplotype sharing, when breeds were created and the results indicate that most breeds were, in fact, created less than 200 years ago.

The to date largest phylogenetic analysis of the relationship between breeds identified 23 clades for 150 different breeds (35). It was found that while haplotype sharing is common among breeds within clades, sharing across clades is less frequent. Thus, it seems that clades represent breed prototypes that existed before modern breeding practises began and that modern breeds were created via selection within the prototypes (35).

2.2.1 Subpopulations

Although there is a great homogeneity within dog breeds, substructures exist due to geography and different selection aims among breeders. There is, for example, a high genetic diversity between golden retriever populations in Europe and North America due to a low degree of genetic flow between the two (36). There are also breeds where a divergence has occurred because breeders have fundamentally different selection criteria, creating different selection lines (breed types) within the breeds. Commonly, this split occurs when there are those that breed for form (dog shows),

modern function (dog sports and pet), or original function. To exemplify, in the herding breeds border collie and kelpie there is a clear diversification between those bred strictly for herding and those bred for show and sports (36, 37). In the kelpie, this has resulted in a separation of the types into two different breeds, Australian kelpie and Australian stock dog/working kelpie. A similar split has taken place in some of the spaniels and retrievers. In both golden and Labrador retriever, for instance, there are two types: one where selection focuses on traits coupled to show and companionability (common type), and one where selection focuses on functionality as gundogs, their original function (field type) (**Paper I, II, III**). See Fig. 1 for pictures of the different types of the two breeds.

Figure 1. Pictures of golden (a and b) and Labrador retrievers (c and d) of the two subpopulations common (a and d) and field type (b and c).

Photo credits:

a) By Golden Retriever Rasead, CC BY-SA 4.0,
commons.wikimedia.org/w/index.php?curid=67193872

b) Amanda Dahlin, published with permission c) CC0

d) By Giuseppe Pitzus, CC BY-SA 4.0, commons.wikimedia.org/w/index.php?curid=58821150

2.3 The dog genome

Domestication and breed formation have formed not only morphology and behaviour, but of course also dog's genome.

During the last 200 years, an intense selection process has taken place, creating the different breeds. Many breeds were created through a small number of founders, a small population size, and high levels of inbreeding. Additionally, as a breed was established, admixture with other breeds has been strictly limited. This breeding practise has caused genetic variation between breeds whereas variation within breeds is very low. Purebred dogs can, for example, be assigned to the correct breed based solely on genotype (17, 34). The breeding has also caused a high linkage disequilibrium in the dog genome where long-range haplotype blocks are inherited together. This makes it possible for genome-wide analyses using relatively few SNPs (single nucleotide polymorphisms). Compared to the human genome, haplotype blocks in the dog genome are about 50 times longer and, therefore, a genome-wide analysis in dogs requires only a fraction of the SNPs needed in humans (4, 38).

Because of their genetic makeup, dogs are a suitable model for genetic studies. Accordingly, the dog genome was one of the first to be sequenced (38). Taking advantage of breed differences in both phenotypic traits and genetics, genes underlying both Mendelian and complex diseases have been identified in dogs, many with human analogies (39). Also, genes have been found for morphological traits such as size (40), colour (41), and coat type (42). Actually, only three genes are responsible for the majority of coat-type variation found in breeds: *FGF5* for long or short, *KRT71* for straight or curly, and *RSPO2* for smooth or wired (42). With the strong selective breeding that we see in dog breeds, this genetic architecture is expected, where few genes have large effects.

3 Dog behaviour

The domestication process and adaptations to a human-dominated niche has formed the behaviour of the modern dog. Low fear of and low aggression towards humans was necessary for domestication, but dogs have traits beyond this that make them successful in the human world, especially their extreme sociability. Furthermore, selection for various functions for different groups of dogs has further formed and differentiated dogs' behaviour.

3.1 Human-directed social behaviours

It can be argued that dogs' natural ecological niche is the anthropogenic environment, and that they are well adapted to it. Human-directed sociability is an important trait in our dogs, and during evolution alongside humans, dogs seem to have acquired an interspecific social competence. Their abilities to communicate, to cooperate, and to form strong social attachments to humans are examples of this (43).

3.1.1 Eye contact, oxytocin, and the dog-human bond

In communication between dog and owner, eye contact is a central part. Both humans and dogs use eye contact in their communication with each other (e.g. 44, 45). In studies of spontaneous eye contact with humans, dogs look more often and for a longer duration than both the wolf (46) and the feral domestic dog, the dingo (47). Actually, already from an age of five weeks, dog puppies seek more human eye contact than wolf puppies (48).

Eye contact is not only important for communication but also for social bonding. Oxytocin is a hormone that facilitates bonding between social partners among both dogs, humans, and other mammals (49). Interestingly, an increase in both the dog and its owner has been observed during interspecific social interactions (50). More specifically, Nagasawa, *et al.* (46) studied eye contact between dogs and their caregivers and concluded that oxytocin in both dog and human increase during eye contact. By administering oxytocin, it was also shown that an increase in oxytocin elicited more eye contact. Thus, eye contact creates a positive oxytocin loop in both dogs and humans. In comparison, the same phenomenon was not found between wolves and their caregivers (46).

Indeed, dogs and their owners form a strong social bond. By using the Ainsworth Strange Situation Procedure, developed to study the attachment between a human child and its parents, Topál, *et al.* (51) concluded that a similar attachment bond exists between dog and owner. Dogs use their owner as a secure base and increase both exploration and playing in their presence. In the absence of their owner they are passive, even with a stranger present. When hand-reared wolf puppies were compared to similarly reared dog puppies, wolves did not show patterns of attachment to their owners whereas dogs did (52).

3.1.2 Understanding of human referential gestures

Another focus of study has been dogs' understanding of referential gestures, for example pointing. Pointing is a commonly used referential gesture in humans. It is used across cultures and both practised and understood by infants (53). Studies have shown that dogs readily and reliably use several human referential cues to find hidden food (see 3, for review, eg. 54, 55).

Commonly, animals are tested on their understanding of referential gestures in an object-choice test where a piece of food is hidden in one of two containers. A referential cue is given as to where the treat is located, for example by pointing at it, before the subject is asked to make its choice. The pointing gestures can vary in difficulty from simple cues like tapping the correct container or holding the gesture while the subject is making its choice, to more subtle signals like briefly pointing towards the container from a distance (momentary distal pointing). The cues can also vary such as gazing or head turning, etc. Dogs do not only understand simple signals but also various other ones (3). They primarily use protruding body parts as cues but can easily generalize between different gestures. For instance pointing with a finger, elbow, or foot, and cross-pointing (56, 57).

Dogs' understanding of referential gestures cannot be explained by simple local enhancement. This is shown by the facts that dogs choose correctly even when the signaller is standing next to the wrong choice (54), or moves away from the correct container (58), and they fail if a stick is used for pointing (57). Neither do dogs learn during the experiment (eg. Paper II; 59). Even young puppies have been shown to understand momentary distal pointing, and environmental factors seems to have little effect (59, 60). Thus, it seems that, to some degree, dogs' skill for understanding human referential gestures is innate.

Interestingly, although not conclusive, there are results that support the hypothesis that dogs understand the sender's intention to communicate. If the pointing is not preceded by an ostensive cue, i.e. a signal that expresses communicative intent, dogs choose at random (45, 61).

Surprisingly, non-human primates, despite being phylogenetically close to humans, have a low comprehension of these gestures and do generally not perform above chance level (62, 63). In fact, in a comparative experiment, Kirchhofer, *et al.* (64) found that dogs outperform chimpanzees. When compared to wolves, dogs' closest relative, they, again, show a better understanding of human communicative gestures (62). With training and intense socialization, wolves can, however, successfully follow human referential cues (65-67). Other species, both domesticated and captive wild species, have been tested in the object-choice paradigm. Dolphins, goats, horses, and cats can use human pointing gestures to find food, but, it does seem that the dog is the most flexible non-human species in this task (3, 68).

3.1.3 Dog's human-directed communication

Not only do dogs understand human communication, they also communicate with us through, for example, attention-seeking and attention-directing signals. Worsley and O'Hara (69) identified 19 intentional signals used by dogs in their human communication that can be classified as referential gestures (e.g. directed towards an object, aimed at a recipient, and that was understood by a recipient). The situations in which dogs use these signals are primarily contexts when they want something, e.g. petting or food. The most common gesture is gaze alternation between a desired object and a recipient (69).

Gaze alternations and eye contact, specifically, have previously been identified in dogs as referential gestures and as important behaviours for social communication. Dogs readily use this to indicate the location of hidden food (44). Interestingly, dogs indicate the location even more frequently when the owner is not present when the desirable object is hidden, suggesting that dogs intend to inform their owner of the location (70).

Dogs are especially prone to show gaze alternations when faced with a problem-solving situation, for example a piece of food in a closed container. In Marshall-Pescini, *et al.* (71) it is shown that dogs perform more gaze alternations when the problem becomes unsolvable compared to solvable, and more gaze alternations in the presence of an attentive person than an inattentive one. These results suggest that

the gesture is both referential and intentional. Moreover, by using a problem-solving paradigm, dog-human communication and attention seeking is stimulated and can be systematically studied (**Paper II, III**).

As with the understanding of human referential gestures, dogs and wolves differ in their behaviour during a problem-solving paradigm. Several studies show that wolves do not seek human contact to the same extent as dogs do and that they rarely seek eye contact (e.g. 72). The difference is clear even when comparing wolves to shelter dogs and free-ranging dogs (73-75). Already at nine weeks of age and with a similar upbringing as the dogs they are compared to, this difference can be observed between the species (48). In turn, wolves show a higher persistency in trying to solve the problem at hand (74, 75), and this they also do in the absence of humans (76).

It has been argued that social behaviours during the problem-solving test may measure persistency rather than sociability. When including the duration spent interacting with the problem in the statistical model, Marshall-Pescini, *et al.* (75) found no difference in gazing behaviour between wolves and dogs. Studies also show negative correlations between test interactions and eye contact (74, 75). At the same time, in **Paper II**, it is the group with highest passivity that have the shortest duration of eye contact (i.e. German shepherd dogs), and principal component analyses consistently form a component for test interactions separate from social behaviours (**Paper II**, 77). It is difficult to disentangle persistent problem solving from sociability in the problem-solving test. Different measurements of duration during the same test may correlate simply because a subject cannot engage in two activities at the same time.

3.1.4 Comparison of human-dog and dog-human communication

As we have seen, the pointing test and the problem-solving test are commonly used to study dogs' understanding of human referential gestures and their human-directed social behaviour, respectively. Because of this, in **Paper II** we wanted to compare dogs' behaviours in the two tests.

Even though the dog as a species systematically differs from other species, there are still differences among dogs. Indeed, in **Paper II**, only 64 out of the 153 dogs reached individual significance in the pointing test (momentary distal cue), but as a group they performed better than chance (Fig. 2). In this study, the same dogs were tested in both a pointing test and a problem-solving test to be able to compare the two. According to our results, none of the behaviours in the problem-solving test

positively correlate with performance in the pointing test. Hence, dogs that understand human referential gestures do not themselves engage more in human-directed behaviours than other dogs. A similar result was found by MacLean, *et al.* (78), where they compared performance in several tests for social cognition. Simply, dogs' social competence is complex and multifaceted.

Figure 2. Histogram of performance in the pointing test for all participants (German shepherd and Labrador retriever dogs) in Paper II. Pink bars represent dogs that reached individual significance (binomial $p < 0.05$). Although not the performance of all individuals can be separated from chance, as a group, dogs understand human pointing.

3.1.5 Human-directed social competence, a domestication effect?

As we have seen, comparative studies in human-directed social competence point out differences between both dog and wolf (62, 72), and dog and non-human primates (64, 78). These differences suggest that dogs have a greater capacity for social competence than the other species. Actually, the social competence of dogs has been argued in some cases to be closer that of human infants than wolves or apes, and developed during a convergent evolution with humans (78, 79).

It is compelling that the social competence of dogs has evolved during domestication (72, 80). It has been pointed out, though, that there are confounding differences between the environment of dogs and the species it is compared to and that these may be more important than evolutionary history (e.g. 81). Intensely socialised and trained wolves do, for example, show understanding for human referential gestures

(66), and dog puppies not socialised before the age of about 14 weeks (i.e. during critical period) cannot form normal relationships with people (82).

Nevertheless, the facts, among others, that 1) dogs show human-directed social skills already from a young age (48, 60), 2) that free-roaming dogs and shelter dogs, presumably with less human interactions during their upbringing, behave as pet dogs in gazing behaviour and differ from wolves (73-75), and 3) the feral dingo behaves intermediately compared to domestic dogs and wolves both in spontaneous eye contact and referential-gesture understanding (47, 83), suggest that the social competence of dogs have been affected by domestication.

When discussing the cause of dogs' social skills, we cannot forget the dog's ancestor. Even though dogs may show a larger interspecific social competence, wolves are highly social animals with a rich intraspecific communication and cooperation. It is likely that the social skills of dogs have evolved on this basis (84) and have been further formed during the domestication process. This has given the individual dog a capacity for human social skills that is developed and shaped during ontogeny in the human world (85).

3.2 Breed differences in behaviour

A dog is a dog and it behaves as a dog. But, among dogs, we also find a large phenotypic variation in behaviour. Not only is there an individual variation, but there is also variation between breeds and breed clusters both in breed-specific behaviours, more general behavioural traits, and human-directed social behaviours (for review: 86).

3.2.1 Breed-specific behaviours

Different groups of dogs have evolved since the beginning of the dog through selection pressures which focused on required function, such as hunting, herding, and guarding, as well as adaptations to different environments (35). These differences clearly stem from selection for the historical function of the breed groups. Although modern breeds are a new concept, breeds still have their roots in ancient types.

One thing that has been modified in dogs bred for different functions is the predatory motor pattern consisting of the behaviours orient, eye stalk, chase, grab-

bite, kill-bite, dissect, and consume. For example, sheepdogs possess a hypertrophied orient-eye stalk-chase sequence, retrievers a hypertrophied orient and grab-bite, and livestock guardians a suppression of the whole sequence (Coppinger and Coppinger, 1996; Mehrkam and Wynne, 2014). It would be difficult to train a sighthound to herd, a terrier to retrieve without crushing the game, and a livestock guardian to hunt, in comparison to how it would be to train a sheepdog, a retriever, and a hound for these respective tasks.

However, there is also an individual variation for breed-specific behaviours, for example in herding behaviours for border collies (87). In some breeds this is due to a relaxed selection for breed-specific behaviours when the breed is no longer bred for function. In the common- and field-type Labrador and golden retrievers, this is apparent when studying data from a Swedish assessment of functionality for retrievers developed by the Swedish kennel club. Field-type dogs clearly show a greater interest for retrieving, both dummy and, especially, game (Fig. 3; (88)). Indeed, today there are many dogs that are mainly companion dogs and for these individuals, there is no longer a reason to breed for historical function.

Figure 3. Comparison of the performance of common type (dark) and field type (pink) in an assessment of retrieving abilities in a test developed by the Swedish kennel club (funktionsbeskrivning retriever, FB-R). Higher scores indicate a hunting dog with greater functionality. During a series of subtests, retrievers are tested on their functionality. A principal component on the behavioural reactions found, among others, components for gripping and retrieving a dummy and gripping and retrieving real game (Louise Brodd, Master thesis, 2016 (88)).

3.2.2 Behavioural traits

We can also study more general behavioural traits. While these, too, differ individually (yes, dogs have personality: 89, 90), behaviours also differ on a group level. Breed differences have been reported for several behavioural traits and in several studies (e.g. **Paper I**, 1, 91, 92).

Differences in behavioural traits between individuals or groups of dogs can be studied through standardised behaviour tests. In Sweden, we have the Dog Mentality Assessment which is a test battery consisting of ten standardised subtests that assess the dog's reaction to, among other things, strangers, play, suddenly appearing dummy, and a sudden loud sound. When correlated, the behaviour reactions represent five or six behavioural traits: curiosity, playfulness, chase interest, sociability (or social curiosity and social greeting), and aggression (**Paper I**; 93).

The Dog Mentality Assessment was developed and is arranged by the Swedish Working Dog Club, and from its start in 1997, more than 100,000 dogs of various breeds have been tested, and all results are registered by the Swedish Kennel Club. An impressive database that can be used in dog behaviour research. In a series of studies, Svartberg found the Dog Mentality Assessment to be both reliable and valid (90, 93-95).

Svartberg (96) found breed differences in the behavioural traits among 31 different breeds. For example, the extremes for the different traits were: playfulness, Malinois (highest) and Swiss mountain dog (lowest); curiosity, Labrador and collie; sociability, flat-coated retriever and Groenendael; aggressiveness, Malinois and Leonberger. In **Paper I**, we used data from the Dog Mentality Assessment to study how selection affects behaviour. We found that there are differences between the breeds Labrador retriever and golden retriever. Labradors are more curious, playful and aggressive in comparison to goldens that, instead, are more sociable and show higher chase interest.

Behavioural traits of dogs can also be gathered from questionnaires, where the owner, or someone else that knows the dog well, can assess how the dog would react in several described scenarios. Two such questionnaires that have been validated and that are commonly used in research, are the Dog Personality Questionnaire (97) and C-BARQ (98). These measure, for example, excitability and trainability, as well as aggression and fear towards both humans and other dogs. Serpell and Duffy (92) used the C-BARQ to compare the 30 most common breeds in USA and, again, a large

variation among breeds was found for each behavioural trait. To mention a few, Siberian husky has the lowest stranger-directed aggression and lowest non-social fear, the toy poodle seeks the most attention from its owner, and the Australian shepherd is the breed that is the most trainable.

In **Paper I**, we were not only interested in comparing the breeds Labrador and golden retriever, we also studied the within-breed differences between the selection lines, common and field type. Our results show that the types differ in all six behavioural traits. To mention but a few, field-type retrievers are less sociable and show less aggression than common-type retrievers. Importantly, for some traits the within-breed differences are even greater than the between-breed difference. Field-type Labradors are, for example, as playful as field-type golden retrievers, whereas common-type Labradors are least playful (**Paper I**). Also previous studies have pointed out behavioural differences between selection lines of breeds, e.g. Labrador retriever and English springer spaniel (99-101).

The consequences of these results are that even though differences in behaviour are found between breeds, there are substantial within-breed differences that should not be neglected. Additionally, Mehrkam and Wynne (86) caution from claims about systematic breed differences because many breeds, especially the less common ones, are underrepresented in research. A systematic review and meta-analysis would be able to teach us more about breed differences and their consistency. Apparently, there are breed-typical behavioural traits, but the behaviour of the individual is much more than its breed.

It has been discussed whether breed differences in general behaviour stems from historical function or if they are rather linked to recent selection. In Svartberg (2006), none of the behavioural traits studied could be related to breeds' historical function. Contrarily, Turcsán, *et al.* (102) could trace boldness and trainability to historical function. According to **Paper I**, the recent selection that has created the diversification within Labrador and golden retriever has not affected the behaviour of two breeds in a similar manner. Even though there has been a recent and similar selection pressure for the two breeds, a field-type Labrador behaves differently from a field-type Golden retriever. As all the four groups in **Paper I**, common and field of the two breeds, share historical function, neither does this explain the differences. Rather, general behaviours not directly targeted by selection may be more affected by genetic processes such as genetic drift, bottlenecks, founder effects, and popular-sire effects, or simply different breeders' preferences, than by either historical function or recent selection.

3.2.3 Human-directed social skills

Group differences can also be found among dogs in their human-directed social competence. It has been suggested that breeds historically bred for working may be more successful in the pointing test than non-working breeds (103). Within working breeds, those with the function of working cooperatively with humans seem more successful than those working independently. During a problem-solving test, the herding/hunting group gazed more at the human than the molosser and primitive group (104), again suggesting a link between human-directed social competence and historical function. In congruence, the performance in pointing understanding in the breeds border collie (herder; high cooperation), Airedale terrier (hunter; medium cooperation), and Anatolian shepherd (livestock guardian; low cooperation), decline in that order (105).

In **Paper II**, we investigate the differences between the breeds German shepherd dog and Labrador retriever, both of which, historically, are two cooperative working breeds. We found that they differ in their human-directed social behaviours. Labrador retrievers performed better in the pointing test and partook in more human eye contact during the problem solving. In the latter test, the German shepherd dogs were instead more passive. A similar difference in gazing behaviour between these two breeds has previously been shown (106). Even though both are comparatively cooperative working breeds, this might still reflect a difference in historical function. It could be argued that a gundog possesses a higher cooperativeness than a herding and guardian dog.

However, maybe it is more relevant to compare based on today's function instead of the historical one. In **Paper II**, we compare the two subpopulations within the Labrador retriever, the common type and the field type. The field type is still bred for their original function as gundogs, in comparison to the common type where selection has changed to include morphological characteristics and behaviours appreciated in a pet dog. Based on this, we would expect a higher cooperativeness in the field type. As expected, the field type gazed more at the humans than the common type during the problem-solving test (even though both types engaged equally in the test setup). On the other hand, both types were equally successful in following human referential signals.

Studies on breed comparisons in this area of research are surprisingly scarce and if we want to know the effects on human-social competence of selection for different traits, more research is needed. Because of the differences we found in **Paper II**, we

can at least suggest that human-directed social behaviours are affected by both ancestral roots and recent selection, and that selection for cooperative tasks may have an effect.

3.3 Behaviour and morphology

Some of the behaviour differences found between breeds may be due to differences in morphology. For example, Horschler, *et al.* (107) show some evidence that brain size accounts for some of the breed differences in cognition. Additionally, by studying behaviours from the Dog Mentality Assessment, Stone, *et al.* (108) found correlations between behaviour and height, weight, and skull size. Among other things, the results correlated shorter skulls (brachycephalic) to higher aggression and sociability, taller body size to higher activity, and heavier weight to higher playfulness.

Correlations between morphology and behaviour may be due to a genetic correlation between the traits, but some breeds are also likely to experience constraints due to morphological features. For example, behaviours of extreme brachycephalic and giant breeds may be affected by a lack of stamina, and those of extreme chondrodystrophic may be affected by restrictions in their movements (92, 109). Moreover, certain traits can affect the dog's perception of their surroundings. Brachycephalic breeds, with short skull and frontally placed eyes, have a region with a very high ganglion cell density, an area centralis, while dolichocephalic breeds with long skulls have a horizontal streak of ganglion cells (110). Thus, a pug might have a higher visual acuity than a whippet, which is expected to affect their way of viewing their world. Indeed, in a study on visual acuity in dogs, a pug outperformed the dolichocephalic individuals (111).

4 Genes and environment affect dog behaviour

Behaviour is always a product of both genetic and environmental factors. Genes control the formation of sensory organs that receive sensory input from the environment, the nervous system that interprets the information, and the muscular apparatus that performs the behaviours (112). Even behaviours that are strongly determined by genetic factors will need environmental input (a herding dog needs something that triggers its herding behaviour), and behaviours strongly determined by environmental factors will need genetic factors (learning how to sit on command).

4.1 Behavioural genetics

For behavioural traits to change through selection, genetic variation is required. Because breeds and selection lines differ in behaviours, and the dog differs from the wolf, it suggests a substantial genetic variation and a genetic basis for the traits. The characteristics of the dog genome together with different behaviour profiles of breeds, make the dog an important subject for studies of behaviour genetics.

There are behaviours that follow Mendelian inheritance. In a genetic cross experiment between basenji, with a high threshold for barking, and cocker spaniel, with a low threshold for that same behaviour, Scott (113) found that the cocker spaniel's low threshold is dominantly inherited and that the trait seems caused by only two genes. However, in general, finding the genetic foundation for behaviours has proven more difficult than for morphological traits and diseases. Behaviours are genetically complex with possible multiple genomic regions who each make a small contribution and are greatly influenced by the environment. Also, behaviours might be hard to phenotype with necessary specificity.

A whole-genome comparison of wolf and dog reveals that those genes and regions that show signs of strong selection are mainly involved in brain function and nervous system development pathways, and thus likely connected to behavioural differences (114). Interestingly, another set of genes that shows selection signals are responsible for starch digestion. This suggests an adaptation to the starch-rich diet of humans rather than the carnivorous diets of wolves (114). vonHoldt, *et al.* (17) found a SNP close to the gene *WBSCR17* that displays signs of strong selection. This gene is responsible for Williams-Beuren syndrome in humans, characterised by, among other things, extreme sociability.

4.1.1 Heritability of behaviour

One method of estimating the amount of genetic contribution to a trait is to calculate the narrow-sense heritability. Narrow-sense heritability (h^2) is an estimate of the proportion of the total phenotypic variation that is caused by additive genetic factors in a population, or, more simply, an estimate of how much of the phenotypic variation can be explained by variation in genes rather than environment. By knowing the relatedness of individuals in a population and having individual data on behavioural traits we can estimate the genetic contribution to behaviour variation. Heritability estimates can be used in selection to predict phenotypic change from one generation to the next:

$$\text{parental deviation from population} \times h^2 = \text{change in offspring}$$

Heritability estimates have been used to find a significant genetic contribution to several behavioural traits in dogs. For example, human-directed social interactions measured during problem solving in a population of beagles had a heritability of 0.22 (77). Thus, 22 % of the variation in the behaviour depended on genetic variation. Also, heritability estimates for behavioural traits from the Dog Mentality Assessment have been reported for the breeds rough collie, Rottweiler, German shepherd dog, Labrador retriever and golden retriever (**Paper I**; 115, 116, 117), and are overall moderate in size (0.1-0.4). For instance, in **Paper II**, the heritability for playfulness was 0.35 and 0.13, in golden and Labrador retrievers, respectively, and for curiosity it was 0.30 and 0.32.

In **Paper I** we identified differences in heritability between the selection lines of the Labrador retriever and golden retriever. This is an indication of differences in selection pressure for the types. Indeed, genetic variation is affected by selection (118). It is important to realise that the heritability estimates may vary between populations, and generations, and differences in heritability estimates between studies are rather expected (119).

4.1.2 Finding the genes

When a trait has a known genetic contribution, it is possible to identify involved genes by associating phenotype with genotype. One way of finding genetic regions of interest is to use genome-wide association studies (GWAS). The heritable and variable traits are tested if they are associated with any genetic markers in the genome, a method the dog genome is well suited for (see section 2.3).

This approach was used in Persson, *et al.* (120) where two genetic regions on chromosome 26 were associated with human-directed social behaviour in laboratory beagles. The SNPs are located within *SEZ6L* and *ARVCF*, and in the same linkage disequilibrium block are also *COMT*, *TXNRD2*, and *TANGO2*. In **Paper III**, we verify that these SNPs are associated with social behaviour in dogs by testing the additional breeds Labrador and golden retriever. Through inclusion of both common- and field-type Labradors, we also know that recent selection has affected allele frequencies for both polymorphisms as it varies between the types (**Paper III**). Previous associations with these genes include autism (*SEZ6L*), schizophrenia (*ARVCF*), and mood regulation (*COMT*) in humans (121-123). Most interestingly, *COMT* SNPs have been linked to fear reactions measured in the Dog Mentality Assessment (124), and to dogs' activity levels (125). In **Paper III**, we tentatively suggest that these regions may have been important during domestication.

As previously stated, vonHoldt, *et al.* (17) identified a genetic region in the dog genome that showed strong signals of selection and that in humans is responsible for Williams-Beuren syndrome. This region was further investigated in vonHoldt, *et al.* (126), and structural variants of the genes *GTF2I* and *GTF2IRD1* (transcription factors) could be strongly linked to human-directed social behaviours in dogs. These genes have previously been linked to hypersocial behaviours in humans with Williams-Beuren syndrome and in mice (127, 128). The variants are transposable elements, an element that has been suggested to have had impact on domestication. Transposons at a low copy number could be present in the ancestral genome and become functional due to rapid amplification as a response to intense selection (129).

There are also other candidate genes that have been indicated for being involved in dogs' social behaviour, for example hormones and neurotransmitters. Polymorphisms in the oxytocin receptor gene (130, 131) and the opioid receptor gene (132) are associated with human-directed social behaviours, and polymorphisms in dopamine and serotonin related genes are associated with human-directed aggression (133).

GWA studies have identified loci of interest for general behaviours as well. Ilkka, *et al.* (134) found several associations between SNPs and behavioural traits in Labrador retrievers, many in or close to genes known to regulate neurological or behavioural functions. For example, a genetic marker associated with agitation is close to the *TH* (tyrosinase hydroxylase) gene, involved in synthesis of a dopamine precursor, and the SNP associated with barking is located close to *CLINT1* (*Epsin 4*), a gene previously associated with schizophrenia. A recent study on noise sensitivity and

fear of strangers in German shepherd dogs found two genetic regions that reached genome-wide significance. Interestingly, these regions include known neuropsychological and hearing-related candidate genes (135).

Importantly, SNPs associated with phenotype are likely not causative. Further studies are required to find the structural variants that are, as was done in vonHoldt, *et al.* (126). However, by locating loci of interest, this will considerably narrow the search for functional elements.

The effects of certain genes may vary across dogs. In **Paper III**, the associations between behaviour and the SNPs differ between Labrador and golden retriever. For example, one of the genetic markers affects physical contact in golden retriever and gazing behaviour in field Labrador retrievers, but not in the common type. Another example shows that genetic effects may be sex specific. Konno, *et al.* (136) linked a specific allele on the androgen receptor gene to aggressive behaviour in akita inu, but only in male dogs as it did not affect female aggression. Thus, even though when genes are found to contribute to a behaviour, they may not affect all dogs. That is why it is important to verify results in other groups, as was done in **Paper III**.

4.2 Environmental effects

An individual's experiences and surroundings will be part of forming its behaviour. Actually, experiences of the parents may already have an effect. In chickens, Ericsson, *et al.* (137) show how stress exposure early in a female's life not only affects its own behaviour as an adult, but also the behaviour of its subsequent offspring.

4.2.1 Early experiences

Effects of early experiences have been shown to have a great effect on later adult behaviour in many species. This is also true in dogs, and for example the mothers' level of care for her puppies has an effect. A higher level of maternal care in military German shepherd dogs positively affected the puppies performance in a suitability test at 18 months (138). Interestingly, the opposite was found in guide dogs where more maternal care was linked to failure of the training program (139). Additionally, early-life variables such as season of birth, the size of the litter it is born in, and the ratio of females and males in the litter impacts adult dog behaviour (140).

Human interactions will affect the puppy as well. As previously mentioned, if dogs have no interactions with humans during the so-called critical period (about 2-14

weeks) they will show strong human-directed fear and struggle to form attachment to humans (82). The level of human handling during the puppies' first weeks will also affect them (141). An extensively handled group outperformed normally handled control puppies in solving a puzzle and showed less anxiety and more human interest when five weeks old. A third group was socially isolated between four and five weeks of age (single housing, darkened room, very limited human contact), and these puppies were hyperactive, had low problem-solving skills, high anxiety, and showed very little human interest. A more recent study found that daily, gentle handling until 3 weeks of age resulted in lower emotional (stress) reactivity in an open-field test at 8 weeks (142).

A comparison between dogs bought as puppies from non-commercial (hobby/home) breeders versus those from pet stores, and thus coming from commercial (puppy farm) breeders, points out the impact early experiences can have. The latter group, raised in a stressful environment with limited human contact, had increased aggression, fear, and separation anxiety compared to puppies from non-commercial breeders (143).

4.2.2 Environmental effects on human-directed social behaviours

There is some evidence that dogs' performance in tests of their human-directed social skills are affected by environment and experiences of the dog. For example, in a comparison of pointing understanding between pet and kennel golden retrievers, the latter group, with comparably limited human contact, understood proximal but not distal gestures, whereas pet dogs were successful in both conditions (144). In a similar cohort of Labrador retrievers tested in the problem-solving paradigm, kennel dogs showed less gazing than pet dogs while spending equal amount of time interacting with the test setup (145). On the contrary, Gácsi, *et al.* (59) found no effect of housing condition or owner-interaction time on the performance in the pointing test.

Whereas the training amount in general does not seem to influence dogs' understanding of referential gestures (**Paper II**), gundog training (retrieving) is linked to a higher performance (**Paper II**; 58). Gundog training involves directional gestures and this is to be expected. Agility training, on the other hand, did not influence pointing success (59), but it seems to affect problem-solving behaviours. In a comparison between pet, agility, and search-and-rescue dogs, Marshall-Pescini, *et al.* (146) found different strategies between the groups. Agility dogs gazed at the

human more, search-and-rescue dogs barked more, and pet dogs exhibited the least gaze alternations.

4.2.3 Owners' effect on dog behaviour

Dogs live in an anthropogenic environment and many times, primarily in the Western world, together with a human owner. Their lives are, to a great extent, decided by their human caretakers. The everyday life of the owner is shared by the dog, and the dogs' daily activity is mainly dependent on the owner. An example of how owner's affect dogs is that aversive training methods have been associated with behaviour problems in dogs (147). There are also more intricate effects based on the human-dog social bond. The owner's attachment and interaction style, as well as aspects of the owner's personality, have been shown to influence the relationship and the dog's behaviour (148-150).

Group-living animals often exhibits behavioural synchronization by doing the same thing, at the same time and place, and this seems to also strengthen bonds within the group (151). Primarily, synchronization is intraspecific, but an interspecific behavioural synchronization has been found within the dog-human dyad. Dogs stay in proximity of their owners, change activity when the owners do, and move at the same pace (152, 153). Additionally, dogs may prefer humans that synchronize their activity to that of the dog, which suggests that behavioural synchrony affects the dog-human relationship (154).

Social referencing also occurs between owners and dogs. Similar to human and chimpanzee infants, dogs take emotional cues from their human caregiver for how to behave in a novel situation (155). When faced with a novel, potentially threatening, stimulus, puppies alternated their gaze between object and caregiver and approached the stimulus more readily if the caregiver showed positive emotions towards it (156).

4.2.4 Stress and dog-human interactions

A stressor, i.e. a potential external or internal threat, will activate a cascade of reactions, for example the neuroendocrinal HPA axis which will result in increased levels of glucocorticoids, cortisol in dogs. Cortisol concentrations in saliva, blood plasma, and hair can therefore be used as a biomarker for stress. Cortisol concentrations, however, also show diurnal rhythm, seasonal effects, and are affected by activity, and should therefore always be interpreted with care. Whereas blood

plasma and saliva only measure the instantaneous stress reaction, hair can be used to study long-term cortisol concentrations as cortisol is incorporated into the hair as the shaft grows (157). By measuring hair cortisol in dogs, dogs' long-term stress levels could be linked to lifestyle (pet, competing, or police dog) in German shepherd dogs (158).

Positive interactions between dogs and humans are associated with wellbeing in both species. In shelter dogs, interactions with humans increase dogs' welfare by decreasing behavioural signs of distress and decreasing plasma cortisol concentrations (159). Interestingly, among a group of German shepherd dogs, owners that played more with their dogs had dogs with lower hair-cortisol concentrations (158). This suggests that positive human interaction has the potential of decreasing stress in dogs. Dogs, in turn, have a positive effect on humans, for example as a social supporter (160), and it has been shown that having a dog in the classroom decreases students' aggressive and hyperactive behaviour and increases their attention towards their teacher (161).

In **Paper IV**, we investigated the emotional contagion between dogs and their owners. Emotional contagion is the mirroring of emotional state between individuals and has frequently been observed within group-living species (162). Stress, for instance, can be highly contagious among individuals of the same species (163), and even interspecifically between dogs and their owners (**Paper IV**; 164). It has been shown that an owner's perceived stress during testing can have a significant impact on their dog's memory performance (165). Also, there is evidence of synchronization of cortisol concentrations between dogs and their owners. Buttner, *et al.* (164) identified this on short-term stress between agility dogs and their handlers. After an agility run, the dyad showed a correlated increase of saliva cortisol. In **Paper IV**, we studied the long-term cortisol by using hair samples from both dogs and their owners. We found that dogs and their owners are synchronized in long-term cortisol (Fig. 4). To my knowledge, this has previously only been seen between human mothers and their children (166, 167). The synchronization was found in both pet and competing dogs, but was stronger among the competing dyads. Importantly, there was no effect of activity on cortisol concentrations. This implies that the mirroring of long-term stress is affected by emotional contagion between the two species and not simply due to lifestyle effects (**Paper IV**).

Paper IV also shows an effect of owner personality on dogs' stress levels, whereas the dog's own personality traits had little effect. Owners scoring high on conscientiousness and openness had dogs with higher cortisol concentrations, and

owners scoring high on neuroticism had dogs with lower concentrations. Interestingly, this is consistent with previous results on how owners' and dogs' personality traits influence short-term stress levels in dogs (168-171). Thus, it seems that the owner has the greater influence on dog cortisol concentrations within the dyad and that dogs reflect the stress of their owners.

Figure 4. Synchronisation in hair cortisol concentrations between dogs (Shetland sheepdog and border collies) and their female owners (58 dog-human dyads).

4.2.5 Are we measuring effects of life-history or differences between genetic isolates?

That a dog's life experience and its owner affect dog behaviour is important to take into account when comparing groups of dogs. There may even be consistent differences in the environments for different breeds, for example in training amount, lifestyle, or owner characteristics. Certainly, breeds' behaviours are inherently different, but this may also attract people with different intentions and lifestyles that potentially will further increase the differences. Indeed, among the Labrador retrievers used in **Paper II** and **III**, owners of the common type declared more often that they got their dog as a companion dog than owners of the field type, who instead stated more often that they got their dogs for training purposes. When Marshall-Pescini, *et al.* (172) compared trained (competition level) to untrained dogs from four different breed groups in cognitive tasks, training seemed to have had a larger effect than breed, but few studies have systematically investigated this.

Potential environmental differences between breeds are quite apparent when comparing extremes. It is intuitive that the life of a Great Pyrenees will likely differ

considerably from the life of a Belgian Malinois, and the English bulldog's life from that of the Karelian bear dog. They may attract people of different lifestyles, interests, and stress-resiliencies. Maybe even of different personalities; for sure, differences in personality have been identified between 'dog people' and 'cat people' (173). This is something for future studies to further explore.

4.3 Epigenetics – the mediator between environment and genes

Epigenetics is the study of those processes that affect the expression of genes without changing the DNA sequence and that are mitotically and/or meiotically heritable (174). Whereas the DNA sequence is static, epigenetic factors are dynamic and can act as the mediator between environment and genes, and contribute to phenotypic plasticity. In response to changes or challenges in the environment there may be epigenetic modifications that could facilitate an adaptive response by gene-expression change (112).

Epigenetics factors include DNA methylation, histone modifications, and non-coding RNAs. DNA methylation, a methyl group at CpG dinucleotides, is the most commonly studied epigenetic factor (Fig. 5). CpG islands, i.e. repetition of CpGs in the DNA sequence, are frequently found in promotor regions of genes, and the methylation may thus alter gene expression, specifically by down-regulating or gene silencing (175, 176).

Figure 5. DNA methylation. A methyl group (molecule to the left) binds to cytosine, one of the nitrogenous bases found in DNA.

4.3.1 Epigenetics and behaviour

We do know that early experiences affect dog behaviour (see section 4.2.1). Whereas the mechanisms for this is not yet known in dogs, there are more studies performed in other species. The level of maternal care, for example, has long-term effects not only in dogs but also in mice. In mice, low maternal care is associated with higher glucocorticoid levels in adult offspring (177). By comparing offspring of high and low caring mothers, a difference in the epigenome in the promoter region of a glucocorticoid receptor was found (178). Specifically, offspring receiving low maternal care have a hypermethylation of the region, and, consequently, a down-regulation of gene expression. Individual experiences may also have transgenerational effects, which has been shown in a variety of species. In chickens, early-life stress in females will alter brain gene expressions not only in her, but also in her offspring (137), and in humans, methylation patterns of the grandchildren to women exposed to violence during pregnancy differ from controls (179).

This is a new area of research, and studies on dog behaviour and epigenetic factors are scarce. As the first of its kind, Cimarelli, *et al.* (180) could associate dog's social behaviour to DNA methylation of the oxytocin receptor gene. In males, a lower methylation level was associated with approaching a threatening stranger and a higher level with hiding behind their owner. Females with higher methylation levels approached the stranger more readily, and in a friendly manner, compared to those with lower levels (180).

Interestingly, Cimarelli, *et al.* (180) also tested if owner interaction style affects methylation of dog's oxytocin receptor gene. They did not find an association between the two. However, based on the results in **Paper IV** where we find a synchronization of cortisol levels between owners and dogs, this area of research is highly intriguing. I expect future explorations of similar associations, perhaps between owner personality and methylation of glucocorticoid receptors, to identify important epigenetic mechanisms on how owners affect their dogs.

4.3.2 Epigenetic modifications and domestication

It has been suggested that epigenetics is an important factor in evolutionary processes, for example in domestication and speciation (181-183). Epigenetic factors affect gene expression and phenotype (and social behavior: 180, e.g. coat color: 184), they have been shown to be heritable (185, 186), and they promote genetic mutations (187).

Few structural differences are found in genome-sequence comparisons of dogs and other canines. However, gene-expression patterns in dogs are markedly different from the conserved patterns of wolves and coyotes (188). Similar findings have been reported between domesticates and ancestors of other species (189). This implies that epigenetics may play an important role in changing the gene expression in domesticated species. Consequently, Nätt, *et al.* (190) compared DNA methylation patterns in promotor regions in brain samples from domesticated chickens and their ancestor, and found differences that could be associated with gene-expression differences.

In **Paper V**, we compared differentially methylated regions between wolf and dog as well as within dogs, i.e. across breeds, using DNA samples from the frontal cortex of the brain. Previous studies have identified methylation differences in blood and buccal samples between wolf and dog (129, 191). Because DNA methylation is highly tissue specific (192), the brain is the main organ of interest from a behavioural perspective.

Paper V showed substantial DNA methylation differences between wolf and dog (Fig. 6) as well as between the dog breeds. In the comparison between wolf and dog, the significant differentially methylated regions were more methylated in wolves than in dogs. The methylation profiles of wolf compared to dog and wolf compared to breeds imply that epigenetic factors have been important in the domestication process from wolf to dogs. Eight different breeds were also compared; beagle, boxer, German shepherd, Great Dane, Labrador retriever, pitbull terrier, Rottweiler, and walker hound. The divergence between the breeds could clearly be seen in the substantial differences of differentially methylated regions. The boxer showed the largest difference in DNA methylation and German shepherd dog had the lowest difference compared to the other breeds. The boxer also showed high levels of hypermethylation, whereas the opposite was found in beagle, Great Dane and pitbull terrier. Because of the large differences between breeds, it seems that DNA methylation has been, and still is, important for domestication as well as breed formation (**Paper V**).

The DNA methylation differences also occur in genes of high interest for the variation found in morphology and behaviour of dogs. Similar to Janowitz Koch, *et al.* (129), in **Paper V** we identified that genes that are involved in neurobiological processes are differentially methylated between the wolves and dogs, for example genes related to neurotransmitters. A change in gene expression of these genes may surely affect behaviour and development. For morphology, differentially methylated

genes include *hox* genes, which are involved in forming appendicular skeleton (193). Pugs have typical vertebral anomalies consistent with altered *hox* (194). Also, genes involved in the *IGF1* signalling pathway were found to be differentially methylated. *IGF1* determines the size of dogs (195), and is associated with anxious behaviour (196).

DNA methylation of the genes identified in **Paper V** may affect gene expression, and consequently, phenotype. Therefore, it is very probable that at least some of the DNA methylation differences that has been identified are a part of the impressive phenotypic variation among dogs.

Figure 6. Volcano plot over the methylated regions that are different between wolf and dog. Colours represent significant levels (from bottom to top: $p > 0.05$, $p < 0.05$, $p < 0.005$, $p < 0.0005$). Differentially methylated regions with a positive log fold change are more methylated in the wolf than the dog. The regions that reach the highest level of significance are listed to the right of the graph.

5 To conclude

This thesis highlights the intricate picture of dogs' behaviour. It is rooted in the behaviour of wolves but formed during domestication. Breed formation has led to consistent behavioural differences between populations, but a dog is more than its breed. Subpopulations within a breed are affected by different selection pressures that will influence their behaviour. The selection pressure will not affect different populations similarly, though, and differences in general behavioural traits seem influenced by differences in genetic architecture rather than recent or historical selection (**Paper I**). Dogs' human-directed hypersociability may be affected by selection, also recent selection, for higher human cooperativeness. Dogs' sociability is multifaceted, however, and a dog good at one social-cognitive task is not necessarily good at the next (**Paper II**). There is also an individual variation in behaviour among populations, and there is a clear association with this variation and genetic variants (**Paper III**). A dog's behaviour is not only explained by genetics, however. The dog's experiences and, maybe especially, its owner will influence the dog greatly (**Paper IV**). Epigenetic factors are influenced by the environment and affects gene expression; therefore, it can also affect behaviour. By epigenetic influence, a dog's behaviour could even be affected by the environment of its great-grandparents. Epigenetic effects surely have their part in variation among dogs (**Paper V**).

Why your dog behaves in a certain way? It is complex.

Summary of papers

Paper I

Similar recent selection criteria associated with different behavioural effects in two dog breeds

Ann-Sofie Sundman, Martin Johnsson, Dominic Wright, Per Jensen, 2016. *Genes, Brain and Behavior*, 15(8), pp.750-756.

<http://doi.org/10.1111/gbb.12317>

Background and aim

During domestication, human selection of dogs resulted in different dog types and later in different breeds with closed genetic pools. However, selection has continued, and, in some breeds, the different aims of the breeders have resulted in different types within the breeds. One example of this is within the golden and Labrador retriever where there are two established types, the common type bred for conformation and as a pet, and the field type bred for hunting abilities. The aim of the present study was to explore behaviour differences between the two retrievers and between the two breed types. By calculating heritability estimates, we could also compare the genetic contribution.

Method

We studied behaviour data from a standardized behavioural test, the Swedish Dog Mentality Assessment (DMA). Results from 902 goldens (689 common and 204 field) and 1672 Labradors (1023 and 649) were used, tested between 2005 and 2014. By studying pedigrees, the dogs were classified as either common or field type.

Results and discussion

Principal component analysis revealed six behavioural components from the DMA data: curiosity, play interest, chase proneness, social curiosity, social greeting and threat display. The effects of breed, type, age and sex on the components were tested by general linear models. Breed and type affected all behaviours. For example, Labradors were more curious than goldens ($F_{1,2568}=360.874$, $p<0.001$) and the field type was more playful than the common type ($F_{1,2565}=86.004$, $p<0.001$). However, there was also an interaction between breed and type for all behaviours but threat display, implying that the differences between the types are not the same across breeds. For example, in the Labrador retriever, the common type showed higher curiosity than the field type ($F_{1,1668}=18.359$; $p<0.001$), while the golden retriever field typed dogs displayed higher curiosity than the common type ($F_{1,897}=65.201$; $p<0.001$). In both breeds, heritability estimates for the behaviours were moderate, indicating that there was a considerable genetic contribution to the variation in behaviours. There was also variation in heritability between the breeds and the breed types, suggesting a difference in genetic variance due to different selection pressures. In conclusion, even though the golden and Labrador retrievers have both a similar origin, are closely related, and have a similar split between a common and a field type, we show that the types behave differently across the breeds. We therefore suggest that the genetic architecture related to behaviour differs between the breeds.

Paper II

Understanding of human referential gestures is not correlated to human-directed social behaviour in Labrador retrievers and German shepherd dogs

Ann-Sofie Sundman, Mia E. Persson, Anna Grozelier, Lise-Lotte Halldén, Per Jensen, Lina S.V. Roth, 2018. *Applied Animal Behaviour Science*, 201, pp.46-53.
<http://doi.org/10.1016/j.applanim.2017.12.017>

Background and aim

During domestication, dogs have developed specific skills when it comes to human communication and they excel at both communicating with humans and following human communicative cues. Two experiments commonly used to study these skills are the problem-solving test, where the dog is tested on how much contact they seek with the human, and the pointing test, where the dog is tested on how well they follow human gestures. The aim of this study was to compare behaviours in the two tests to see if dogs that seek more human contact also understand humans' communicative cues better. Additionally, we were interested in investigating breed differences and breed type differences.

Method

153 dogs of the breeds German shepherd dog (GSD; 16 females and 16 males) and Labrador retriever (63 females and 58 males) were included in the study. Labradors were divided into breed types common (35 females and 25 males) and field type (28 females and 33 males) based on ancestry. The problem-solving apparatus was a plate with three identical compartments containing treats, two with lids that could be opened and one that could not be opened by the dogs. The dogs' test-oriented and human-oriented behaviours were scored. In the pointing test, we used a momentary distal pointing cue and measured their success rate in 20 trials.

Results and discussion

The behaviours scored during the problem-solving test were reduced using a principal component analysis which revealed four components: Passivity, Experimenter Contact, Owner Contact and Eye Contact. Differences between the GSD and the Labrador were found for all four components. The GSD scored higher for Passivity and Experimenter and Owner Contact while Labradors sought more Eye Contact. The Labrador breed types differed for Owner Contact and Eye Contact where the field type scored higher. In the pointing test, all groups of dogs performed better than chance, but the Labradors performed better than the GSD. There was no difference between the breed types in pointing performance. In the comparison of the two tests, neither Passivity, Experimental Contact or Eye Contact had an effect on pointing performance. For Owner Contact, there was a negative correlation to pointing performance, but this was only present in the common-typed Labradors. Because we found no positive correlations between the two tests, we conclude that human-directed behaviour during a problem-solving situation does not affect the ability to follow human pointing cues and suggest that these two tests measure different aspects of dogs' social behaviour. The breed and type differences found show that human-directed social behaviour have been affected by both early selection (breed) and recent selection (breed types).

Paper III

Sociality genes are associated with human-directed social behaviour in golden and Labrador retriever dogs

Mia E. Persson, Ann-Sofie Sundman, Lise-Lotte Halldén, Agaia J. Trottier, Per Jensen, 2018. *PeerJ*, 6, p.e5889.

<http://doi.org/10.7717/peerj.5889>

Background and aim

There is a genetic contribution to individual variation in dogs' human-directed social behaviour during a problem-solving test and previous research has found two single nucleotide polymorphisms associated with social behaviours during a problem solving in beagles. The aim of this study was to investigate if these genetic regions are also associated with social behaviours in other breeds. We also compared allele frequencies between groups by sequencing this region in both dogs and wolves and two different breed types.

Methods

A total of 161 dogs were tested in a problem-solving test and genotyped. The dogs were of the two breeds Golden retriever (34 females and 27 males) and Labrador retriever (52 females and 48 males). The Labradors were further divided into the breed types common type (28 females and 24 males) and field type (24 females and 24 males). We also genotyped 21 wolves (7 females and 14 males). The problem-solving test consisted of a plate with three compartments containing treats, two of which with lids that could be opened by the dogs and one that could not be opened. The dogs' human-directed social behaviours were scored. DNA from dogs (buccal swabs) and wolves (blood or brain tissue) was genotyped on two of the previously identified SNPs, BICF2G630798942 (SNP1) and BICF2S23712114 (SNP2), by pyrosequencing.

Results and discussion

Associations between the two SNPs and social behaviours during the problem-solving test were found in both dog breeds and for different behaviours. In goldens, SNP1 was associated with physical contact variables. SNP2 was associated with several of the measured behaviours, for example gazing frequency in both breeds (goldens: $F_{2,55} = 6.330$, $p = 0.003$; Labradors: $F_{1,93} = 5.209$, $p = 0.025$). Moreover, there was a genetic variation for both SNPs in both dog breeds whereas wolves were fixed for SNP1. Common and field type Labradors differed in allele frequencies for both SNP1 and SNP2, which suggests that these SNPs have been affected by recent selection. These results show that these genetic regions are not only associated with human-directed social behaviour in beagles, but in other dog breeds also. We tentatively suggest that these regions have been important during domestication.

Paper IV

Long-term stress levels are synchronized in dogs and their owners

Ann-Sofie Sundman, Enya Van Poucke, Ann-Charlotte Svensson-Holm, Åshild Faresjö, Elvar Theodorsson, Per Jensen, Lina S.V. Roth, 2019. *Submitted manuscript*.

Background and aim

Emotional contagion occurs not only within species but may also occur between species, for example between dogs and humans. Previous research shows a synchronization in short-term stress within the dog-human dyad, and since owners often share everyday life and environment with their dogs this may lead to an interspecific long-term stress hormone synchronization. Our aim in this study was to investigate interspecific contagiousness of long-term stress by using dogs and their owners.

Methods

58 dog-human dyads were included in the study and hair from both dogs and their owners were analysed for their concentration of hair cortisol, which gives a measure of long-term stress. The hair cortisol was extracted using radioimmunoassay. Hair was taken on two different occasions, reflecting cortisol levels during summer and winter months. The dogs were of two different breeds, Shetland sheepdog (18 females and 15 males) and border collies (13 females and 12 males). The dogs were grouped into either companion (15 Shetland sheepdog and 11 border collies) or competing dogs (18 Shetland sheepdogs and 14 border collies). Owners' and dogs' personalities were assessed through owner-completed Dog Personality Questionnaire and Big Five Inventory. To investigate the activity levels of the dogs they wore a PetPace™ smart collar for seven days that continuously recorded activity data.

Results and discussion

The results show a clear synchronization in hair cortisol concentrations between dogs and their owners on two separate occasions (summer: $N=57$, $\chi^2=23.697$, $P<0.001$, $\beta=0.235$; winter: $N=55$, $\chi^2=13.796$, $P<0.001$, $\beta=0.027$). The synchrony was affected by sex and lifestyle. Female dogs and their owners showed a stronger synchronization than male dogs and their owners, and the synchrony was stronger in competing dyads than pet dyads. Furthermore, the cortisol concentration was not affected by dogs' activity levels nor their personality. However, the personality of the owners had an effect on the dogs' long-term stress. Higher scores for Neuroticism in the owner had a negative association and scores on Conscientiousness and Openness had a positive association with dogs' hair cortisol concentrations. Since the personality of the owners, but not the personality of the dogs, was related to long-term cortisol concentrations in the dogs, the results imply that dogs mirror the stress levels of their owners.

Paper V

DNA methylation in canine brains is related to domestication and dog-breed formation

Ann-Sofie Sundman, Fábio Pértile, Carlos Guerrero-Bosagna, Luiz Lehmann Coutinho, Elena Jazin, Per Jensen. *Manuscript*.

Background and aim

Epigenetic factors, e.g. DNA methylation, acts as mediators in the interaction between genome and environment. Variation in the epigenome can both affect phenotype and be inherited, and has therefore been suggested to be an important factor in the evolutionary process, e.g. in domestication. Dogs are not only our oldest domesticated species, they also show an extreme diverse in morphology and behavior. Thus, canines are a good model for studies of epigenetic involvement in diversification. The aim of this study was to explore DNA methylation in wolf, dog, and different dog species.

Methods

In this study, genomic DNA from the frontal cortex of 38 dogs of eight breeds and of three wolves were extracted. We optimized a combined method of genotype-by-sequencing (GBS) and methylated DNA immunoprecipitation (MeDIP) for application in canines. From this, we get a reduced representation of the canine genome. Two libraries (GBS and GBS-MeDIP) were prepared and sequenced on Illumina HiSeq2500 platform and were analyzed by bioinformatic tools.

Results and discussion

By performing a neighbor-joining tree analysis, we conclude that our reduced sample is representative of the dog genome as wolf and dog breeds cluster according to group. In the methylation analysis, substantial DNA methylation differences between wolf and dog and between the dog breeds are found. The methylation profiles of the different groups suggest that epigenetic factors have been important in the speciation from dog to wolf and also in the divergence into dog breeds. We highlight methylation differences in genes related to behavior and morphology, that may alter gene expression and phenotype. We hypothesize that these differences are involved in the phenotypic variation found among dogs.

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To my dogs, for reminding me what is real.

if you need a companion
well just go right to the pound
and find yourself a hound
and make that doggie proud
'cause that's what it's all about

The Dog Song by Nellie McKay
Photo: Leona Örtenberg

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