Behav Genet (2017) 47:88–101
DOI 10.1007/s10519-016-9815-1

ORIGINAL RESEARCH

Genetics of Interactive Behavior in Silver Foxes (*Vulpes vulpes*)

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Received: 1 December 2015 / Accepted: 27 August 2016 / Published online: 18 October 2016

Abstract Individuals involved in a social interaction exhibit different behavioral traits that, in combination, form the individual's behavioral responses. Selectively bred strains of silver foxes (*Vulpes vulpes*) demonstrate markedly different behaviors in their response to humans. To identify the genetic basis of these behavioral differences we constructed a large F_2 population including 537 individuals by cross-breeding tame and aggressive fox strains. 98 fox behavioral traits were recorded during social interaction with a human experimenter in a standard four-step test. Patterns of fox behaviors during the test were evaluated using principal component (PC) analysis. Genetic mapping identified eight unique significant and suggestive QTL. Mapping results for the PC phenotypes from different test steps showed little overlap suggesting that different QTL are involved in regulation of behaviors exhibited in different behavioral contexts. Many individual behavioral traits mapped to the same genomic regions as PC phenotypes. This provides additional information about specific behaviors regulated by these loci. Further, three pairs of epistatic loci were also identified for PC phenotypes suggesting more complex genetic architecture of the behavioral differences between the two strains than what has previously been observed.

Keywords Behavior genetics · Social behavior · Quantitative trait loci · Domestication · Aggression · Epistasis · *Vulpes vulpes* · *Canis familiaris*

Edited by Stephen Maxson.

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Electronic supplementary material The online version of this article (doi:10.1007/s10519-016-9815-1) contains supplementary material, which is available to authorized users.

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Introduction

The heritability of inter-individual differences in aggression and affiliation has been established in many mammalian species (Roubertoux et al. 2005; Albert et al. 2009; McGraw and Young 2010; Champoux et al. 1997; Fairbanks et al. 2004; Brent et al. 2013) but the identification of underlying loci and genes has been proved to be extremely difficult. The rodent models are powerful for the analysis of candidate genes using reverse genetics (Freudenberg et al. 2016) and functional studies (King et al. 2016; Barrett et al. 2013) but there are only a few studies in none-human mammals that used genome-wide analysis to identify the genetic basis of aggressive and affiliative behaviors (Brodkin et al. 2002; Roubertoux et al. 2005; Nehrenberg et al. 2010; Takahashi et al. 2014, 2015; Dow et al.

2011; Albert et al. 2009; Heyne et al. 2014). This is associated at least in part with a shortage of well-established animal models for genetic mapping studies of social behavior.

Animal domestication provides a compelling example of the influence of genetics on behavior (Price 2008; Anholt and Mackay 2009). Differences in aggressive and affiliative behaviors between domesticated species and their wild ancestors are well recognized, and some genomic regions implicated in animal domestication have been identified (von Holdt et al. 2010; Axelsson et al. 2013; Wang et al. 2013; Carneiro et al. 2014; Montague et al. 2014; Groenen 2016). However, the domestication took place so long time ago (Savolainen et al. 2002; Lindblad-Toh et al. 2005; Driscoll et al. 2007; Hu et al. 2014; Groenen 2016) and domesticated animals were selected for so many different traits that it is difficult to distinguishing those genomic domestication signals directly involved into behavioral differences from those that are linked to other, non-behavioral traits.

In contrast to the domestic dog which was domesticated historically, the red fox (*Vulpes vulpes*) was domesticated in a controlled experiment in the second part of the 20th century. The red fox, a close relative of the dog, and a member of the same Canidae family, has been selected as a model species for an experimental reconstruction of early stages of canid domestication at the Institute of Cytology and Genetics of the Russian Academy of Sciences (Belyaev 1979; Trut 1999; Trut et al., 2012). Starting in 1959 with a population of conventional farm-bred foxes and selecting against fear and aggression and for a friendly response to humans the tame strain of foxes has been produced. The tame foxes are playful with humans and other foxes, develop close attachment to their owners if kept in human homes, and as skillful as pet dogs at using human point gestures to find hidden food (Hare et al. 2005).

To study the genetics of tameness it was important to have a population with contrasting behavior. Selection of conventional farm-bred foxes for aggressive behavior was started in 1970. Here, the main selection criteria were the critical distance between a fox and an experimenter when the fox first expressed an aggressive response, and the intensity of this response. The tame and aggressive foxes remained outbred during the entire program, and the differences in behavior between the two strains have been retained and increased for many decades and generations. The genetic inheritance of their behaviors has been confirmed in multiple experiments including embryo transplantation, cross-fostering of pups between tame and aggressive mothers, and cross-breeding of tame and aggressive strains (Trut 1980, Trut et al. 2009; Kukekova et al. 2008, 2011). As a result, the fox experiment not only revealed the importance of selection for behavior during animal domestication (Trut 1999) but also led to the development of a promising animal model for studying genetics of affiliative and aggressive behaviors.

In previous studies we developed a standardized system for measuring fox response to a human observer using a standard four-step test (Kukekova et al. 2008, 2011). Foxes, similar to dogs, exhibit an array of postures, sounds, and movements when they interact with humans. Formally, individual's responses are expressed as presence or absence of behavioral traits. A few examples of such simple, easy to score traits include moving forward or backwards, changing postures, standing in one place without movement for a duration of time, touching an object, and producing sounds. Since each of these traits can be expressed in different contexts (for instance, an individual moves forward to explore, to greet, or to attack), the behavioral response leading to a specific outcome depends on the expression of a combination of traits. To identify main axes of fox behavior formed by sets of correlated traits at each test step and to measure behavior of each individual fox we used principal component analysis (Kukekova et al. 2008, 2011, 2012).

These fox strains were developed several decades ago but only with the growth of genomic resources for the dog it became feasible to undertake genetic mapping of their behavioral phenotypes. In previous studies we adapted dog microsatellite markers for the fox, constructed the fox meiotic linkage map, and identified behavioral quantitative trait loci (QTL) on fox chromosome 12 (Kukekova et al. 2004, 2007, 2011, 2012). In the current study we developed a larger F_2 population to increase power of the QTL analysis. Although the growth of next generation sequencing technologies now allows the comparison of the genomes of two strains and the identification of regions of extreme divergence between tame and aggressive foxes (Johnson et al. 2015) we expect that only a subset of such regions are targets of selection for behavior while others may arise through random fixation. Therefore, genetic mapping of behavioral phenotypes remains critical for the identification of behavioral loci. The fox model thus provides an opportunity to apply a combination of genetic and genomic approaches to study social behaviors intermediate in complexity between rodent and primate models. These advantages may allow studies in foxes to benefit genetic studies of aggressive and affiliative behaviors in dogs and other mammals, including humans.

Materials and methods

Animals and pedigrees

The foxes studied in this project were bred and maintained at the experimental farm of the Institute of Cytology and Genetics (ICG) in Novosibirsk, Russia. All animal procedures at the ICG complied with standards for humane care

and use of laboratory animals by foreign institutions. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois at Urbana-Champaign.

Fox F_2 pedigrees were constructed in course of 3 years (2006—90 offspring; 2007—160 offspring; 2009—287 offspring) by cross-breeding tame and aggressive foxes to produce an F_1 population, and subsequently mating F_1 individuals to each other. The F1 population was produced by breeding of 32 foxes from the tame strain and 30 foxes from the aggressive strain in reciprocal manner with respect to parental gender and population of origin. In total, 537 F_2 offspring were used in this study. These includes 250 F_2 offspring used in Kukekova et al. (2011) study and 287 F_2 offspring which have not been previously analyzed. The pedigree information is provided in Supplementary Table 1 and the number of offspring contributed by each grandparent is presented in Supplementary Table 2.

Blood samples were collected from all F_2 individuals, as well as the F_1 parents and F_0 grandparents. DNA was extracted using Qiagen Maxi Blood kits following the manufacturer's instructions (Qiagen, Valencia, CA) or using phenol–chloroform extraction method (Gilbert and Vance 1994).

Genotyping fox pedigrees and map construction

All F_2 pedigrees, including parents and grandparents, were genotyped using microsatellite markers adapted from the dog genome. In total, 273 markers (Supplementary Table 3) well spaced throughout the fox genome were amplified with fluorescently labeled primers as previously described (Kukekova et al. 2007, 2011, 2012). All markers except three (VV1172, 26749b, and CM6.75) were previously used for construction of the fox meiotic linkage map (Kukekova et al. 2007, 2011, 2012). Four to seven microsatellites were combined, post PCR, in multiplex sets and resolved on an ABI3730 Genetic Analyzer (PE Biosystems, Foster City, CA). PCR product sizes were determined using an internal size standard and the ABI Genemapper 3.5 software package (PE Biosystems, Foster City, CA).

All genotypes were evaluated for data clarity, number of alleles, peak height, and percentage of failed data points. Genotypes that passed the initial evaluation criteria were checked for Mendelian segregation using the prepare option of MultiMap (Matise et al. 1994) and manually corrected for errors. Polymorphism information contents (PIC) was calculated for all markers using Eq. 2.3 from Ott (1999):

$$PIC = 1 - \sum_{i=1}^{a} p_i^2 - \sum_{i=1}^{a-1} \sum_{j=i+1}^{a} 2(p_i p_j)^2$$

where p_i is the frequency of the p^{th} allele and a is the number of alleles for the marker.

The framework map with LOD 3.0 support was generated using CRI-MAP (Green et al. 1990) as previously described (Kukekova et al. 2007, 2011, 2012). To produce a map for QTL mapping, the fox framework map was saturated with previously unmapped markers without statistical support (LOD 0.0) (Supplementary Table 3). The map spans 16 autosomes and X chromosome with average intermarker distance 6.2 cM (SE 0.32). The total length of the current map is 1590.7 cM, which is very similar to the versions of the fox map which were reported in previous studies (Kukekova et al. 2007, 2011, 2012).

Analyses of behavioral phenotypes in the F_2 population

The behavior of all F_2 offspring was tested using a standard test designed to evaluate fox responses to humans in situations with different levels of interaction between the experimenter and tested animal (Kukekova et al. 2008, 2011). The test included four steps, each of which was 1 min long:

Step A. Observer stands calmly near the closed cage but does not deliberately try to attract the animal's attention (Approach);
Step B. Observer opens the cage door, remains nearby but does not initiate any contact with the fox (Stay);
Step C. Observer attempts to touch the fox (Contact);
Step D. Observer closes the cage door, then stays calmly near the closed cage (Exit).

In short, the behavior of each fox in the standard test was tested twice at 5.5–6 months of age. All foxes were tested in their home cages by the same observer and each test was videotaped. After the test was completed, the video records were used to analyze the behavior of the fox by scoring the presence or absence of 98 recordable observations (traits) (Supplementary Table 4).

The kappa statistics was used to estimate reproducibility of 98 traits between tests 1 and 2 in F_2 population (Supplementary Table 4). The calculations were performed using *kappa2* function in R. The judgment of the estimated kappa for the extent of agreement was done following Landis and Koch (1977): "If kappa is less than 0-"no agreement", if 0-0.2- "slight agreement", if 0.2-0.4- "fair agreement", if 0.4-0.6- "moderate agreement", if 0.6-0.8-

"substantial agreement", if 0.8-1.0- "almost perfect agreement".

For each F_2 individual, the mean of the tests was calculated for each trait. The behavioral scores from the F_2 population were then combined with the scores of foxes from tame (83), aggressive (80), F_1 (93), backcross-to-tame (293), and backcross-to-aggressive (202) populations from our earlier study (Kukekova et al. 2011). The trait matrix for all 1287 foxes was used in a principal component (PC) analysis using the *prcomp* function in R as previously described (Kukekova et al. 2011).

Two PC analyses were performed, one for each of the four individual test steps (denoted A.PC, B.PC, C.PC and D.PC ending with digit for principal components) and one for all test steps together. The PC analysis of the individual test steps identified less complex axes of fox behavior and the first three PCs calculated for each test step were selected as phenotypes for QTL mapping. In each PC analysis the first three PCs explained at least 5 % of the variation in fox behavior (Table 1). The behavioral axes defined by each PC were characterized by the traits that had the highest absolute loading to the corresponding PC (traits with loading in the top 20th and bottom 20th percentiles) (Table 2; Supplementary Table 5). Distributions for the first three PCs calculated for each individual test step are presented in Supplementary Figures 1 and 2.

Correlation analysis of the 98 behavioral traits was performed using scores for all 1287 individuals included in PC analysis. Spearman Rank correlation was calculated using the *cor* function in R.

Covariates included

Two cofactors were included in the QTL regression model: sex and matings. Sex was included for several PC traits (A.PC2, A.PC3, B.PC1, B.PC3, and D.PC3) which showed significant differences between males and females. Matings was included to account for any possible environmental effects shared within full-sib families. A mating number was assigned to each mating pair, and was defined as a factor. Hence, matings included the mother and the father effects and the year. There were 110 matings. Ten of these resulted in one offspring and consequently these 10 observations were deleted in the analysis of the main effects.

To verify that the modeled family effect picks up potential polygenic effects, we performed a simulation study using pedigree information (Supplementary File 1; Supplementary Table 1). Without family effects, an animal model including the additive relationship matrix gives an estimate of the heritability of around 0.5 when a heritability of 0.5 was simulated. After correcting the phenotypes by estimated family effects (fixed effects in an ordinary linear model) the estimated heritability was reduced to below 0.01 when an animal model was fitted. These results indicate that the family effects adjust for most of the polygenic effects.

Table 1 The percentage of variation in behavior explained by the first three Principal Components calculated for individual test steps

Step A	A.PC1	A.PC2	A.PC3
Proportion of variance	0.46	0.13	0.06
Cumulative proportion	0.46	0.59	0.65
Step B	B.PC1	B.PC2	B.PC3
Proportion of variance	0.41	0.13	0.07
Cumulative proportion	0.41	0.54	0.61
Step C	C.PC1	C.PC2	C.PC3
Proportion of variance	0.45	0.15	0.05
Cumulative proportion	0.45	0.60	0.65
Step D	D.PC1	D.PC2	D.PC3
Proportion of variance	0.50	0.10	0.07
Cumulative proportion	0.50	0.59	0.66

Interval mapping for a single QTL

The line-origin QTL genotype probabilities of the F_2 individuals were calculated across the genome at 1 cM intervals using the triM algorithm (Crooks et al. 2011) in the software MAPfastR (Nelson et al. 2013). This algorithm estimates the probability of a particular F_2 individual having the QTL genotypes AA (both QTL alleles from the Aggressive-line), AT (one from the Aggressive—and one from the Tame line) or TT (both from the Tame-line) at 1 cM intervals across the genome. The triM algorithm uses a hidden Markov model and inheritance information of all the markers to infer these genotype probabilities (Crooks et al. 2011).

From the QTL genotype probabilities, additive (a) and dominance (d) indicator regression variables, c_a and c_d, were calculated for each cM location as: $c_a = -p(AA) - p(TT)$ and $c_d = p(AT)$ where $p(x)$ indicates the line origin probability. Hence, c_a and c_d would be (−1, 0, 1) and (0 ,1, 0) for individuals that are (homozygous tame, heterozygous, homozygous aggressive), respectively, at the locus. As a result of this coding, negative estimates of the corresponding additive and dominance effects indicate that the tame line have higher values for the phenotype than the aggressive line, and vice versa for positive estimates. Then, a linear model was fitted at each cM across the genome:

$$y = c_a \times a + c_d \times d + cofactors + e \quad (1)$$

where y is the tested phenotype, c_a and c_d the indicator regression variables for the additive and dominant effects as specified above, a and d the estimates for the additive

Table 2 Behavioral axes defined by the first three Principal Components calculated for each individual test step

PC	Behavioral category	Traits
A.PC1	Avoiding the front part of the cage	A25, A52
	Low moving activity	**A31**, A32, **A40**
	Located or moving to the front part of the cage	A22, A24, A28
	Exploratory behavior	A5, A6
A.PC2	Avoiding the front part of the cage	A23
	Moving activity	**A34**, **A36**, A37, A38
	Located or moving to the front part of the cage	A24, A27
	Low moving activity	**A31**, A32, **A40**
A.PC3	Avoiding the front part of the cage	A25
	Exploratory behavior	A8, A9
	Tame behavior	A2, A7
	Located or moving to the front part of the cage	A24, A28, A29
	Low moving activity	A48
	Exploratory behavior	A6
B.PC1	Avoiding the front part of the cage	B2, B12, B13, B29, B30
	Low moving activity	B42
	Located or moving to the front part of the cage	B11, B28, B31
	Exploratory behavior	B7, B10, B15
B.PC2	Located or moving to the front part of the cage	B1, B31
	Low moving activity	B42
	Aggression	**B25**
	Avoiding the front part of the cage	B30
	Tame behavior	**B3**
	Located or moving to the front part of the cage	B11
	Moving activity	B39, **B40**
	Neutral or curiosity	B48
	Exploratory behavior	B14, B15
B.PC3	Moving activity	**B40**
	Tame behavior	B19, B20, B21
	Avoiding the front part of the cage	B2, B29
	Located or moving to the front part of the cage	B11, B28
	Neutral or curiosity	B48
	Low moving activity	B42
	Exploratory behavior	B8, B15
C.PC1	Tame behavior	C8, C12, C13, C14, **C15**, **C16**
	Aggression	C30, **C31**, C32, **C34**, **C36**, **C37**
C.PC2	Avoiding the front part of the cage	C3, **C4**, C7, C38, C55
	Fear	C35
	Located or moving to the front part of the cage	C19, C39
	Exploratory behavior	C29
	Tame behavior	C12, C17, C24
C.PC3	Located or moving to the front part of the cage	C19, C39
	Avoiding the front part of the cage	C7, C55
	Neutral or curiosity	C50
	Exploratory behavior	C29
	Located or moving to the front part of the cage	C2
	Avoiding the front part of the cage	C38
	Tame behavior	C8, C12, C24, C25
D.PC1	Avoiding the front part of the cage	D31
	Aggression	D39
	Neutral or curiosity	D13, D33
	Located or moving to the front part of the cage	D1, **D2**, D17
	Exploratory behavior	D7
D.PC2	Moving activity	D28, D29
	Located or moving to the front part of the cage	D24, D25
	Exploratory behavior	D4, **D32**
	Avoiding the front part of the cage	D31
	Tame behavior	D6
D.PC3	Located or moving to the front part of the cage	**D2**, D17, D24
	Exploratory behavior	D7
	Moving activity	D28, D29
	Tame behavior	D3
	Neutral or curiosity	D33

For each PC the traits with highest absolute loadings (top 20th percentiles and bottom 20th percentiles) and their behavioral categories are listed. The behavioral categories and traits highlighted in gray define an opposite end of a PC axis relative to the behavioral categories and traits which are not highlighted. See Supplementary Table 5 for details. PCs and traits for which significant QTL were identified are in bold. PCs for which suggestive QTL were identified are underlined

and dominance effects, *cofactors* the additional effects included in the model ("Sex" and "Matings") and e the residual error. The fit of the models were evaluated by calculating F ratios at each tested cM location and visualized in genome-wide QTL profiles.

Significance-testing was based on empirical significance-thresholds obtained using randomization testing (Churchill and Doerge 1994). The phenotypes and associated cofactors where permuted 1000 times relative to the genotype to maintain the same data structure. Genome scans were performed in each permuted dataset to obtain a genome-wide significance threshold of 5 %. Significant QTL were recorded when the test-statistic at a genomic location exceeded the threshold and the peak location for each QTL was chosen as the position with the highest F ratio above the threshold. We evaluated a 10 cM window on both sides of each peak to collapse multiple statistically inseparable signals into a single QTL. To quantify the contribution of each QTL to the analyzed trait, we calculated the variance explained by each locus using the genotype information at the peak location. The significance threshold used to infer suggestive QTL was set to an F-value of 6.5, which corresponds to a genome-wide p value of approximately 0.2 across all the PC-phenotypes.

In addition to the PC phenotypes, we also performed QTL analyses for the binary behavioral traits used to calculate the PCs. For this, the same linear model was fitted across the genome (Model 1), where y in this case was the binary phenotypes (Supplementary Table 4) with sex and mating as cofactors.

Epistatic QTL analysis

To explore whether more of the phenotypic variance for the behavioral traits could be explained by also accounting for genetic interactions, we performed a two-dimensional genome-scan to detect pair-wise epistasis. We used the same genotype probabilities calculated by the MAPfastR software and fitted the following linear model to all possible pairs in the genome:

$$y = c_{a1} \times a_1 + c_{d1} \times d_1 + c_{a2} \times a_2 + c_{d2} \times d_2 + i_{aa}\,(aa) + i_{ad}\,(ad) + i_{da}\,(da) + i_{dd}\,(dd) + sex + e \quad (2)$$

The additive and dominance indicator regression variables, c_a *and* c_d, were the same as in the interval mapping for a single QTL. These were then used to calculate the indicator regression variables for each possible interaction between the additive and dominance genetic effects for the pair, i_{aa}, i_{ad}, i_{da}, and i_{dd}, to be used in the estimation of the additive by additive (aa), additive by dominance (ad),

dominance by additive (da) and dominance by dominance (dd) effects (Carlborg et al. 2003). The "matings" co-factor was excluded in the epistatic model since fitting this effect with 110 different levels corresponding to the individual matings led to numerical instabilities when fitted jointly with an epistatic genetic model. We do, however, not expect that the exclusion of this effect will make any major impact on the results obtained.

For each pair the residual sum of squares (RSS) for the fitted model was recorded. Significance testing was done in three steps as described in (Carlborg et al. 2000; Carlborg and Andersson 2002; Carlborg et al. 2003). Briefly, during the first step the significance for epistatic interactions amongst QTL with significant main effects in the interval mapping for a single QTL is evaluated using randomization testing. This is a 4 df joint test for the interaction effects i_{aa}, i_{ad}, i_{da} and i_{dd}. Then, the significance for QTL pairs where one of the loci has significant marginal effect in the interval mapping for a single QTL is evaluated. Here, a 6 df test is performed, where the joint significance for the marginal effects of the insignificant locus in the single QTL analysis (a_2, d_2) and the interaction effects for the pair (i_{aa}, i_{ad}, i_{da} and i_{dd}) is evaluated using a randomization test. For the pairs that were significant in this test, the 4 df test was also performed to evaluate the significance for the interaction effects only. Lastly, the significance for the QTL pairs where none of the QTL had significant marginal effects were tested using an 8 df randomization test for all genetic effects jointly. For the pairs that were significant in this test, the significance of the interaction was evaluated using the 4 df test. A unique significance threshold was calculated for each PC phenotype.

Results

QTL mapping of step-specific PC traits

In all, we mapped 5 genome-wide significant (Fig. 1) and 5 suggestive QTL for 7 of the 12 analyzed step-specific PC traits (Table 3; Table 4). These QTL represent 8 unique loci (Supplementary Figure 4). Per trait, the detected QTL explained between 2.81 and 12.42 % of the phenotypic variance (Table 3).

Additive and dominance effects were estimated for all QTL (Table 3). As a result of coding, negative estimates of the additive and dominance effects indicate that the tame line have higher values for the phenotype than the aggressive line, and vice versa for positive estimates. E.g. for the first PC at the step "Approach" (A.PC1), the estimates for the additive (-0.169 ± 0.074) and dominance (-0.316 ± 0.114) effects in Table 3 mean that the expected phenotype for an individual having both QTL alleles inherited from the tame line is 0.169, i.e. E(y|TT) = 0.169, whereas E(y|AA) = −0.169 and for the heterozygotes E(y|TA) = -0.316. All estimates for the additive effects were negative and consistent with the behaviors of the selected strains (values for these PC traits were higher if two alleles were inherited from the tame population). Over-dominance, when dominance is higher than additivity, was observed for five traits (four QTL) (Table 3). Dominance was observed in both directions, e.g. negative over-dominance for the QTL on Chr 15 (13 cM) leads to lower A.PC1 values while positive over-dominance for the QTL on Chr 15 (67 cM) leads to higher D.PC1 values.

QTL for step-specific PC1 traits

The PC1 calculated for each individual test step explain the largest percent of variation in behavior during the test step and clearly differentiate the tame and aggressive populations from each other (Table 1; Supplementary Figure 1). The QTL identified for each of the step-specific PC1 phenotypes do not overlap, suggesting that PC1 at each individual test step measures different context-specific behaviors that are controlled by different genetic loci (Table 3). The first PCs for the test steps "Approach" and "Stay" (A.PC1 and B.PC1) describe rather similar behavioral patterns, both differentiate foxes that avoid the front part of the cage and show low moving activity (anxiety-like behavior) versus foxes which spend most time in the front part of the cage and show exploratory behavior (Table 2). However, these behavioral patterns are expressed in the different situations: at the step A ("Approach") the cage is closed but at the step B ("Stay") the cage is open. Despite the similarity of the behaviors described by the two PCs, the A.PC1 and B.PC1 map to different regions in the genome. Suggestive QTL for A.PC1 and B.PC1 were identified on Chr 15 (13 cM) and Chr 12 (111 cM), respectively, indicating that similar behaviors in different contexts can be regulated by different loci (Table 3). QTL for A.PC1 and B.PC1 explain similar percent of variance for both the phenotypic variance in F_2 population (2.81 and 2.93 %, respectively; Table 3) and the difference between the parental populations (15.20 and 16.34 %, respectively; Supplementary Table 6).

Step C ("Contact") is the step when the investigator makes an attempt to touch the fox, making it the test step with the most intense interaction between the fox and an experimenter. At this step the differentiation between tame and aggressive behaviors is the strongest, as evidenced by the presence of two groups of traits with opposite effects on behavior and their high contribution to C.PC1 (Table 2; Supplementary Figure 2; Supplementary Table 5). Two

Fig. 1 Significant main effect QTL identified for PC phenotypes B.PC2 and C.PC1. QTL plot with significant ($p = 0.05$) and suggested ($p \approx 0.20$) QTL thresholds indicated. *Vertical dashed lines* indicate boundaries of fox autosomes

significant QTL (Chr 1, 64 cM and Chr 5, 48 cM) were identified for C.PC1) which explain 7.03 % of the phenotypic variance in F_2 population (Table 3) and 34.03 % of the difference between the parental populations (Supplementary Table 6).

At step D ("Exit") the fox cage is closed again. D.PC1 differentiates foxes coming to the front part of the cage and showing exploratory behavior, often trying to attract attention of an experimenter staying near the front part of the cage, and foxes which avoid the front part of the cage and show neutral or aggressive behavior. The suggestive QTL identified for D.PC1 maps to a unique region in the genome (Chr 15, 67 cM). The QTL explains 2.85 % of the phenotypic variance in F_2 population (Table 3) and 16.13 % of the difference between the parental populations (Supplementary Table 6).

Correlations between PC1 traits across the four test steps

An analysis of the correlations between step-specific PC1 phenotypes in the F_2 population found the lowest correlation for two pairs of phenotypes: A.PC1 to C.PC1 ($|r| = 0.15$) and B.PC1 to C.PC1 ($|r| = 0.28$). Low correlation between A.PC1 and C.PC1 was also observed in tame, aggressive, and backcross-to-aggressive populations (Supplementary Table 7). The correlation between B.PC1

Table 3 Summary of QTL and QTL effects for PC defined phenotypes

Phenotype	Number of QTL	Chromosome*	F value	Estimated additive effect ± se	P	Estimated dominance effect ± se	P	Phenotypic variance**
A.PC1	1	15 (13 cM)	7.016	-0.169 ± 0.074	0.024	*-0.316 ± 0.114*	0.006	2.81%
A.PC2	1	13 (49 cM)	8.313	-0.098 ± 0.042	0.021	*0.171 ± 0.063*	0.007	3.33%
B.PC1	1	12 (111 cM)	7.510	-0.206 ± 0.057	0.000	-0.123 ± 0.083	0.139	2.93%
B.PC2	3	*8 (26 cM)*	9.743	-0.142 ± 0.035	0.000	-0.080 ± 0.052	0.121	12.42%
		14 (57 cM)	9.838	-0.153 ± 0.035	0.000	-0.019 ± 0.052	0.717	
		15 (13 cM)	10.564	-0.048 ± 0.036	0.185	*-0.230 ± 0.055*	0.000	
C.PC1	2	*1 (65 cM)*	8.780	-0.333 ± 0.083	0.000	-0.037 ± 0.121	0.761	7.03%
		5 (48 cM)	9.489	-0.164 ± 0.085	0.054	*-0.477 ± 0.126*	0.000	
C.PC3	1	14 (52 cM)	7.859	-0.114 ± 0.030	0.000	-0.056 ± 0.046	0.225	3.02%
D.PC1	1	15 (67 cM)	6.807	-0.194 ± 0.063	0.002	*0.200 ± 0.101*	0.048	2.85%

The table includes significant and suggestive QTL (F value >6.5, see also Supplementary Figure 4). Significant QTL are underlined. QTL that overlap between phenotypes are marked by color. PC1 phenotypes for each individual test step are in bold and italics. The additive and the dominance effects were estimated as difference in PC values of foxes homozygous for the tame-strain allele or heterozygous, respectively, from the mean of the two homozygotes, *se* standard error. QTL effect estimates indicating over-dominance are in italic

*The most significant position is shown. **Phenotypic variance explained by all QTL identified for this phenotype (i.e. F value >6.5)

and C.PC1 was also low in aggressive, and backcross-to-aggressive population but significant in tame and backcross-to-tame population (Supplementary Table 7). These results indicate that selection in tame population was favoring combinations of specific behaviors across the test steps (e.g. presence in the front part of the cage at step B and positive response to a tactile contact at step C) but in the F_2 cross these patterns became reshuffled. This led to a relatively low correlation between PC1 phenotypes, particularly between the steps at which fox behavior is tested in significantly different contexts (for example, where in step B the observer stays calmly near the open cage whereas in step C the observer is actively trying to touch the fox; Supplementary Figure 7).

QTL mapping of lower order PCs calculated based on individual steps

QTL were also mapped for the three lower order PCs (A.PC2, B.PC2, and C.PC3) (Table 3). The A.PC2 differentiates foxes which are located in the front part of the cage and show low moving activity versus foxes avoiding the front part of the cage and demonstrating active moving during the step "Approach" (Table 2). A suggestive QTL for A.PC2 was identified on Chr 13 (49 cM) and this does not overlap with other identified QTL (Table 3). B.PC2 differentiates foxes showing active moving behavior and curiosity versus foxes showing low moving activity and friendly or aggressive behavior at step "Stay" (Table 2). Three significant QTL were identified for B.PC2 (Table 3), one of which overlaps with the QTL identified for A.PC1 (Chr 15, 13 cM) and another with the QTL identified for C.PC3 (Chr 14, 52 cM). Similar to B.PC2, the C.PC3 differentiates foxes that show tame versus neutral or exploratory behavior at the step "Contact". All QTL identified for A.PC2, B.PC2, and C.PC3 have significant additive effects and two of these QTL (Chr 13, 49 cM and Chr 15, 13 cM) are over-dominant (Table 3).

QTL analysis of individual traits and their genetic contribution to PC phenotypes

We found 23 genome-wide significant, and 27 suggestive QTL for the 98 individual trait phenotypes (Table 4; Supplementary Figure 5). For 18 traits a single suggestive or significant QTL was identified, for 13 traits two QTL were identified, and for one trait three QTL were identified (Table 4).

Out of the 23 genome-wide significant QTL identified for the individual traits, 18 overlap with six QTL identified for PC phenotypes (Table 4; Supplementary Figure 6). All traits which were mapped to the same genomic regions as corresponding PC phenotypes contributed to these PCs with an absolute loading of 0.1 or higher (Supplementary Table 5). The mapping data for individual traits strongly support mapping results for PC phenotypes pinpointing the

Table 4 Summary of QTL identified for PC phenotypes and individual traits

For Chromosome	Step-specific PC	Chromosome and position	Trait	Trait description	Chromosome and position	F-value
Chr 1						
	C.PC1	1 (65 cM)	*C15*	Allows to touch nose	1 (65 cM)	9.590
			C16	Allows to touch head	1 (64 cM)	8.558
			C31	Attack alert	1 (65 cM)	8.157
			C32	Pinned ears (aggr.)	1 (59 cM)	7.456
			C34	Follows the hand (aggr.)	1 (64 cM)	9.182
			C36	Triangle ears directed back (aggr.)	1 (59 cM)	9.457
			C37	Aggressive sounds	1 (64 cM)	8.904
	None		*A31#*	Lie in any zone longer than 30"	1 (52 cM)	8.400
	None		*A34#*	Changed place at least once	1 (52 cM)	8.088
	None		*A40#*	Keep same posture and place at least for 40"	1 (50 cM)	8.008
Chr 3						
	None		*B39§*	Changed place at least 2-4 times	3 (7 cM)	6.629
	None		*B40§*	Changed place at least 5 times	3 (7 cM)	7.129
Chr 4						
	None		C24	Loud breathing	4 (62 cM)	7.646
Chr 5						
	C.PC1	5 (48 cM)	*C15*	Allows to touch nose	5 (49 cM)	8.875
			C16	Allows to touch head	5 (49 cM)	9.866
			C31	Attack alert	5 (49 cM)	9.597
			C34	Follows the hand (aggr.)	5 (49 cM)	9.172
			C36	Triangle ears directed back (aggr.)	5 (49 cM)	9.124
			C37	Aggressive sounds	5 (49 cM)	9.176
	None		B20	Tail wagging	5 (52 cM)	6.821
	None		*B3*	Touch hand for at least 40"	5 (12 cM)	11.521
	None		B31	Spend in zone 1-2 at least 40"	5 (38 cM)	7.235
Chr 6						
	None		A47	Tail is up for at least 3"	6 (64 cM)	7.449
	None		D1	Come to the zone 2 during first 5"	6 (40 cM)	8.262
Chr 7						
	None		*A8**	Lean on the door	7 (78 cM)	6.918
	None		A9*	Lean on the right wall in zone 2	7 (75 cM)	7.251
Chr 8						
	B.PC2	8 (26 cM)	*B25*	Pinned ears (aggr.)	8 (26 cM)	9.485
			B39	Changed place at least 2-4 times	8 (27 cM)	7.832
Chr 9						
	None		C4	Spend more than 30" in zones 3-4-5-6	9 (69 cM)	9.379
Chr 12						
	B.PC1	12 (111 cM)	B28	Spend in zone 1-2-3-4 at least 40"	12 (109 cM)	7.456
	None		*B25*	Pinned ears (aggr.)	12 (67 cM)	8.551
	None		C30	Attack	12 (36 cM)	6.951
Chr 13						
	A.PC2	13 (49 cM)	None			
	None		*A8*	Lean on the door	13 (68 cM)	7.656
	None		B10	Come to hand and sniffing	13 (19 cM)	7.735
Chr 14						
	B.PC2	14 (57 cM)	*B40*	Changed place at least 5 times	14 (61 cM)	11.310
	C.PC3	14 (52 cM)	C55	Leaning on side or back walls in zones 5-6	14 (58 cM)	6.642
	None		*A8*	Lean on the door	14 (1 cM)	7.221
	None		*D2*	Spends in zones 1-2 at least 30"	14 (53 cM)	7.408
Chr 15						
	A.PC1	15 (13 cM)	*A31*	Lie in any zone longer than 30"	15 (16 cM)	9.338
			A22	Moving forward for at least one zone during first 15"	15 (14 cM)	6.878
			A32	Lie in any zone a whole minute	15 (17 cM)	7.811
			A34	Changed place at least once	15 (19 cM)	8.548
			A36	Changed place at least 2-4 times	15 (17 cM)	8.352
			A40	Keep same posture and place at least for 40"	15 (17 cM)	9.719
	B.PC2	15 (13 cM)	*B39*	Changed place at least 2-4 times	15 (14 cM)	7.548
			B42	Keeping same posture and place for at least 40"	15 (13 cM)	7.828
	None		*B3*	Touch hand for at least 40"	15 (26 cM)	7.185
	D.PC1	15 (67 cM)	*D2*	Spends in zones 1-2 at least 30"	15 (67 cM)	8.948
			D28	Changes place at least 5 times	15 (66 cM)	7.311
Chr 16						
	None		D32	Leaning on right wall in zone 2	16 (36 cM)	8.499

The table includes significant (F value >8.3, underlined) and suggestive QTL (F value >6.5) for individual traits and QTL for PC phenotypes listed in Table 3 (see also Supplementary Figures 5 and 6). Individual trait QTL which support QTL for the correspondent PCs are listed next to the PC mapping data. QTL for both PC phenotypes and traits are listed by genomic regions. The PCs and traits that maps to more than one location are in italic. The QTL for individual traits which do not overlap with QTL for PC phenotypes but overlap with QTL identified for other traits are marked by symbols specific for each genomic region: #*§. Genomic regions identified for PC phenotypes only or for PC phenotypes and traits with significant contribution to these PCs are highlighted in gray. "None" indicates that QTL identified for a trait does not overlap with QTL identified for PCs to which this trait has a significant contribution

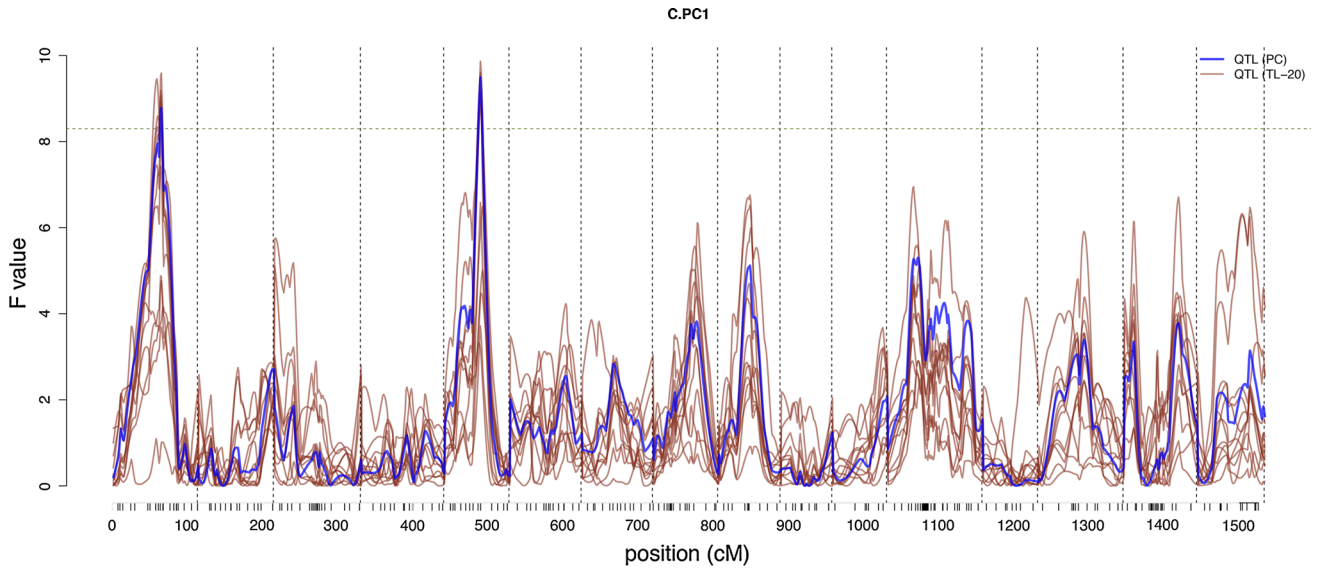

Fig. 2 QTL plot for C.PC1 and the associated traits. QTL plot for the C.PC1 phenotype is in *blue*. Associated traits in the 20th percentile (see Table 2 for details) indicated in *red* (TL-20). Genome-wide significance threshold ($p = 0.05$) is indicated (F value of 8.3). See also Supplementary Figure 6 and for combined PC and trait profiles for all PCs

genomic regions that affect particular aspects of fox behavior.

Significant QTL for three traits from the step "Approach" (A31, A34, and A40) were identified on Chr 1 at 50–52 cM. These traits are correlated and, when shown, indicate a low moving activity of a fox (Supplementary Figures 3 and 5). The identified QTL do not overlap with QTL intervals for PC phenotypes but these results strongly suggest an involvement of this genomic region into regulation of fox moving activity during the step A (Table 4). The other four significant QTL identified for individual traits do not overlap with the loci identified for PC phenotypes or other individual traits (Table 4).

We found several groups of individual traits with QTL in the same locations in the genome (Table 4). When trait correlation coefficients are grouped by test steps and sorted by step-specific PC1s it is evident that behavior is structured in a way such that a specific set of strongly correlated traits is expressed at each step (Supplementary Figure 3). As expected, these step-specific clusters are largely composed of traits that make significant contribution to the corresponding step-specific PCs. The correlation is weaker between similar traits recorded at different steps, which is a likely explanation for why QTL for apparently similar traits but measured in different test steps may map to different locations (Table 4).

QTL for C.PC1 on fox chromosomes 1 and 5

The significant QTL identified for C.PC1 phenotype (Chr 1 at 65 cM and Chr 5 at 48 cM) overlap with the largest number of QTL for the individual traits (Fig. 2; Table 4). Five individual traits contributing to this PC have significant QTL, and two traits have suggested QTL, within the 10 cM QTL interval on Chr 1 (Table 4). Further, six individual traits have significant QTL on Chr 5 at 49 cM (Fig. 2; Table 4). Six traits making a significant contribution to C.PC1 also have very similar QTL profiles (C15, C16, C31, C34, C36, and C37) and detect both C.PC1 QTL: Chr 1 at 65 cM and Chr 5 at 48 cM (Table 4). The QTL for C.PC1 on Chr 1 (65 cM) has significant additive effect and very small dominance effect (Table 3). The direction of the additive effect is as expected, F_2 individuals caring tame alleles have higher values for C.PC1 phenotype and for the traits associated with tame behavior (Supplementary Figure 8). The QTL for C.PC1 on Chr 5 (48 cM) display an over-dominant inheritance. This means that heterozygous (AT) F_2 individuals have higher values for C.PC1 phenotype, and for the traits associated with tame behavior, and lower values for the traits associated with aggressive behavior when compared to homozygous (AA or TT) F_2 individuals (Table 3; Supplementary Figure 9).

Mapping of epistatic QTL pairs

We identified three genome-wide significant epistatic QTL pairs for two PC phenotypes (B.PC2 and C.PC1) (Table 5; Supplementary Figure 10). The total phenotypic variance explained for each PC phenotype was estimated by fitting a joint model including all the main and interaction effects for the QTL pairs that were significantly associated with

Table 5 Summary of epistatic QTL identified for PC phenotypes

Phenotype	Number of QTL pairs	Phenotypic variance (%)	RSS threshold	QTL 1	QTL 2	RSS	P value*
B.PC2	2	3.73	147.854	1 (71 cM)	14 (55 cM)	147.501	0.049
				7 (14 cM)	15 (8 cM)	147.781	0.001
C.PC1	1	3.20	881.794	1 (52 cM)	1 (64 cM)	880.671	<0.001

The table includes all QTL that have significant interaction terms as well as the phenotypic variance explained by the interaction terms. The chromosome and cM position of each QTL are indicated (see also Supplementary Figure 10)

* P value for testing H0: aa = ad = da = dd = 0

the phenotype. The estimates for the traits ranged between 3.20 and 3.73 % of the PC variance (Table 5).

When comparing the epistatic QTL with the main effect QTL, no pairs are detected where both QTL have significant marginal effects in the interval mapping for a single QTL for the same trait. A partial overlap is observed (Table 5), where one of the epistatic QTL is also found in the single QTL scan via its marginal additive and/or dominance effects. For example, a QTL with significant marginal effects on the trait C.PC1 located on Chr 1 at 65 cM. In the interaction analyses, this QTL was involved in two pairs: with a QTL on Chr 14 at 55 cM (B.PC2), and a QTL on Chr 1 at 52 cM (C.PC1) (Table 5). Also the QTL with marginal effects on B.PC2 on Chr 14 at 57 cM and Chr 15 at 13 cM were involved in epistatic pairs for this trait with loci on Chr 1 at 71 cM and Chr 7 at 14 cM, respectively. Thus, out of the six loci involved in the epistatic interactions only two (Chr 1 at 52 cM and Chr 7 at 14 cM) were novel to the epistatic analysis.

Discussion

Our QTL mapping of behavioral PC phenotypes in a large F_2 population identified eight unique significant and suggestive loci involved in regulation of fox behavior. The analyses of the PC1 phenotypes from the individual test steps did not overlap, suggesting that different loci regulate fox responses to a human experimenter in different behavioral contexts. Three of these main effect QTL were also identified as part of epistatic pairs of loci (Table 5), suggesting a more complex genetic architecture of fox behavioral phenotypes than previously considered.

Seven QTL identified for step specific PC phenotypes are supported by mapping of individual traits (Table 4; Supplementary Figure 6). The largest number of traits mapped to the same genomic regions as a PC phenotype was observed for PC1 at the step "Contact" (C.PC1). The behavioral meaning of the mapped traits clearly indicates that identified loci (Chr 1, 65 cM and Chr 5, 48 cM) regulate fox tolerance to touch versus active aggressive response to an experimenter leading to different outcomes of the test.

Another large cluster of individual and PC traits (A.PC1 and B.PC2) at steps "Approach" and "Stay" were mapped to Chr 15 at 13–19 cM (Table 4). The description of these traits indicates that identified loci are involved in regulation of fox moving activity during the first two test steps. This QTL have over-dominance effect for both PC traits (Table 3) and it is a part of one of the epistatic pairs identified for B.PC2.

Several smaller sets of traits were also mapped as clusters. The traits associated with moving activity at step A ("Approach") (traits A31, A34, A40) were mapped to Chr 1, 50-52 cM; moving activity at step B ("Stay") (traits B39, B40) to Chr 3, 7 cM; location close to an experimenter and soliciting a contact (traits A8, A9) to Chr 7, 75-78 cM; aggression and moving activity (traits B25, B39) to Chr 8, 26 cM; moving and avoidance behavior (traits B40 and C55) to Chr 14, 58-61 cM, and interest in continuation of a contact with an experimenter and moving activity at the step D ("Exit") (traits D2 and D28) to Chr 15, 67 cM (Table 4).

The traits from the same behavioral categories measured at the same test step in general have higher correlations than traits from different steps with a few exceptions. For example, traits relevant to exploratory behaviors (A5, A6, A28, A29, B7, B10, B11, B15, C19, C29, D1, D7, D24) are correlated across steps: the foxes that tend to come close to the cage entrance and spend more time exploring the situation in the beginning of the test tend to do so during the following stages as well (Supplementary Figure 3). However, suggestive QTL were found only for two of these traits (B10 and D1) and QTL profiles of these traits do not overlap (Supplementary Figure 5). Another example where the behavior is correlated across steps is provided by a set of traits describing tame behavior. The significant correlation is observed for traits "tame ears" (B21), "wagging tail" (A2, B20, C25, D3) and "loud breathing" (B19, C24). The correlations between these three groups of traits associated with greeting behavior are stronger than the correlations between any of these traits and the traits that control sensitivity to touch (C13–C16), indicating that

these two sets of phenotypes are different although both are classified as tame-specific (Supplementary Figure 3). Only one trait from the step B "Stay" (B20) was mapped to the same genomic region (Chr 5 at 48 cM) as the traits from the step C "Contact" (C5 and C16). These results are in line with the observations made in course of mapping PC1 phenotypes, which revealed that different loci are involved in regulation of rather similar behaviors in different contexts.

Studies of personality and behavioral syndromes in different species have identified correlations among different behaviors. For example, in sticklebacks boldness correlates with inter-male aggression (Bell 2005). In our tame and aggressive fox strains, the selection criteria was designed to favor selection for tame behavior and boldness in the tame strain and defensive aggressive behavior and boldness in the aggressive strain (Trut 1980; Kukekova et al. 2014). Although both populations were under selection for many generations, a stronger correlation was identified between tameness and boldness in tame and backcross-to-tame populations (correlation between B.PC1 and C.PC1) than between aggression and boldness in aggressive and backcross-to-aggressive populations. (Supplementary Table 7; Supplementary Fig. 7). This observations are in line with the results of behavior analysis in dogs, which found correlation between such traits as "curiosity/fearlessness" and"sociability" but not between "curiosity/fearlessness" and "aggression" (Svartberg and Forkman 2002). The observation that B.PC1 and C.PC1 show relatively low correlation in the F_2 population, and that the detected QTL map to different genomic regions, suggests a different genetic regulation for tameness, aggression, and boldness.

In an earlier study, we identified several significant loci on Chr 12 associated with tame behavior in both backcross-to-tame and a smaller F_2 population (Kukekova et al. 2011). In the current experiment only one QTL on the distal part of Chr 12 (111 cM) reached the suggestive QTL threshold for B.PC1 phenotype and a suggestive QTL for the trait B28 (Table 4). In the previous study the strongest support was found for QTL located in the beginning and the middle part of the Chr 12 (Kukekova et al. 2011) while in the current study only two individual traits were mapped to these genomic regions (Table 4). We expect that the differences in the mapping profiles between these two studies are due to a fact that less than a half of F_2 individuals in this study had same grandparents as F_2 in the previous study and there were overall smaller number of individuals in the new F_2 population which demonstrated tame behavior.

Four main QTL identified in the current study showed over-dominance effects (Table 3) and it may explain why these loci were not identified in the previous study focused on QTL mapping in backcross pedigrees (Kukekova et al. 2011). One locus with an over-dominance effect (Chr 15, 8 cM) was also identified as part of an epistatic pair. Identification of over-dominant effects and interacting loci may indicate that selection of fox strains for behavior led not only to increased homozygosity in the genomic regions involved in regulation of behavior but also to more complex allelic compositions.

The resolution of the current QTL mapping study in an F_2 population does not provide sufficient resolution to pinpoint specific positional candidate genes. A larger number of markers, as well as haplotype analyses of the identified intervals to utilize historical recombinations in the parental lines for increasing the resolution, are needed to fine map these loci. Fortunately, the growth of next generation sequencing technologies now makes feasible the large-scale identification of SNP markers in the fox (Johnson et al. 2015). Analysis of allele frequency differences between the tame and aggressive populations using genotyping-by-sequencing (GBS) data identified 30 regions of interest in the fox genome. One of such regions including 10 SNPs with significant allele frequency differences between the two populations is located on Chr 14 and overlaps with the interval for a significant QTL identified for B.PC2 (Chr 14, 57 cM) and suggestive QTL for C.PC3, B40, and C55. This region is syntenic to the 1.3 Mb interval on dog chromosome 3 (CFA3: 43,357,310–44,632,651 bp) and contains one gene, orphan nuclear receptor *COUP-TFII* (*NR2F2*) (Johnson et al. 2015). Two other individual SNPs with significant allele frequency differences between the two populations (SNP S1_1712828536 on fox Chr 5 and SNP S1_1272088483 on Chr 15) were found to be located near QTL peaks on Chr 5 at 48 cM and Chr 15, at 67 cM (Johnson et al. 2015). Additional SNP markers in these regions need to be analyzed to identify critical haplotypes and positional candidate genes.

Using synteny between the fox and rat genomes we compared genomic positions of fox QTL with genomic positions of five QTL for tameness mapped in rat model of animal domestication (Albert et al. 2009). No overlap between QTL of two species was observed suggesting that tameness in foxes and rats is regulated through different sets of loci. Two fox QTL overlap with canine domestication regions (CDRs) identified in Axelsson et al. study (2013). The QTL on fox chromosome 14 for phenotypes B.PC2 and C.PC3 (Table 3) overlap with three CDRs (6, 7, and 8) located on dog chromosome 3. The QTL on fox chromosome 15 for phenotype D.PC1 overlap with two CDRs (32 and 33) located on dog chromosome 28. Although current fox QTL intervals are broad and contains many genes, future sequencing of the genomes of tame and aggressive foxes will allow refined analysis of fox QTL

and identification of targets of selection for behavior. The comparative analysis of the regions which differentiate tame versus aggressive foxes and domestic dogs versus gray wolves may also shed light on an intriguing question: Is domesticated behavior in canids regulated through similar genomic regions and gene networks?

In the current study we found that fox behavior in a standard four-step test is comprised by context specific behavioral patterns regulated through multiple genetic loci. Although, the four step standard test represents a relatively simple interaction between an experimenter and a caged fox, we identified significant differences in genetic regulation of fox behavior among the test steps. These findings suggest that a response to the same social stimulus can be regulated through different genetic loci when the stimulus is presented in different contexts. Different behavioral phenotypes may also have different genetic architectures. The fox model provides an opportunity to dissect patterns of interactive behavior into more simple components and get insight into the genetic architecture of the affiliative, aggressive, and anxiety-related behaviors. These findings should be of interest for genetic studies of social behavior in other mammals including humans.

Acknowledgments We are grateful to Irina V. Pivovarova, Tatyana I. Semenova, and all the animal keepers at the ICG experimental farm for research assistance. We thank K. Gordon Lark and Kevin Chase for advice and important discussions. The project was supported by National Institutes of Health Grant MH077811, NIH FIRCA Grant TW008098, USDA Federal Hatch Project #538922, Program of the Siberian Branch of the Russian Academy of Sciences #0324-2015-0007, Grant #13-04-00420 from the Russian Fund for Basic Research, and Campus Research Board Grant from the University of Illinois at Urbana-Champaign.

Conflict of interest Ronald M. Nelson, Svetlana V. Temnykh, Jennifer L. Johnson, Anastasiya V. Kharlamova, Anastasiya V. Vladimirova, Rimma G. Gulevich, Darya V. Shepeleva, Irina N. Oskina, Gregory M. Acland, Lars Rönnegård, Lyudmila N. Trut, Örjan Carlborg, Anna V. Kukekova declare that they have no conflict of interest.

Human and animal rights and informed consent All institutional and national guidelines for the care and use of laboratory animals were followed. All animal procedures at the Institute of Cytology and Genetics of the Russian Academy of Sciences complied with standards for humane care and use of laboratory animals by foreign institutions.

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