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1 Genetic characterisation of dog personality traits

2

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16

17 **Abstract**

18 Personality or individual consistency in behavioural responsiveness to stimuli and situations,
19 is recognized in a wide range of animal species, including dogs. These traits are important for
20 determining how well a dog fits its role (e.g. as pet or working dog) and can also influence
21 the dog's psychological well-being. The distinct behavioural characteristics of individual dog
22 breeds suggest a strong genetic component to personality in this species and there is also
23 evidence for within-breed variation. However, it is a challenge to gather sufficiently large
24 datasets to dissect the genetic basis of complex traits such as behaviour, which are both time-
25 consuming and logistically difficult to measure, and known to be influenced by non-genetic
26 factors. In this study, we exploited the knowledge that owners have of their own dogs to
27 generate a large dataset of 12 personality traits in Labrador Retrievers, the most popular
28 breed in the UK and various other countries. While accounting for key environmental factors,
29 we demonstrate that genetic variance can be detected for dog personality traits assessed using
30 questionnaire data. We identified substantial genetic variance for several traits, including
31 fetching tendency and fear of loud noises, while other traits, such as owner-directed
32 aggression, revealed negligibly small heritabilities. For comparison, an alternative set of 14
33 traits developed in previous studies were also analysed; differences between the heritabilities
34 of corresponding traits in the two sets indicate that the method of grouping questionnaire data
35 into behavioural factors may influence estimates of heritability. Genomic analyses indicated
36 that these traits are mainly polygenic, such that individual genomic regions have small
37 effects, and suggested chromosomal associations for eight of the traits. Our results
38 demonstrate that dissection of genetic and non-genetic factors that influence dog personality
39 traits can be facilitated using data provided by owners.

40 **Author summary**

41 Unravelling the factors influencing complex biological traits is one of the major goals of
42 modern biology. Behavioural traits are among the most challenging due to the recognised
43 influences of both genetic and non-genetic factors and equally, to the difficulties and costs of
44 assembling sufficiently large sample sizes to provide reasonable statistical power. However,
45 these traits are also among the most interesting in that they are integral to distinguishing
46 species, breeds and individuals from each other. By exploiting the knowledge that dog
47 owners have of their own dogs' behaviour, a large dataset was generated, suitable for genetic
48 investigation. We demonstrated a substantial genetic component associated with a range of
49 personality characteristics assessed using a standard dog behaviour questionnaire and
50 associations with chromosomal regions were suggested for several of the traits.

51

52 **Introduction**

53 The distinct behavioural predispositions of individual dog breeds clearly indicate a strong
54 genetic component to dog personality (understood as the individual consistency in
55 behavioural responsiveness to stimuli and situations; [1]), further strengthened by estimates
56 of substantial within-breed genetic variance found for a variety of behavioural traits across
57 studies.

58 In the past, the majority of dog behaviour studies were carried out on working dogs and used
59 standardized tests, where the effects of the environment at the time of the test could be clearly
60 characterized. These standardized tests in controlled environments provide estimates of
61 moderate heritability for some tested behaviours, e.g. heritability of “gun shyness” has been
62 estimated at 0.56 (SE 0.09) [2]. However, the majority of the reported heritability estimates
63 for these traits fall below 0.4 (e.g. [2-5]), with various management and lifestyle factors being
64 shown to affect behaviour (e.g. training practices, [6]). Thus large datasets are required for
65 accurate decomposition of the variance in these traits into genetic and non-genetic
66 components. Generating such datasets requires substantial infrastructure which, in practice,
67 may be unattainable for most pet dog populations. Thus, even though personality traits are
68 extremely important for the well-being of both the dog and its owner, their heritabilities for
69 pet dogs are still largely unknown.

70 Genomic methodologies like GWAS that assess markers across the genome have been used
71 to determine associations between traits and particular genetic variants. However, substantial
72 datasets are required to identify genomic associations or to use genomic prediction techniques
73 when a large number of small genetic effects are involved, as is expected to be the case for
74 behavioural traits [7]. As a result, few genomic analyses have been applied to dog behaviour
75 traits so far and thus, little is known about the genetic architecture or the individual genes
76 involved. Variation in a few functional candidate genes (e.g. *DRD4*, *TH*, *OXTR*, *SLC6A*) has
77 been shown to be associated with behaviour in dogs ([8-11]). However, these detected
78 associations are only a starting point in the process of understanding the molecular genetic
79 basis of dog behaviour.

80 Thus, the size of available datasets is a limiting factor to the dissection of the variance
81 components of behavioural traits, as well as to the characterisation of their genetic
82 architecture. An alternative approach to using data from standardised tests would be to
83 exploit the knowledge that pet owners and dog breeders have of their own dogs in everyday

84 situations, in order to accumulate large datasets suitable for dissection of behavioural traits.
85 The size of these datasets could then overcome the lack of standardised assessment and at the
86 same time, avoid possible interactions between the behaviour and the somewhat artificial
87 conditions of the test environment.

88 A survey-based approach has been now utilized in a number of studies on dog behaviour,
89 where the dog owner's answers to validated questionnaires, such as Canine Behavioral
90 Assessment and Research Questionnaire (C-BARQ), were used to assess the personality traits
91 of the dog. C-BARQ was developed at the University of Pennsylvania originally as a method
92 for evaluating and predicting the success of guide dogs [12]. The reliability and validity of C-
93 BARQ has been shown by the developers of the method and others (e.g. [13]) and
94 subsequently, it has been applied in studies of dog behaviour by various groups (e.g. [14,
95 15]). The C-BARQ survey contains 101 questions regarding the dog's behavioural response
96 to various situations, with answers marked on a 5-step scale. The particular items of the C-
97 BARQ questionnaires are then typically grouped into factors describing a personality trait. In
98 most studies (e.g. [16, 17]), the grouping and number of resulting traits are largely based on
99 the definitions derived by the developers of the questionnaire [18, 19], who used factor
100 analysis to define 11 (and later, 14) behavioural traits. In a previous study of Labrador
101 Retrievers, we used multivariate statistical techniques to define 12 personality traits from C-
102 BARQ data [20], some of which overlapped the previous grouping while others were novel.
103 In this paper we used quantitative genetic and genomic approaches to investigate the genetic
104 contribution to everyday life behaviour in the Labrador Retriever breed.

105

106 **Methods**

107 Personality trait characterisation

108 The data used in the study were a subset of a larger study on genetics of complex traits in
109 dogs, and consisted of owner-supplied responses to C-BARQ as well as a separate
110 demographic questionnaire. The dataset was limited to UK Kennel Club-registered Labrador
111 Retrievers. We previously applied a combination of Principal Components Analysis and
112 correlation structure to derive 12 behaviour traits (subsequently referred to as "SetA traits"):
113 Agitated when Ignored (Agitated), Attention-seeking (Attention), Barking Tendency
114 (Barking), Excitability, Fetching, Human and Object Fear (HOFear), Noise Fear (NoiseFear),

115 Non-owner-directed Aggression (NOAggression), Owner-directed Aggression
 116 (OAggression), Separation Anxiety (SepAnxiety), Trainability and Unusual Behaviour
 117 (Unusual) [20]. The 12 trait values were calculated as averages of the responses observed in
 118 each associated group, where the number of questions in the group ranged from 1 (Barking,
 119 Fetching) to 20 (Unusual) (Supplementary Table 3 in [20]). The final dataset used in the
 120 current analyses included 1,975 animals. The numbers of observations and the range of
 121 scores observed for each of the SetA traits are presented in Table 1. For comparison, we also
 122 calculated values for the 14 traits previously defined for C-BARQ data (subsequently referred
 123 to as “SetB traits”) [18, 19], for the same data as in SetA.

124

125 Table 1. Description of the 12 SetA personality traits analysed 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

126

127 Demographic factors

128 Factors included as fixed effects and covariates in the mixed linear animal models were based
 129 on information on management and physical traits recorded from a separate questionnaire
 130 sent to the dog owners [20, 21]. The fixed effects included sex and neuter status, housing,
 131 coat colour, health status, exercise per day and “Role” (based on the activities of the dog), as
 132 described in Table 2. The latter was determined using a stringent criterion such that in case of
 133 uncertainty, the value was recoded as missing. The age of the dog in days (760 – 3,380 days)
 134 was fitted as a covariate. Thus, seven demographic factors were fitted in the models (Table
 135 2). All of these factors were shown to be associated with one or more traits in the previous
 136 analysis (Lofgren et al., 2014). Records with missing values (either trait values or fixed

137 effects) were removed from the analyses, thus resulting in variable numbers of observations
 138 for each trait.

139 Table 2. Description of factors included as fixed effects in genetic models. These include sex and
 140 neuter status (four levels), housing (three levels), coat colour (three levels), health status (two levels:
 141 healthy or having had some health problem during their lifetime), exercise per day (four levels) and
 142 “Role” (three levels).

Factors	Categories	No. observations
Coat colour	Black	1144
	Yellow	521
	Chocolate	310
	missing	0
Exercise per day (hours)	<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

143

144 Mixed linear models analysis

145 The pedigree used in the analysis was spread over 29 generations and included 28,943 dogs:
 146 9,040 sires (from 3,837 paternal grand-sires and 6,524 paternal grand-dams) and 17,975 dams
 147 (from 6,555 maternal grand-sires and 12,272 maternal grand-dams). Approximately 70% of

148 the sires had only one offspring with phenotypes. The maximum number of phenotyped
149 offspring per sire was 37 (for one sire).

150 *Univariate Analysis*

151 For both SetA and SetB traits, the estimation of the variance components, heritability and
152 significance of fixed effects was carried out by fitting mixed linear models in ASReml [22].
153 The mixed linear models can be described as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

154 Where \mathbf{y} is the vector of observations, $\boldsymbol{\tau}$ is a vector of fixed effects, \mathbf{X} is an incidence matrix
155 referring the observations pertaining to fixed effect levels described further below, \mathbf{u} is a
156 vector of breeding values treated as random effects, \mathbf{Z} is an incidence matrix referring
157 observations to their corresponding random effects, and \mathbf{e} is a vector of residual effects,
158 assumed to be normally distributed according to the distribution $N(0, \sigma_e^2 \mathbf{I})$, where σ_e^2 is the
159 residual variance and \mathbf{I} is the identity matrix.

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

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

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

170 *Bivariate analysis*

171 Genetic and environmental correlations between SetA traits with $h^2 > 0.2$ were obtained by
172 fitting bivariate models to their records. The general model behind bivariate analyses is
173 similar to that presented in univariate analyses, but with \mathbf{u} assumed to be $MVN(0, \mathbf{V} \otimes \mathbf{A})$,
174 where \mathbf{V} is a (co)variance matrix of the two trait terms. The fixed effects fitted to each trait in

175 the bivariate analyses were the same as those fitted in the final model derived for each trait in
176 the univariate analyses. The phenotypic, genetic and environmental correlations were
177 calculated as:

$$r = \frac{cov_{XY}}{\sqrt{var_X var_Y}}$$

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

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

182

183 SNP genotyping and marker quality control

184 The genomic data was collected as part of a larger project [21, 23] where genotypes were
185 obtained using the Illumina Canine High Density Beadchip containing 173,662 SNPs
186 (http://www.illumina.com/documents/products/datasheets/datasheet_caninehd.pdf; accessed
187 [27/04/16](http://www.illumina.com/documents/products/datasheets/datasheet_caninehd.pdf)). Extraction of DNA from buccal swabs was performed according to standard
188 protocols. DNA was resuspended in water and quantified using a Nanodrop and stored at 4°C
189 until use. Filtering criteria have been previously applied to samples based on call rate and
190 excessive genotyping errors [21]. Of the 1,179 animals that satisfied these quality control
191 criteria, 885 were included in the set of 1,975 with C-BARQ assessments and thus were
192 retained for the current study. Filtering criteria have also been previously applied to markers
193 [21]. Using Genome Studio software
194 ([http://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-](http://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html)
195 [design/genomestudio.html](http://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html); accessed 27/04/16), 59,260 markers were discarded due to low
196 call rate (<98%), low reproducibility (GTS < 0.6) and low or confounded signal (ABR
197 mean < 0.3). Further quality control was applied using PLINK [24], removing SNPs on the
198 sex chromosomes and those deviating from Hardy-Weinberg equilibrium (threshold of
199 $p < 4.48E-7$ applying a Bonferroni correction). Additional quality control involved the
200 removal of markers with low minor allele frequency (MAF < 0.01) in the dataset of 885 dogs.
201 The final set of 103,623 SNPs were assigned genomic positions according to the CanFam 2.0
202 assembly.

203

204 Genomic analyses

205 Genome-wide association analyses of the SetA traits were performed using GEMMA [25],
206 accounting for population stratification by fitting the genomic relationship matrix (GRM, \mathbf{G}).
207 The linear mixed models were assumed as follows:

208

$$209 \mathbf{y} = \mathbf{W}\boldsymbol{\alpha} + \mathbf{x}\boldsymbol{\beta} + \mathbf{u} + \mathbf{e},$$

210

211 where \mathbf{y} is the vector of phenotypes, \mathbf{W} is the matrix of covariates with the $\boldsymbol{\alpha}$ vector of
212 associated fixed effects (including the intercept) and \mathbf{x} is the vector of marker genotypes
213 (coded as 0/1/2) with $\boldsymbol{\beta}$ representing the regression coefficient of the marker genotype on the
214 phenotype. The vectors of random polygenic effects, \mathbf{u} , and residual errors, \mathbf{e} follow
215 multivariate normal (MVN) distributions given by $\mathbf{u} \sim \text{MVN}(0, \sigma_g^2 \mathbf{G})$ and $\mathbf{e} \sim \text{MVN}(0, \sigma_e^2 \mathbf{I})$,
216 where σ_g^2 and σ_e^2 are the variances associated with random polygenic (\mathbf{u}) and residual (\mathbf{e})
217 terms, respectively. Fixed effects were determined for each trait separately, based on results
218 from the pedigree-based analysis (described above), with minor changes in coding. Thus,
219 effects used were: sex (2 degrees of freedom, df), neuter status (2 df), Role (2 df, Gundog and
220 Pet/Showdog) and exercise (1 df). Animals for which one or more fixed effects or covariates
221 were missing were removed from the analysis, such that the number of animals included in
222 the analysis varied across the traits (range: 778-878; analyses of nine of the 12 traits
223 incorporated 802-807 animals) (Table 1).

224

225 The statistical significance for each marker was assessed using a Wald t-test. Due to the
226 possibility of inflation of $-\log(p)$ as a result of differences in allele frequencies (cryptic
227 population stratification) or genotyping errors, a correction to the p-values by the inflation
228 factor λ was also performed using the method suggested by Amin et al. [26] under the
229 assumption that the inflation is roughly constant across the genome. Following Bonferroni
230 correction for multiple testing resulting from the large number of markers, significance
231 thresholds (based on the corrected p-values) were $p < 4.825\text{E-}7$ for genome-wide ($p < 0.05$)
232 and $p < 9.650\text{E-}6$ for suggestive (one false positive per genome scan) levels.

233

234 Estimations of the variance explained by the full set or subsets of SNPs were performed in
235 GCTA [27, 28] using the same models as for the GWAS.

236

237 **Results**

238 Mixed linear models

239 The number of significant demographic factors affecting a personality trait differed between
240 the SetA traits, ranging from just one significant effect detected for Barking to five effects
241 detected for Unusual (Table 3). The factors with largest impact on personality traits were
242 Role (11 traits) and sex-neuter status (8 traits). Exercise levels and coat colour were also
243 associated with several traits (5 and 4 traits, respectively). Health status, housing and age
244 were associated with the fewest traits (2, 2 and 1, respectively). Analysis of the SetB traits
245 showed similar results, with sex-neuter status, Role and exercise levels having effects on the
246 largest number of traits (Table 3).

247

248 Table 3. Summary of fixed effects and covariates found to be significantly ($p < 0.05$) associated with
 249 personality traits using mixed linear models.

Trait	Factor						
SetA	Age	Coat colour	Gender/ Neuter	Health	Housing	Exercise	Role
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						√	√

250

251 The h^2 estimates from the best-fitting models for the SetA traits varied from 0.03 (SE 0.04)
252 for OAggression to 0.38 (SE 0.08) for Fetching (Table 4). Heritabilities greater than 0.20
253 were found for six traits (shown in Table 4 in bold). All traits except OAggression and
254 SepAnxiety were found to have genetic variance significantly greater than 0.

255 Table 4. Pedigree-based (SetA and SetB) and genomic (SetA) heritability estimates and associated standard errors for trait-specific models (fixed effects and
 256 covariates fitted as shown in Table 2). Values ≥ 0.20 shown in bold.

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)
Agitated (2)	0.22 (0.07)	0.02 (0.03)	2	Attachment (6)	0.13 (0.06)
Attention (3)	0.14 (0.06)	0.00 (0.05)	3		
Barking (1)	0.15 (0.07)	0.10 (0.07)			
Excitability (5)	0.10 (0.06)	0.00 (0.05)	5	Excitability (6)	0.11 (0.06)
Fetching (1)	0.38 (0.08)	0.18 (0.08)			
HOFear (15)	0.08 (0.05)	0.13 (0.06)	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)	2	Non-social fear (6)	0.25 (0.08)
NOAggression (14)	0.29 (0.08)	0.20 (0.07)	8	Stranger-directed aggression (9)	0.26 (0.07)
			4	Dog-directed aggression (4)	0.17 (0.07)
OAggression (7)	0.03 (0.04)	0.05 (0.06)	7	Owner-directed aggression (8)	0.02 (0.03)
SepAnxiety* (8)	0.06 (0.05)	0.00 (0.04)	8	Separation-related behaviour* (8)	0.00 (0.02)
Trainability (7)	0.28 (0.07)	0.20 (0.07)	7	Trainability (8)	0.15 (0.06)
Unusual (20)	0.25 (0.08)	0.11 (0.07)	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)

257

258 * These two traits had the same definition but heritability estimates were slightly different due to different rules regarding treatment of missing values for
 259 individual CBARQ responses.

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

264 Only six out of 44 of the SetA trait pairs were found to be significantly genetically correlated
265 (Supplementary Table S1; the genetic correlation for NoiseFear-HOFear could not be
266 estimated due to a singularity in the average information matrix computed by the ASREML
267 algorithm). Four of these involved Unusual Behaviour (with Agitated, NoiseFear,
268 NOAggression and Trainability). The other significant genetic correlations involved
269 NOAggression (with Fetching and HOFear). The significant correlations were mostly
270 moderate and positive, with the exception of that between Unusual and Trainability. In
271 contrast, more than half of the residual correlations (28 out of 44) between the SetA traits
272 were found to be significant, suggesting shared environmental influences. The residual
273 correlations varied in sign and magnitude, with the strongest negative correlation found for
274 Trainability and Unusual ($r_e = -0.36$, SE 0.06) and the strongest positive correlation found for
275 Excitability and Unusual ($r_e = 0.42$, SE 0.05).

276 Genetic correlations between SetA and SetB traits are given in Supplementary Table S2 (the
277 analysis failed for the NoiseFear (SetA) - Non-social Fear (SetB) pair due to a singularity in
278 the average information matrix computed by the ASREML algorithm). For some related trait
279 pairs, the genetic correlation was very high (e.g. SetA-SetB: Excitability-Excitability,
280 $r_g = 0.98$, SE 0.01; NOAggression-Stranger-directed-aggression, $r_g = 0.98$, SE 0.03) while it
281 was not as high for others (e.g. Trainability-Trainability, $r_g = 0.55$, SE 0.18). Another notable
282 estimate was between Unusual (SetA) and Chase (SetB) ($r_g = 0.88$, SE 0.07).

283

284 Genomic Analyses

285 The proportion of the phenotypic variance explained by the full set of SNPs (“genomic
286 heritabilities”), based on a smaller dataset than that of the pedigree-based heritabilities,
287 ranged from 0.00 (Attention, Excitability, SepAnxiety; SE ~0.04) to 0.23 (NoiseFear; SE
288 0.07) (Table 4). Ten of the traits showed lower genomic heritabilities than the pedigree-based
289 estimates; for the majority of these traits, the SNP data explained less than half of the
290 pedigree-based heritability, although for two traits (Trainability and NoiseFear), the SNP data

291 explained >70% of the pedigree-based heritability. For HOFear and OAggression, the
292 genomic heritabilities were higher than the pedigree heritabilities, although the differences
293 were not significant.

294 GWAS detected one genome-wide significant SNP for SepAnxiety (CFA3:94,526,955,
295 $\beta=0.3279$, SE 0.06081) (Figure 1). We also identified 27 SNPs (in 16 genomic regions)
296 showing suggestive significance (“suggestive SNPs”) for eight out of the twelve SetA traits:
297 Agitated (CFA18), Barking (CFA4), Fetching (CFA1, 4 and 22), NoiseFear (CFA20),
298 NOAggression (CFA9), OAggression (CFA5, 14, 28 and 31), SepAnxiety (CFA3, 20) and
299 Unusual (CFA2) (Table 5; Supplementary Figure 1). A visual inspection of Quantile-Quantile
300 (Q-Q) plots revealed that the lambda-correction procedure adequately corrected for
301 unexplained population structure in the sample (Supplementary Figure 2).

302 The genomic region bounded by the significant and suggestive SNPs on CFA3 explained
303 ~0.03 of the phenotypic variance of SepAnxiety (approximately half of the pedigree-based
304 heritability), despite its estimated genomic heritability of 0.00. However, the significant SNP
305 has a very low minor allele frequency (0.01) and the minor homozygote is absent (also true
306 for the other two SNPs in this region), which may have compromised the estimate of its
307 effect size. The proportion of the variance explained by the individual suggestive SNPs
308 across the genome ranged from 0.022 to 0.041 across the traits (Table 5).

309

310 **Figure 1.** Results from genome-wide association analysis of SepAnxiety. A. $-\log(p)$ values
311 for all SNPs across the genome. The genome-wide threshold (red line) corresponds to the
312 Bonferroni correction for a nominal P-value = 0.05. The suggestive threshold (blue line)
313 corresponds to one false positive per genome scan. B. Q-Q plot of Expected versus Observed
314 p-values.

315
316Table 5. SNPs exceeding suggestive level threshold in genome-wide association analysis (SNP showing genome-wide significance shown in **bold**).

Trait	Chrom	Position*	SNP	Effect size (β) [‡] (SE)	Corrected p-value	Proportion of variance explained [§]
Agitated	18	50359100	BICF2P964118	-0.2541 (0.05)	2.22e-06	0.029
Barking	4	55645061	BICF2P696817	-0.2251 (0.05)	8.52e-06	0.029
Fetching	1	84905345	BICF2G630792579	-0.2925 (0.06)	6.60E-06	0.029
	4	91287944	BICF2P844921	-0.3267 (0.07)	9.58E-07	0.031
	4	91442298	BICF2P456276	-0.3600 (0.08)	1.91E-06	0.029
	4	91453025	BICF2P73495	-0.3627 (0.08)	1.99E-06	0.029
	4	91475109	BICF2P519369	-0.4005 (0.09)	4.19E-06	0.026
	22	35218609	BICF2S2314224	-0.6586 (0.15)	7.01E-06	0.023
NoiseFear	20	31482825	BICF2P846231	0.3961 (0.09)	5.86e-06	0.028
NOAggression	9	28762604	BICF2G630832223	-0.1212 (0.03)	8.67e-06	0.027
OAggression	5	19381324	BICF2S2362330	0.1174 (0.03)	4.98E-06	0.025
	5	19420165	BICF2P935231	0.08785 (0.02)	1.30E-06	0.030
	5	39447402	BICF2G630184924	0.06829 (0.01)	1.94E-06	0.030
	5	41496464	BICF2G630186215	0.05384 (0.01)	5.09E-06	0.027
	5	41528401	BICF2G630186251	0.0530 (0.01)	6.61E-06	0.026
	5	41575639	BICF2G630186301	0.05375 (0.01)	4.79E-06	0.027
	5	41596934	BICF2G630186303	0.05247 (0.01)	7.32E-06	0.026
	5	41690223	BICF2G630186310	0.05613 (0.01)	2.43E-06	0.028
	14	22804558	BICF2P319167	0.1186 (0.03)	6.86E-06	0.024
	21	45731572	BICF2P1339075	0.1437 (0.03)	1.36E-06	0.041
	28	11671986	BICF2S23541632	0.0732 (0.02)	6.71E-06	0.025
	31	32326939	BICF2G630739766	0.0932 (0.02)	8.72E-06	0.023
SepAnxiety	3	93609499	BICF2P186901	0.2848 (0.06)	2.75E-06	0.023
	3	94469321	BICF2S2323991	0.2847 (0.06)	2.76E-06	0.023
	3	94526955	BICF2G630362033	0.3279 (0.06)	2.52E-08	0.033
	20	15556881	BICF2P1395346	0.1840 (0.04)	3.81E-06	0.022
Unusual	2	77975665	BICF2P612229	0.3294 (0.07)	6.65e-06	0.027

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*SNP positions according to CanFam2.0

‡ Additive effect of the minor allele

§ Proportion of variance explained: $2pq\beta^2/\sigma_p^2$, where p (q) = minor (major) allele frequency, σ_p^2 = phenotypic variance (these values are very similar to those estimated using GCTA)

322 Discussion

323 The analysis of C-BARQ answers collected from owners of Labrador Retrievers in the UK
324 revealed a significant genetic variance present for most of the behavioural traits examined.
325 The magnitude of the estimates significantly different from 0 for the SetA traits ranged
326 between 0.08 (HOFear) and 0.38 (Fetching), showing consistency with the range of
327 heritabilities previously reported for behavioural traits in dogs [4, 29-31] (see also review by
328 [32]). For most traits, genomic heritabilities were considerably lower than pedigree-based
329 estimates, however, genome-wide association analysis identified several genomic regions
330 showing suggestive associations with C-BARQ traits. While C-BARQ has been used in a
331 large number of studies on dog behaviour, the genetic analysis of the traits derived from the
332 questionnaire is still in its infancy, with only a handful of heritability estimates published to
333 date (e.g. [15, 16]). The results presented in this study show that there is a consistency in
334 detection of the genetic variance and detectable genomic associations for traits derived from
335 C-BARQ, but also that quantification of the genetic component of C-BARQ-based traits is
336 sensitive to how these behavioural factors are extracted from the questionnaire responses.

337

338 Heritability estimates and trait definition for C-BARQ data

339 The SetA traits with highest heritability were Fetching and NoiseFear. Our estimate for the
340 latter falls within the range of previous reports based on standardised tests, with heritabilities
341 of “reaction to gunfire” ranging between 0.23 and 0.56 [2, 33]. The heritability estimate for
342 Non-social fear (SetB) was similar to NoiseFear for this dataset and somewhat lower than
343 found previously for Rough Collies ($h^2=0.36$, SE 0.06) [16]. Thus it appears that genetic
344 variation for this trait exists in various breeds, including gun dogs.

345 Fetching was only considered as a separate trait for SetA. In SetB, the question related to
346 fetching ability was included in Trainability ($h^2=0.15$, SE 0.06). Treating Fetching and
347 Trainability as separate traits resulted in higher heritability estimates for both: $h^2=0.38$ (SE
348 0.08) for Fetching and $h^2=0.28$ (SE 0.07) for Trainability, with a positive but small genetic
349 correlation between the traits ($r_g=0.26$, SE 0.18). Heritabilities for Trainability (SetB) have
350 been previously estimated at 0.15 (SE 0.04) for Rough Collies [16] and 0.25 (SE 0.04–0.06)
351 across 14 breeds (not including either Labrador Retrievers or Rough Collies) [29]. The
352 genetic correlations between SetA and SetB traits demonstrate the large influence of fetching
353 ability on SetB Trainability for this population such that they are higher for Fetching (SetA) –

354 Trainability (SetB) ($r_g = 0.78$, SE 0.11) than for Trainability (SetA) – Trainability (SetB) (r_g
355 $= 0.55$, SE 0.18). These results suggest, at least in Labrador Retrievers, some degree of
356 distinction between the genetic basis for fetching ability and other trainability characteristics.

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

363 In contrast to the above-mentioned traits, Unusual (SetA) was constructed from a much larger
364 number of questions (20) than any of the SetB traits. The significant genetic correlations
365 between Unusual and several other SetA traits confirm the multidimensionality of this trait.
366 Its estimate of heritability of 0.25 (SE 0.08) invites further investigation, as the questions
367 incorporated in this trait cover a wide range of behaviours and not all were expected to share
368 genetic variance. However, several of the questions that are included in Unusual and show
369 substantial variation between dogs refer to chasing behaviours, thus the genetic variance may
370 largely reflect these characteristics. This is supported by a large, significant and positive
371 genetic correlation between Unusual and Chasing (SetB) ($r_g = 0.88$, SE 0.07).

372 Moderate heritability estimates were found for several other SetA traits, including
373 NOAggression, which included questions related to aggression towards unfamiliar humans as
374 well as dogs. Its heritability was very similar to that for Stranger-directed aggression (SetB)
375 and further, the two traits showed a high genetic correlation, not significantly different from 1
376 ($r_g = 0.98$, SE 0.03). The C-BARQ questions relating to aggression, particularly aggression
377 directed toward strangers, show good consistency across studies [16, 19, 34]. Aggressive
378 behaviours in dogs have been shown to fall into different categories based on the target, e.g.
379 owner, child, stranger or dog [35]. Moderate heritability detected for NOAggression and
380 Stranger-directed Aggression are similar to estimates for Stranger-directed Aggression in
381 Rough Collies ($h^2 = 0.24$, SE 0.05) [16] and other breeds ($h^2 \sim 0.21$, SE not given) [29]. In
382 contrast to aggression directed towards strangers and other dogs, our estimate of heritability
383 for owner-directed aggression was not significantly different from 0, in accordance with
384 previous reports showing low or no genetic variance, most likely due to strong selection
385 intensity against this trait, particularly in breeds of large size [29, 36].

386 While the questions contained in the C-BARQ questionnaire seem to capture the variance of
387 the behavioural traits, the method of grouping into behavioural factors may influence
388 estimates of heritability, as was shown above for Trainability and also suggested for Agitated.
389 One alternative approach to trait definition could involve grouping questions based on their
390 genetic, rather than phenotypic, covariances. Such an approach has been shown in the context
391 of standardised behavioural tests to improve the estimates of the behavioural dimensions of
392 the temperament test used by the Swedish Armed Forces, especially when items with 0
393 genetic variance were removed from the factor [3]. Evaluating the genetic variance of
394 individual C-BARQ questions has only been carried out once to our knowledge, based on
395 data for young (6 and 12 months old) guide dog candidates [37]. Using a similar approach, it
396 would be interesting to examine the heritabilities of particular questions, as well as their
397 genetic correlations, using data collected from adult dogs.

398 In considering how to interpret results of genetic studies on behavioural traits, it is important
399 to recognise that dog breeds may differ in terms of the meaningfulness (and thus heritability)
400 of behavioural constructs, as is suggested by differences between heritability estimates for
401 Labrador Retrievers (our study) and Rough Collies [16], which could be due to differences in
402 breed history or the intensity of selection for specific traits. Depending on the scientific
403 question or practical application, researchers may need to make a choice between using the
404 same trait definitions across breeds but accepting that their meaning differs between breeds or
405 alternatively, developing breed-specific trait definitions that show similar levels of genetic
406 variation.

407 Along with illustrating how trait definition may influence estimates of genetic variance,
408 results from this study emphasize the important role of lifestyle and management factors on
409 behavioural traits. Due to the strong associations with these factors, it would be prudent in the
410 future to develop a standardized questionnaire detailing the possible sources of the
411 environmental effects on the dog's behaviour; this could accompany the C-BARQ
412 questionnaire and would allow a more standardized decomposition of the trait variance across
413 populations.

414

415 Genomic regions showing associations with personality traits

416 The limited number of molecular genetic studies of canine behaviour mainly comprise
417 candidate gene studies or studies targeted at clinical behavioural disorders, which tend to

418 have more clearly defined phenotypes than everyday life behaviours. The few studies using
419 genomic techniques to address everyday life behaviour have primarily implemented between-
420 breed comparisons based on breed-average phenotypes (e.g. [38, 39]). This approach has
421 limitations in that behavioural and physical traits distinguishing breeds are often confounded,
422 making it difficult to identify which trait is associated with a particular genomic region.
423 Analysis of within-breed genotypic and phenotypic variation avoids this problem although
424 the variants (genes) that contribute to behavioural differences within breeds may not be the
425 same as those that account for between-breed behavioural variation.

426 Based on results in mice, behavioural traits are suspected to be largely polygenic, with a
427 strong environmental component [7, 40], thus, difficulties are expected in detecting genomic
428 associations. Our results were consistent with a model of polygenic inheritance for most
429 traits, nevertheless, one significant association and several suggestive associations were
430 identified, albeit only explaining small proportions of the phenotypic variance. Based on the
431 shape of the GWAS peaks (i.e. the number of suggestive SNPs within the identified regions),
432 the most convincing genomic associations were identified for Fetching (CFA4) and
433 OAggression (CFA5). The largest effect sizes were seen for Fetching (CFA4 and CFA22)
434 and NoiseFear (CFA20).

435 Although SepAnxiety showed very low genetic variance, the genomic analyses indicated a
436 significant association with the CFA3 region, which may be due to limitations of heritability
437 estimation if the assumptions of the infinitesimal model are not met and the effect size is
438 small. Alternatively this discrepancy may reflect a problem of estimation of effect size for
439 such a rare haplotype, i.e. a false positive association. A similar situation may apply to
440 OAggression, for which the estimated heritability was not significantly greater than 0 but
441 several markers on CFA5 showed suggestive associations; however, minor allele frequencies
442 for these markers (4-6%) were greater than those associated with SepAnxiety, such that
443 estimates of effect sizes will have been more accurate. Additional data will be required to
444 confirm and resolve the identified genomic associations.

445 Several SNPs showing suggestive or significant associations with the CBARQ traits were
446 found close to genes with known neurological or behavioural functions. The *TH* (tyrosinase
447 hydroxylase) gene, whose enzyme product is involved in the synthesis of L-DOPA, the
448 precursor of the neurotransmitter dopamine, is located ~1 Mb from the SNP on CFA18
449 associated with Agitated. Dopamine plays numerous functions and several distinct dopamine

450 pathways are found in the brain. Furthermore, conditions in humans involving inattention and
451 impulsivity, such as attention deficit hyperactivity disorder (ADHD), are associated with
452 decreased dopamine activity [41]. Polymorphism in *TH* has previously been associated with
453 activity, impulsivity and inattention in two dog breeds [9, 11]. Studies have also shown an
454 association between *TH* polymorphisms in humans and “neuroticism” (tendency to
455 experience negative emotions) and “extraversion” (characterized by sociability and
456 excitability) [42, 43], two personality traits associated with impulsivity [44].

457 Genes in the suggestive or significant GWAS peak regions on CFA3, CFA4 and CFA20 have
458 also been associated with neurological functions. *CPLX1*, which encodes complexin 1, is
459 located in the CFA3 region associated with SepAnxiety (~49 kb from the genome-wide
460 significant SNP). Complexins are cytoplasmic neuronal proteins that regulate
461 neurotransmitter release [45]. Complexin genes have been proposed as candidate loci for
462 associations with psychiatric disease due to their neurobiological functions [46] and knock-
463 out mice lacking complexins have been shown to exhibit unusual behaviours [47, 48]. The
464 SNP on CFA4 associated with Barking is located ~5 kb from *CLINT1* (*Epsin 4*), a gene for
465 which mutations have been associated with susceptibility to schizophrenia [49]. Finally, the
466 SNP associated with NoiseFear is located ~0.27 Mb from *CADPS2* on CFA20. *CADPS2* is a
467 member of a gene family encoding calcium binding proteins that regulate the exocytosis of
468 neuropeptide-encompassing (dense-core) vesicles from neurons and neuroendocrine cells.
469 The gene and its variants have been associated with autism in humans [50, 51] and with
470 various behavioural and neurological phenotypes in mice [52]. An association with noise
471 phobia on CFA20 (position not given) was previously reported for dogs [53].

472

473 **Conclusions**

474 The analysis of an owner-evaluated behavioural questionnaire, C-BARQ, together with a
475 questionnaire examining demographic factors, revealed significant genetic variation for most
476 of the behavioural traits studied in a population of Labrador Retrievers. While C-BARQ
477 questionnaires are thus confirmed as a valuable tool in detecting genetic variance in everyday
478 life behaviours of dogs across different lifestyles, it has been shown that the grouping of the
479 questions into behavioural factors may have a considerable effect on the magnitude of the
480 genetic variance detected. Further work is needed to devise the optimal method of extracting
481 information about the genetic background of the behaviours from the questionnaire

482 responses. A model of polygenic inheritance with small effect sizes is consistent with most
483 traits investigated in this study. Chromosomal regions associated with some traits were
484 suggested by genomic analyses, however, additional data will be required to confirm and
485 resolve the genomic associations.

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491 assistance.

492

493 **Supplementary material**

494

495 Figure S1. Results from genome-wide association analysis of all 12 personality traits: $-\log(p)$
496 values for all SNPs across the genome. The genome-wide threshold (red line) corresponds to
497 the Bonferroni correction for a nominal P-value = 0.05. The suggestive threshold (blue line)
498 corresponds to one false positive per genome scan.

499

500 Figure S2. Results from genome-wide association analysis of all 12 personality traits: Q-Q
501 plot of Expected versus Observed p-values.

502

503 Table S1. Results from bivariate analysis for pairs of SetA traits with significant genetic
504 variance determined by univariate analyses (the genetic correlation for NoiseFear-HOFear
505 could not be estimated, see text); factors included in the model were the same as those fitted
506 in the final models derived for each trait in the univariate analyses (see Table 4). Above
507 diagonal: additive genetic correlations (standard errors); below diagonal: residual correlations
508 (standard errors). Those shown in bold are significantly greater than 0 ($p < 0.05$).

509

510 Table S2. Genetic correlations between SetA and SetB traits with significant genetic variance
511 determined by univariate analyses (the genetic correlation for NoiseFear-Non-social Fear
512 could not be estimated, see text); factors included in the model were the same as those fitted
513 in the final models derived for each trait in the univariate analyses (see Table 4). Those
514 shown in bold are significantly greater than 0 ($p < 0.05$).

515

516

517

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