Genetic Characterization of Dog Personality Traits

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ABSTRACT The genetic architecture of behavioral traits in dogs is of great interest to owners, breeders, and professionals involved in animal welfare, as well as to scientists studying the genetics of animal (including human) behavior. The genetic component of dog behavior is supported by between-breed differences and some evidence of within-breed variation. However, it is a challenge to gather sufficiently large datasets to dissect the genetic basis of complex traits such as behavior, which are both time-consuming and logistically difficult to measure, and known to be influenced by nongenetic factors. In this study, we exploited the knowledge that owners have of their dogs to generate a large dataset of personality traits in Labrador Retrievers. While accounting for key environmental factors, we demonstrate that genetic variance can be detected for dog personality traits assessed using questionnaire data. We identified substantial genetic variance for several traits, including fetching tendency and fear of loud noises, while other traits revealed negligibly small heritabilities. Genetic correlations were also estimated between traits; however, due to fairly large SEs, only a handful of trait pairs yielded statistically significant estimates. Genomic analyses indicated that these traits are mainly polygenic, such that individual genomic regions have small effects, and suggested chromosomal associations for six of the traits. The polygenic nature of these traits is consistent with previous behavioral genetics studies in other species, for example in mouse, and confirms that large datasets are required to quantify the genetic variance and to identify the individual genes that influence behavioral traits.

KEYWORDS canine genetics; genome-wide association; heritability; personality; temperament

DOGS play important roles as companions and helpers for humans, and dog personality influences their ability to carry out these functions (Jones and Gosling 2005), where personality refers to individual consistency in behavioral responsiveness to stimuli and situations. The distinct behavioral predispositions of individual dog breeds clearly indicate a strong genetic component to dog personality, which is further strengthened by estimates of substantial within-breed genetic variance found for a variety of dog behavioral traits across studies (*e.g.*, Wilsson and Sundgren 1997; Saetre *et al.* 2006; Meyer *et al.* 2012; Arvelius *et al.* 2014a; Persson *et al.* 2015).

The majority of dog behavior studies have been carried out on working dogs and have used standardized tests, where the effects of the environment at the time of the test could be clearly characterized. These standardized tests in controlled environments provide estimates of moderate heritability for some tested behaviors, *e.g.*, heritability of "gun shyness" has been estimated at 0.56 (SE 0.09) in Labrador Retrievers (van der Waaij *et al.* 2008). However, the majority of the reported heritability estimates for these traits fall below 0.4 (*e.g.*, Wilsson and Sundgren 1997; Saetre *et al.* 2006; van der Waaij *et al.* 2008; Arvelius *et al.* 2014b), with various management and lifestyle factors (*e.g.*, training practices, Haverbeke *et al.* 2010) shown to affect behavior. Thus, large datasets are required for accurate decomposition of the variance in these traits into genetic and nongenetic components. Generating such datasets requires substantial infrastructure, which, in practice, may be unattainable for most pet dog populations. Thus, even though personality traits are extremely important for the well-being of both the dog and its owner, their heritabilities for pet dogs, usually not subjected to any formalized behavior testing, are still largely unknown.

Genomic methodologies like the genome-wide association study (GWAS) that assess markers across the genome have been used to determine associations between traits and particular genetic variants. However, again substantial datasets are required to identify genomic associations or to obtain genomic predictions when a large number of small genetic effects are involved, as is expected to be the case for behavioral traits (Willis-Owen and Flint 2006). As a result, few genomic analyses have been applied to dog behavior traits so far and thus, little is known about their genetic architecture or the individual genes involved. Variation in a few functional candidate genes (*e.g.*, *DRD4*, *TH*, *OXTR*, *SLC6A*) has been shown to be associated with behavior in dogs (Våge *et al.* 2010; Kubinyi *et al.* 2012; Wan *et al.* 2013; Kis *et al.* 2014). However, these detected associations are only a starting point in the process of understanding the molecular genetic basis of dog behavior.

Thus, the size of available datasets is a limiting factor to the dissection of the variance components of behavioral traits as well as to the characterization of their genetic architecture. An alternative approach to using data from standardized tests would be to exploit the knowledge that pet owners and dog breeders have of their own dogs in everyday situations, in order to accumulate sufficiently large datasets. The size of these datasets could then overcome the lack of standardized assessment and at the same time, avoid possible interactions between the behavior and the somewhat artificial conditions of the test environment.

A survey-based approach has now been utilized in a number of studies on dog behavior, where the dog owner's answers to validated questionnaires, such as the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), were used to assess the personality traits of the dog. C-BARQ was developed at the University of Pennsylvania originally as a method for evaluating and predicting the success of guide dogs (Serpell and Hsu 2001). The reliability and validity of C-BARQ demonstrated by the developers of the method and others (*e.g.*, Hsu and Serpell 2003; Svartberg 2005; Duffy and Serpell 2012) as well as the relationship between C-BARQ responses and standardized test scores (Arvelius *et al.* 2014a) support its use as a tool in behavioral research (Wiener and Haskell 2016). Subsequently, it has been applied in studies of dog behavior by various groups (*e.g.*, Liinamo *et al.* 2007; Kutsumi *et al.* 2013). The C-BARQ survey contains 101 questions regarding the dog's behavioral response to various situations, with answers marked on a five-step scale. The particular items of the C-BARQ questionnaires are then typically grouped into factors describing a personality trait. In most studies (*e.g.*, Arvelius *et al.* 2014a; Asp *et al.* 2015), the grouping of questions and number of resulting traits are largely based on the definitions derived by the developers of the questionnaire (Hsu and Serpell 2003; Duffy and Serpell 2012), who used factor analysis to define 11 (and later, 14) behavioral traits. In a previous study of Labrador Retrievers, we used multivariate statistical techniques to define 12 personality traits from C-BARQ data (Lofgren *et al.* 2014), some of which overlapped the previous grouping while others were novel.

In this paper we used quantitative genetic and genomic approaches to investigate the genetic contribution to everyday life behavior, as assessed by C-BARQ data, in the Labrador Retriever breed.

Methods

Personality trait characterization

The data used in the study were a subset of a larger study on genetics of complex traits in dogs, and consisted of owner-supplied responses to C-BARQ as well as a separate demographic questionnaire. The dataset was limited to UK Kennel Club-registered Labrador Retrievers. We previously applied a combination of Principal Components Analysis and correlation structure to derive 12 behavior traits (subsequently referred to as "SetA traits"): Agitated when Ignored (Agitated), Attention-seeking (Attention), Barking Tendency (Barking), Excitability, Fetching, Human and Object Fear (HOFear), Noise Fear (NoiseFear), Non-owner-directed Aggression (NOAggression), Owner-directed Aggression (OAggression), Separation Anxiety (SepAnxiety), Trainability, and Unusual Behavior (Unusual) (Lofgren *et al.* 2014). The 12 trait values were calculated as averages of the responses observed in each associated group, where the number of questions in the group ranged from 1 (Barking, Fetching) to 20 (Unusual) (supplementary table 3 in Lofgren *et al.* 2014), as long as at least half of the questions were answered (otherwise, the dog's record for that trait was treated as missing). The final dataset used in the current analyses included 1975 animals. The numbers of observations and the range of scores observed for each of the SetA traits are presented in Table 1. Two of the traits (OAggression and SepAnxiety) showed highly skewed distributions, with most dogs showing no evidence of these behavioral characteristics. For comparison, we also calculated values for the 14 traits previously defined for C-BARQ data (subsequently referred to as "SetB traits") (Hsu and Serpell 2003; Duffy and Serpell 2012), for the same dogs as in SetA.

Demographic factors

Factors included as fixed effects and covariates were based on information on management and physical traits recorded from a separate questionnaire sent to the dog owners (Lofgren *et al.* 2014; Sánchez-Molano *et al.* 2014). The fixed effects included sex and neuter status, housing, coat color, health status, exercise per day, and "Role" (based on the activities of the dog), as described in Table 2. The latter was determined using a stringent criterion such that in case of uncertainty, the value was recoded as missing. The age of the dog in days (760–3380 days) was fitted as a covariate. All of these factors were shown to be associated with one or more traits in the previous analysis (Lofgren *et al.* 2014). Records with missing values (either trait values or fixed effects) were removed from the analyses, thus resulting in variable numbers of observations for each trait.

Table 1 Description of the 12 SetA personality traits analyzed in the study

Trait	Pedigree analysis		Genomic analysis	
	Range	No. observations	Range	No. observations
Agitated	1–5	1901	1–5	780
Attention	1–5	1942	1–5	792
Barking	1–5	1955	1–5	795
Excitability	1–5	1962	1–5	777
Fetching	1–5	1953	1–5	798
HOFear	0.7–5	1970	0.73–3.33	776
NoiseFear	1–5	1942	1–5	788
NOAggression	1–3.86	1971	1–3.86	802
OAggression	1–2.43	1967	1–2.14	801
SepAnxiety	1–3	1947	1–2.75	856
Trainability	1–5	1969	2–5	799
Unusual	1–3.55	1968	1–3.55	800

Mixed linear models analysis

The pedigree used in the analysis was spread over 29 generations and included 28,943 dogs: 9040 sires (from 3837 paternal grand-sires and 6524 paternal grand-dams) and 17,975 dams (from 6555 maternal grand-sires and 12,272 maternal grand-dams). Approximately 70% of the sires had only one offspring with phenotypes. The maximum number of phenotyped offspring per sire was 37 (for one sire). As most of the trait distributions were skewed, we first attempted to transform the traits using standard approaches (log, inverse, square root). However, most of the distributions were not improved by transformation and some were considerably worsened. We therefore decided to analyze the untransformed traits.

While other methods (*e.g.*, Bayesian approaches) can be used for heritability estimation of non-normally-distributed traits, the Mixed Linear Model (REML) approach has been shown to be asymptotically consistent, *i.e.*, it approaches the true value for the genetic variance as the size of the dataset increases, independent of trait distributions (Jiang 1996), and furthermore, does not depend on assumptions about prior distributions. In a range of studies of non-normally-distributed traits, REML has performed well in comparison with other methods, both in terms of variance component estimation and predictive accuracy (Olesen *et al.* 1994; Matos *et al.* 1997; Koeck *et al.* 2010; de Villemereuil *et al.* 2013). We therefore implemented REML using ASReml software (Gilmour *et al.* 2009) for heritability estimation.

Univariate analysis: For both SetA and SetB traits, the estimation of the variance components, heritability, and significance of fixed effects was carried out by fitting mixed linear models in ASReml (Gilmour *et al.* 2009). The mixed linear models can be described as:

$$y = X\tau + Zu + e$$

where y is the vector of observations, τ is a vector of fixed effects, X is an incidence matrix referring the observations to fixed effect levels described further below, u is a vector of breeding values treated as random effects, Z is an incidence matrix referring observations to their corresponding random effects, and e is a vector of residual effects, assumed to be normally distributed according to the distribution $N(0, \sigma_e^2 I)$, where σ_e^2 is the residual variance and I is the identity matrix.

The direct additive genetic effect of the dogs was fitted as the only random effect. In the animal model, the vector of random effects u is assumed to be normally distributed according to the distribution $N(0, \sigma_A^2 A)$, where σ_A^2 is the additive genetic variance and A is the pedigree-based numerator relationship matrix. The heritability was estimated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2}$$

The choice of effects included in the best-fitting model was based on their *P*-value. The model was constructed through backward elimination, *i.e.*, by first fitting all effects, followed by stepwise subtraction of the term with highest *P*-value from the model. Model construction was performed separately in each trait, being carried out until all effects included were significant. Thus, the final model was defined as the most comprehensive model in which all fixed effects and covariates had a *P*-value below 0.05.

The analyses were run until the likelihood and parameters converged, which, through a default setting in ASReml, was determined when the variance component estimates changed by no more than 1% between iterations and the change in the likelihood was <0.002*current iteration number (Gilmour *et al.* 2015). The significance of the additive genetic component of the variance was tested via a log-likelihood ratio test, with the parameter deemed significant when twice the difference between the log-likelihood value of the model containing it and a simpler model with no additive variance exceeded 3.84.

Because the log-likelihood ratio tests performed to assess significance of heritability may have been influenced by the non-normal nature of the traits, we carried out an alternative permutation-based approach to assess significance, which was independent of trait distribution. We first fitted a fixed effects model, *i.e.*, including only the relevant fixed effects, to obtain the residuals for each trait (fixed effects were fitted separately so that these did not need to be considered in the permutation process). We then randomized the residuals 100 times with respect to the animal IDs and reran the variance components analysis for each permuted dataset, using the correct pedigree. This procedure randomized the relationship between the traits and pedigree relationships. Thus, we derived a null distribution of h^2 values under the assumption of no effect of pedigree relationships on the phenotype (*i.e.*, $h^2 = 0$), without any assumption of normality. We then compared the actual estimates of h^2 with this distribution, such that significance was concluded if the

Table 2 Description of factors included as fixed effects in genetic models

Factors	Categories	No. observations
Coat color	Black	1144
	Yellow	521
	Chocolate	310
	missing	0
Exercise per day (hr)	<1	315
	1 – <2	972
	2 – <4	565
	≥4	118
	missing	5
Health	Some health problem during lifetime	1697
	No health problems	278
	Missing	0
Housing	Primarily inside	1578
	Both inside and outside	170
	Primarily outside	176
	Missing	51
Role	Gundog	840
	Pet	817
	Showdog	140
	Missing	178
Sex/neutered status	Male entire	451
	Male neutered	59
	Female entire	1028
	Female neutered	426
	Missing	11

These include sex and neuter status (four levels), housing (three levels), coat color (three levels), health status (two levels: healthy or having had some health problem during their lifetime), exercise per day (four levels), and "Role" (three levels).

actual estimate exceeded the 95th percentile of the permutation results.

Bivariate analysis: Genetic and environmental correlations between SetA traits with significant heritabilities were obtained by fitting bivariate models to their records. The general model behind bivariate analyses is similar to that presented in univariate analyses, but with u assumed to be MVN($0, V \otimes A$), where V is a (co)variance matrix of the two trait terms. The fixed effects fitted to each trait in the bivariate analyses were the same as those fitted in the final model derived for each trait in the univariate analyses. The phenotypic, genetic, and environmental correlations were calculated as:

$$r = \frac{\text{cov}_{XY}}{\sqrt{\text{var}_X \, \text{var}_Y}}$$

where cov_{XY} is the covariance between the particular components of traits X and Y, and var_X and var_Y are the given variance components.

Bivariate analyses were also conducted between SetA and SetB traits for which a significant genetic variance was detected in the univariate analyses.

SNP genotyping and marker quality control

The genomic data were collected as part of a larger project (Sánchez-Molano *et al.* 2014, 2015) where genotypes were obtained using the Illumina Canine High Density Beadchip containing 173,662 SNPs (http://www.illumina.com/documents/products/datasheets/datasheet_caninehd.pdf; accessed April 27, 2016). Filtering criteria were previously applied to samples based on call rate and excessive genotyping errors, detected as inconsistencies between the genomic and pedigree relatedness of individuals or between recorded sex and sex determined from the genotyping (Sánchez-Molano *et al.* 2014). Of the 1179 animals that satisfied these quality control criteria, 885 were included in the set of 1975 with C-BARQ assessments and thus were retained for the current study. Filtering criteria were also previously applied to markers (Sánchez-Molano *et al.* 2014). Using Genome Studio software (http://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html; accessed April 27, 2016), 59,260 markers were discarded due to low call rate (<98%), low reproducibility (GenTrain score, GTS, <0.6, where GTS measures the shape of the genotype clusters and their relative distance to each other) and low or confounded signal [ABR mean <0.3, where ABR is the normalized intensity (R) of the heterozygote cluster]. Further quality control was applied using PLINK (Purcell *et al.* 2007), removing markers with low minor allele frequency (MAF <0.01) and subsequently, those showing a significant excess of heterozygotes compared to that predicted under Hardy–Weinberg equilibrium (HWE) (thresholds of $P < 4.59\text{E}{-}7$ for autosomal markers and $P < 1.80\text{E}{-}5$ for X-linked markers, applying Bonferroni correction); regarding deviations from HWE, we made the decision to only exclude markers showing significant excess of heterozygotes since a deficit of heterozygotes may represent a true effect of inbreeding while a highly significant excess of heterozygotes is likely to be an indicator of genotyping errors. The final set of 108,829 autosomal and 2772 X-linked SNPs were assigned genomic positions according to the CanFam 2.0 assembly.

Genomic analyses

Genome-wide association analyses of the SetA traits with significant heritabilities were performed using GEMMA (Zhou and Stephens 2012), accounting for population stratification by fitting the genomic relationship matrix (GRM, G). The linear mixed models were assumed as follows:

$$y = W\alpha + x\beta + u + e,$$

where y is the vector of phenotypes, W is the matrix of covariates with the α vector of associated fixed effects (including the intercept), and x is the vector of marker genotypes (coded as 0/1/2) with β representing the regression coefficient of the marker genotype on the phenotype. The vectors of random polygenic effects, u, and residual errors, e, follow multivariate normal (MVN) distributions given by $u \sim \text{MVN}(0, \sigma_g^2 G)$ and $e \sim \text{MVN}(0, \sigma_e^2 I)$, where σ_g^2 and σ_e^2 are the variances

associated with random polygenic (u) and residual (e) terms, respectively. Fixed effects were determined for each trait separately, based on results from the pedigree-based analysis (described above), with minor changes in coding. Thus, the following effects were used: sex (only for autosomal markers) (two classes), neuter status (two classes), Role (two classes, Gundog and Pet/Showdog) and exercise (covariate, 1–4), health (two classes), housing (covariate, 1–3), and age (covariate). Unlike the pedigree-based analysis, coat color was not included as a fixed effect under the assumption that this factor would be accounted for by markers linked to the genes encoding coat color (i.e., *MC1R*, *TYRP1* genes). It is likely that Role incorporates both genetic and lifestyle factors, based on analysis of genetic structure in this population (unpublished data, P. Wiener and E. Sánchez-Molano). The genomic relationship matrix should account for much of the genetic component. Animals for which one or more fixed effects or covariates were missing were removed from the analysis, such that the number of animals included in the analysis varied across the traits (range: 778–878; analyses of nine of the 12 traits incorporated 802–807 animals) (Table 1). For X-linked markers, analyses were conducted separately for males and females.

The statistical significance for each marker was assessed using a Wald *t*-test. Due to the possibility of inflation of –log(P) as a result of differences in allele frequencies (cryptic population stratification) or genotyping errors, a correction to the P-values by the inflation factor λ was also performed using the method suggested by Amin *et al.* (2007) under the assumption that the inflation is roughly constant across the genome. For X-linked markers, P-values were first calculated separately for males and females. The weighted Z-test was then used to combine these into an overall P-value (Whitlock 2005). Following Bonferroni correction for multiple testing resulting from the large number of markers, significance thresholds (based on the corrected P-values) were $P < 4.480E-7$ for genome-wide ($P < 0.05$) and $P < 8.961E-6$ for suggestive (one false positive per genome scan) levels.

Estimations of the variance explained by the full set or subsets of SNPs were performed in GCTA (Yang *et al.* 2010, 2011) using the same models as for the GWAS. Genetic variances (V_G) explained by the autosomes and X chromosome were calculated separately for each trait (using the "–make-grm" and "–make-grm-xchr" options, respectively). Autosomal and X-linked genomic heritabilities for each trait were reported.

Data availability

Data are available at Dryad repository: doi: 10.5061/dryad.171q5.

Results

Mixed linear models

The number of significant demographic factors affecting a personality trait differed between the SetA traits, ranging from just one significant effect detected for Barking to five effects detected for Unusual (Table 3). The factors with largest impact on personality were Role (11 traits) and sex-neuter status (eight traits). Exercise levels and coat color were also associated with several traits (five and four traits, respectively). Health status, housing, and age were associated with the fewest traits (2, 2, and 1, respectively). The results for Role were similar to those found in the previous study (Lofgren *et al.* 2014), such that Gundogs were generally different from Pets and/or Showdogs, while Pets and Showdogs showed fewer differences, Analysis of the SetB traits showed similar results, with sex-neuter status, Role, and exercise levels having effects on the largest number of traits (Table 3).

The h^2 estimates from the best-fitting models for the SetA traits varied from 0.03 (SE 0.04) for OAggression to 0.38 (SE 0.08) for Fetching (Table 4). Heritabilities >0.20 were found for six traits. Based on the log-likelihood ratio test (LRT), all traits except OAggression and SepAnxiety were found to have genetic variance significantly >0 (Table 4). The permutation test results (Supplemental Material, Table S1 in File S1) were in good agreement with the LRT results with two exceptions: HOFear was significantly different from 0 according to the LRT but not the permuted h^2 values, and SepAnxiety was not significantly different from 0 according to the LRT but it was for the permuted h^2 values. Thus, we conclude that nine traits showed significant heritability, OAggression showed no evidence of genetic variance, and significance could not be confirmed for HOFear or SepAnxiety.

The range of heritability estimates for the SetB traits were somewhat lower than for the SetA traits (Table 4), with similarities between some related traits (e.g., NoiseFear and Nonsocial Fear, NOAggression and Stranger-directed aggression, Unusual and Chasing) but also some notable differences (e.g., SetA_Trainability greater than SetB_Trainability).

Only five out of 36 of the SetA trait pairs were found to be significantly genetically correlated (Table S2 in File S1). Four of these involved Unusual Behavior (with Agitated, NoiseFear, NOAggression, and Trainability); the other significant genetic correlation was for NOAggression – Fetching. The significant correlations were mostly moderate and positive, with the exception of that between Unusual and Trainability. In contrast, more than half of the residual correlations (22 out of 36) between the SetA traits were found to be significant, suggesting shared environmental influences. The residual correlations varied in sign and magnitude, with the strongest negative correlation found for Trainability and Unusual ($r_e = -0.36$, SE 0.06) and the strongest positive correlation found for Excitability and Unusual ($r_e = 0.42$, SE 0.05).

Genetic correlations between SetA and SetB traits are given in Table S3 in File S1 (the analysis failed for the NoiseFear (SetA) – Non-social Fear (SetB) pair due to a singularity in the average information matrix computed by the ASREML algorithm). For some related trait pairs, the genetic correlation was very high (e.g., SetA-SetB: Excitability-Excitability, $r_g = 0.98$, SE 0.01; NOAggression-Stranger-directed-aggression,

Table 3 Summary of fixed effects and covariates found to be significantly associated with personality traits using mixed linear models

Trait	Age	Coat color	Sex/Neuter	Health	Housing	Exercise	Role
SetA							
Agitated		*					***
Attention			***				***
Barking							***
Excitability					*	***	***
Fetching	**	*					***
HOFear			**		*		***
NoiseFear			***				***
NOAggression			*	*		***	***
OAggression			*				**
SepAnxiety		*	***	*		***	
Trainability			*			***	***
Unusual		**	*			**	***
SetB							
Attachment			***			*	*
Chasing			***			**	***
Dog-directed aggression			**				***
Dog-directed fear						**	***
Dog rivalry							
Energy level	**	*	***				
Excitability					**	***	***
Non-social fear			***				***
Owner-directed aggression						*	*
Separation-related behavior			***	*		***	
Stranger-directed aggression			***			***	**
Stranger-directed fear			**				
Touch sensitivity					*		***
Trainability						**	***

$* \, P < 0.05, \; ** P < 0.01, \; *** P < 0.001.$

$r_g = 0.98$, SE 0.03) while it was not as high for others (*e.g.*, Trainability-Trainability, $r_g = 0.55$, SE 0.18). Another notably high genetic correlation was between Unusual (SetA) and Chasing (SetB) ($r_g = 0.88$, SE 0.07).

Genomic analyses

The proportion of the phenotypic variance explained by the full set of SNPs ("genomic heritabilities," considering the total of the autosomal and X-linked estimates), based on a smaller dataset than that of the pedigree-based heritabilities, ranged from 0.00 (Attention, Excitability) to 0.31 (NoiseFear) (Table 4). Nine of the traits showed lower genomic heritabilities than the pedigree-based estimates; for four of these traits, the SNP data explained less than half of the pedigree-based heritability, although for three traits (Barking, NOAggression, and Trainability), the proportion explained by the SNP data was >70% of the pedigree-based heritability. For HOFear, NoiseFear, and OAggression, the genomic heritabilities were higher than the pedigree-based heritabilities, although the differences were small.

GWAS was carried out for the nine SetA traits with pedigree-based heritabilities significantly different from 0 according to both log-likelihood ratio and permutation tests (excluding HOFear, OAggression, and SepAnxiety, Table 4). No SNPs were found to show genome-wide significance; however, we identified 11 SNPs (in eight genomic regions) showing suggestive significance ("suggestive SNPs") for six traits: Agitated (CFA18), Barking (CFA4), Fetching (CFA1, 4 and 22), NoiseFear (CFA20), NOAggression (CFA9), and Unusual (CFA2) (Figure S1 and Table 5). A visual inspection of Quantile-Quantile (Q-Q) plots revealed that the lambda-correction procedure adequately corrected for unexplained population structure in the sample for the autosomes, although it was slightly less effective for the *X* chromosome (Figure S2). The proportion of the variance explained by the individual suggestive SNPs across the genome ranged from 0.022 to 0.043 across the traits (Table 5).

Discussion

The analysis of C-BARQ responses collected from owners of Labrador Retrievers in the UK revealed a significant genetic variance present for most of the behavioral traits examined. The magnitude of the estimates significantly different from 0 (according to both log-likelihood ratio and permutation tests) for the SetA traits ranged between 0.10 (Excitability) and 0.38 (Fetching), showing consistency with the range of heritabilities previously reported for behavioral traits in dogs [Strandberg *et al.* 2005; Saetre *et al.* 2006; Meyer *et al.* 2012; Eken Asp *et al.* 2014; also see review by Hall and Wynne (2012)]. For 9 out of 12 traits, genomic heritabilities were lower than pedigree-based estimates; however, genome-wide

Table 4 Pedigree-based (SetA and SetB) and genomic (SetA) heritability estimates and associated SEs for trait-specific models

Trait (SetA) (number of questions on which it was based)	h^2 (SE)	Genomic h^2 (SE)		Number of questions in common	Traits (SetB) (number of questions on which it was based)	h^2 (SE)
		Autosomal markers	X-linked markers			
Agitated (2)	**0.22 (0.07)**	0.02 (0.03)	**0.03 (0.03)**	2	Attachment (6)	**0.13 (0.06)**
Attention (3)	**0.14 (0.06)**	0.00 (0.04)	0.00 (0.02)	3		
Barking (1)	**0.15 (0.07)**	**0.10 (0.07)**	0.01 (0.02)			
Excitability (5)	**0.10 (0.06)**	0.00 (0.04)	0.00 (0.02)	5	Excitability (6)	**0.11 (0.06)**
Fetching (1)	**0.38 (0.08)**	**0.19 (0.08)**	**0.05 (0.03)**			
HOFear (15)	**0.08 (0.05)**	**0.12 (0.06)**	0.02 (0.02)	4	Stranger-directed fear (4)	**0.14 (0.06)**
				4	Dog-directed fear (4)	**0.07 (0.05)**
NoiseFear (2)	**0.30 (0.08)**	**0.23 (0.07)**	**0.08 (0.03)**	2	Non-social fear (6)	**0.25 (0.08)**
NOAggression (14)	**0.29 (0.08)**	**0.19 (0.07)**	0.02 (0.02)	8	Stranger-directed aggression (9)	**0.26 (0.07)**
				4	Dog-directed aggression (4)	**0.17 (0.07)**
OAggression (7)	0.03 (0.04)	0.04 (0.06)	0.00 (0.02)	7	Owner-directed aggression (8)	0.02 (0.03)
SepAnxiety[a] (8)	0.06 (0.05)	0.00 (0.04)	0.01 (0.02)	8	Separation-related behavior[a] (8)	0.00 (0.02)
Trainability (7)	**0.28 (0.07)**	**0.20 (0.07)**	**0.04 (0.02)**	7	Trainability (8)	**0.15 (0.06)**
Unusual (20)	**0.25 (0.08)**	**0.12 (0.07)**	0.01 (0.02)	3	Chasing (4)	**0.26 (0.07)**
					Dog rivalry (4)	**0.11 (0.06)**
					Energy level (2)	**0.15 (0.06)**
					Touch sensitivity (3)	**0.18 (0.08)**

Fixed effects and covariates fitted as shown in Table 2. Values significantly >0 based on a log-likelihood ratio test shown in bold.
[a] These two traits had the same definition but heritability estimates were slightly different due to different rules regarding treatment of missing values for individual C-BARQ responses.

association analysis identified several genomic regions showing suggestive associations with C-BARQ traits. While C-BARQ has been used in a large number of studies on dog behavior, the genetic analysis of the traits derived from the questionnaire is still in its infancy, with only a handful of heritability estimates published to date (*e.g.,* Liinamo *et al.* 2007; Arvelius *et al.* 2014a). The results presented in this study show that there is a consistency in detection of the genetic variance and detectable genomic associations for traits derived from C-BARQ, but also that quantification of the genetic component of C-BARQ-based traits is sensitive to how these behavioral factors are extracted from the questionnaire responses.

Heritability estimates and trait definition for C-BARQ data

The SetA traits with highest heritability were Fetching and NoiseFear. Our estimate for the latter falls within the range of previous reports based on standardized tests, with heritabilities of "reaction to gunfire" ranging between 0.23 and 0.56 (Ruefenacht *et al.* 2002; van der Waaij *et al.* 2008). The heritability estimate for Non-social fear (SetB) was similar to NoiseFear for this dataset and somewhat lower than found previously for Rough Collies (h^2 = 0.36, SE 0.06) (Arvelius *et al.* 2014a). Thus, it appears that genetic variation for this trait exists in various breeds, including gun dogs.

Fetching was only considered as a separate trait for SetA. In SetB, the question related to fetching ability was included in

Trainability (h^2 = 0.15, SE 0.06). Treating Fetching and Trainability as separate traits resulted in higher heritability estimates for both: h^2 = 0.38 (SE 0.08) for Fetching and h^2 = 0.28 (SE 0.07) for Trainability, with a positive but small genetic correlation between the traits (r_g = 0.26, SE 0.18). Heritabilities for Trainability (SetB) have been previously estimated at 0.15 (SE 0.04) for Rough Collies (Arvelius *et al.* 2014a) and 0.25 (SE 0.04–0.06) across 14 breeds (not including either Labrador Retrievers or Rough Collies) (Eken Asp *et al.* 2014). The genetic correlations between SetA and SetB traits demonstrate the large influence of fetching ability on SetB Trainability for this population such that their estimates are higher for Fetching (SetA) – Trainability (SetB) (r_g = 0.78, SE 0.11) than for Trainability (SetA) – Trainability (SetB) (r_g = 0.55, SE 0.18). These results suggest, at least in Labrador Retrievers, some degree of distinction between the genetic basis for fetching ability and other trainability characteristics, and illustrate the effects of trait grouping on resulting heritability estimates.

Agitated and Attention were considered as separate traits in SetA but together contributed to Attachment in SetB. The heritability estimate for Attention (SetA) was very similar to that for Attachment (SetB), with a high genetic correlation (r_g = 0.86, SE 0.08). The estimate of heritability for Agitated (SetA) was higher than the estimate for Attachment (SetB), with a lower genetic correlation (r_g = 0.62, SE 0.17). These results suggest that there may be differences between the genetic influences on Agitated and Attention.

Table 5 SNPs exceeding suggestive level threshold in genome-wide association analysis

Trait	Chrom	Position[a]	SNP	Effect size (β)[b] (SE)	Corrected P-value	Proportion of variance explained[c]
Agitated	18	50359100	BICF2P964118	−0.2540 (0.05)	2.60e−06	0.028
Barking	4	55645061	BICF2P696817	−0.2256 (0.05)	7.98e−06	0.023
Fetching	1	84905345	BICF2G630792579	−0.2914 (0.06)	7.56e−06	0.031
	4	91287944	BICF2P844921	−0.3270 (0.07)	9.19e−07	0.030
	4	91442298	BICF2P456276	−0.3596 (0.08)	1.88e−06	0.027
	4	91453025	BICF2P73495	−0.3623 (0.08)	1.98e−06	0.027
	4	91475109	BICF2P519369	−0.4003 (0.09)	4.06e−06	0.023
	22	35218609	BICF2S2314224	−0.6587 (0.15)	7.00e−06	0.022
NoiseFear	20	31482825	BICF2P846231	0.3945 (0.09)	6.08e−06	0.043
NOAggression	9	28762604	BICF2G630832223	−0.1210 (0.03)	8.76e−06	0.039
Unusual	2	77975665	BICF2P612229	0.3290 (0.07)	6.74e−06	0.023

[a] SNP positions according to CanFam2.0.
[b] Additive effect of the minor allele.
[c] Calculated using GCTA.

In contrast to aggression directed toward strangers and other dogs, which showed moderate heritability, our estimate of heritability for owner-directed aggression was not significantly different from 0, in accordance with previous reports showing low or no genetic variance, most likely due to strong selection intensity against this trait, particularly in breeds of large size (Duffy *et al.* 2008; Eken Asp *et al.* 2014).

While the questions contained in the C-BARQ questionnaire seem to capture the variance of the behavioral traits, the method of grouping into behavioral factors may influence estimates of heritability, as was shown above for Trainability and also suggested for Agitated. One alternative approach to trait definition could involve grouping questions based on their genetic, rather than phenotypic, covariances. Such an approach has been shown in the context of standardized behavioral tests to improve the estimates of the behavioral dimensions of the temperament test used by the Swedish Armed Forces, especially when items with 0 genetic variance were removed from the factor (Arvelius *et al.* 2014b). Evaluating the genetic variance of individual C-BARQ questions has only been carried out once to our knowledge, based on data for young (6 and 12 months old) guide dog candidates (Schiefelbein 2012). Using a similar approach, it would be interesting to examine the heritabilities of responses to particular questions, as well as their genetic correlations, using data collected from adult dogs.

In considering how to interpret results of genetic studies on behavioral traits, it is important to recognize that dog breeds may differ in terms of the meaningfulness (and thus heritability) of behavioral constructs, as is suggested by differences between heritability estimates for Labrador Retrievers (our study) and Rough Collies (Arvelius *et al.* 2014a), which could be due to differences in breed history or the intensity of selection for specific traits. Depending on the scientific question or practical application, researchers may need to make a choice between using the same trait definitions across breeds but accepting that their meaning differs between breeds or

alternatively, developing breed-specific trait definitions that show similar levels of genetic variation.

Genomic analysis of personality traits

The limited number of molecular genetic studies of canine behavior mainly comprise candidate gene studies or studies targeted at clinical behavioral disorders, which tend to have more clearly defined phenotypes than everyday life behaviors. The few studies using genomic techniques to address everyday life behavior have primarily implemented between-breed comparisons based on breed-average phenotypes (*e.g.*, Jones *et al.* 2008; Vaysse *et al.* 2011; Zapata *et al.* 2016). This approach has limitations in that behavioral and physical traits distinguishing breeds are often confounded, making it difficult to identify which trait is associated with a particular genomic region. Analysis of within-breed genotypic and phenotypic variation, such as in the current study, avoids this problem although the variants (genes) that contribute to behavioral differences within breeds may not be the same as those that account for between-breed behavioral variation.

Based on results in mice, behavioral traits are suspected to be largely polygenic, with a strong environmental component (Flint 2003; Willis-Owen and Flint 2006), thus, difficulties are expected in detecting genomic associations. Our results were consistent with a model of polygenic inheritance for most traits; nevertheless, several suggestive associations were identified, albeit only explaining small proportions of the phenotypic variance. Based on the number of suggestive SNPs within the identified regions, the most convincing genomic association was identified for Fetching (CFA4). The largest effect sizes were seen for Fetching (CFA4 and CFA22) and NoiseFear (CFA20). The finding that pedigree-based heritability estimates were generally higher than genomic estimates was consistent with the common finding of "missing heritability" in other recent studies that have estimated genomic heritability for complex traits and compared them to pedigree-based heritability. A possible explanation for underestimation of the genomic variance is that rare

variants of large effect may not be well-tagged by the analyzed SNPs, supported by the fact that the extent of missing heritability for human height has decreased with increases in sample size and the application of genotype imputation, leading to improved variant characterization (Yang *et al.* 2015). Studies of human traits have also demonstrated that family-based heritability estimates may be inflated when shared environmental factors are not accounted for (Zaitlen *et al.* 2013; Yang *et al.* 2015; Munoz *et al.* 2016), but this is likely to be less of a problem for pedigree dogs as puppies are generally sold and distributed to multiple households and thus spend much less time with their littermates than do human siblings.

Several SNPs showing suggestive associations with the C-BARQ traits were found close to genes with known neurological or behavioral functions. The *TH* (tyrosinase hydroxylase) gene, whose enzyme product is involved in the synthesis of L-DOPA, the precursor of the neurotransmitter dopamine, is located ~1 Mb from the SNP on CFA18 associated with Agitated. Dopamine plays numerous functions and several distinct dopamine pathways are found in the brain. Furthermore, conditions in humans involving inattention and impulsivity, such as attention deficit hyperactivity disorder (ADHD), are associated with decreased dopamine activity (Volkow *et al.* 2009). Polymorphism in *TH* has previously been associated with activity, impulsivity, and inattention in two dog breeds (Kubinyi *et al.* 2012; Wan *et al.* 2013). Studies have also shown an association between *TH* polymorphisms in humans and "neuroticism" (tendency to experience negative emotions) and "extraversion" (characterized by sociability and excitability) (Persson *et al.* 2000; Tochigi *et al.* 2006), two personality traits associated with impulsivity (Whiteside and Lynam 2001).

Genes in the suggestive GWAS peak regions on CFA4 and CFA20 have also been associated with neurological functions. The SNP on CFA4 associated with Barking is located ~5 kb from *CLINT1* (*Epsin 4*), a gene for which mutations have been associated with susceptibility to schizophrenia (Pimm *et al.* 2005). Furthermore, the SNP associated with NoiseFear is located ~0.27 Mb from *CADPS2* on CFA20. *CADPS2* is a member of a gene family encoding calcium-binding proteins that regulate the exocytosis of neuropeptide-encompassing (dense-core) vesicles from neurons and neuroendocrine cells. The gene and its variants have been associated with autism in humans (Cisternas *et al.* 2003; Bonora *et al.* 2014) and with various behavioral and neurological phenotypes in mice (Sadakata *et al.* 2013). An association with noise phobia on CFA20 (position not given) was previously reported for dogs (Hakosalo *et al.* 2015).

Conclusions

The analysis of an owner-evaluated behavioral questionnaire, C-BARQ, together with a questionnaire examining demographic factors, revealed significant genetic variation for most of the behavioral traits studied in a population of Labrador Retrievers. While owner-assessed questionnaires are thus confirmed as a valuable tool in detecting genetic variance in everyday life behaviors of dogs across different lifestyles, it has also been shown that the grouping of the questions into behavioral factors may have a considerable effect on the magnitude of the genetic variance detected. A model of polygenic inheritance with small effect sizes is consistent with most traits investigated in this study. Chromosomal regions associated with some traits were suggested by genomic analyses; however, additional data will be required to fully capture the genomic variance and to confirm and resolve the genomic associations.

Literature Cited

Amin, N., C. M. van Duijn, and Y. S. Aulchenko, 2007 A genomic background based method for association analysis in related individuals. PLoS One 2: e1274.

Arvelius, P., H. E. Asp, W. F. Fikse, E. Strandberg, and K. Nilsson, 2014a Genetic analysis of a temperament test as a tool to select against everyday life fearfulness in Rough Collie. J. Anim. Sci. 92: 4843–4855.

Arvelius, P., E. Strandberg, and W. F. Fikse, 2014b The Swedish Armed Forces temperament test gives information on genetic differences among dogs. J. Vet. Behav. 9: 281–289.

Asp, H. E., W. F. Fikse, K. Nilsson, and E. Strandberg, 2015 Breed differences in everyday behaviour of dogs. Appl. Anim. Behav. Sci. 169: 69–77.

Bonora, E., C. Graziano, F. Minopoli, E. Bacchelli, P. Magini *et al.*, 2014 Maternally inherited genetic variants of CADPS2 are present in autism spectrum disorders and intellectual disability patients. EMBO Mol. Med. 6: 795–809.

Cisternas, F. A., J. B. Vincent, S. W. Scherer, and P. N. Ray, 2003 Cloning and characterization of human CADPS and CADPS2, new members of the Ca2+-dependent activator for secretion protein family. Genomics 81: 279–291.

de Villemereuil, P., O. Gimenez, and B. Doligez, 2013 Comparing parent-offspring regression with frequentist and Bayesian animal models to estimate heritability in wild populations: a simulation study for Gaussian and binary traits. Methods Ecol. Evol. 4: 260–275.

Duffy, D. L., and J. A. Serpell, 2012 Predictive validity of a method for evaluating temperament in young guide and service dogs. Appl. Anim. Behav. Sci. 138: 99–109.

Duffy, D. L., Y. Hsu, and J. A. Serpell, 2008 Breed differences in canine aggression. Appl. Anim. Behav. Sci. 114: 441–460.

Eken Asp, H., P. Arvelius, W. F. Fikse, K. Nilsson, and E. Strandberg, 2014 Genetics of aggression, fear and sociability in everyday life of Swedish dogs. Proceedings, 10th World Congress of Genetics Applied to Livestock Production, Vancouver, Canada.

Flint, J., 2003 Analysis of quantitative trait loci that influence animal behavior. J. Neurobiol. 54: 46–77.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson, 2009 *ASReml User Guide Release 3.0*. VSN International Ltd, Hemel Hempstead, UK.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson, 2015 *ASReml User Guide Release 4.1 Structural Specification*. VSN International Ltd, Hemel Hempstead, UK.

Hakosalo, O., K. Tiira, R. Sarviaho, M. Sillanpaa, J. Kere *et al.*, 2015 Identification of novel candidate genes in canine noise phobia – a model for human phobias. Biol. Psychiatry 77: 80S–81S.

Hall, N. J., and C. D. L. Wynne, 2012 The canid genome: behavioral geneticists' best friend? Genes Brain Behav. 11: 889–902.

Haverbeke, A., C. Rzepa, E. Depiereux, J. Deroo, J.-M. Giffroy *et al.*, 2010 Assessing efficiency of a human familiarisation and training programme on fearfulness and aggressiveness of military dogs. Appl. Anim. Behav. Sci. 123: 143–149.

Hsu, Y. Y., and J. A. Serpell, 2003 Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. J. Am. Vet. Med. Assoc. 223: 1293–1300.

Jiang, J. M., 1996 REML estimation: asymptotic behavior and related topics. Ann. Stat. 24: 255–286.

Jones, A. C., and S. D. Gosling, 2005 Temperament and personality in dogs (Canis familiaris): a review and evaluation of past research. Appl. Anim. Behav. Sci. 95: 1–53.

Jones, P., K. Chase, A. Martin, P. Davern, E. A. Ostrander *et al.*, 2008 Single-nucleotide-polymorphism-based association mapping of dog stereotypes. Genetics 179: 1033–1044.

Kis, A., M. Bence, G. Lakatos, E. Pergel, B. Turcsan *et al.*, 2014 Oxytocin receptor gene polymorphisms are associated with human directed social behavior in dogs (Canis familiaris). PLoS One 9: e83993.

Koeck, A., B. Heringstad, C. Egger-Danner, C. Fuerst, and B. Fuerst-Waltl, 2010 Comparison of different models for genetic analysis of clinical mastitis in Austrian Fleckvieh dual-purpose cows. J. Dairy Sci. 93: 4351–4358.

Kubinyi, E., J. Vas, K. Hejjas, Z. Ronai, I. Bruder *et al.*, 2012 Polymorphism in the Tyrosine Hydroxylase (TH) gene is associated with activity-impulsivity in German Shepherd dogs. PLoS One 7: e30271.

Kutsumi, A., M. Nagasawa, M. Ohta, and N. Ohtani, 2013 Importance of puppy training for future behavior of the dog. J. Vet. Med. Sci. 75: 141–149.

Liinamo, A.-E., L. van den Berg, P. A. J. Leegwater, M. B. H. Schilder, J. A. M. van Arendonk *et al.*, 2007 Genetic variation in aggression-related traits in golden retriever dogs. Appl. Anim. Behav. Sci. 104: 95–106.

Lofgren, S. E., P. Wiener, S. C. Blott, E. Sánchez Molano, J. A. Woolliams *et al.*, 2014 Management and personality in the labrador retriever dogs. Appl. Anim. Behav. Sci. 156: 44–53.

Matos, C. A. P., D. L. Thomas, D. Gianola, M. Perez-Enciso, and L. D. Young, 1997 Genetic analysis of discrete reproductive traits in sheep using linear and nonlinear models. 2. Goodness of fit and predictive ability. J. Anim. Sci. 75: 88–94.

Meyer, F., P. Schawalder, C. Gaillard, and G. Dolf, 2012 Estimation of genetic parameters for behavior based on results of German Shepherd dogs in Switzerland. Appl. Anim. Behav. Sci. 140: 53–61.

Munoz, M., R. Pong-Wong, O. Canela-Xandri, K. Rawlik, C. S. Haley *et al.*, 2016 Evaluating the contribution of genetics and familial shared environment to common disease using the UK Biobank. Nat. Genet. 48: 980.

Olesen, I., M. Perezenciso, D. Gianola, and D. L. Thomas, 1994 A comparison of normal and nonnormal mixed models for number of lambs born in Norwegian sheep. J. Anim. Sci. 72: 1166–1173.

Persson, M. E., L. S. V. Roth, M. Johnsson, D. Wright, and P. Jensen, 2015 Human-directed social behaviour in dogs shows significant heritability. Genes Brain Behav. 14: 337–344.

Persson, M. L., D. Wasserman, E. G. Jonsson, H. Bergman, L. Terenius *et al.*, 2000 Search for the influence of the tyrosine hydroxylase (TCAT)(n) repeat polymorphism on personality traits. Psychiatry Res. 95: 1–8.

Pimm, J., A. McQuillin, S. Thirumalai, J. Lawrence, D. Quested *et al.*, 2005 The Epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. Am. J. Hum. Genet. 76: 902–907.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira *et al.*, 2007 PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81: 559–575.

Ruefenacht, S., S. Gebhardt-Henrich, T. Miyake, and C. Gaillard, 2002 A behaviour test on German Shepherd dogs: heritability of seven different traits. Appl. Anim. Behav. Sci. 79: 113–132.

Sadakata, T., Y. Shinoda, M. Oka, Y. Sekine, and T. Furuichi, 2013 Autistic-like behavioral phenotypes in a mouse model with copy number variation of the CAPS2/CADPS2 gene. FEBS Lett. 587: 54–59.

Saetre, P., E. Strandberg, P. E. Sundgren, U. Pettersson, E. Jazin *et al.*, 2006 The genetic contribution to canine personality. Genes Brain Behav. 5: 240–248.

Sánchez-Molano, E., J. A. Woolliams, R. Pong-Wong, D. N. Clements, S. C. Blott *et al.*, 2014 Quantitative trait loci mapping for canine hip dysplasia and its related traits in UK labrador retrievers. BMC Genomics 15: 833.

Sánchez-Molano, E., R. Pong-Wong, D. N. Clements, S. C. Blott, P. Wiener *et al.*, 2015 Genomic prediction of traits related to canine hip dysplasia. Front. Genet. 6: 97.

Schiefelbein, K. M., 2012 *Estimation of Genetic Parameters for Behavioral Assessment Scores in Labrador Retrievers, German Shepherd Dogs and Golden Retrievers*. Department of Animal Sciences & Industry, College of Agriculture, Kansas State University, Manhattan, Kansas.

Serpell, J. A., and Y. Y. Hsu, 2001 Development and validation of a novel method for evaluating behavior and temperament in guide dogs. Appl. Anim. Behav. Sci. 72: 347–364.

Strandberg, E., J. Jacobsson, and P. Saetre, 2005 Direct genetic, maternal and litter effects on behaviour in German shepherd dogs in Sweden. Livest. Prod. Sci. 93: 33–42.

Svartberg, K., 2005 A comparison of behaviour in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. Appl. Anim. Behav. Sci. 91: 103–128.

Tochigi, M., T. Otowa, H. Hibino, C. Kato, T. Otani *et al.*, 2006 Combined analysis of association between personality traits and three functional polymorphisms in the tyrosine hydroxylase, monoamine oxidase A, and catechol-O-methyltransferase genes. Neurosci. Res. 54: 180–185.

Våge, J., C. Wade, T. Biagi, J. Fatjo, M. Amat *et al.*, 2010 Association of dopamine- and serotonin-related genes with canine aggression. Genes Brain Behav. 9: 372–378.

van der Waaij, E. H., E. Wilsson, and E. Strandberg, 2008 Genetic analysis of results of a Swedish behavior test on German Shepherd dogs and labrador retrievers. J. Anim. Sci. 86: 2853–2861.

Vaysse, A., A. Ratnakumar, T. Derrien, E. Axelsson, G. Rosengren Pielberg *et al.*, 2011 Identification of genomic regions associated with phenotypic variation between dog breeds using selection mapping. PLoS Genet. 7: e1002316.

Volkow, N. D., G.-J. Wang, S. H. Kollins, T. L. Wigal, J. H. Newcorn et al., 2009 Evaluating dopamine reward pathway in ADHD clinical implications. JAMA 302: 1084–1091.

Wan, M., K. Hejjas, Z. Ronai, Z. Elek, M. Sasvari-Szekely et al., 2013 DRD4 and TH gene polymorphisms are associated with activity, impulsivity and inattention in Siberian Husky dogs. Anim. Genet. 44: 717–727.

Whiteside, S. P., and D. R. Lynam, 2001 The five factor model and impulsivity: using a structural model of personality to understand impulsivity. Pers. Individ. Dif. 30: 669–689.

Whitlock, M. C., 2005 Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. J. Evol. Biol. 18: 1368–1373.

Wiener, P., and M. J. Haskell, 2016 Use of questionnaire-based data to assess dog personality. J. Vet. Behav. 16: 81–85.

Willis-Owen, S. A. G., and J. Flint, 2006 The genetic basis of emotional behaviour in mice. Eur. J. Hum. Genet. 14: 721–728.

Wilsson, E., and P. E. Sundgren, 1997 The use of a behaviour test for selection of dogs for service and breeding. 2. Heritability for tested parameters and effect of selection based on service dog characteristics. Appl. Anim. Behav. Sci. 54: 235–241.

Yang, J., B. Benyamin, B. P. McEvoy, S. Gordon, A. K. Henders et al., 2010 Common SNPs explain a large proportion of the heritability for human height. Nat. Genet. 42: 565–569.

Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher, 2011 GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88: 76–82.

Yang, J., A. Bakshi, Z. Zhu, G. Hemani, A. A. E. Vinkhuyzen et al., 2015 Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. Nat. Genet. 47: 1114.

Zaitlen, N., P. Kraft, N. Patterson, B. Pasaniuc, G. Bhatia et al., 2013 Using extended genealogy to estimate components of heritability for 23 quantitative and dichotomous traits. PLoS Genet. 9: e1003520.

Zapata, I., J. A. Serpell, and C. E. Alvarez, 2016 Genetic mapping of canine fear and aggression. BMC Genomics 17: 572.

Zhou, X., and M. Stephens, 2012 Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44: 821–824.

Communicating editor: S. F. Chenoweth