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

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Background. Dogs have human-directed social skills that allow them to communicate and cooperate with humans. We have previously identified two loci on chromosome 26 associated with human contact-seeking behaviours during an unsolvable problem task in laboratory beagles (Persson et al., 2016). The aim of the present study was to verify the SNPs in additional dog breeds and investigate possible associations to other social skills. We also studied how the allele frequencies have changed during domestication and recent selection.

Methods. Dogs of two breeds, 61 golden retrievers and 100 Labrador retrievers, were phenotyped and genotyped, and 19 wolves were genotyped. The Labrador retrievers were divided into common and field type by pedigree data to make it possible to study the effects of recent selection. All dogs were tested in an unsolvable problem task where human-directed social behaviours were scored. DNA from dogs (buccal swabs) and wolves (blood or brain tissue) was analysed for genotype on two of the previously identified SNP markers, BICF2G630798942 (SNP1) and BICF2S23712114 (SNP2), by pyrosequencing.

Results. There was genetic variation for SNP1 in both dog breeds whereas the wolves were fixed for this polymorphism, and for SNP2 there was variation in both dogs and wolves. For both SNPs, Labrador retriever types differed significantly in allele frequencies. We found associations between SNPs and human-directed social behaviour in both dog breeds. In golden retrievers, SNP1 was associated with physical contact variables, for example with the duration of physical contact with the owner ($F_{2,56} = 4.389, p = 0.017$). SNP2 was associated with several behavioural variables in both breeds, among others owner gazing frequency in both golden retrievers ($F_{2,55} = 6.330, p = 0.003$) and Labradors ($F_{1,93} = 5.209, p = 0.025$).

Discussion. Our results verify the association between the previously identified SNPs and human-directed social behaviour scored in an unsolvable problem task. Differences in allele frequencies suggest that these loci have been affected by selection. The results suggest that these genomic regions are involved in human-directed social behaviour in not only beagles but in other dog breeds as well. We hypothesise that they may have been important during dog domestication.
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ABSTRACT

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Results. There was genetic variation for SNP1 in both dog breeds whereas the wolves were fixed for this polymorphism, and for SNP2 there was variation in both dogs and wolves. For both SNPs, Labrador retriever types differed significantly in allele frequencies. We found associations between SNPs and human-directed social behaviour in both dog breeds. In golden retrievers, SNP1 was associated with physical contact variables, for example with the duration of physical contact with the owner ($F_{2,56} = 4.389, p = 0.017$). SNP2 was associated with several behavioural variables in both breeds, among others owner gazing frequency in both golden retrievers ($F_{2,55} = 6.330, p = 0.003$) and Labradors ($F_{1,93} = 5.209, p = 0.025$).
Discussion. Our results verify the association between the previously identified SNPs and human-directed social behaviour scored in an unsolvable problem task. Differences in allele frequencies suggest that these loci have been affected by selection. The results suggest that these genomic regions are involved in human-directed social behaviour in not only beagles but in other dog breeds as well. We hypothesise that they may have been important during dog domestication.

INTRODUCTION

Social behaviours are complex traits affected by environmental factors as well as by many genes, each with small effects. The genetics of complex traits such as social behaviour is difficult to study in humans as it requires a standardized environment, dense genotyping as well as phenotyping of a large number of individuals. The dog (*Canis familiaris*) on the other hand, has a genome with large haplotype blocks and is thus more convenient for genetic mapping (Lindblad-Toh et al. 2005). In addition, their human-like social skills could make them a suitable model species for human social behaviour and disorders.

Through sharing our ecological niche for thousands of years, dogs have developed social talents that, in some cases, have been reported to surpass the skills of our closest relative, the chimpanzee (*Pan troglodytes*), as well as their own wolf ancestor (*Canis lupus*) (Hare & Tomasello 1999; Hare & Tomasello 2005). For example, dogs are able to comprehend human ostensive cues and referential gestures such as pointing and gazing (Lakatos et al. 2012; Soproni et al. 2001). They are also able to communicate with humans through intentional communicative referential gestures involving both attention-seeking and directional-showing behaviour.
(Marshall-Pescini et al. 2013; Miklosi et al. 2000; Passalacqua et al. 2011). Furthermore, dogs have been demonstrated to discriminate between human emotions when viewing facial expressions (Muller et al. 2015). Not even socialised wolves are as prone as dogs to seek human attention (Gacsi et al. 2009; Topal et al. 2005), or to communicate with humans through mutual gazing (Nagasawa et al. 2015). Different hypotheses for how these differences between the wolf and the dog evolved has been proposed. For example, Hare and Tomasello (2005) suggested that selection against fear and aggression towards humans also mediated the evolution of dogs’ social skills. More recently, however, Range and Viranyi (2015) showed that wolves are as attentive to both human and conspecific actions as dogs are. They propose the canine cooperation hypothesis, suggesting that wolf-wolf cooperation established the basis for the evolution of dog-human cooperation.

Human-directed contact-seeking behaviours have specifically been studied using a problem-solving paradigm. Hare and Tomasello (2005) and Miklósi et al. (2003) showed that dogs and socialised wolves differ in their human-directed contact-seeking behaviour when faced with an unsolvable task. Whereas wolves were more oriented towards the task, dogs quickly gave up and turned to a nearby human in a help-seeking manner. Hence, behavioural tests involving unsolvable tasks that stimulate communication and attention-seeking towards humans can be used to study dog-human social interactions, including variation between and within breeds (Persson et al. 2015; Persson et al. 2017; Sundman et al. 2018).

When studying dogs’ human-directed social behaviour it is important to take into account that dogs form strong attachment bonds with their owners (Topal et al. 1998). It has been suggested...
that owners function as a secure base influencing persistence in cognitive tasks (Horn et al. 2013), because the presence of the owner affects dogs’ duration of task manipulation. Additionally, these authors found that dogs spend more time in proximity of their owner and the presence or absence of the owner affected dogs’ interactions towards the unfamiliar experimenter. It is therefore relevant to analyse social interactions towards an unfamiliar experimenter and the familiar owner separately if both are present during a cognitive task. Consistent with this, we previously found that dogs’ social behaviours directed at owners and those directed at unfamiliar experimenter were separated into different components in a principal component analysis (Sundman et al. 2018).

The dog is not only well-suited for studies on social behaviour, the species is also well-suited for studies of the genetics of both simple and complex traits such as social behaviours. The structure of the dog genome with long regions of linkage disequilibrium is particularly suitable for genome-wide studies identifying candidate regions for traits of interest (Lindblad-Toh et al. 2005; Sutter et al. 2004). Regarding human-directed social behaviour, heritability estimates show a significant genetic component underlying variation in behaviours as measured in an unsolvable problem task (Persson et al. 2015). Furthermore, genome-wide association studies (GWAS) have identified two candidate regions on chromosome 26 associated with human-directed social behaviour in laboratory beagles, with single nucleotide polymorphisms (SNPs) located within the SEZ6L and ARVCF genes (Persson et al. 2016). Interestingly, these genes have previously been associated with human social disorders such as autism for SEZ6L (Chapman et al. 2015) and schizophrenia for ARVCF (Sim et al. 2012). Additionally, Persson et al. (2016) found that the SNP associated with the gene ARVCF is in linkage with three other genes of interest for dogs’
sociability, e.g., the *COMT* gene that has previously been associated with mood regulation in humans (Qayyum et al. 2015).

These earlier findings suggest a possible cross-species genetic basis for social behaviour shared between dogs and humans. However, further studies are necessary to verify that the same SNP markers are associated with human-directed contact seeking in other dog breeds. In the present study, we utilised behavioural data collected in two different experiments in two other breeds, each experiment using similar methods for studying dog-human interactions in a standardised behavioural test. In both breeds we collected DNA and analysed associations with the previously reported candidate SNPs. The aim of the study was thus to investigate whether there are associations between human-directed social behaviour, as measured in an unsolvable problem task, and two candidate SNP markers in groups of golden and Labrador retrievers. It is possible that genetic variants affecting social behaviour have been under selection during domestication and more recent breed formations. To provide some tentative data in relation to this, we genotyped wolves and included a dog breed that has undergone recent selection for its cooperative bond with humans, the Labrador retriever. The Labrador retriever has recently been split into a common and a hunting type differing in many aspects of social behaviour (Sundman et al. 2016).

**MATERIALS AND METHODS**

**Ethical note**

These studies were carried out in accordance with the relevant guidelines and the ethical permit approved by the regional ethical committee for animal experiments in Linköping, Sweden.
(permit number: 51-13). All owners had given their informed consent for their dogs’ participation. Wolf samples were donated by the veterinarians at Kolmården Wildlife Park and Borås Animal Park in Sweden. All wolf samples were collected in connection with veterinary motivated procedures and no particular ethical licence was therefore required for them.

**Subjects**

Dogs of the breeds golden and Labrador retrievers were recruited to participate by finding dog owners through social media, local radio and advertisements. In total, 61 golden retrievers (34 females and 27 males) and 100 Labrador retrievers (52 females and 48 males) were tested in the same unsolvable problem task and additionally genotyped for the two candidate SNPs. All dogs were originally recruited for and used in other studies. Golden retrievers participated in a study involving intranasal oxytocin treatment and its effect on behaviours in an unsolvable problem task (Persson et al. 2017) and, due to this, they were required to be at least 4 months of age (mean age 5 ± SE 0.5 years; range 4 months-12 years) and not pregnant or lactating. Labrador retrievers participated in a study on correlations between the unsolvable problem task and other behaviour assessments (Sundman et al. 2018) and were required to be at least 1 and not older than 4 years of age (mean age 2.43 ± 0.195). Participating dogs also had to be registered as purebred by the Swedish Kennel Club.

In addition, the same candidate SNPs were genotyped in 21 Scandinavian wolf samples (*Canis lupus lupus*, 7 females and 14 males) (Table S1). Out of these, eighteen blood samples were donated by Kolmården Wildlife Park, Sweden. These blood samples were old samples collected between 2008-2016 upon routine procedures for veterinarian purposes. The wolves at Kolmården
Wildlife Park were originally born in captivity and originated from 5 different animal parks in Scandinavia. Three brain samples, collected after death from reasons unrelated to any scientific studies, were donated by Borås Animal Park, Sweden. One of the latter three was wild-caught and the others born in captivity at the zoo. For more information on the wolf samples, see Table S1.

By use of pedigree information, Labrador retrievers were divided into two types: common and field. If ancestors for at least three generations back were bred for field work, which can be seen in titles of ancestors e.g., field-trial champion, the dogs were classified as field-type Labrador retrievers. If ancestors instead had show titles, they were classified as common-type Labrador retrievers. Labrador retrievers with mixed ancestry were not included in the study. We used the Swedish Kennel Club’s online registry (Hunndata, http://hundar.skk.se/hunndata/) and k9data.com (http://www.k9data.com/) for pedigrees. Based on the pedigree analysis we classified 52 Labrador retrievers as common (28 females and 24 males) and 48 as field type (24 females and 24 males). For golden retrievers, we did not have sufficient information to perform a similar division, and they were therefore all treated as one single breed.

Procedure

Upon arrival at the testing site, owners were informed of the testing procedure. Buccal DNA samples were collected either prior to the testing (golden retrievers) or after the testing (Labrador retrievers). To assure sufficient food motivation in the dogs, their willingness to eat the treats used in the unsolvable problem task was confirmed as described in Persson et al. (2015). Briefly, dogs were presented with three quarter-pieces of Frolic© on a plastic plate of the same material
as the problem-solving device but without a lid. The treats were presented one at the time. When
the dog ate a treat, another was placed until the dog had consumed the three treats. All subjects
consumed all pieces within 20 seconds and were therefore not considered to differ in their
willingness to eat the treats.

Two female experimenters tested the dogs, one person tested the golden retrievers and one the
Labrador retrievers. The video analyses were performed by the two experimenters.

Subjects of the breed golden retriever were part of a parallel study investigating effects of
oxytocin treatment on dogs’ human directed social behaviour (Persson et al. 2017). Therefore, as
part of this parallel study, subsequently to DNA sampling half of the females and half of the
males received an intranasal dose of 20 IU oxytocin 45 minutes prior to the behavioural test.
Individuals that were not given an oxytocin treatment were instead given saline as a control
treatment. After the food motivation test, these dogs were taken for a 30-minute walk followed
by 10 minutes of resting in the car immediately prior to the behaviour test. Treatment was taken
into account in the later analyses, as described below. The experimenter who tested the golden
retrievers was blinded to which hormone treatment the dogs received until after the behavioural
video analysis. Subjects of the breed Labrador retriever had been subjected to a standardized test
battery (Behaviour and Personality test for Dogs, Swedish Kennel Club) before testing them in
the unsolvable problem task. Lastly, they were tested in a pointing test.

Unsolvable problem task
Testing was carried out at ten different locations in Sweden during the autumn of 2014 (Labrador retrievers) and autumn of 2015 (golden retrievers). Results from the behaviour test have been previously published in Sundman et al. (2018) (Labrador retrievers) and in Persson et al. (2017) (golden retrievers). The unsolvable problem task was performed in the same way in both breeds. To have a uniform setting, testing took place in a 3 x 3 m marquee tent without the presence of any other dogs. The tent had three walls and no flooring. A mesh fence was placed at the open side to keep the dogs within the tent (testing area). An HD camcorder (Canon Legria HF G25) was placed on a camera stand approximately 3 meters from the testing area to record the behaviour of each dog.

Dogs were tested with the unsolvable problem task thoroughly described in Persson et al. (2015) with the addition of the presence of the owner. The device used consists of a plastic tray (55 x 25 cm) with three identical circular wells (7 cm in diameters) covered with plexiglass lids with odour ports (Fig. 1). Three quarter-pieces of Frolic© dog treats were placed underneath each lid. The dogs could easily access the treats in two of the three wells by sliding the lids to the side. However, the lid in the middle could not be opened hence making the task unsolvable. The experimenter cleaned, prepared and placed the unsolvable problem task on the ground approximately 15-30 cm from the middle of the back wall prior to the arrival of each dog.

Upon arrival at the testing arena, owners were reminded to stand passively immediately close to the fence at the front right corner inside the tent, facing the problem task. The experimenter was standing in the same position but on the opposite side of the tent (in the front left corner). After closing the fence gate, the owner was asked to unleash the dog that then could freely move
around inside the testing area from this point onwards. The owner had been instructed to not interact with the dog unless it was attempting to escape. If the dog tried to leave the tent, the owner was allowed to interrupt and call the dog back. Behaviours were not recorded during this interruption. If the dog had not opened any of the lids within 60 seconds, the experimenter opened both solvable lids halfway and immediately went back to her original position. The duration of the behaviour test was three minutes.

The behaviours scored from the behaviour test were the human-directed social behaviours proximity, physical contact and gazing in relation to owner and experimenter. Duration and frequency were scored for each of these behaviours as described in the ethogram (Table 1). In addition to the social behaviours, we scored the duration of the time the dog spent in close proximity of the test-setup. The behaviours were scored from the video recordings using the Observer XT 10, Noldus software.

**DNA sampling, extraction and genotyping**

Buccal cells were collected from the dogs by rubbing a cotton swab on the inside of their cheek for approximately 20 seconds. Buccal samples were stored at 4 °C and wolf samples (blood and serum) were stored at -20 °C until DNA extraction. The standard protocol of the Isohelix DDK-50 kit was used to extract DNA from buccal swabs, with the exception that samples were kept in Lysis Buffer and proteinase K for 48 hours prior to continuing with the protocol. Single 50 µl elusions were used. DNA was also extracted from fifteen whole blood and three serum wolf samples using the QIAGEN DNeasy® Blood and Tissue Kit and from three wolf brain samples using QIAGEN AllPrep DNA/RNA/miRNA Universal Kit, both by standard protocol.
Subsequently, DNA yield was quantified using a Nanodrop ND-1000 and all isolated samples were stored at -20 °C until further use.

Genotyping was performed on the two SNPs identified in Persson et al. (2016), BICF2G630798942 (rs23313128, chr26:20025266C/A) and BICF2S23712114 (rs23317526, chr26:29319675A/G). Hereafter, BICF2G630798942 will be referred to as SNP1 and BICF2S23712114 as SNP2. Polymerase chain reaction (PCR) and subsequent pyrosequencing were used to genotype both wolf and dog samples for the two SNPs. Primers were designed using the PyroMark Assay Design software by QIAGEN. The primers used for SNP1 were:

- Forward biotinylated in 5’ CTGCCAGGGACTCCTGAG, reverse CTCAAGGCAGCCCATCCT and sequencing reverse GGAGGCTTGCTGCCG. For SNP2 the primers used were: forward biotinylated in 5’ CATGTCACAGTTGAGGGGATAGGT, reverse TCTTCAGACAGCCCACCCA and sequencing reverse CAGTCCAGGAAGGAATA. For each sample, the PCR-mixture contained 0.12 μl DreamTaq™ DNA Polymerase 5 u/ μl (Thermo Scientific), 2.5 μl of 10X DreamTaq™ Buffer (Thermo Scientific), 0.5 μl dNTP 10 mM (2.5 mM each, BIOLINE), 0.5 μl of each primer diluted to 5 μM (Invitrogen), 19.9 μl of nuclease free water and approximately 100-200 ng of DNA template. The final PCR volume was 25 μl for each sample and the reaction was run on the Palmcycler PCR by Corbett. The PCR cycle consisted of an initial denaturation at 95°C for 3 min, 40 cycles of 30 s denaturation at 95°C, 30 s annealing at 63°C for the SNP1 primers and 61°C for SNP2 primers, 30 s extension at 72°C and a 10 min final extension at 72°C. Pyrosequencing was performed on the entire PCR product according to the PyroMark Q24 Vacuum Workstation Quick-Start Guide found at www.qiagen.com. The results were analysed using the PyroMark Q24 2.0.6 software.
Genotyping of the SNP1 marker was successful in all 61 golden retriever samples, in 97 Labrador retriever samples (genotyping failed in one male and two female samples) and in 19 wolf samples (two female samples failed). SNP2 was successfully genotyped in 60 golden retrievers (one female sample failed), in 98 Labrador retrievers (two female samples failed) and in 19 wolves (two female samples failed).

Statistical analysis

Except for Hardy-Weinberg Estimates (HWE), all statistical analyses were carried out using IBM SPSS statistics software version 22 and 25. Behaviour data was checked for normality both visually and with the Kolmogorov-Smirnov test, and, if necessary, transformed using log10(x+1). To analyse behaviour data, Generalized Linear Mixed Models were used. For golden retriever analyses, statistical models contained oxytocin treatment, sex and SNP genotype and for Labrador retriever analyses, the models contained type, sex and SNP genotype. The two SNPs were tested separately together with the other fixed variables and Bonferroni correction was used to account for multiple testing in post-hoc comparisons. Data distribution was set to normal with a link function or gamma with a log function depending on data distribution. Best model-distribution fit was determined by Akaike measurements comparisons. Final models for each behaviour including F and P statistics can be found in Table 2 for golden retrievers and Table 3 for Labrador retrievers. Inter-observer reliability analysis was done for all behaviours in 10% of the individuals with correlation coefficients ranging from 0.901 – 0.999 (Pearson) and 0.803 – 1 (Spearman) (Table S2). Fisher’s exact test was used to compare genotype frequencies. To calculate HWE, the exact test incorporated in the “genetics” R-package was used.
RESULTS

Variation was found in SNP1 in golden retrievers (HWE: p = 0.769) and in Labrador retrievers (HWE: p = 0.153) but all wolves were fixed for the C allele (Fig. 2A). For SNP2, variation was found in both golden retrievers (HWE: p = 1), in Labrador retrievers (HWE: p = 1) and in wolves (HWE: p = 1) (Fig. 2B). Additionally, when looking at the two types of Labrador retriever separately, there was a variation in both SNPs for both the common and the field type and the HWE was not significant for any of them (for SNP1 p = 0.580 and p = 1 and for SNP2 p = 1 and p = 1 for common and field, respectively) (Fig. 2).

The Fisher’s exact test showed a significant difference in genotype frequencies between the common and field Labrador retrievers for both SNP1 (p < 0.001) and SNP2 (p = 0.031) (Fig. 2).

For SNP1, AC was the most common genotype in common type Labrador retrievers whereas CC was the most common in the field type. In SNP2, AA was the most frequent genotype for both types, but the proportion between AA and AG differed between the types. In the common type a larger proportion was of the AG genotype than in the field type.

In the unsolvable problem task, there were no associations between the SNPs and the time spent close to the test-setup. However, we found effects of both SNPs on behavioural variables related to social behaviour in both breeds. The final models for each social behaviour variable as well as F and P statistics for all behaviours can be found in Table 2 and 3. Figures of all genotype-behaviour associations are presented in supplementary Figure S1. In the golden retriever, the genotype of SNP1 was significantly associated with physical contact, both on the frequency with
the experimenter (Fig 3A, F_{1,56} = 4.339, p = 0.018) and on frequency (Fig 3B, F_{2,56} = 6.996, p = 0.002) and duration (Fig 3C, F_{2,56} = 4.389, p = 0.017) with the owner. Golden retrievers with the AA genotype had a higher frequency of physical contact with the experimenter than individuals with AC. The AC genotype had instead more frequent physical contact with their owners than both AA and CC, and genotypes AA and AC had contact for a longer duration than individuals with the CC genotype.

In the Labrador retrievers we found a significant interaction between genotype of SNP1 and breed type for the duration of gazing at owner (Fig 4, F_{1,90} = 10.394, p = 0.002). In the field type, dogs with CC genotype gazed longer at their owner than those with AC genotype, whereas there were no differences between the genotypes among the common type.

SNP2 was significantly associated with several human-directed social behaviours in both breeds. In the golden retrievers, AG individuals spent less time in the experimenter zone than AA and GG dogs (Fig 5A, F_{2,55} = 3.304, p = 0.044) and AA dogs gazed at the experimenter more frequently than GG dogs (Fig 5B, F_{2,55} = 3.243, p = 0.047). Individuals carrying the AA and AG genotype gazed at their owners more frequently (Fig 5C, F_{2,55} = 6.330, p = 0.003) and with a longer duration (Fig 5D, F_{1,55} = 4.477, p = 0.016) than GG dogs. Also, golden retrievers with the AG genotype had longer duration of physical contact with their owner than those with AA (Fig 5E, F_{2,55} = 14.809, p < 0.001).

In the Labrador retrievers, AA individuals at SNP2 spent more time in the experimenter zone (Fig. 6A, F_{1,94} = 6.252, p = 0.014) and visited it more frequently (Fig. 6B, F_{1,94} = 6.860, p =...
0.010, as well as spent more time in the owner zone (Fig. 6C, $F_{1,94} = 5.945$, $p = 0.017$) and
visited it more frequently (Fig. 6D, $F_{1,94} = 13.846$, $p < 0.001$). AA individuals also gazed more
often at their owner (Fig. 6E, $F_{1,93} = 5.209$, $p = 0.025$) whereas AG individuals instead gazed at
their owner for a longer duration (Fig. 6F, $F_{1,93} = 7.209$, $p = 0.009$). For the duration of gazing
towards experimenter (Fig 7A) and the frequency of gazing towards the owner (Fig 7B) there
was an interaction between genotype and type in the Labrador retrievers ($F_{1,93} = 7.796$, $p = 0.006$
and $F_{1,93} = 3.947$, $p = 0.05$, respectively). In the field type, AA individuals gazed longer at the
experimenter and more often at their owner than AG individuals whereas there were no
differences in the common type.

In golden retrievers, sex and intranasal oxytocin treatment (part of the experiment for which the
dogs were originally recruited) were included in the models. In the analyses for both SNP1 and
SNP2, sex had an effect on the duration dogs spent in the owner zone where males spent
significantly more time with their owner than females ($F_{1,56} = 6.590$, $p = 0.013$ for SNP1 model
and $F_{1,55} = 7.374$, $p = 0.009$ for SNP2 model; males $35.02 \pm 7.986$ vs. females $16.72 \pm 3.921$).
Additionally, in analyses for both SNPs, intranasal oxytocin treatment significantly decreased the
duration ($F_{1,56} = 15.797$, $p < 0.001$ and $F_{1,55} = 22.377$, $p < 0.001$; oxytocin $0.24 \pm 0.109$ vs.
control $1.75 \pm 0.591$) and frequency ($F_{1,56} = 9.705$, $p = 0.003$ and $F_{1,55} = 12.305$, $p = 0.001$;
oxytocin $0.97 \pm 0.481$ vs. control $4.20 \pm 1.603$) of experimenter physical contact seeking as well
as the frequency of owner physical contact ($F_{1,56} = 8.137$, $p = 0.006$ and $F_{1,55} = 5.437$, $p = 0.023$;
oxytocin $0.71 \pm 0.377$ vs. control $1.67 \pm 0.615$).
For the Labradors, sex and type were included in the models. In the models for both SNP1 and SNP2, the difference between males and females for the duration of gazing at their owner was significant where males looked for a longer time ($F_{1,90} = 7.667$, $p = 0.004$ and $F_{1,93} = 13.995$, $p < 0.001$; $10.19 \pm 2.72$ vs. $4.42 \pm 0.73$ seconds for males and females respectively). There were many differences between the types. In the models for both SNPs, the frequency of owner and experimenter zone were significant as well as the duration of gazing at the owner and frequency and duration of owner physical contact. Field type Labradors visited the experimenter zone at a higher frequency ($F_{1,92} = 9.046$, $p = 0.003$ and $F_{1,94} = 4.821$, $p = 0.031$; $3.27 \pm 0.37$ vs. $2.12 \pm 0.22$) as well as the owner zone ($F_{1,92} = 18.153$, $p < 0.001$ and $F_{1,94} = 10.559$, $p = 0.002$; $4.21 \pm 0.43$ vs. $2.51 \pm 0.23$ times). The field type gazed longer at their owner ($F_{1,91} = 10.475$, $p = 0.002$ and $F_{1,93} = 25.596$, $p < 0.001$; $5.74 \pm 0.92$ vs. $3.16 \pm 0.50$ seconds) and were in physical contact with their owner both more often ($F_{1,92} = 8.065$, $p = 0.006$ and $F_{1,94} = 20.862$, $p < 0.001$; $0.77 \pm 0.17$ vs. $0.29 \pm 0.10$) and for a longer time ($F_{1,90} = 15.910$, $p < 0.001$ and $F_{1,94} = 9.025$, $p = 0.003$; $0.70 \pm 0.19$ vs. $0.09 \pm 0.04$). Additionally, in the model for SNP1, there were significant differences between the types in frequency and duration of gazing at the experimenter as well as frequency of owner gazing. The field type gazed both longer and more often at the experimenter ($F_{1,91} = 10.475$, $p = 0.002$ and $F_{1,91} = 7.679$, $p = 0.007$) and more often at their owner ($F_{1,91} = 20.208$, $p > 0.001$). The means of the duration and frequency for looking at experimenter were $5.74 \pm 0.92$ seconds and $4.02 \pm 0.49$ times in field type versus $3.16 \pm 0.50$ seconds and $2.63 \pm 0.34$ in the common type. For the frequency of looking at owner, the means were $6.52 \pm 0.82$ versus $3.00 \pm 0.33$ times.

**DISCUSSION**
In a previous genome-wide association study on beagles tested in the unsolvable problem paradigm, Persson et al. (2016) identified two SNPs on chromosome 26, BICF2G630798942 (SNP1) and BICF2S23712114 (SNP2), associated with social interactions directed towards humans. Here we show that both SNPs are also associated with human-directed social behaviour in two additional dog breeds, golden and Labrador retriever. We also show that genotype frequencies for the two SNPs differ between wolves and dogs, between breeds and between recently selected breed types. Thus, these loci could have been affected by selection during domestication as well as during breed formations. This suggests that selection of favourable alleles in the genomic region of the SNPs may have been an important part of dog domestication.

In a population of laboratory beagles, Persson et al. (2016) found a significant association between SNP1 and the duration of physical contact and duration of human proximity. Additionally, a suggestive association was found between SNP2 and the duration of human proximity. It is important to stress that these SNPs are not causative for the behaviour differences but rather linked to the specific causal loci. SNP1 and SNP2 are both located on chromosome 26, and within the linkage disequilibrium regions five possible associated genes are present. The marker SNP1 is located in an intron of the gene SEZ6L and there are no other genes present in the same linkage block. SNP2 is located in an intron of the gene ARVCF and three additional genes are in linkage: COMT, TXNRD2, and TANGO2. Previously, SEZ6L, ARVCF, COMT and TXNRD2 have been associated with social disorders and schizophrenia in humans (Chapman et al. 2015; Mas et al. 2010; Sanders et al. 2005; Xu et al. 2013).
In the present study, we verify that the association between these genomic regions and human-directed social behaviour are not specific to the previously studied beagles. Similar effects were found in two other breeds, Labrador and golden retrievers, tested in the same unsolvable problem task as the beagles. The genotype of SNP1 had an effect on physical contact seeking with both experimenter and owner in the golden retriever and was associated with a difference in owner gazing between Labrador types. The genotype of SNP2 was primarily associated with eye contact seeking with the experimenter as well as owner in both golden and Labrador retrievers. In the Labradors this effect differed between types where the only field type individuals carrying the AA-genotype were gazing more. Additionally, SNP2 was associated with experimenter proximity seeking in both breeds and owner proximity seeking in only Labradors. Finally, an association was found between SNP2 and owner physical contact seeking in golden retrievers but not in Labradors. Genotype and sex interactions were identified in the beagles for SNP1 (Persson et al. 2016) but this was not present in the retrievers.

In addition to SEZ6L, ARVCF and COMT, there are other genes suggested to be associated with dogs’ human-directed social behaviour. Previous research has found associations between polymorphisms in the oxytocin receptor gene and social behaviour both during problem solving and in additional situations (Kis et al. 2014; Persson et al. 2017). The oxytocin receptor gene has also been associated with successful training of detection dogs (Konno et al. 2018). Another gene of interest is the dopamine receptor D4 gene that has been associated with gazing towards humans (Hori et al. 2013). Additionally, vonHoldt et al. (2017) investigated a candidate region associated to Williams-Beuren syndrome (WBS) in humans, which is causing hyper-social
behaviour amongst other effects. It was found that structural variants in this region were also associated with extreme sociability in dogs.

During the course of domestication, dogs seem to have evolved impressive interspecific cooperation skills with humans (Jensen et al. 2016; Miklósi & Topál 2013). For example, comparative studies have shown that dogs have a higher sociability in general than wolves (Bentosela et al. 2016) and, specifically, that dogs seek more human contact when faced with a problem than wolves do (Heberlein et al. 2016; Miklósi et al. 2003; Udell 2015). It seems that genetic variants contributing to human-directed social abilities have been selected during domestication and, thus, the two genomic regions investigated in the present study may have been targeted. Variation in sociability towards humans has a significant genetic component, which has been shown by heritability estimates (Persson et al. 2015; Sundman et al. 2016; van der Waaij et al. 2008; Wilsson & Sundgren 1998) and this is a requirement for selection. Wolves were found to be fixed for the C-allele on SNP1 whereas there was a variation in SNP2. The former polymorphism may thus not exist or is rare among wolves and could even have appeared after the split with dogs, whereas the latter is present in both species. However, as this current study has investigated the SNP genotypes in only a very limited sample of Scandinavian wolves, these results should be cautiously interpreted. A recent study describing the genetic architecture of a dog x wolf crossbreed, the Czechoslovakian Wolfdog, found regions containing excess of wolf and dog ancestry genes respectively (Caniglia et al. 2018). The Czechoslovakian Wolfdog derives from a cross between Carpathian wolves and German shepherd dogs and, even though it shares many morphological features with the wolf, it shows mostly dog-like behavioural
phenotypes. Interestingly the SEZ6L, ARVCF and COMT genes were all detected within regions of excess dog ancestry.

When using an unsolvable problem paradigm to study human-directed social behaviour we should also consider the dogs’ persistence to solve the problem and their food motivation. Previous studies have, for example, found a negative correlation between persistence and eye-contact duration (Brubaker et al. 2017), and that more persistent dogs show a longer latency until they gaze at the present human (Marshall-Pescini et al. 2017). On the other hand, in both Persson et al. (2015) and Sundman et al. (2018), test-setup interactions form a component separate from social behaviours in a principal component analysis. It is difficult to disentangle sociability from persistence and food motivation when using the unsolvable problem paradigm. However, neither of the two SNPs in the present study were associated with the duration the dogs spent in proximity of the test-setup, and thus, they seem to be associated with human-directed social behaviour rather than persistence.

Previous studies have reported breed differences in behaviours related to human communication (Passalacqua et al. 2011; Sundman et al. 2018; Udell et al. 2014). We can study recent selection by examining established breeds recently diverged into two types due to different breeding goals; for example, dogs suitable for field-work versus pet and conformation dogs. The Labrador retriever is a breed clearly divided into two types. Pedigrees as well as morphology and behaviour distinguish the common type from the field type (Sundman et al. 2018; Sundman et al. 2016). Specifically, Sundman et al. (2018) compared the types in the unsolvable problem task and found several behavioural differences. During recent selection, it seems that the field type
has increased its human-contact seeking behaviour in comparison to the common type, although environmental causes cannot be discarded. Likewise, in the present study, we found differences in allele frequencies between the types for both SNP1 and SNP2. These genetic markers have thus been affected by recent selection in two selection lines of dogs that also differ in social abilities, lending further support to the association between the genomic region and human-directed social behaviour.

CONCLUSIONS

Our results verify the associations between human-directed social behaviour and the SNPs BICF2G630798942 and BICF2S23712114 on canine chromosome 26. We suggest that these loci could have been affected by domestication and selection for sociality in dogs and that genetic variants linked to the SNPs may have been targeted during domestication. Hence, genes within the linkage disequilibrium of these genetic markers are of high interest for further investigation of the genetics behind the impressive social skills of dogs.

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REFERENCES

Andics A, Gabor A, Gacsi M, Farago T, Szabo D, and Miklosi A. 2016. Neural mechanisms for lexical processing in dogs. Science 353:1030-1032. 10.1126/science.aaf3777

Bentosela M, Wynne CD, D’Orazio M, Elgier A, and Udell MA. 2016. Sociability and gazing toward humans in dogs and wolves: Simple behaviors with broad implications. Journal of the Experimental Analysis of Behavior 105:68-75. 10.1002/jeab.191

Brubaker L, Dasgupta S, Bhattacharjee D, Bhadra A, and Udell MA. 2017. Differences in problem-solving between canid populations: Do domestication and lifetime experience affect persistence? Anim Cogn 20:717-723.

Caniglia R, Fabbri E, Hulva P, Bolfikova BC, Jindrichova M, Stronen AV, Dykyy I, Camatta A, Carnier P, Randi E, and Galaverni M. 2018. Wolf outside, dog inside? The genomic make-up of the Czechoslovakian Wolfdog. BMC Genomics 19. ARTN 533 10.1186/s12864-018-4916-2

Chapman NH, Nato AQ, Jr., Bernier R, Ankenman K, Sohi H, Munson J, Patowary A, Archer M, Blue EM, Webb SJ, Coon H, Raskind WH, Brkanac Z, and Wijsman EM. 2015. Whole exome sequencing in extended families with autism spectrum disorder implicates four candidate genes. Hum Genet. 10.1007/s00439-015-1585-y

Gacsi M, Gyori B, Viranyi Z, Kubinyi E, Range F, Belenyi B, and Miklosi A. 2009. Explaining Dog Wolf Differences in Utilizing Human Pointing Gestures: Selection for Synergistic Shifts in the Development of Some Social Skills. Plos One 4. ARTN e6584 DOI 10.1371/journal.pone.0006584

Hare B, and Tomasello M. 1999. Domestic dogs (Canis familiaris) use human and conspecific social cues to locate hidden food. J Comp Psychol 113:173-177. Doi 10.1037/0735-7036.113.2.173

Hare B, and Tomasello M. 2005. Human-like social skills in dogs? Trends Cogn Sci 9:439-444. 10.1016/j.tics.2005.07.003
Heberlein MTE, Turner DC, Range F, and Virányi Z. 2016. A comparison between wolves, Canis lupus, and dogs, Canis familiaris, in showing behaviour towards humans. *Animal Behaviour* 122:59-66. http://dx.doi.org/10.1016/j.anbehav.2016.09.023

Hori Y, Kishi H, Inoue-Murayama M, and Fujita K. 2013. Dopamine receptor D4 gene (DRD4) is associated with gazing toward humans in domestic dogs (Canis familiaris). *Open Journal of Animal Sciences* 3:54.

Horn L, Huber L, and Range F. 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. *Plos One* 8. ARTN e65296 DOI 10.1371/journal.pone.0065296

Jensen P, Persson ME, Wright D, Johnsson M, Sundman A-S, and Roth LSV. 2016. The Genetics of How Dogs Became Our Social Allies. *Current Directions in Psychological Science* 25:334-338. 10.1177/0963721416657050

Kis A, Bence M, Lakatos G, Pergel E, Turcsan B, Pluijmakers J, Vas J, Elek Z, Bruder I, Foldi L, Sasvari-Szekely M, Miklosi A, Ronai Z, and Kubinyi E. 2014. Oxytocin Receptor Gene Polymorphisms Are Associated with Human Directed Social Behavior in Dogs (Canis familiaris). *Plos One* 9. ARTN e83993 DOI 10.1371/journal.pone.0083993

Konno A, Inoue-Murayama M, Yabuta S, Tonoike A, Nagasawa M, Mogi K, and Kikusui T. 2018. Effect of Canine Oxytocin Receptor Gene Polymorphism on the Successful Training of Drug Detection Dogs. *Journal of Heredity* 1:7.

Lakatos G, Gácsi M, Topál J, and Miklósi Á. 2012. Comprehension and utilisation of pointing gestures and gazing in dog–human communication in relatively complex situations. *Anim Cogn* 15:201-213. 10.1007/s10071-011-0446-x

Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ, Zody MC, Mauceli E, Xie XH, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F, Smith
DR, deJong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, DeCaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SMJ, Sutter NB, Thomas R, Webber C, Lander ES, and Plat BIGS. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438:803-819. Doi 10.1038/Nature04338

Marshall-Pescini S, Colombo E, Passalacqua C, Merola I, and Prato-Previde E. 2013. Gaze alternation in dogs and toddlers in an unsolvable task: evidence of an audience effect. Anim Cogn 16:933-943. Doi 10.1007/S10071-013-0627-X

Marshall-Pescini S, Rao A, Virányi Z, and Range F. 2017. The role of domestication and experience in ‘looking back’ towards humans in an unsolvable task. Sci Rep 7:46636.

Mas S, Bernardo M, Gasso P, Alvarez S, Garcia-Rizo C, Bioque M, Kirkpatrick B, and Lafuente A. 2010. A functional variant provided further evidence for the association of ARVCF with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B:1052-1059. 10.1002/ajmg.b.31073

Miklósi Á, Kubinyi E, Topál J, Gácsi M, Virányi Z, and Csányi V. 2003. A simple reason for a big difference: wolves do not look back at humans, but dogs do. Current Biology 13:763-766.

Miklosi A, Polgardi R, Topal J, and Csanyi V. 2000. Intentional behaviour in dog-human communication: an experimental analysis of "showing" behaviour in the dog. Anim Cogn 3:159-166.

Miklósi Á, and Topál J. 2013. What does it take to become ‘best friends’? Evolutionary changes in canine social competence. Trends Cogn Sci 17:287-294.

Muller CA, Schmitt K, Barber AL, and Huber L. 2015. Dogs can discriminate emotional expressions of human faces. Current Biology 25:601-605. 10.1016/j.cub.2014.12.055

Nagasawa M, Mitsui S, En S, Ohtani N, Ohta M, Sakuma Y, Onaka T, Mogi K, and Kikusui T. 2015. Social evolution. Oxytocin-gaze
positive loop and the coevolution of human-dog bonds. *Science* 348:333-336. 10.1126/science.1261022

Passalacqua C, Marshall-Pescini S, Barnard S, Lakatos G, Valsecchi P, and Previde EP. 2011. Human-directed gazing behaviour in puppies and adult dogs, Canis lupus familiaris. *Animal Behaviour* 82:1043-1050. Doi 10.1016/J.Anbehav.2011.07.039

Persson ME, Roth LSV, Johnsson M, Wright D, and Jensen P. 2015. Human-directed social behaviour in dogs shows significant heritability. *Genes Brain and Behavior* 14:337-344. 10.1111/gbb.12194

Persson ME, Trottier AJ, Belteky J, Roth LSV, and Jensen P. 2017. Intranasal oxytocin and a polymorphism in the oxytocin receptor gene are associated with human-directed social behavior in golden retriever dogs. *Hormones and Behavior* 95:85-93. 10.1016/j.yhbeh.2017.07.016

Persson ME, Wright D, Roth LS, Batakis P, and Jensen P. 2016. Genomic Regions Associated With Interspecies Communication in Dogs Contain Genes Related to Human Social Disorders. *Sci Rep* 6:33439. 10.1038/srep33439

Qayyum A, C CZ, Hirata Y, A KT, Cheema S, Nowrouzi B, Beitchman JH, and Kennedy L. 2015. The Role of the Catechol-o-
Methyltransferase (COMT) Gene Val158Met in Aggressive Behavior, a Review of Genetic Studies. *Curr Neuropharmacol* 13:802-814.

Range F, and Viranyi Z. 2015. Tracking the evolutionary origins of dog-
human cooperation: the "Canine Cooperation Hypothesis". *Frontiers in Psychology* 5. ARTN 1582 10.3389/fpsyg.2014.01582

Sanders AR, Rusu I, Duan J, Vander Molen JE, Hou C, Schwab SG, Wildenauer DB, Martinez M, and Gejman PV. 2005. Haplotypic association spanning the 22q11.21 genes COMT and ARVCF with schizophrenia. *Mol Psychiatry* 10:353-365. 10.1038/sj.mp.4001586
Sim K, Chan WY, Woon PS, Low HQ, Lim L, Yang GL, Lee J, Chong SA, Sitoh YY, Chan YH, Liu J, Tan EC, Williams H, and Nowinski WL. 2012. ARVCF genetic influences on neurocognitive and neuroanatomical intermediate phenotypes in Chinese patients with schizophrenia. *J Clin Psychiatry* 73:320-326. 10.4088/JCP.10m06491

Soproni K, Miklosi A, Topal J, and Csanyi V. 2001. Comprehension of human communicative signs in pet dogs (Canis familiaris). *J Comp Psychol* 115:122-126. Doi 10.1037//0735-7036.115.2.122

Sundman A-S, Persson ME, Grozelier A, Halldén L-L, Jensen P, and Roth LSV. 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*. [https://doi.org/10.1016/j.applanim.2017.12.017](https://doi.org/10.1016/j.applanim.2017.12.017)

Sundman AS, Johnsson M, Wright D, and Jensen P. 2016. Similar recent selection criteria associated with different behavioural effects in two dog breeds. *Genes Brain and Behavior* 15:750-756. 10.1111/gbb.12317

Sutter NB, Eberle MA, Parker HG, Pullar BJ, Kirkness EF, Kruglyak L, and Ostrander EA. 2004. Extensive and breed-specific linkage disequilibrium in Canis familiaris. *Genome Research* 14:2388-2396.

Topal J, Gacsi M, Miklosi A, Viranyi Z, Kubinyi E, and Csanyi V. 2005. Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies. *Animal Behaviour* 70:1367-1375. Doi 10.1016/J.Anbehav.2005.03.025

Topal J, Miklosi A, Csanyi V, and Doka A. 1998. Attachment behavior in dogs (Canis familiaris): A new application of Ainsworth's (1969) Strange Situation Test. *J Comp Psychol* 112:219-229. Doi 10.1037/0735-7036.112.3.219

Udell MA. 2015. When dogs look back: inhibition of independent problem-solving behaviour in domestic dogs (Canis lupus
familiaris) compared with wolves (Canis lupus). *Biology Letters* 11:20150489.

Udell MAR, Ewald M, Dorey NR, and Wynne CDL. 2014. Exploring breed differences in dogs (Canis familiaris): does exaggeration or inhibition of predatory response predict performance on human-guided tasks? *Animal Behaviour* 89:99-105. 
http://dx.doi.org/10.1016/j.anbehav.2013.12.012

van der Waaij EH, Wilsson E, and Strandberg E. 2008. Genetic analysis of results of a Swedish behavior test on German Shepherd Dogs and Labrador Retrievers. *J Anim Sci* 86:2853-2861.
10.2527/jas.2007-0616

Wilsson E, and Sundgren PE. 1998. Behaviour test for eight-week old puppies - heritabilities of tested behaviour traits and its correspondence to later behaviour. *Applied Animal Behaviour Science* 58:151-162. Doi 10.1016/S0168-1591(97)00093-2

vonHoldt BM, Shuldiner E, Koch IJ, Kartzinel RY, Hogan A, Brubaker L, Wanser S, Stahler D, Wynne CDL, Ostrander EA, Sinsheimer JS, and Udell MAR. 2017. Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Science Advances* 3. 10.1126/sciadv.1700398

Xu C, Mullersman JE, Wang L, Bin Su B, Mao C, Posada Y, Camarillo C, Mao Y, Escamilla MA, and Wang KS. 2013. Polymorphisms in seizure 6-like gene are associated with bipolar disorder I: evidence of gene x gender interaction. *J Affect Disord* 145:95-99. 10.1016/j.jad.2012.07.017
Table 1 (on next page)

Ethogram of the behaviours analysed in the unsolvable problem task.

For each behaviour, duration and frequency were scored. The behaviours were not mutually exclusive.
Table 1. Ethogram of the behaviours analysed in the unsolvable problem task. Duration and frequency of the behaviours were scored. Zone behaviours are mutually exclusive.

| Behaviour                  | Description                                                                 |
|----------------------------|-----------------------------------------------------------------------------|
| Experimenter zone          | Dogs’ head is within one body length of the experimenter                    |
| Owner zone                 | Dogs’ head is within one body length of the owner                            |
| Experimenter gaze          | The dog directs its eyes towards the face of the experimenter                |
| Owner gaze                 | The dog directs its eyes towards the face of the owner                       |
| Experimenter physical contact | The dog is in physical contact with the experimenter                        |
| Owner physical contact     | The dog is in physical contact with the owner                                |
| Duration test-setup        | The duration of time (s) the dogs’ head is within one body length of the test-setup |
Table 2 (on next page)

Generalized linear mixed models analysis for behavioural variables and SNP genotype in golden retrievers

The models also included sex and treatment (intranasal oxytocin) as fixed factors.
Table 2. Generalized linear mixed models analysis for behavioural variables and SNP genotype in golden retrievers. The models also included sex and treatment (intranasal oxytocin) as fixed factors.

| Behaviour                      | Df1 | Df2 | SNP1 | Sex | Treatment | SNP1 * sex | SNP1 * treatment | Distribution |
|--------------------------------|-----|-----|------|-----|-----------|------------|------------------|--------------|
| Duration experimenter look     | 1   | 56  | 1,723| 0,188| 7,382     | 0,058      | 0,81            | NA           |
| Frequency experimenter look    | 1   | 56  | 0,575| 0,566| 1,537     | 0,615      | 0,436           | NA           |
| Duration experimenter zone     | 1   | 56  | 0,096| 0,908| 1,742     | 0,488      | 0,488           | NA           |
| Frequency experimenter zone    | 1   | 56  | 0,292| 0,748| 1,112     | 0,085      | 0,771           | NA           |
| Duration experimenter contact  | 1   | 56  | 2,117| 0,13 | 0,569     | 0,454      | 0,013           | NA           |
| Frequency experimenter contact | 1   | 56  | 4,339| 0,018| 0,697     | 0,407      | 9,705           | NA           |
| Frequency owner look           | 1   | 55  | 1,68 | 0,196| 0,006     | 0,938      | 0,274           | NA           |
| Frequency owner look           | 2   | 56  | 1,26 | 0,292| 0,002     | 0,965      | 2,356           | NA           |
| Frequency owner zone           | 1   | 56  | 0,451| 0,639| 6,59      | 0,013      | 1,194           | NA           |
| Frequency owner zone           | 1   | 55  | 1,956| 0,151| 2,197     | 0,144      | 0,292           | NA           |
| Frequency owner contact        | 2   | 56  | 4,389| 0,017| 1,095     | 0,3        | 2,93            | NA           |
| Frequency owner contact        | 2   | 56  | 6,996| 0,002| 3,175     | 0,08       | 8,137           | NA           |
| Duration test-setup            | 2   | 56  | 0,457| 0,636| 3,246     | 0,077      | 0,217           | NA           |

| Behaviour                      | Df1 | Df2 | SNP2 | Sex | Treatment | SNP2 * sex | SNP2 * treatment | Distribution |
|--------------------------------|-----|-----|------|-----|-----------|------------|------------------|--------------|
| Duration experimenter look     | 1   | 55  | 1,023| 0,366| 7,682     | 0,013      | 0,909           | NA           |
| Frequency experimenter look    | 2   | 55  | 3,243| 0,047| 2,957     | 0,771      | 0,384           | NA           |
| Duration experimenter zone     | 2   | 55  | 3,304| 0,044| 1,655     | 0,552      | 0,461           | NA           |
| Frequency experimenter zone    | 1   | 55  | 0,97 | 0,386| 1,185     | 0,051      | 0,822           | NA           |
| Duration          | Frequency          |接触次数 |频率 |时间 |测试次数 | p值 |统计量 |显著性 |参数 |标志 |描述 |
|-------------------|--------------------|--------|-----|-----|---------|-----|-------|-------|-----|-----|------|
| experimenter      | contact            | 1      | 55  | 1,113| 0,336   | 0,099| 0,754 | >0,00 | NA  | NA  | Gamma log |
| Frequency         | experimenter       | 1      | 55  | 2,711| 0,075   | 0,125| 0,725 | 12,30 | 0,001| NA  | Gamma log |
| owner look        |                    | 1      | 55  | 4,477| 0,016   | 0,319| 0,575 | NA    | NA  | NA  | Gamma log |
| Frequency         | owner look         | 2      | 55  | 6,33 | 0,003   | 0,129| 0,72  | 3,658 | 0,061| NA  | Gamma log |
| owner zone        |                    | 1      | 55  | 0,14 | 0,87    | 8,106| 0,006 | 2,42  | 0,126| NA  | Gamma log |
| Frequency         | owner zone         | 1      | 55  | 1,536| 0,224   | 2,347| 0,131 | 1,529 | 0,222| NA  | Gamma log |
| owner contact     |                    | 2      | 55  | 14,80 | >0,00   | 3,802| 0,056 | 5,438 | 0,023| NA  | Gamma log |
| Frequency         | owner contact      | 1      | 55  | 0,028| 0,972   | 4,285| 0,043 | 3,318 | 0,074| NA  | Gamma log |
| test-setup        |                    | 2      | 55  | 0,099| 0,906   | 2,882| 0,095 | 0,483 | 0,490| NA  | Gamma log |
Generalized linear mixed models analysis for behavioural variables and SNP genotype in Labrador retrievers.

The models also included sex and type (common or field) as fixed factors.
Table 3. Generalized linear mixed models analysis for behavioural variables and SNP genotype in Labrador retrievers. The models also included sex and type (common or field) as fixed factors.

| Behaviour                  | Df1  | Df2   | SNP1 | Sex  | Type           | SNP1 * sex       | SNP1 * type | Distribution |
|---------------------------|------|-------|------|------|----------------|------------------|-------------|--------------|
| Duration experimenter look| 2    | 91    | 2,472| 0,09 | 0,043          | 0,837            | 0,09        | Gamma log    |
| Frequency experimenter look| 2    | 91    | 1,719| 0,185| 0,148          | 0,702            | 0,08        | Gamma log    |
| Duration experimenter zone| 1    | 92    | 1,031| 0,351| 0,113          | 0,737            | NA          | Gamma log    |
| Frequency experimenter zone| 1    | 92    | 1,09 | 0,34 | 0,698          | 0,406            | NA          | Gamma log    |
| Duration experimenter contact| 1   | 92    | 1,582| 0,211| 0,047          | 0,828            | NA          | Gamma log    |
| Frequency experimenter contact| 1   | 92    | 1,085| 0,342| 0,131          | 0,719            | NA          | Gamma log    |
| Duration owner look       | 2    | 90    | 0,953| 0,39 | 8,82           | 0,004            | 0,00        | Gamma log    |
| Frequency owner look      | 2    | 91    | 1,242| 0,294| 2,897          | 0,092            | 0,10        | Gamma log    |
| Duration owner zone       | 1    | 92    | 0,026| 0,975| 0,085          | 0,772            | NA          | Normal identity (logged) |
| Frequency owner zone      | 1    | 92    | 1,432| 0,244| 0,029          | 0,866            | NA          | Gamma log    |
| Duration owner contact    | 1    | 90    | 0,034| 0,966| 0,211          | 0,647            | NA          | Gamma log    |
| Frequency owner contact   | 1    | 92    | 0,268| 0,765| 0,177          | 0,675            | NA          | Gamma log    |
| Duration test-setup       | 2    | 92    | 0,020| 0,980| 0,211          | 0,647            | NA          | Gamma log    |

| Behaviour                  | Df1  | Df2   | SNP2 | Sex  | Type           | SNP2 * sex       | SNP2 * type | Distribution |
|---------------------------|------|-------|------|------|----------------|------------------|-------------|--------------|
| Duration experimenter look| 2    | 93    | 3,365| 0,07 | 0,087          | 0,769            | 0,00        | Gamma log    |
| Frequency experimenter look| 2    | 93    | 3,025| 0,085| 0,0  | 0,992          | 0,728        | NA          | Gamma log    |
| Duration experiment zone | 1 94 | 6,252 0,014 0,312 0,578 0,135 0,714 NA | Gamma log |
|--------------------------|------|--------------------------------------------------|-----------|
| Frequency experiment zone| 1 94 | 6,86 0,01 0,643 0,425 4,821 0,031 NA | Gamma log |
| Duration experimenter contact| 1 94 | 3,316 0,072 0,283 0,596 0,462 0,498 NA | Gamma log |
| Frequency experimenter contact| 1 94 | 3,594 0,061 0,398 0,53 0,348 0,557 0,06 | Gamma log |
| Duration owner look | 2 93 | 7,209 0,009 13,99 >0,00 25,59 >0,00 3,509 4 | Gamma log |
| Frequency owner look | 2 93 | 5,209 0,025 1,729 0,192 1,807 0,182 3,947 0,05 | Gamma log |
| Duration owner zone | 1 94 | 5,945 0,017 0,302 0,584 2,387 0,126 3,947 0,05 Normal identity (logged) |
| Frequency owner zone | 1 94 | 13,84 >0,00 0,104 0,747 9 0,002 10,55 | Gamma log |
| Duration owner contact | 1 94 | 1,48 0,227 0,068 0,794 20,86 >0,00 1 | Gamma log |
| Frequency owner contact | 1 94 | 2,879 0,093 0,043 0,836 9,025 0,003 | Gamma log |
| Duration test-setup | 1 94 | 2,942 0,090 0,479 0,491 2,141 0,147 NA | Gamma log |
Figure 1

The unsolvable task

A) Golden retriever dog interacting with the unsolvable task. B) The plastic tray measures 55 x 25 cm, circular wells 7 cm in diameter and the plexiglass lids 10 x 15 cm. The left and right lid can be opened to access the treats. The middle lid cannot be opened, hence making the task unsolvable. Photo credit: Mia E. Persson
Figure 2

Allele frequencies

Allele frequencies for (A) the SNP BICF2G630798942 (SNP1) and (B) the SNP BICF2S23712114 (SNP2) for Labrador retrievers (common and field type), golden retrievers and wolves.
Figure 3

Associations between SNP1 and behaviours in golden retrievers

Associations between BICF2G630798942 (SNP1) and the frequency/duration for behaviours scored in the unsolvable problem task in golden retrievers. There was a significant difference between the genotypes in (A) the frequency of physical contact with the experimenter, and (B) and (C) the frequency and duration of physical contact with the owner. Graphs show mean frequency/duration for each of the genotypes. Error bars show ± 1 SE. * P < 0.05
Figure 4

Associations between SNP1 and behaviours in Labrador retrievers

For the duration of owner gaze there was an interaction between genotype and type. The SNP BICF2G630798942 (SNP1) was associated with owner gaze in the field type but not in the common type. Graph shows mean duration for each of the genotypes. Error bars show ± 1 SE. * P < 0.05
Figure 5

Associations between SNP2 and behaviours in golden retrievers

Associations between the genotype on the SNP BICF2S23712114 (SNP2) and the frequency/duration for behaviours scored in the unsolvable problem task in golden retrievers. There was a significant difference between the genotypes in (A) the duration in the experimenter zone, (B) the frequency of gazing at the experimenter, (C) and (D) the frequency and duration of gazing at the owner, and (E) the duration of physical contact with the owner. Graphs show mean frequency/duration for each of the genotypes. Error bars show ± 1 SE. * P < 0,05
Figure 6

Associations between SNP2 and behaviours in Labrador retrievers

Associations between the genotype on the SNP BICF2S23712114 (SNP2) and the frequency/duration for behaviours scored in the unsolvable problem task in Labrador retrievers. There was a significant difference between the genotypes in (A) the duration in the experimenter zone, (B) the frequency of gazing at the experimenter, (C) and (D) the frequency and duration of gazing at the owner, and (E) the duration of physical contact with the owner. Graphs show mean frequency/duration for each of the genotypes. Error bars show ± 1 SE. * P < 0.05
A) Duration experimenter zone (s)  C) Frequency experimenter zone (count)  B) Duration owner zone (s)

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D) Frequency owner zone (count)  E) Frequency owner gaze (count)  F) Duration owner gaze (s)

SNP2
Interactions between SNP2 and type in Labrador retrievers

For (A) the duration of experimenter gaze, and (B) the frequency of owner gaze, there was an interaction between the SNP BICF2S23712114 (SNP2) and type (common or field) in Labrador retrievers. While genotype was associated with both behaviours in the field type Labradors, they were not associated with the behaviours in the common type. Graphs show mean frequency/duration for each of the genotypes. Error bars show ± 1 SE. * P < 0.05