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Genetic Architecture of Tameness in a Rat Model of Animal Domestication

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ABSTRACT

A common feature of domestic animals is tameness—*i.e.*, they tolerate and are unafraid of human presence and handling. To gain insight into the genetic basis of tameness and aggression, we studied an intercross between two lines of rats (*Rattus norvegicus*) selected over >60 generations for increased tameness and increased aggression against humans, respectively. We measured 45 traits, including tameness and aggression, anxiety-related traits, organ weights, and levels of serum components in >700 rats from an intercross population. Using 201 genetic markers, we identified two significant quantitative trait loci (QTL) for tameness. These loci overlap with QTL for adrenal gland weight and for anxiety-related traits and are part of a five-locus epistatic network influencing tameness. An additional QTL influences the occurrence of white coat spots, but shows no significant effect on tameness. The loci described here are important starting points for finding the genes that cause tameness in these rats and potentially in domestic animals in general.

ANIMAL domestication marked a turning point in human prehistory (Diamond 2002), and domestic animals have been the subject of research for many years (Darwin 1868). Recently, genetic studies have shed light on when, where, and how often a range of animal species were domesticated (Troy *et al.* 2001; Vila *et al.* 2001; Savolainen *et al.* 2002; Larson *et al.* 2005; Driscoll *et al.* 2007; Eriksson *et al.* 2008; Naderi *et al.* 2008). With the exception of coat color (*e.g.*, Pielberg *et al.* 2008) and skin pigmentation (Eriksson *et al.* 2008), little is known about *what* occurred genetically during animal domestication. At what genes were allelic variants selected for by would-be practitioners of animal husbandry? Although domestic animals differ from each other in many ways, they all share the trait of tameness—*i.e.*, they tolerate and sometimes even seek human presence and handling. Almost nothing is currently known about the genetic basis of tameness.

In a series of studies initiated by D. K. Belyaev, researchers at the Institute for Cytology and Genetics in Novosibirsk (Russia) have subjected several mammalian species to a process of experimental domestication (Trut 1999). These studies, some of them ongoing for several decades, involve selection for tame and aggressive behavior in lines of animals derived from wild populations. They include a fox population that has been "domesticated" to such an extent that the tame foxes are now similar to dogs in some respects (Hare *et al.* 2005). They also include a population of wild-caught rats (*Rattus norvegicus*) that was selected for either reduced or enhanced aggression toward humans over >60 generations (Belyaev and Borodin 1982). To select the animals, their response to an approaching human hand was observed, and the rats showing the least and the most aggressive behavior were allowed to mate within the two lines, respectively. The initial response to selection was rapid and then slowed, so that little change in behavior from generation to generation has been observed in the last 10–15 generations, although the selection regime has been continued to the present. Today, the "tame" rats are completely unafraid of humans, they tolerate handling

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and being picked up, and they sometimes approach a human in a nonaggressive manner. By contrast, the "aggressive" rats ferociously attack or flee from an approaching human hand.

To study the genetic basis of tameness we have established populations of both rat lines in Leipzig. In their new environment, the rats maintained their behavioral differences in response to humans, and these differences were not influenced by postnatal maternal factors (Albert *et al.* 2008). In addition, the rat lines differ in a number of other behavioral, anatomical, and physiological traits, raising the question whether these traits are influenced by the same loci as tameness and aggression toward humans.

Many domestic animals display conspicuous coat color variations not found in their wild relatives. Prominent examples include the white color variants in dogs, pigs, cows, horses, and chickens. In laboratory rats, it has been proposed that "coat color genes" may account for many of the differences associated with domestication (Keeler and King 1942). It is thus interesting that individuals with white spots appeared in both the tame foxes (Trut 1999) and the tame rats (Trut *et al.* 2000) at higher frequency than in the corresponding aggressive lines, although they were absent or rare in the founding fox and rat populations, and although they were not selected for. The rat populations studied here provide an excellent opportunity to examine whether tameness is influenced by the same loci as white coat spotting.

In this study, we crossed the two rat lines and bred >700 intercross animals. A broad set of behavioral, anatomical, and physiological traits was measured, and a genomewide set of genetic markers was used to identify genomic regions (quantitative trait loci, QTL) that influence tameness as well as other traits that differ between the lines, including white spots.

MATERIALS AND METHODS

Additional materials and methods, as well as data files containing genotype and phenotype data collected for this study, can be found in the accompanying supporting information, File S1 and File S2.

Animals: The tame and the aggressive rat (*R. norvegicus*) lines derive from one population of wild-caught rats, which has been bidirectionally selected at every generation since 1972 at the Institute of Cytology and Genetics at the Siberian Branch of the Russian Academy of Sciences in Novosibirsk, Russia. About 30% of the animals from each generation were selected on the basis of the level of tameness and defensive aggression they displayed in response to humans on a five-point scale (Naumenko *et al.* 1989; Plyusnina and Oskina 1997). Inbreeding was kept at a minimum by avoiding mating closely related individuals.

The pedigree described in this study was initiated from four tame and four aggressive individuals (2 females each; the "F minus one", or "F_{-1}", generation) from the 64th generation of selection. The F_{-1} animals did not have common parents and at most one common grandparent. They were mated within line to yield 11 (5 tame and 6 aggressive rats, one male each) F_0 animals. These were crossed reciprocally, and six hybrid F_1 males were repeatedly mated to 37 F_1 females to produce 733 F_2 rats (383 females). A separate set of 47 F_1 animals (25 females) derived from different F_0 crosses was used for characterizing the F_1 generation in behavioral tests. Phenotypic data from F_0 animals discussed in this article are the same as presented in Albert *et al.* (2008). Animals were maintained under standard laboratory conditions, under an artificial 12-hr light cycle (lights off at 1:00 pm). The light cycle allowed behavioral testing during the dark phase, when rats are more active. Cages were equipped with sliding doors to allow for transfer without handling. During all caretaking procedures and experiments, animals from different lines and generations were treated identically. The study was approved by the regional government of Saxony (TVV Nr. 29/05).

Behavioral testing: F_2 rats were tested in a standardized series of behavioral paradigms. We measured the animals' level of tameness/aggression with the "glove test," by confronting them with an approaching human hand and attempting to handle them (see Albert *et al.* 2008 for details on the testing procedure). Beginning 2 weeks after the glove test, rats performed an open-field test, a light–dark test, and a startle response test, which provide various measures of exploratory and anxiety-related behavior. A total of 470 (64%) of the F_2 rats performed a second glove test trial. F_1 rats were tested once with the glove test at 12–14 weeks of age and then followed the testing schedule described in Albert *et al.* (2008). All tests were performed with minimal handling following procedures described in Albert *et al.* (2008). Glove test trials were videotaped and later analyzed by two independent observers (5% of trials only by one observer). Experimenters and observers were blind to the animals' identity and to the further data processing. A set of 11 behaviors (*e.g.*, "attack" or "tolerate handling") was scored (see Albert *et al.* 2008 for detailed descriptions of the behaviors), and each score was converted to a numeric measure (*e.g.*, "number of occurrences" or "duration in seconds"; Table 1).

Blood and tissue sampling: Dissections were performed on 733 F_2 (383 females) and 37 F_1 animals (16 females). Within 2 weeks after the last behavioral test, animals were killed between 2:00 and 6:00 pm. F_2 animals had been starved for 24 hr prior to dissection. Animals were weighed, anesthetized with CO_2, and killed by cervical dislocation. Blood was collected rapidly after death by heart puncture and separated into serum and blood cells by centrifugation 30 min after sampling. Serum was stored at −20° and later transferred to −80° until analysis. Liver (small section, not weighed), spleen, kidney, adrenal gland, lung, and heart were removed rapidly, weighed, snap-frozen in liquid N_2, and stored at −80°. From F_1 animals, kidney and spleen were weighed and stored.

Serological phenotypes: Serum was analyzed in 684 F_2 rats (357 females). Electrolytes, metabolites, immunological parameters, enzymes, and hormones were analyzed in serum according to the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine. Measurements for all serum traits but corticosterone were performed using a Hitachi PPE-Modular analyzer (Roche Diagnostics, Mannheim, Germany). Corticosterone was measured using a commercial ELISA assay (IDS, Frankfurt, Germany).

Statistical phenotype analyses: We sought to control for possible confounding effects, such as observer in the glove test or an animal's litter. We constructed mixed linear models of the phenotypes, estimated effect sizes using restricted maximum likelihood, and adjusted the phenotypes for the respective effects specified in Table S1. Sex and covariates were not adjusted; we instead included them as fixed effects into the QTL analyses. For glove test measures, we separately adjusted

observations from different trials and observers and then averaged the available observations from each rat. To summarize a rat's behavior in the glove test, we performed principal component analysis (PCA) on the individual glove test measures. We used only F_2 animals in the PCA and calculated scores of F_0 and F_1 animals on the basis of the obtained regression coefficients.

Comparisons of non-glove test phenotypes between the F_0, F_1, and F_2 generations, as well as tests for sex differences (Wilcoxon's rank test), were performed on phenotypes adjusted for covariates. Correlations between phenotypes were calculated using Pearson's product-moment correlation, on phenotypes adjusted for all effects (including sex and covariates) listed in Table S1. All analyses were performed using the software R (R Development Core Team 2008) and the nlme (Pinheiro *et al.* 2008) and lme4 (Bates 2007) packages.

Genotyping: Animals were genotyped with 152 microsatellite and 49 single-nucleotide polymorphism (SNP) markers. Markers were selected for maximum allele frequency differences between the outbred rat lines, as determined from preliminary genotyping of a panel of F_0 animals. Preliminary genotyping of microsatellites was performed as described below, while SNPs were screened as described in Saar *et al.* (2008). All markers used in the QTL mapping are listed in Table S2. DNA was isolated either from lung or from spleen tissue. Polymerase chain reaction (PCR) for microsatellite markers was performed using the M13-primer PCR system (Schuelke 2000) and analyzed on an ABI3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Microsatellite genotypes were determined using the software GeneMapper Version 4.0 (Applied Biosystems), and all genotypes were manually double checked. SNPs were genotyped using TaqMan chemistry (Applied Biosystems). SNP genotypes were called automatically as part of the scanning process, and genotype plots were inspected visually.

Pedigree construction: Genotype data for individual markers were tested for Mendelian pedigree errors, using the program PedCheck (O'Connell and Weeks 1998). Allele calls for inconsistent genotypes were rechecked manually and either they were corrected for obvious genotyping errors or the marker was excluded from further analysis if genotypes could not be determined unambiguously. We found that some rats yielded inconsistent genotypes for several markers, although the respective genotypes appeared to have been called correctly. We interpreted these individuals as having incorrect pedigree records. Initially, genotypes had been obtained from F_0 and F_2 animals. To clarify the pedigree structure, we typed all markers in all F_{-1} and F_1 animals for which samples were available, as well as in an extended panel of F_0 animals. Using the software "Cervus" 3.0 (Kalinowski *et al.* 2007), we determined the most likely parental pair for all genotyped F_0, F_1, and F_2 individuals, on the basis of a subset of 107 microsatellite markers with unambiguous genotype patterns. Rats for which the inferred parents differed from those in our records were either reassigned to the inferred parents or excluded from further analysis if no unique parental pair could be identified. The final pedigree used in the QTL analyses showed no Mendelian errors and comprised 8 F_{-1} rats (all genotyped), 11 F_0 rats (8 genotyped), 43 F_1 rats (30 genotyped), and 706 F_2 rats (all genotyped).

Linkage map construction: We constructed a sex-averaged linkage map, using a version of the program crimap (Green *et al.* 1990) modified to handle large pedigrees by the University of California Davis, Veterinary Genetics Laboratory. On chromosome 6, we found the markers D6rat213 and D6rat68 to be inverted on our linkage map relative to their physical positions. On chromosome 13, the markers D13rat5 and D13rat64 were found to be inverted and to map very closely (1.2 cM) to each other, despite a physical distance of 21 Mb. These cases may reflect chromosomal rearrangements in our wild-derived rats compared to the genome sequence of the inbred Brown Norway laboratory strain (Gibbs *et al.* 2004). We used our inferred linkage maps in further analyses (Table S2). We estimated information content at autosomal marker positions on the basis of the fraction of individuals whose alleles could be unambiguously traced to the F_{-1} generation.

Single-QTL mapping: A standard model of a phenotype y influenced by a single QTL can be written as

$$y = \beta_0 + FZ + \beta_{1_j} a_j + \beta_{2_j} d_j + \varepsilon_j,$$

where β_0 is the population mean, F is a matrix of regression coefficients for fixed effects and covariates (see Table S1 for the effects we included in the QTL models for various phenotypes), Z is an incidence matrix relating observations in F to individual observations, β_{1_j} and β_{2_j} are the additive and dominance effects at genomic position j, a_j and d_j are indicator variables relating these genetic effects to individuals, and ε_j is the residual error. We estimated the parameters β_0, β_1, and β_2 using a variation of the least-squares regression framework (Haley and Knott 1992; Haley *et al.* 1994). In this framework, F_2 animals are grouped at a given genomic position according to whether they carry two, one, or zero alleles originated from the tame (allele "T") or aggressive (allele "A") line, forming the genotype classes TT, TA, and AA. Missing F_0 genotypes can lead to a loss of power because some alleles in F_2 animals might not be reliably traced to parental lines. This limitation can be overcome by including the genotyped parents of F_0 animals (the F_{-1}) in the analysis and by tracing alleles back to them. Hence, we inferred missing genotypes of F_0 and F_1 animals on the basis of their ancestors' and offspring genotypes (File S1). Next, we calculated, in steps of 1 cM throughout the genome, the probability of an F_2 animal belonging to the TT, TA, or AA genotype classes on the basis of genotypes of flanking markers, using methods described in Pong-Wong *et al.* (2001) and Besnier and Carlborg (2007). The genotype probabilities were used to compute the indicator variables a_j and d_j (Haley *et al.* 1994). Finally, we fitted the parameters β_0, β_1, and β_2 using least-squares regression. High F-values obtained from the regression point to the presence of a putative QTL at the respective location. The difference in mean between the two homozygous classes TT and AA corresponds to twice the additive effect β_1 of the QTL, while the deviation of the heterozygous TA class from the mean of the TT and AA classes measures the degree of dominance β_2. We searched for QTL by following a forward selection procedure (Figure S1). After the initial scan, we included the effect and position of the most significant QTL that reached genomewide significance as a fixed effect in the model. The scan was then repeated, and QTL were added until no additional significant QTL were identified. For each phenotype, we performed 1000 permutations of phenotypes with regard to genotypes to determine the F-value threshold that corresponds to a genomewide significance level of $P = 0.05$ (Churchill and Doerge 1994). We express variance components attributable to QTL as a fraction of the residual phenotypic variance, *i.e.*, the variance in phenotype after fixed effects and covariates have been factored out.

We analyzed the X chromosome using the software QxPak (Perez-Enciso and Misztal 2004) (see File S1 for details). Since the permutation-based significance thresholds derived for the autosomes cannot be directly applied to the X chromosome, we assumed QTL with a nominal P-value <0.001 (0.005) to be significant (suggestive) at a genomewide level, as suggested in the QxPak manual. We tested for linkage to the Y chromosome, using ANOVA as described in File S1.

Mapping of epistatic QTL: We searched for epistatic QTL using an extension of the least-squares regression model for single QTL, following a search strategy described and applied earlier (Carlborg and Andersson 2002; Carlborg *et al.* 2003, 2004, 2006; Wright *et al.* 2006). Here, we first describe the regression model underlying our epistatic analyses and then go through the steps of the search strategy. A schematic representation of the approach is shown in Figure S1.

The standard extension of the model for a single QTL to incorporate two epistatic QTL is

$$y = \beta_0 + FZ + \beta_{1_j} a_j + \beta_{2_j} d_j + \beta_{3_k} a_k + \beta_{4_k} d_k + \beta_{5_{jk}} aa_{jk} + \beta_{6_{jk}} ad_{jk} + \beta_{7_{jk}} da_{jk} + \beta_{8_{jk}} dd_{jk} + \varepsilon_{jk}.$$

The additional parameters are the additive (β_{3_k}) and dominance effects (β_{4_k}) of the second QTL at position k, the epistatic effects between the two loci ($\beta_{5_{jk}}$–$\beta_{8_{jk}}$), and the corresponding indicator variables. The indicator variables for the interaction terms (aa_{jk}, ad_{jk}, da_{jk}, dd_{jk}) were computed by multiplying the respective additive and dominance indicator variables for the single QTL (Haley and Knott 1992). We estimated the effects β_0–β_8 at a given pair of loci using least-squares regression.

The search strategy to find epistatic pairs of QTL involves three steps (see Figure S1 and Carlborg *et al.* 2004 for further details). First, we searched for single QTL as described above. Second, we performed a genomewide search for putative epistatic pairs of loci. Third, each pair is tested for the existence of epistasis.

For each pair proposed by the pairwise search in step two, we assigned significance in one of three ways. If both loci were significant by themselves in the single-QTL analysis, no further testing is necessary, and the pair is declared significant. If one locus in the putative pair was a significant single QTL, we need to test only whether the second locus is significant (*i.e.*, whether its inclusion in the model already containing the first locus improves model fit). To derive the corresponding threshold, we created, for each single QTL, 1000 randomized data sets by permuting only the indicator variables of the second QTL (a_k, d_k), as well as those of the interaction effects (aa_{jk}, ad_{jk}, da_{jk}, dd_{jk}), while the first QTL was kept in the model as a fixed effect (permutation test "Type I" in Figure S1). In each permuted data set, we searched for the best fitting pair including the known QTL and a second QTL. We then compared the model fit obtained from the putative pair with the model fits obtained from the permutations. Finally, if neither QTL in the putative pair was significant as a single QTL, we need to test whether the joint inclusion of both loci improves the model fit significantly. We performed 1000 phenotype/genotype permutations and searched each randomized data set for its best fitting pair (permutation test "Type II" in Figure S1). For increased efficiency, this was done using a genetic search algorithm (Carlborg *et al.* 2000). Significance of the putative pair was assigned by comparing its model fit to the fits obtained from the permutations. Throughout, we used a cutoff of $P < 0.05$ for "significant" pairs of QTL.

So far, we have detected pairs of QTL, but not yet tested whether there is significant epistasis between the members of a given pair. To do this, we generated 1000 randomized data sets for each pair by permuting only the interaction indicator variables (aa_{jk}, ad_{jk}, da_{jk}, dd_{jk}), while keeping the additive and dominance effects of the two loci in the pair constant (Carlborg and Andersson 2002) (permutation test "Type III" in Figure S1). Epistasis is assumed if the putative epistatic pair is in the top 5% of model fits obtained from the permutations.

To construct the network influencing tameness, we consider loci (single or as part of a pair) with overlapping confidence intervals to be the same locus. We show these loci as circles in Figure 5, with lines between them indicating significant epistatic interactions for that given pair. We did not fit a model incorporating interactions between more than two loci. To visualize the directions of the epistatic interactions (Figure 5, B–F), we grouped F_2 animals according to their genotypes at the respective loci. For each of the resulting nine two-locus genotype groups, we calculated the mean and the standard error of the mean of the respective animals' level of tameness.

Because some loci in the tameness network are part of more than one epistatic interaction, we cannot calculate the residual phenotypic variance explained by the whole network by simply adding up the variances explained by the respective pairs (Table 4). Instead, we used the NOIA model of genetic effects (Alvarez-Castro and Carlborg 2007) as implemented in Le Rouzic and Alvarez-Castro (2008), with analyses restricted to at most pairwise interactions. NOIA is specifically designed to estimate parameters, including genetic variances, in multilocus networks (Alvarez-Castro and Carlborg 2007).

RESULTS

A cross between tame and aggressive rats: To create an intercross between the tame and aggressive rats, we mated one tame and one aggressive male to 5 aggressive and 4 tame females, respectively. In the resulting F_1 generation, we repeatedly mated six males with a total of 37 females to produce an F_2 population of 733 animals (362 females). Details of the mating scheme are described in materials and methods.

Analyses of phenotypes: We recorded a total of 45 phenotypic traits in the F_2 animals, including measures from four behavioral tests, anatomical parameters, and serum levels of hormones, enzymes, and other serum components (Table 1). To measure the level of tameness and aggression, we used a paradigm that closely mimics the test used to select the two rat lines over the past 36 years. In this "glove test," a gloved human hand approaches a rat in an experimental cage and attempts to touch it and to pick it up (Figure 1A). Various aspects of the rat's behavior are recorded (Albert *et al.* 2008). When testing F_1 animals in this test, we found that the extreme levels of tameness and aggression observed in the original F_0 lines were absent (Figure S2). By contrast, F_2 animals displayed the full range of behaviors found in the original lines (Figure S2). A few F_2 animals even exceeded the levels of tameness and aggression observed in the tame and aggressive lines.

A PCA of the behaviors recorded in the glove tests of the F_2 animals confirms these observations. The first principal component (PC1) corresponds to behaviors such as attacks, screaming, and (with inverse loading) the toleration of touch or handling (Table 2). PC1 explains 26% of the variance in behavior of F_2 rats. Of the PCs explaining >10% of the variance, PC1 most clearly separates the tame from the aggressive F_0 animals (Wilcoxon's rank test: PC1, $P < 10^{-15}$; PC2, $P = 0.003$; PC3, $P = 0.07$), although these animals were not included in the PCA (see materials and methods).

TABLE 1

Traits measured in F_2 animals

Trait	Unit	No. F_2	Higher trait value	Comments
Glove test		700		
Approach	Count		Tame	No. of occurrences
Attack	Count		Aggressive	No. of occurrences
Escape	Count		Aggressive	No. of occurrences
Flight	Count		Aggressive	No. of occurrences
Move and leave	Count		Tame	No. of occurrences
Squeak	Count		Aggressive	No. of occurrences
Boxing	sec		Aggressive	Duration
Freeze	sec		—	Duration
Scream	sec		Aggressive	Duration
Tolerate handling	sec		Tame	Duration
Tolerate touch	sec		Tame	Duration
Open field test		689		
Time spent in center	%		Tame	
Time spent in corner	%		Aggressive	
Time spent moving	%		Tame	
Time spent rearing	sec		Tame	
Locomotion speed	cm/sec		Tame	
Fecal boli	Count		—	
Light–dark test		690		
Time spent in light compartment	%		Tame	
Time spent moving	%		—	
Time spent rearing	sec		Tame	
Locomotion speed in light compartment	cm/sec		—	
Fecal boli	Count		—	
Startle response test		700		
Startle response	g		Aggressive	Mean startle response across 10 trials
Anatomy				
Body weight	g	700	—	
Adrenal gland weight	g	664	Aggressive	
Heart weight	g	669	—	
Kidney weight	g	669	Tame	
Lung weight	g	668	—	
Spleen weight	g	668	Tame	
Testis weight	g	321	—	
White coat spotting	Yes/no	573	Tame	
Serum traits		638		
Hormones				
Corticosterone	ng/ml		Aggressive	
fT3	pmol/liter		—	
fT4	pmol/liter		Aggressive	
Enzymes				
ALAT	μkat/liter		Aggressive	
AP	μkat/liter		Aggressive	
ASAT	μkat/liter		Aggressive	
Substrates				
Creatinine	μmol/liter		Aggressive	
Glucose	μmol/liter		Aggressive	
Urea	mmol/liter		Tame	
Serum protein				
Total protein	g/liter		Aggressive	
Albumin	g/liter		Tame	
Electrolytes				
Ca^{2+}	mmol/liter		Tame	
Cl^{-}	mmol/liter		Aggressive	
$Fe^{2+/3+}$	μmol/liter		Tame	

ALAT, alanine aminotransferase; AP, alkaline phosphatase; ASAT, aspartate aminotransferase; fT3, free triiodthyronine; fT4, free thyroxine.

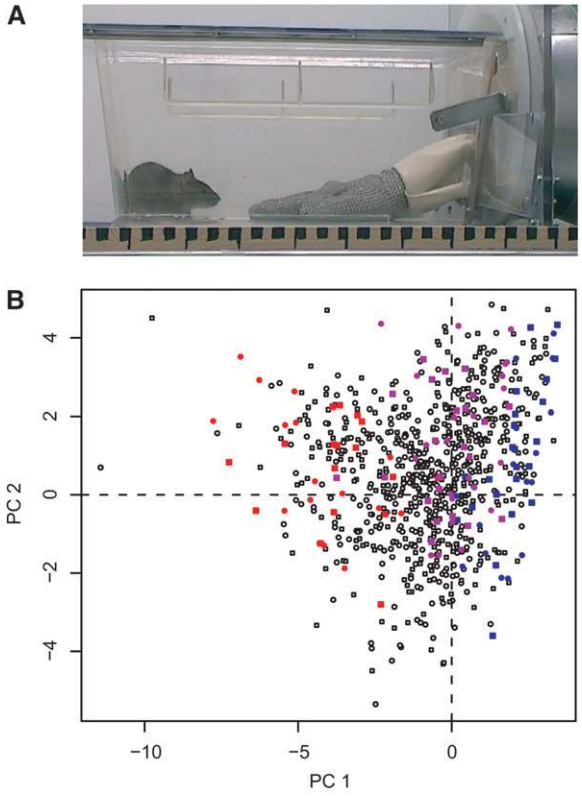

FIGURE 1.—Behavior in the glove test. (A) The glove test measures the level of tameness or aggression toward an approaching hand. (B) PCA scores derived from glove test behaviors of tame F_0 (blue), aggressive F_0 (red), F_1 (purple), and F_2 (black) animals. Circles, females; squares, males.

Of the F_1 rats, 94% (44/47) had PC1 scores between the medians of the tame and the aggressive rats. Of the F_2 rats, 79% (551/700), fell into this intermediate range, while 6% (43) of the F_2 rats had more tame and 16% (109) had more aggressive PC1 scores than defined by the respective F_0 medians (Figure 1B). This indicates that there is substantial variation in tameness in the F_2 rats and suggests that PC1 is a useful measure of this variation. The glove tests were repeated in 470 F_2 rats (MATERIALS AND METHODS). The correlation between the PC1 scores obtained for the two trials was 0.44 (Pearson's r, $P < 10^{-15}$).

We also performed an open-field test, a light–dark test, and a startle response test, which measure traits related to anxiety and fear as well as general activity (Table 1). The values of all these traits in F_2 animals overlapped substantially with those in F_0 animals (Figure S3). This was also true for body weight, for the weight of six organs, and for 8 of 14 serum traits (Figure S4 and Figure S5). By contrast, >75% of the F_2 rats had higher (corticosterone, creatinine, glucose, chloride) or lower (alanine aminotransferase, ALAT; alkaline phosphatase, AP) values in these measures than >75% of the tame and aggressive rats (Figure S5).

Sex differences were apparent for many traits in the F_2 animals (Table S3). When males and females were considered separately, most phenotypes were approximately normally distributed in the F_2 generation (Figure S6, Figure S7, Figure S8, and Figure S9). By contrast, raw glove test measures had highly skewed distributions, with prominent peaks at zero counts/durations.

TABLE 2

Principal component analysis of behavior of F_2 animals in the glove test

Measure	PC1	PC2	PC3
Attacks (count)	−0.43		
Boxing posture (duration)	−0.36		
Escapes (count)	−0.32		
Flights (count)	−0.39		
Screaming (duration)	−0.38		
Freezing (duration)		0.46	−0.36
Move and leave (count)		−0.42	−0.61
Squeaks (count)		−0.35	0.31
Approaches (count)		−0.43	
Tolerate handling (duration)		0.41	0.50
Tolerate touch (duration)	0.46		
% variance	26	15	11

The loadings shown for the respective principal components (PCs) indicate the degree to which a trait contributes to the respective PC. Only PCs that explain ≥10% of the variance and loadings with absolute values ≥0.3 are shown.

Correlations between phenotypes: Earlier work revealed a multitude of phenotypic differences between the tame and the aggressive rats, including behavioral, anatomical, hormonal, and neurochemical differences (NAUMENKO *et al.* 1989; PLYUSNINA and OSKINA 1997; POPOVA *et al.* 2005; ALBERT *et al.* 2008). If these differences are caused by the same genetic loci, they should be correlated in the F_2 animals. We did observe significant correlations among parameters recorded in the same test. For example, correlations between different measures in the glove test are reflected in their contributions to PC1 (Table 2). In contrast, parameters from different tests were generally not, or only weakly, correlated (Figure 2, Table S4). Notably, two measures of "anxiety" (the percentage of time spent in the light compartment of the light–dark test and the percentage of time spent in the center of the open field) were not correlated ($r = 0.03$, $P = 0.48$). There were significant but weak correlations between tameness and aspects of behavior in the open-field and light–dark tests. High levels of tameness were also correlated with low corticosterone levels ($r = -0.08$, $P = 0.04$), but not with the weight of the adrenal glands ($r = -0.06$, $P = 0.10$).

QTL for tameness and associated traits: We typed the animals in the pedigree for 201 genetic markers (152 microsatelites and 49 single-nucleotide polymorphisms) that were selected to be polymorphic between the parental strains and to provide coverage of most of the genome (MATERIALS AND METHODS).

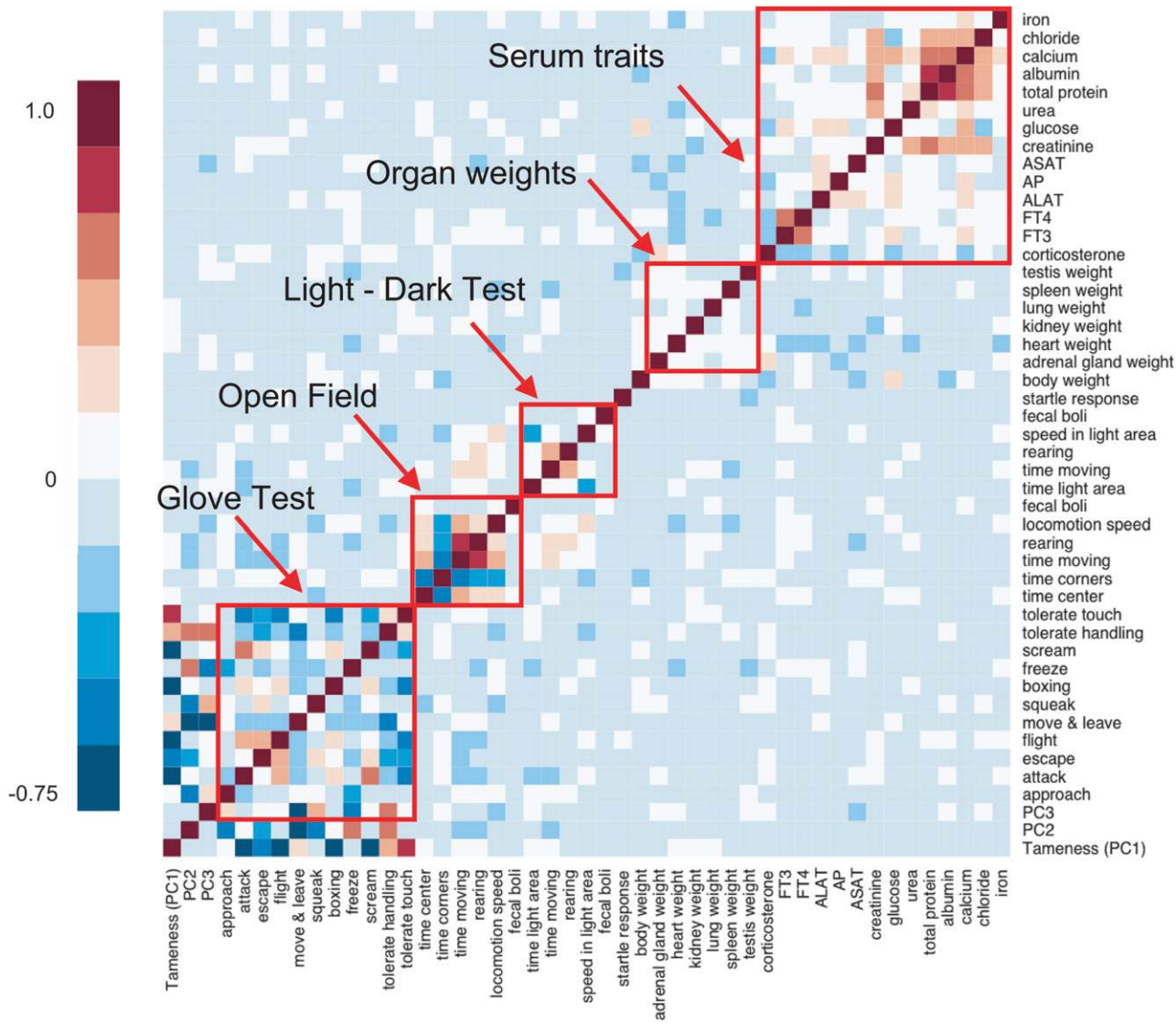

Figure 2.—Correlations between phenotypes. Positive correlation coefficients are shown in red and negative ones in blue. Red boxes mark correlations within the same test or group of traits.

The phenotypic and genetic data were used to identify QTL for the traits measured in the F_2 animals. A total of 23 significant and 125 suggestive autosomal QTL, and one significant QTL on the X chromosome, were identified when analyzing both sexes together (Table 3 and Table S5 and Table S6). All but two serum traits (fT3 and calcium levels) showed at least suggestive QTL. In what follows, we focus on QTL for tameness and overlapping QTL, as well as one QTL for coat color.

Two significant QTL for tameness (measured as PC1) were identified (Figure 3A). The strongest of these (termed "*Tame-1*") is located at 58 cM on chromosome 1. The difference in tameness between homozygous genotypes at *Tame-1* corresponds to ~20% of the difference between the tame and the aggressive line (1.2 of 6.4 units of PC1). *Tame-1* explains 5.1% of the phenotypic variance in tameness. The second locus ("*Tame-2*") is located at 78 cM on chromosome 8. Both the tameness difference between homozygous genotypes (~10% of the line difference) and the portion of residual phenotypic variance in tameness it explains (2.3%, are about half of those of *Tame-1*.

The region encompassed by *Tame-1* also contains significant QTL for rearing in the open field and for adrenal gland weight, as well as a suggestive QTL for the time spent moving in the open field (Figure 3B). The effects of these QTL are in the expected direction—*i.e.*, alleles from the tame line influence the phenotype in the direction expected from the comparison between tame and aggressive animals (*e.g.*, causing higher tameness scores and lower adrenal gland weight). However, *Tame-1* also overlaps with a significant QTL that influences spleen weight. This QTL is transgressive—*i.e.*, the alleles from the tame line reduce spleen weight although the rats from the tame line have 30% heavier spleens on average (Albert *et al.* 2008).

TABLE 3

Autosomal QTL identified at genomewide significance

Trait	Chr[a]	Peak[b]	1 LOD C.I.[b,c]	*F*	Additive effect	Dominance	% variance[d]	Females[e]	Males[e]
Glove test									
Tameness (PC1)	1	58	54–64	19	0.60	0.43	5.1	Significant	Suggestive
Tameness (PC1)	8	78	68–85	9	0.37	0.43	2.3	—	Suggestive
Attack	1	60	51–73	14	−0.55	−0.48	3.8	Suggestive	—
Flight	1	48	42–64	11	−0.36	−0.08	3.2	Significant	—
Screaming	1	69	64–80	10	−0.80 sec	−0.97 sec	3.8	—	Significant
Tolerate touch	1	53	50–60	17	1.6 sec	1.0 sec	4.6	Significant	—
Other behavioral tests									
Time in corner (OF)	8	90	85–96	8	−0.05%	−0.02%	2.4	Suggestive	Suggestive
Rearing (OF)	1	72	66–83	10	3.1 sec	−0.4 sec	2.7	Suggestive	—
Startle response	10	68	57–78	11	−66 g	−7.5 g	3.0	—	Suggestive
Anatomy									
Adrenal gland	1	55	52–60	42	−3.8 mg	−0.6 mg	11.3	Significant	Significant
Adrenal gland	20	34	21–42	10	−0.6 mg	−2.0 mg	2.5	Suggestive	Suggestive
Body weight	7	30	14–46	10	11 g	5.3 g	2.9	Suggestive	Suggestive
Body weight	8	96	92–97	9	10 g	1.4 g	2.3	Suggestive	—
Heart	3	83	74–90	14	39 mg	64 mg	3.9	Suggestive	Suggestive
Spleen	10	68	58–72	16	25 mg	1.1 mg	4.6	Suggestive	Suggestive
Spleen	1	85	60–93	11	−20 mg	7.8 mg	3.0	Suggestive	Suggestive
Spleen	5	114	107–114	9	−17 mg	−10 mg	2.4	—	—
White spotting	14	26	21–33	22	0.20	−0.07	7.1	Significant	Significant
Serum traits									
Albumin	6	87	75–93	10	−0.6 g/liter	−0.3 g/liter	3.1	—	Suggestive
AP	11	22	4–35	9	0.07 μkat/liter	−0.02 μkat/liter	2.8	Suggestive	—
AP	5	114	98–114	8	−0.060 μkat/liter	0.008 μkat/liter	2.5	—	Significant
Creatinine	2	90	82–108	11	1.3 μmol/liter	0.3 μkat/liter	3.3	—	Suggestive

OF, open field test.
[a] Chromosome.
[b] Centimorgans.
[c] Confidence interval.
[d] Residual phenotypic variance explained after accounting for fixed effects.
[e] Significance level when analyzing females/males separately.

In the proximity of *Tame-2* several other QTL are found, two of which are significant. At these QTL, the alleles from the tame line increase body weight and decrease the time an animal spent in the corners of the open field, respectively. These effects are in the expected direction. Among suggestive QTL, those for time spent in the center, time spent moving and the number of rears in the open field, kidney weight, and serum aspartate aminotransferase (ASAT) all have effects in the expected direction. There were also two transgressive suggestive QTL, where the tame allele increases adrenal gland weight and the magnitude of the startle response, respectively.

We found white coat spotting to be linked to a significant QTL on chromosome 14, but no aspect of tameness mapped to this region (Figure 4). White coat spotting did not show association to *Tame-1*, *Tame-2* (Figure S10), or any other QTL for behavior during the glove test. Further, the tameness levels of individuals carrying white ventral spots did not differ significantly from those without them (*t*-test, $P = 0.17$, Figure 4A).

To assess whether the QTL we identified might be specific to one sex, we analyzed all traits using only female or male F_2 animals, respectively (Table 3 and Table S5 and Table S7). For tameness, *Tame-1* reached genomewide significance in females and chromosomewide significance in males, where the *F*-value (8.6) was close to the genomewide significance threshold (8.8). *Tame-2* reached chromosomewide significance only in males. The QTL for adrenal gland weight on chromosome 1 and for white spotting on chromosome 14 were significant in both sexes. Several QTL were suggestive in both sexes (*e.g.*, spleen weight on chromosomes 1 and 10), whereas for several others we found significant or suggestive linkage only in one sex. All individual behaviors in the glove test, but not PC1, fall in the latter category. For example, at *Tame-1*, only males showed a significant QTL for screaming, whereas only females had significant QTL for flights and toleration of touch. Toleration of touch yielded one additional significant sex-specific QTL in females and males, respectively (Table S5).

Epistatic interactions: Epistatic interactions can have large effects on phenotypic traits. Hence, we searched the genome for interacting pairs of loci for all traits described in this study. Fifteen epistatic pairs affecting

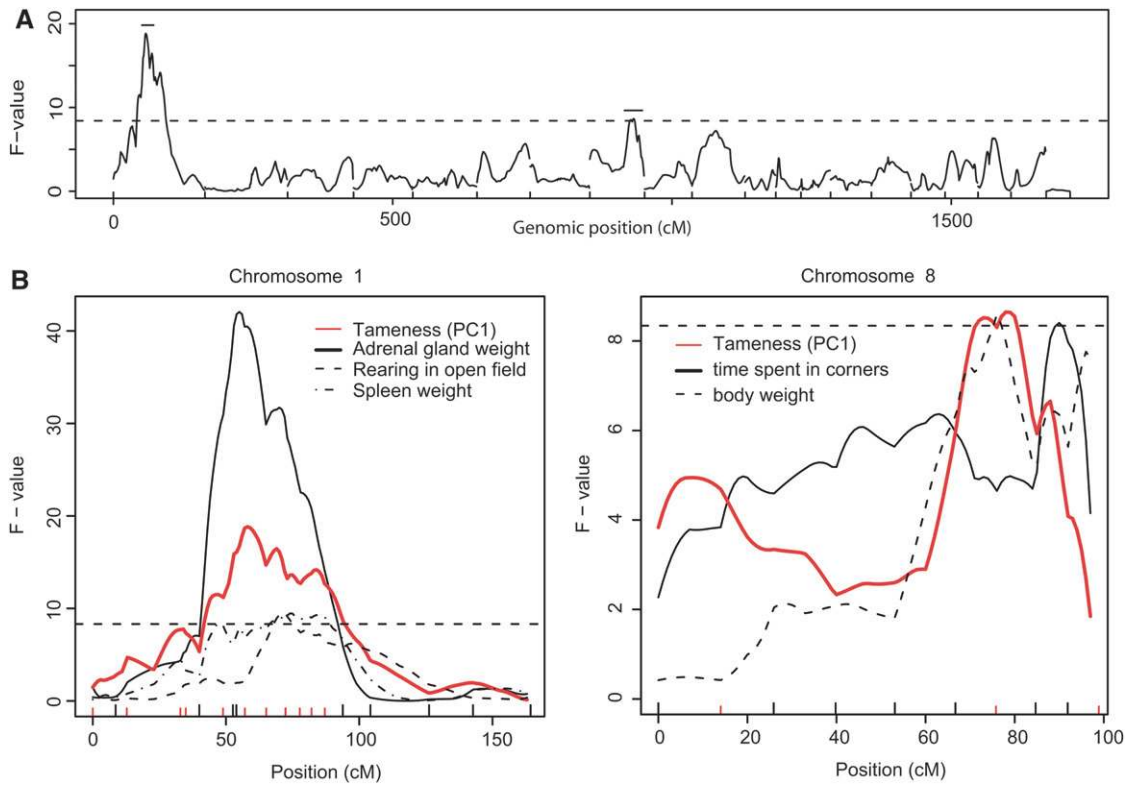

FIGURE 3.—QTL for tameness and aggression. (A) Evidence for linkage to tameness across the genome. High *F*-values indicate the presence of a QTL. The dashed horizontal line represents the genomewide significance threshold. Solid horizontal bars are 2-LOD drop confidence intervals for QTL position. Chromosome boundaries are indicated by upward tick marks on the *x*-axis. (B) Several traits map to the two QTL for tameness. Only significant QTL are shown. Black and red upward tick marks are microsatellite and SNP marker positions, respectively.

9 traits reached genomewide significance ($P < 0.05$), exceeding the random expectation of <3 pairs for 48 analyzed traits. Most pairs were found for tameness, forming an interconnected network of 5 loci (Figure 5A). The network explained 14% of the residual phenotypic variance, compared to 7.4% explained by *Tame-1* and *Tame-2* individually. It is discussed below, while epistatic pairs for the remaining traits are given in Table 4.

The tameness network comprises five pairwise interactions between five loci (Figure 5A). Two loci in the network had significant individual effects (*Tame-1* and *Tame-2*). When considering these loci simultaneously, the tame allele (T) at locus *Tame-1* increases tameness regardless of the *Tame-2* genotype (Figure 5D). The effect is, however, strongest when *Tame-2* is homozygous (AA) for the allele from the aggressive line (A). The effect of the tame allele at *Tame-2* is smallest when *Tame-1* is homozygous for the allele from the tame line (TT), where the difference between the three *Tame-2* genotypes (AA, AT, and TT) is not significant.

Both *Tame-1* and *Tame-2* interact with a locus on chromosome 19 (denoted *Tame-3*) that reached only suggestive significance in the scan for single QTL. The tame allele at *Tame-1* has a significant effect on tameness only in a heterozygous (AT) background of *Tame-3* (Figure 5B). There is no additive effect of *Tame-3* on tameness, but strong overdominance for aggression in the *Tame-1* AA genetic background and a strong overdominance for tameness in the *Tame-1* TT genetic background. This interesting shift in the direction of the dominance effect deserves further investigation. When considering *Tame-3* and *Tame-2* together, the only genotype with a deviating phenotype is the AAAA double homozygote that significantly increases aggression (Figure 5C). In addition, *Tame-1* and *Tame-2* interact significantly with one additional locus each. The effect of the tame allele at *Tame-1* is strongest when a locus on chromosome 4 (denoted *Tame-4*) is TT, and the tame allele at *Tame-4* has a significant effect on tameness only in the AA *Tame-1* genotype (Figure 5E). In this background, it is transgressive, increasing aggression. The *Tame-2* genotype also has a major effect on a locus on chromosome 6 (denoted *Tame-5*), in that *Tame-5* affects tameness only when *Tame-2* is AA (Figure 5F).

DISCUSSION

A polygenic basis for tameness: To uncover the genetic basis for tameness, we analyzed >700 F_2 animals produced by cross-breeding two strains of rats that differ drastically in their response to humans. Tameness levels,

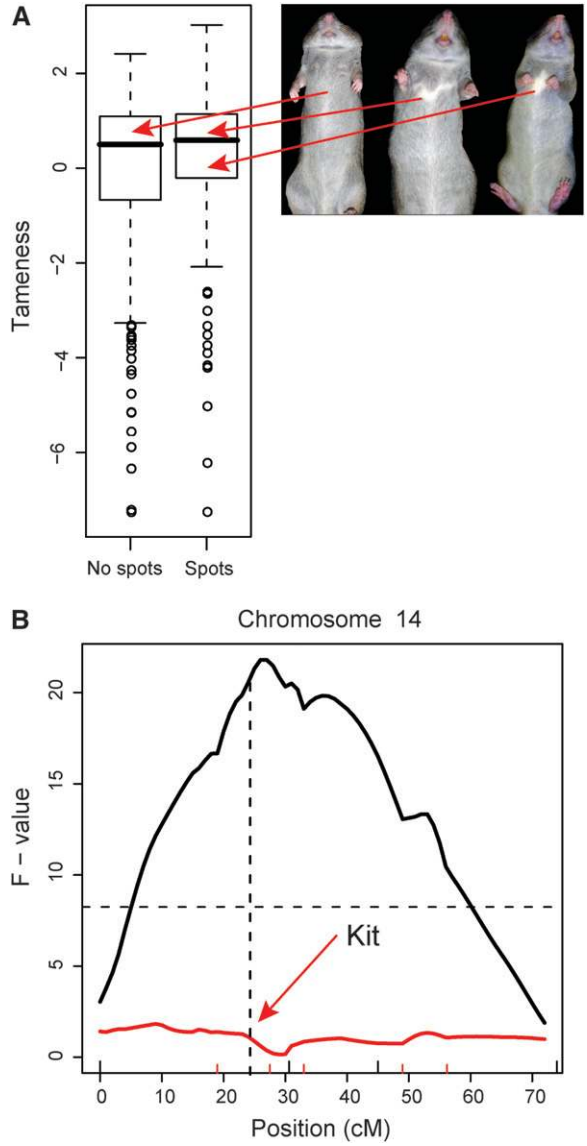

Figure 4.—White coat spotting and tameness. (A) Tameness level of F_2 animals with ($n = 190$) and without ($n = 393$) white ventral coat spots. (B) A QTL for spotting (black line) does not show linkage to tameness (red line). The vertical dashed line indicates the location of the Kit gene. Black and red upward tick marks are microsatellite and SNP positions, respectively.

as measured by the glove test in the F_1 and F_2 rats, approximate a normal distribution with the mean centered between those of the parental lines (Figure 1), suggesting a polygenic basis for tameness and aggression in the rats (Lynch and Walsh 1998).

The linkage study confirms this. The largest QTL, *Tame-1*, explains 5.1% of the residual phenotypic variance, while the remaining loci each explain smaller fractions when considered individually. These estimates are in line with the generally small effect sizes reported for QTL for other rodent behaviors (Flint 2003). Across 45 measures and three principal component scores, our study identified 23 significant and 125 suggestive QTL. We note that this greatly exceeds the number of QTL expected to be observed by chance. For 48 traits, we expect <3 significant QTL at a genomewide significance level of 5% and 48 suggestive QTL with a significance level of 5% at each of 20 chromosomes. Given the sample size of >700 F_2 animals, we consider it unlikely that other unidentified loci with large individual effects exist in these lines.

Overlap of QTL for tameness-associated traits: Previous studies have identified a multitude of phenotypic differences between the tame and the aggressive lines of rats (Naumenko *et al.* 1989; Plyusnina and Oskina 1997; Popova *et al.* 2005; Albert *et al.* 2008). However, the behavioral response to humans was the only criterion used during selection. Are the loci influencing the nonselected traits the same as those contributing to the difference in tameness?

If phenotypic traits are influenced by the same loci one would expect them to show some degree of correlation. It is thus noteworthy that correlations between the traits we measured in the F_2 population were weak at best, often failing to reach significance in spite of the fact that hundreds of animals were analyzed and that some of the uncorrelated traits were markedly different between the parental strains (Albert *et al.* 2008). However, the power to detect a correlation caused by shared loci may be limited given that the effect sizes associated with the alleles are small and perhaps obscured by nongenetic influences.

In the QTL analyses, a number of traits mapped to the same regions. This is especially obvious on chromosome 1 where weight of the adrenal gland maps to a region overlapping *Tame-1* with virtually identical confidence intervals (Figure 3B). It thus seems plausible that alleles of a single gene with pleiotropic effects underlie both *Tame-1* and adrenal gland size variation. Alternatively, a causal relationship might exist between tameness and adrenal gland size. For example, sudden increases of plasma corticosterone, which is produced by the adrenal cortex, promote aggressive behavior in rats (Kruk *et al.* 2004), while chronically high levels of glucocorticoids seem to inhibit aggressive behavior in several vertebrate species (Summers *et al.* 2005). However, *postmortem* corticosterone levels did not map to any locus linked to tameness or aggression. It is thus equally possible that other hormonal activities of the adrenal glands are directly or indirectly linked to the alleles that affect tameness in the rats. The identification of the gene or genes underlying *Tame-1* as well as adrenal gland size variation will eventually clarify this.

Tame-1 was obtained by mapping a principal component score summarizing, among other traits, attacks, screaming, flights, and the toleration of touch (Table 2). The fact that, at *Tame-1*, these individual behaviors yielded QTL apparently specific to one sex (Table 3) may suggest that the causative alleles underlying *Tame-1* influence the two

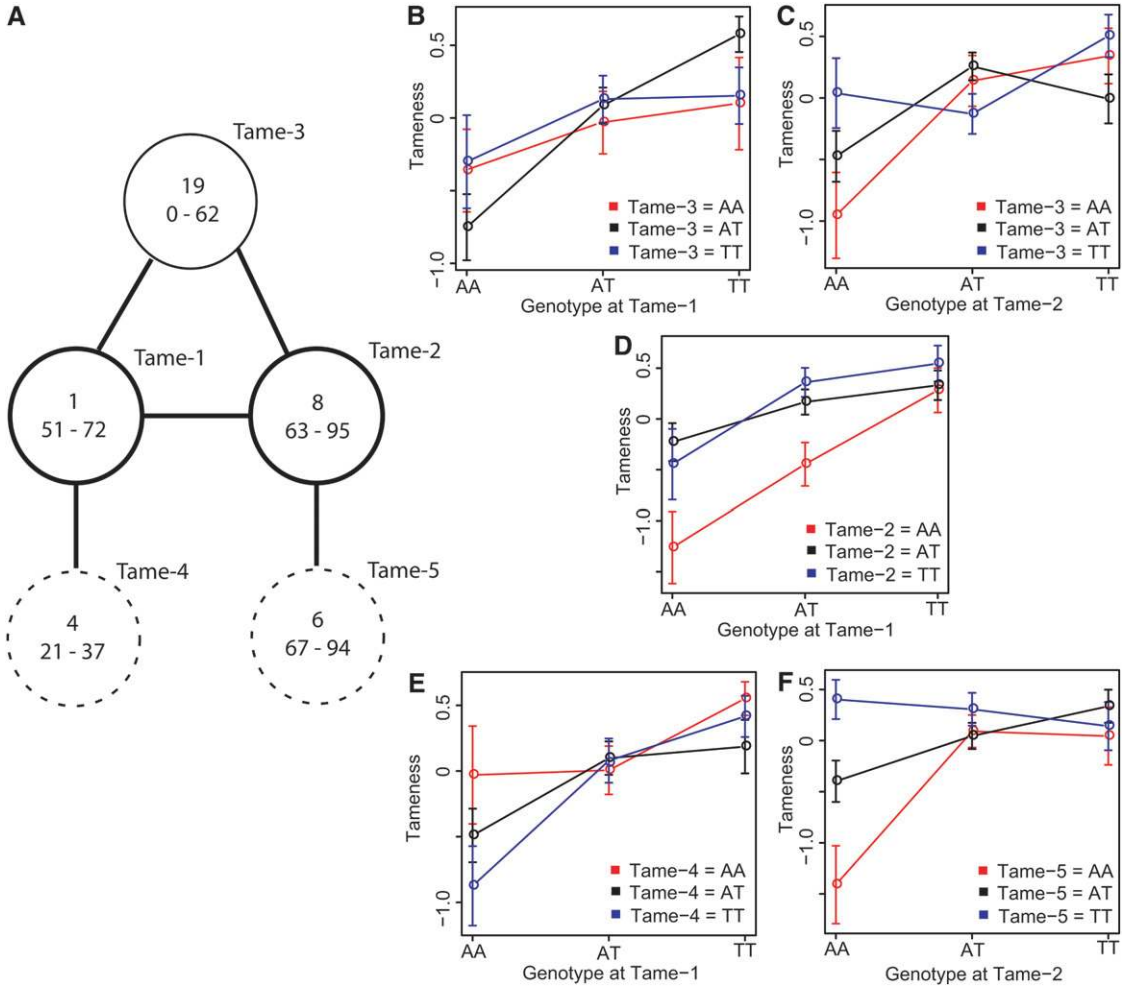

Figure 5.—An epistatic network for tameness. (A) Overview of QTL (circles) and epistatic interactions (lines). Only QTL in pairs with significant epistatic interactions are shown. Bold (nonbold) solid circles: the QTL was significant (suggestive) in the scan for single loci. Dashed circles: the QTL was significant only as part of an epistatic pair. Numbers in circles indicate QTL chromosome and position (centimorgans). (B–F) Phenotypes for two-locus genotypes. Circles indicate the mean phenotype for the respective two-locus genotypes; error bars show the standard error of the mean. The strength of the QTL effect at the first locus in the pair is indicated by the slope of the line connecting the homozygous genotypes [both alleles from the tame line (TT) vs. both alleles from the aggressive line (AA)]. An effect at the second locus in the pair is indicated by nonoverlapping allele effects at a given genotype of the first locus.

sexes differently. However, since the QTL for the principal component at *Tame-1* is found in both sexes, it may be the case that the absence of signals for the individual traits reflects lower power due to using half the number of individuals. In addition, individual traits are likely to have less power than the principal component they contribute to. Further work is warranted to clarify whether *Tame-1* and other loci truly act in a sex-specific manner.

To our knowledge, this study is the first genetic mapping of tameness and defensive aggression against humans in any species. However, several studies in rats have identified QTL for traits potentially related to those studied here. *Tame-1* overlaps with earlier identified QTL influencing several anxiety-related traits (Terenina-Rigaldie *et al.* 2003), rearing behavior (Fernandez-Teruel *et al.* 2002), and adrenal gland weight (Solberg *et al.* 2006). *Tame-2* overlaps with two QTL for activity and anxiety-related behaviors (Terenina-Rigaldie *et al.* 2003; Conti *et al.* 2004). It is reassuring that phenotypes similar to some of those studied here show linkage to similar genomic locations. However, in the absence of information on the molecular basis of these QTL, it cannot presently be determined whether alleles at the same genes are responsible.

A genetic network for tameness: Epistasis affects the expression of numerous traits (Phillips 2008). For behavioral quantitative traits, however, epistatic networks identified by genome scans remain rare (for exceptions, see Wright *et al.* 2006; Bailey *et al.* 2008). In this cross, a network of *Tame-1* and *Tame-2* and three additional loci that were identified only as part of these epistatic pairs influence tameness (Figure 5A).

The additive effect of *Tame-1* was robust across genetic backgrounds (Figure 5), suggesting that *Tame-1* is a major

TABLE 4

Epistatic pairs of QTL identified at genomewide significance

	Locus 1			Locus 2			
Trait	Chr[a]	Peak[b]	Position[b]	Chr[a]	Peak[b]	Position[b]	% variance[c]
Tameness (PC1)	1	58	42–96	8	89	44–97	9.6
	1	69	67–75	4	25	21–37	8.7
	1	69	67–71	19	53	47–61	8.7
	6	82	67–94	8	73	67–81	6.3
	8	71	66–80	19	52	43–61	6.8
Flight	1	45	43–48	9	8	0–14	6.9
Boxing	1	75	73–79	8	95	88–97	6
Time spent moving (LDT)	8	86	83–88	9	84	82–84	5.4
Fecal boli (LDT)	1	10	5–10	10	59	49–71	6.6
	4	8	1–15	13	22	17–27	6.1
Startle response	2	48	33–55	10	69	63–86	6.5
Body weight	7	55	22–68	8	97	95–97	6.3
	8	96	94–97	18	16	12–17	6
Adrenal gland weight	3	0	0–2	11	43	38–48	5.2
Corticosterone	5	25	18–35	6	82	73–92	6.2

LDT: light–dark test.
[a] Chromosome.
[b] Centimorgans.
[c] Residual phenotypic variance explained after accounting for fixed effects.

locus influencing tameness. In contrast, *Tame-2* seems to act as a capacitor of other loci. When it is homozygous for the allele from the aggressive line, it magnifies the effects of all three loci it interacts with (Figure 5, C, D, and F). This is reminiscent of epistatic loci underlying growth in chicken (Carlborg *et al.* 2006). In addition, the effect of *Tame-2* itself is highly dependent on other loci. In a tame background, *i.e.*, where the other loci are homozygous for the allele from the tame line, *Tame-2* has at most a small effect on tameness.

The epistatic network raises interesting questions about the role of *Tame-1* and *Tame-2* during selection for tameness and aggression. Due to the relatively invariant effect of *Tame-1*, it can be selected for in many genetic backgrounds, driving alternative alleles rapidly to fixation. On the other hand, the homozygous aggressive genotype at *Tame-2* might have had an initial role in selection by magnifying the effects of other loci, allowing them to become more prominent targets of selection, while the allele from the tame line would have decreased the response of other loci to selection. The increased frequency of homozygous tame genotypes at loci other than *Tame-2* will, however, decrease the selective advantage of the tame allele at *Tame-2*, due to its small effect in this background. Given the intricate interactions between *Tame-2* and the other loci, it is an intriguing possibility that *Tame-2* might harbor multiple alleles in the current tame and aggressive rat lines. Thus, the single tame and aggressive alleles might in fact be average effects across several alleles. A more in-depth analysis of patterns of polymorphism at *Tame-2* and other loci might shed light on this.

White coat color and tameness: Many domestic animals across a wide range of species are distinguished from their wild relatives by conspicuous coat color variants. Possible explanations include direct selection for coat color variants by humans (*e.g.*, Pielberg *et al.* 2008) and removal of selective pressures for camouflage. It is further conceivable that coat color variation is a pleiotropic effect of alleles influencing other traits and particularly behavior, including the level of tameness (Keeler and King 1942; Cottle and Price 1987; Hayssen 1997).

The F_2 rats provide an excellent opportunity to test whether loci influencing tameness also affect white coat spotting. If the same genes are responsible, F_2 animals carrying coat spots should be more tame than those without. However, this was not observed (see results). Similarly, the QTL for coat spotting shows no linkage to tameness or any other trait (Figure 4B), and neither *Tame-1* nor *Tame-2* is linked to coat spotting (Figure S10). Hence, we find no evidence for white spotting being caused by the same loci that contribute to tameness. Pleiotropic effects linking tameness and coat color may occur in other species, or even in other lines of rats, but such scenarios are clearly not strengthened by these results.

It is noteworthy that the QTL for white coat spots on chromosome 14 contains at its center the *Kit* gene

(RefSeq NM_022264), which encodes a tyrosine-kinase receptor involved in melanoblast migration (Yoshida *et al.* 2001). Allelic variants of homologs of rat *Kit*, or of the gene for the *Kit ligand* (*Kitl*; RefSeq NM_021843), are known to cause white coat color variants in mice (Jackson 1994), pigs (Marklund *et al.* 1998), horses (Haase *et al.* 2007), and stickleback fish (Miller *et al.* 2007). Thus, *Kit* is an excellent candidate for causing the white coat spots in the rats studied here.

Conclusions: We present a genetic analysis of traits associated with tameness in a rat model of animal domestication. Tameness is found to be influenced by two major loci, which are part of a five-locus epistatic network. A possibility not explored here are epistatic interactions involving more than two loci. Such interactions are, however, very difficult to detect given the sample size limitations in mammals.

The confidence intervals for the two tameness loci contain 744 (*Tame-1*) and 339 (*Tame-2*) genes annotated in the Ensembl database, respectively. Since few genes underlying QTL for any behavior have been identified (for two notable exceptions, see Yalcin *et al.* 2004 and Watanabe *et al.* 2007), and none of them are located in *Tame-1* or *Tame-2*, it seems premature to speculate about what genes underlie *Tame-1* and *Tame-2*. Rather, fine-mapping approaches such as advanced intercross lines, as well as other approaches, are needed to clarify what genes are involved.

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Genetic Architecture of Tameness in a Rat Model of Animal Domestication

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FILE S1

Materials and Methods

Mapping pedigree: The pedigree described in this study was initiated from four unrelated tame and four aggressive individuals (two females each; the "F_{-1}" or "F minus one" generation) mated within line to yield 11 (five tame and six aggressive rats, one male each) F_0 animals. The F_{-1} animals did not have common parents and at most one common grandparent. The F_0 animals were crossed reciprocally, i.e. both sexes were used from each line. Two out of four tame females and two out of five aggressive females were full siblings of the respective F_0 founder males. Six hybrid F_1 males (three sired by the tame, and three sired by the aggressive F_0 male) were repeatedly mated to 37 F_1 females to produce a total of 733 F_2 rats (383 females). F_1 animals sired by the aggressive F_0 male were mated only to F_1 animals sired by the tame F_0 male, and vice versa.

Behavioral testing: A list of all traits we collected can be found in Table 1. At six to eight weeks of age, F_2 rats performed the "glove test", which measures an animal's level of tameness/aggression by confronting it with an approaching human hand and attempts at handling (see (ALBERT *et al.* 2008) for details on the testing procedure). Within two weeks, but at least after a four-day break, rats performed an open-field test, followed three days later by a light-dark test. These tests provide various measures of exploratory and anxiety-related behavior. After nine days, rats performed a startle response test, which measures the behavioral response to a sudden acoustic stimulus. 470 (64%) of the F_2 rats then performed a second glove test trial after maximally two weeks. F_1 rats were tested once in the glove test at 12 – 14 weeks of age, and then followed the testing schedule described in (ALBERT *et al.* 2008). All tests were performed with minimal handling following the procedures described in (ALBERT *et al.* 2008). Open field, light-dark and startle response test data were recorded using automated measuring technology (TSE Systems, Bad Homburg, Germany). Glove test trials were videotaped and later analyzed by two independent observers (5% of trials only by one observer) using the software "Interact" (Mangold Software, Arstorf, Germany). Experimenters and observers were blind to the animals' identity and to further data processing. A set of 11 behaviors (e.g. "attack" or "tolerate handling") were scored (see (ALBERT *et al.* 2008) for detailed descriptions of the behaviors), and each converted to a numeric measure (e.g. "number of occurrences" or "duration in seconds"; Table 1).

Serological phenotypes: We measured a series of serum traits, most of which had earlier been found (ALBERT *et al.* 2008) to differ between the tame and the aggressive rats (Table 1). Serum was analyzed in 684 F_2 rats (357 females) as described in the main text. Corticosterone levels reflect the rats' response to handling and sacrifice, rather than baseline (undisturbed) levels. This is consistent with their high values compared to basal measures reported in the literature (VAHL *et al.* 2005). Twenty-two F_2 individuals were excluded from further analysis of all serum traits because they appeared to be outliers with respect to the remaining F_2 population: two individuals with chloride values < 90 mmol / l and 16 individuals with chloride values > 110 mmol / l, and four individuals with triglyceride levels > 4 mmol / l.

Glove test analyses: To summarize a rat's behavior in the glove test, we performed principal component analysis (PCA) on the individual glove test measures. The resulting principal component (PC) scores represent a linear, weighted combination of individual behaviors. Combining animals from several generations when performing PCA could potentially distort the results. Hence, we used only F_2 animals in the analysis.

F_0 and F_1 animals performed only one glove test trial and were scored by a single observer. For comparing F_2 animals' glove tests to those of the F_0 and F_1 generations, we used only observations from the first trial, and from the same observer who scored the F_0 and F_1 animals. We applied the PCA regression coefficients obtained in F_2 animals to the single observations from F_0, F_1, and the comparable subset of single F_2 observations. A small number of F_2 animals had PC scores that exceeded the range defined by the most tame and the most aggressive F_0 animals. Since QTL mapping can be sensitive to phenotypic outliers, we set these extreme individuals' phenotypes to the most extreme values observed in F_0 animals. Adjusting outliers this way did not greatly alter the mapping results.

Marker ascertainment and genotyping procedures: Animals were genotyped with 152 microsatellite and 49 single nucleotide polymorphism (SNP) markers. SNPs were added after a preliminary scan for QTLs using only the microsatellite data (not shown), to improve genome coverage and to obtain more data in putative QTL regions. Microsatellite markers were ascertained from public databases. SNP markers were ascertained from a panel of 16,927 SNPs under study at the CEA/IG-Centre National de Genotypage, Evry, France. All markers were selected based on their segregation patterns in the outbred tame and aggressive rat lines as determined from preliminary genotyping of a panel of F0 animals. All markers used in the QTL mapping are listed in Table S2. DNA was isolated either from lung tissue according to the NucPrep Protocol for animal tissues (Applied Biosystems, Foster City, CA, USA) or from spleen tissue using the DNeasy Blood & Tissue Kit (Quiagen GmbH, Hilden, Germany) according to the manual's section "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)". The extraction protocols were slightly modified. For NucPrep, digestion at 65 °C for 1 h was preceded by overnight incubation of the tissue samples at room temperature in NucPrep Digestion Buffer and Proteinase K (both Applied Biosystems), and the pre-filtration step was skipped. For DNeasy, two final elution steps were performed, each with 100 µl of Buffer AE. For all markers, polymerase chain reaction (PCR) was performed in 384-well format. Microsatellite marker PCRs were performed using the M13-primer PCR system (SCHUELKE 2000). 15 ng of dried DNA per sample were used in a 10 µl PCR mix containing (per sample) 1.0 µl of PCR buffer (Quiagen), 0.5 µl DMSO (Merck KG, Darmstadt, Germany), 0.4 µl dNTPs (5 mM) (Amersham Biosciences, Buckinghamshire, England), 0.2 µl forward and reverse primer in a 1 / 10 ratio (1 / 10 µM) (Thermo Electron, Ulm, Germany), 0.2 µl M13-tail (10 µM) labeled with one of three fluorophores [6-fam (Thermo Electron), Ned or Vic (both Applied Biosystems)], 7.66 µl of water and 0.039 µl of HotStarTaq® Plus DNA polymerase (5 U / µl) (Quiagen). The M13-tail used in all reactions was 5'-[fluorophore]-CACGACGTTGTAAAACGAC. PCR was performed on Eppendorf Mastercycler ep384 thermocyclers (Eppendorf, Hamburg, Germany) according to the following touch-down PCR protocol: 95 °C for 5 min., 44 cycles of each at 94 °C for 30 s, a variable temperature for 30 s

and 72 °C for 30 s. The variable temperature was 65 °C in the first cycle and decreased by 1 °C in the following 5 cycles, then decreased by 0.5 °C for 17 cycles and then held constant at 52 °C for the remaining 21 cycles. PCR products were held at 8 °C after program completion. Up to 10 PCR products of different expected lengths and labeled with different dyes were pooled and analyzed on an ABI3730 DNA Analyzer (Applied Biosystems). Microsatellite genotypes were determined using the software GeneMapper Version 4.0 (Applied Biosystems) and all genotypes were manually double-checked. SNP assays were ordered from ABI Applied Biosystems. PCR cocktails containing 10 ng of dried DNA / sample were prepared according to the assay manufacturer's specifications, but using "ABgene Absolute QPCR Rox Mix X2" (Applied Biosystems) instead of "TaqMan Universal PCR Master Mix". PCR was performed on Eppendorf Mastercycler ep384 thermocyclers (Eppendorf) as follows: 10 min. at 95 °C, followed by 40 cycles of 15 s at 92 °C and 1 min. at 60 °C; reactions were held at 8 °C after program completion. Intensity scans of PCR products were performed on an ABI 7900HT Sequence Detection System v2.2 (Applied Biosystems). SNP genotypes were called automatically as part of the scanning process, and genotype plots inspected visually. Preliminary genotyping of 16,927 SNPs had been performed using the Illumina GoldenGate assay according to manufacturer's instruction in an Illumina Beadlab, as described (SAAR *et al.* 2008).

Details on linkage map construction: We constructed sex-averaged linkage maps for each chromosome using a version of the program crimap (GREEN *et al.* 1990) modified to handle large pedigrees by the University of California Davis, Veterinary Genetics Laboratory. We used the option FIXED to estimate marker distances in centiMorgan (cM) on markers ordered according to their physical chromosomal locations as annotated in the UCSC Genome Browser. Marker order was confirmed using crimap's FLIPS option. Markers that were not annotated in the genome browser were placed on the linkage map using the option ALL. We used the option CHROMPIC to identify inferred double recombination events. For all such events, genotype data was double-checked for errors.

Computation of linkage map information content: We estimated the information content at marker positions from the respective gametic identical-by-decent (IBD) matrices X_g (see below for the matrix estimation procedure). For a given position, the IBD matrices specify which individuals have inherited identical alleles. At informative positions, many allele pairs can be assigned unambiguously (IBD = 1 or IBD = 0). At less informative positions, some estimated IBD estimates will tend towards 0.5. We estimated the information content at a position as $2\frac{1}{n^2}\sum\left|0.5 - X_g\right|$, where n is the number of alleles in the pedigree. The information content statistic takes a value of one for perfectly informative markers, and zero if no allele can be traced to its pedigree origin. On the X-chromosome, we calculated a lower limit for the information content at marker positions as the average difference in allele frequency between tame and aggressive F_{-1} founders, weighted by the respective allele frequency across all F_{-1} founders.

Haplotype-based inference of missing genotypes: Missing F_0 genotypes can lead to a loss of power in QTL mapping because some alleles in F_2 animals might not be traced reliably to parental lines. We sought to overcome this limitation by including the genotyped parents of F_0 animals (the F_{-1}) in the analysis, and by tracing alleles back to them. This way, we sought to infer missing genotypes of F_0 and F_1 animals based on their ancestors' and offspring genotypes. We first determined the phase of informative markers for all individuals in the pedigree. Then, we used a genetic algorithm to iteratively generate chromosome segments carrying putative haplotypes. For each such segment, the algorithm calculates the likelihood that the segment is compatible with the phase of the informative markers, and keeps the most likely haplotype from each iteration until it converges to an optimum. We then inferred the missing genotype information in F_0 and F_1 animals from the haplotypes of related F_{-1} and F_2 individuals. Details of this procedure for inference of haplo- and genotypes will be published elsewhere.

Single QTL mapping on sex chromosomes: The analyses described thus far were designed to handle autosomal data. To analyze the X-chromosome, we used the software QxPak (PEREZ-ENCISO and MISZTAL 2004), which is able to handle sex chromosomal data appropriately. For each trait, we first fitted an additive QTL using the option 'fix_a'. Where this yielded a significant ($p < 0.005$) QTL, we then tested for dominance using option 'fix_ad'. Since the permutation-based significance thresholds derived for the autosomes cannot be directly applied to the X chromosome, we assumed QTLs with a nominal p-value < 0.001 (0.005) to be significant (suggestive) at a genome-wide level, as suggested in the QxPak manual.

To test for linkage to the Y chromosome, we note that F_2 males in our pedigree carry one of only two Y chromosomes, one derived from the tame F_0 male, one from the aggressive F_0 male. Due to the design of our cross, F_2 males carrying the Y chromosome from one parental line always carry mitochondria (and potentially other maternally inherited factors) from the same line. However, while F_2 females share such maternal factors with F_2 males, they do not carry the Y chromosomes. This allows us to disentangle the effect of the Y chromosome by comparing the phenotypes of F_2 males carrying the Y chromosome inherited from the tame F_0 male, of males carrying the Y chromosome inherited from the aggressive F_0 male, and of F_2 females that were sired by the respective F_1 males. A Y-linked locus should lead to a phenotype difference between the two groups of F_2 males, while the two groups of F_2 females should not differ. In an analysis of variance (ANOVA), we tested for interactions between the sex of F_2 animals and the strain origin of the Y chromosome carried by their fathers. Covariates were included in the ANOVA as listed in Table S6. We assumed linkage to the Y chromosome if the interaction term was significant ($p < 0.005$) and if a post-hoc T-test showed a significant ($p < 0.05$) difference between F_2 males, but not between F_2 females.

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FILE S2

A compressed folder is available at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

This folder contains four text files that contain raw data (as described below). This description is also available in the **Read Me.txt** file located in the compressed folder.

1. "genotypes.txt" – contains genotypes and marker info
    - first line contains marker names
    - second line contains markers' chromosomes
    - third line contains markers' genetic position on a given chromosome
    - the rest of the file contains the genotypes:
        - first column is animal id
        - there are *two* columns per marker, one for each allele
        - allele codes are arbitrary numbers, and do not by themselves contain information about strain origin or the genotype
        - the two allele columns per marker do *not* indicate whether an allele was inherited from the mother or the father
        - missing genotypes are coded as '0'
        - on the X-chromosome, '9' indicates the "missing" allele in hemizygous males

2. "pedigree_fixed_effects_covariates.txt"
    - first column is animal id
    - second column is an animal's father ('0' for the first generation)
    - third column: is an animal's mother ('0' for the first generation)
    - fourth column is sex: males are '1', females are '2'
    - fifth column indicates an animals strain (tame or aggressive) or the respective generation in the cross (F1 or F2)
    - remaining columns are numerical covariates (measured only in F2 animals)

3. "phenotypes.txt"
    - first column is animal id
    - only F2 animals were measured (beginning with animal ID 63), and only F2s are listed in this file
    - with the exception of glove test traits, all traits are "raw", i.e. not adjusted for family effects or covariates
    - glove test behaviors in this file *are* adjusted for observer, trial and family effects - see "raw_glove_data.txt" for raw glove test data

4. "raw_glove_data.txt"
    - data from the glove test, directly as scored from the video tapes
    - this data forms the basis for the principal components used to calculate tameness and aggression scores
    - only F2 animals are listed (beginning with animal ID 63)

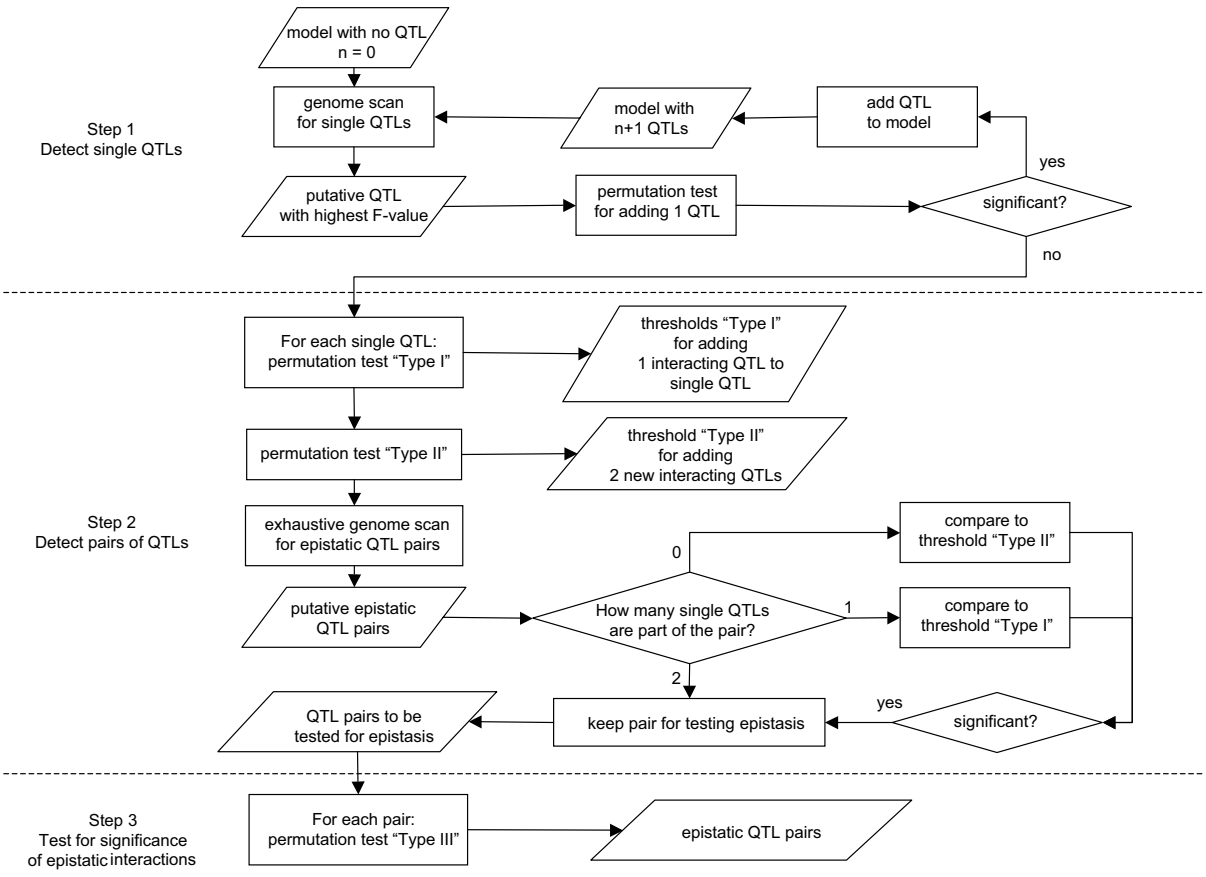

FIGURE S1.—Flow diagram of the search strategy for single QTLs and epistatic QTL pairs.

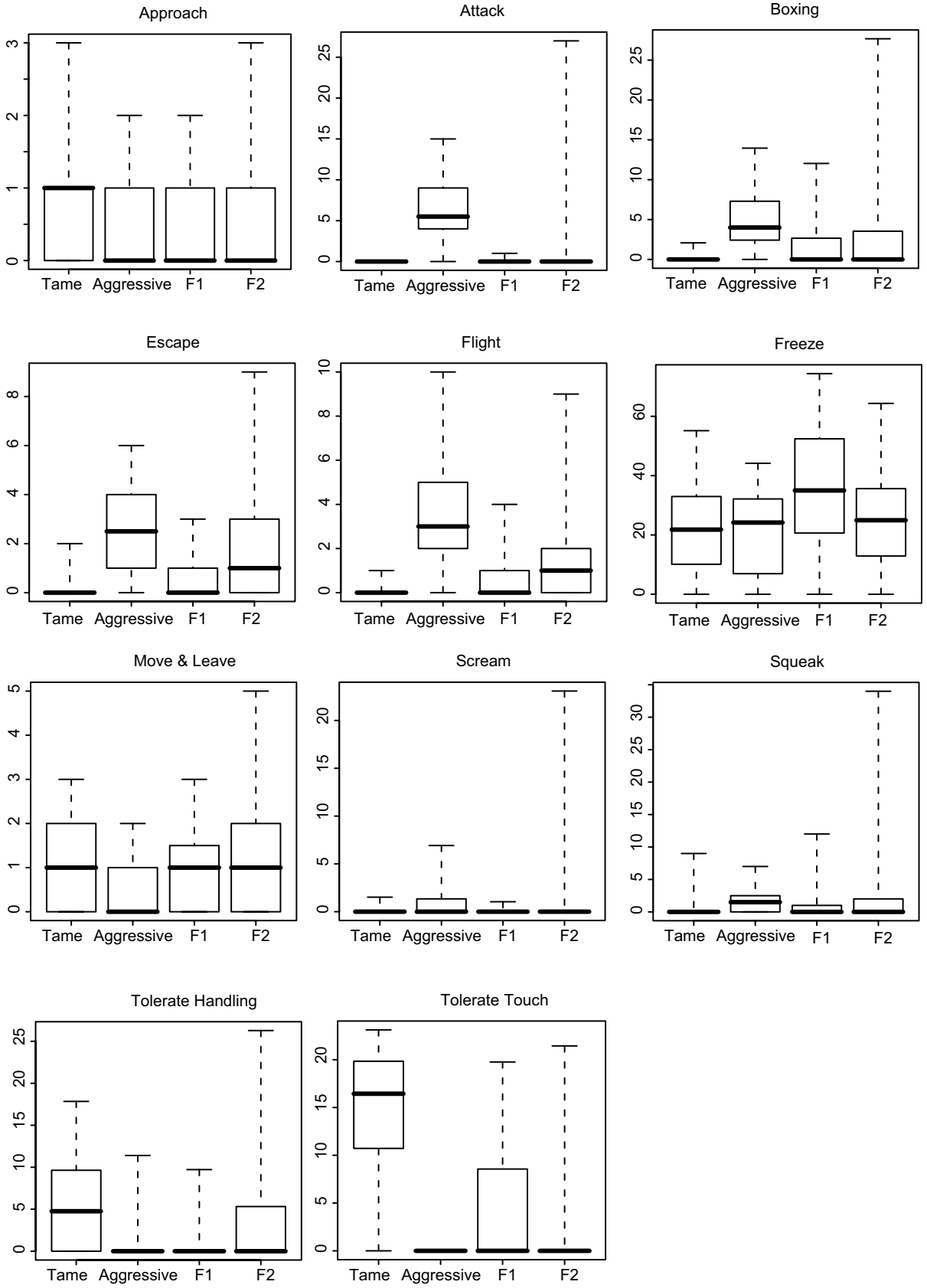

FIGURE S2.—Phenotype ranges for traits recorded in the glove test. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values.

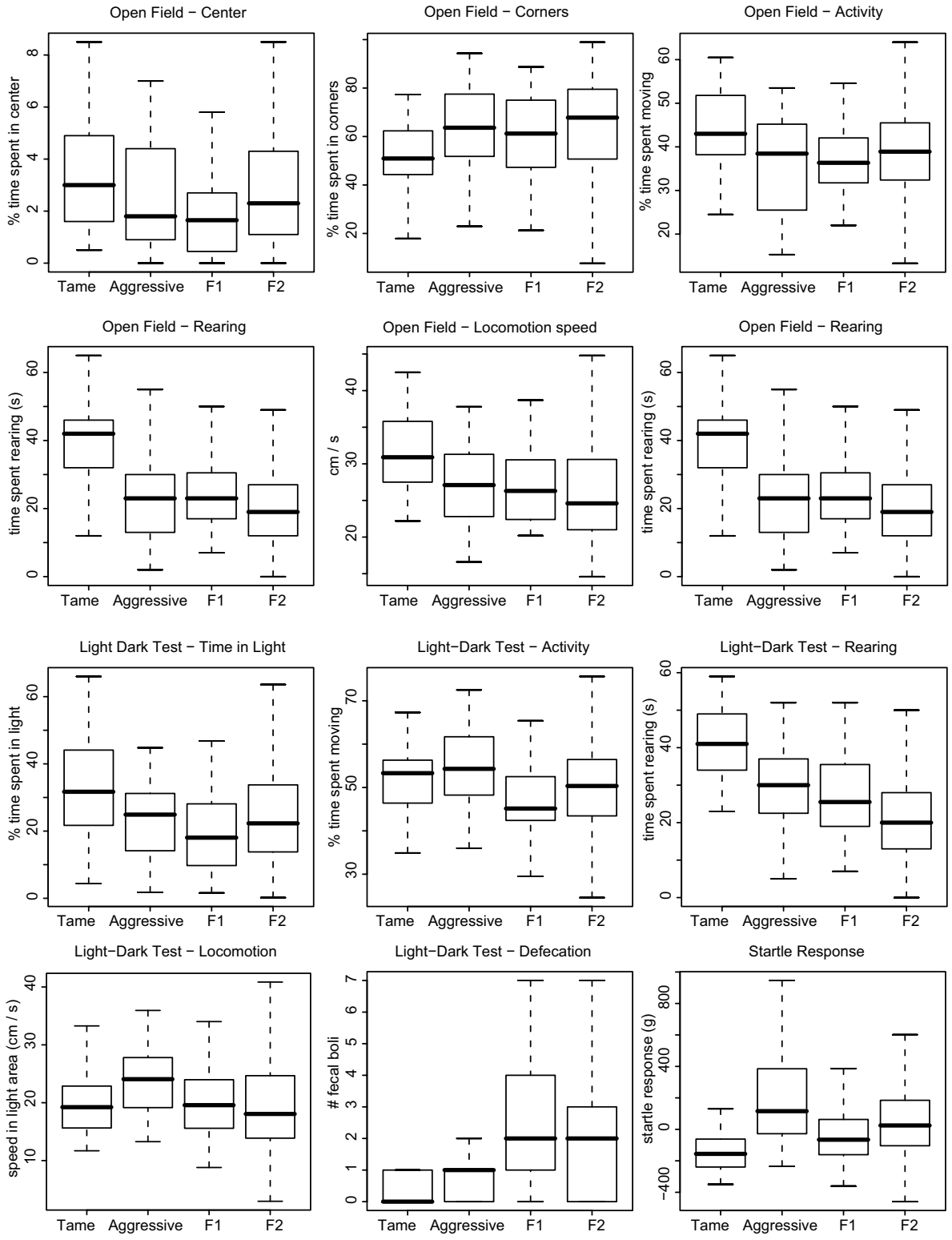

FIGURE S3.—Phenotype ranges for traits recorded in the open field, light-dark and startle response tests. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

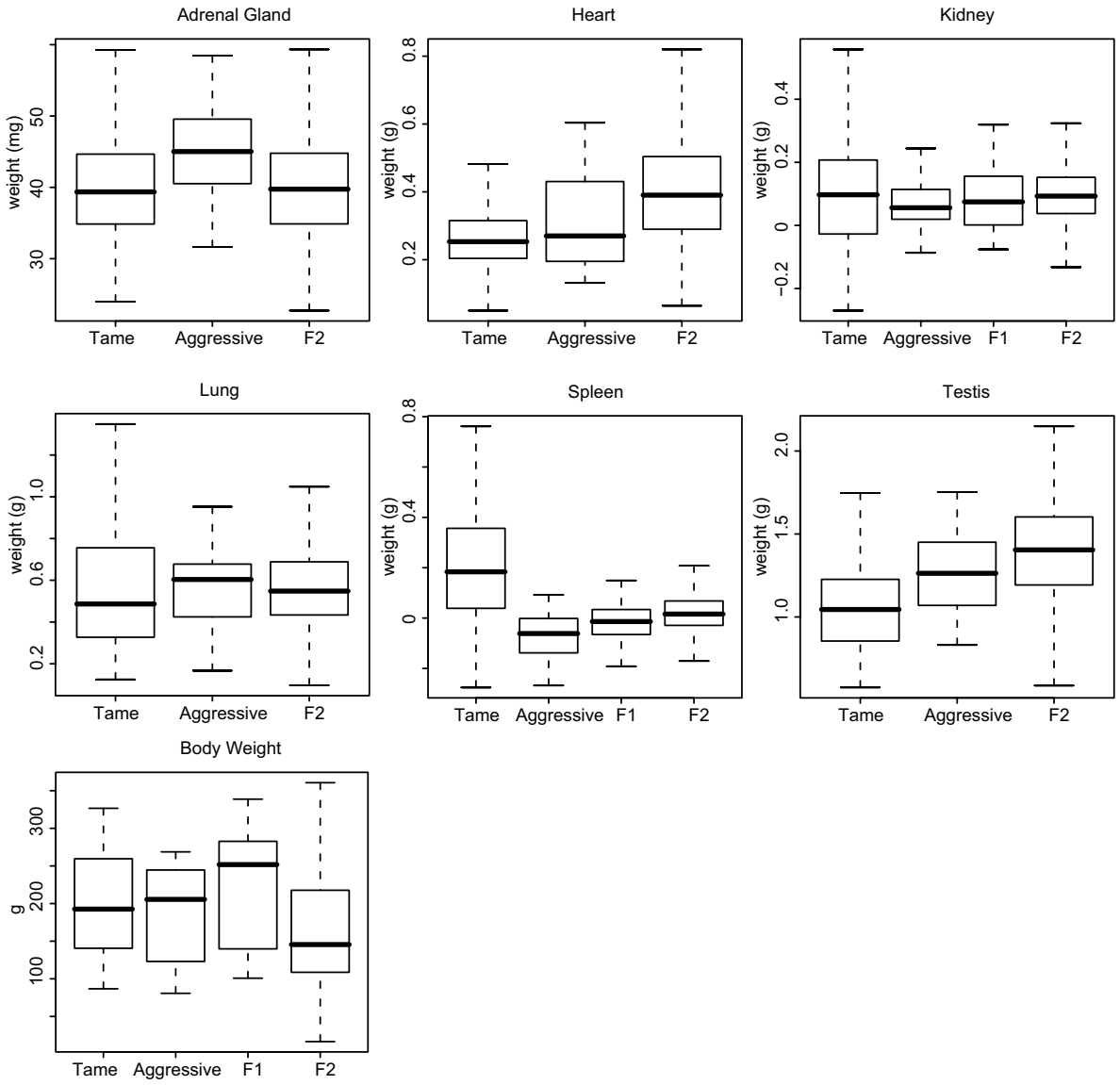

FIGURE S4.—Phenotype ranges for body and organ weights. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

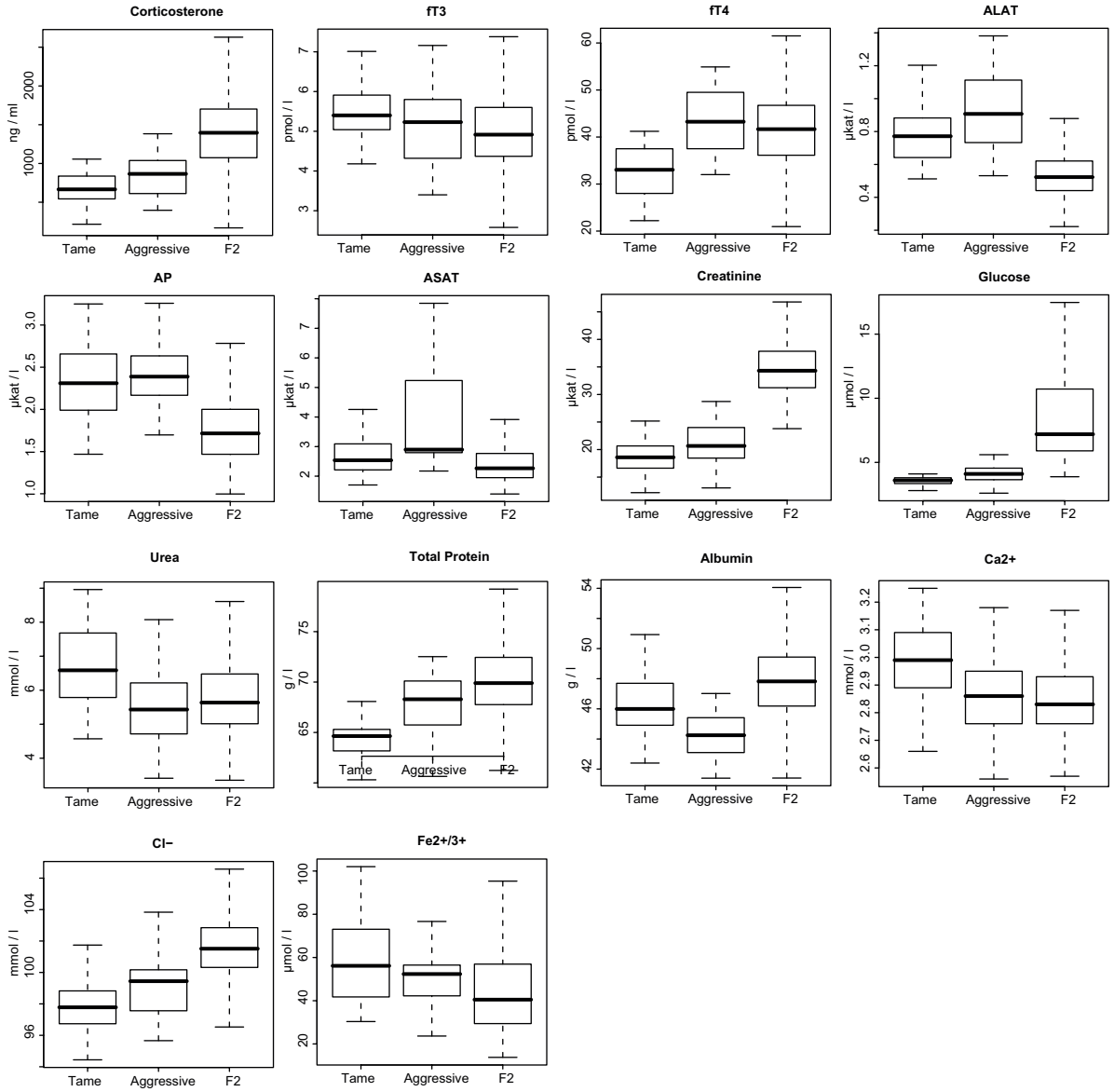

FIGURE S5.—Phenotype ranges for serum traits. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

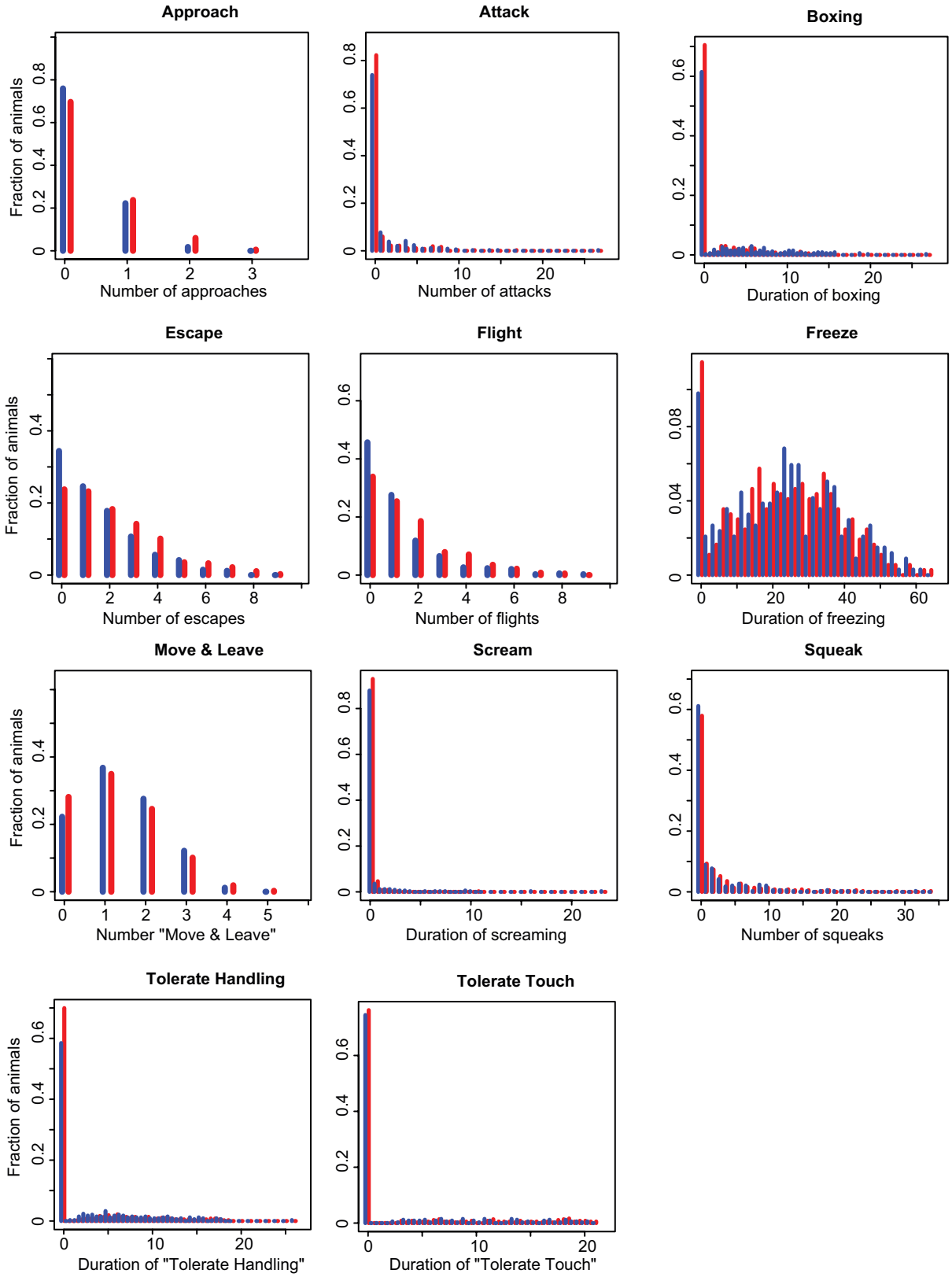

FIGURE S6.—Phenotype distributions for traits recorded in the glove test. Red: female F2 rats, blue: male F2 rats

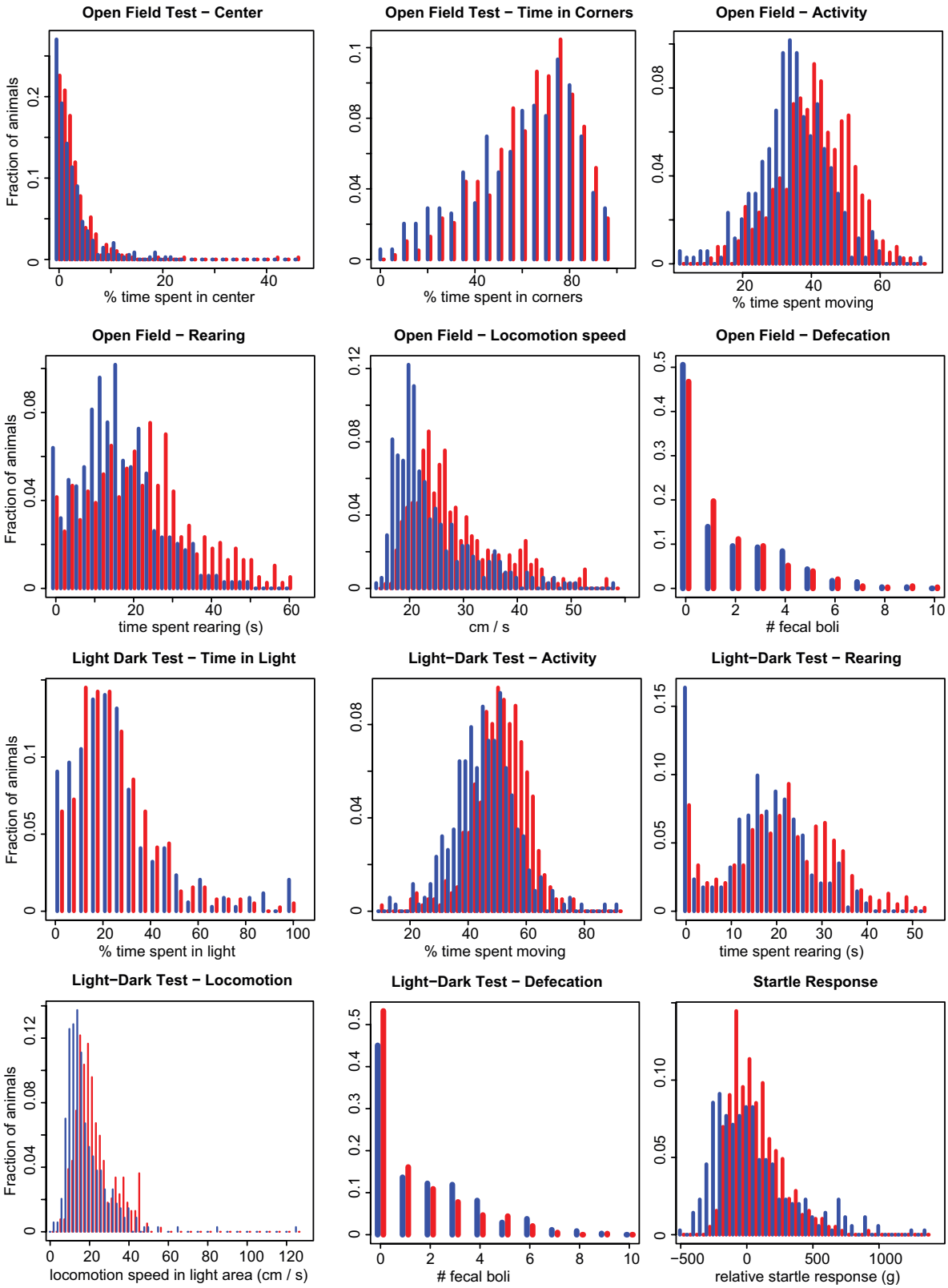

FIGURE S7.—Phenotype distributions for traits recorded in the open field, light-dark, and startle response tests. Red: female F2 rats, blue: male F2 rats.

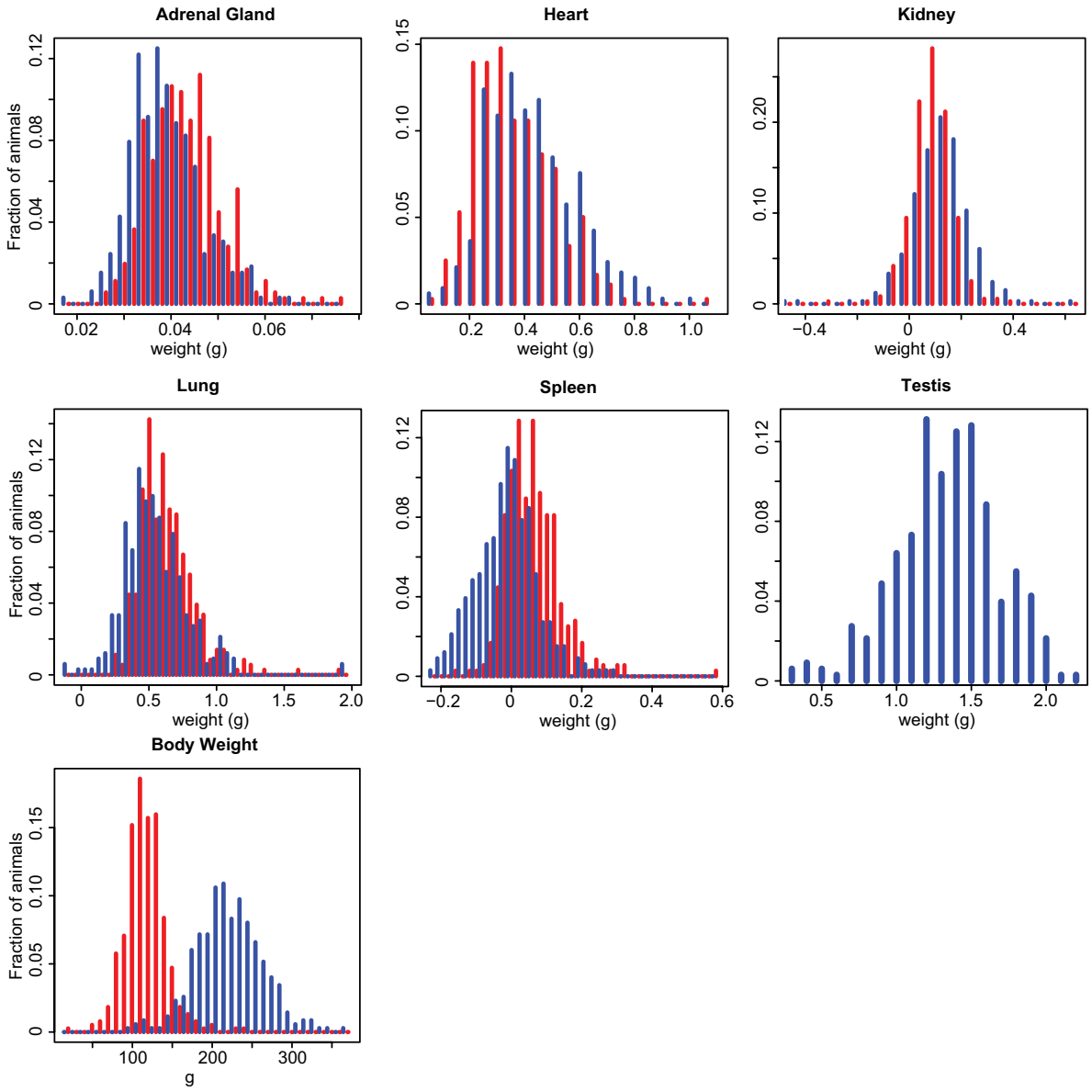

FIGURE S8.—Phenotype distributions for body and organ weights. Organ weights are shown relative to body weight. Red: female F2 rats, blue: male F2 rats

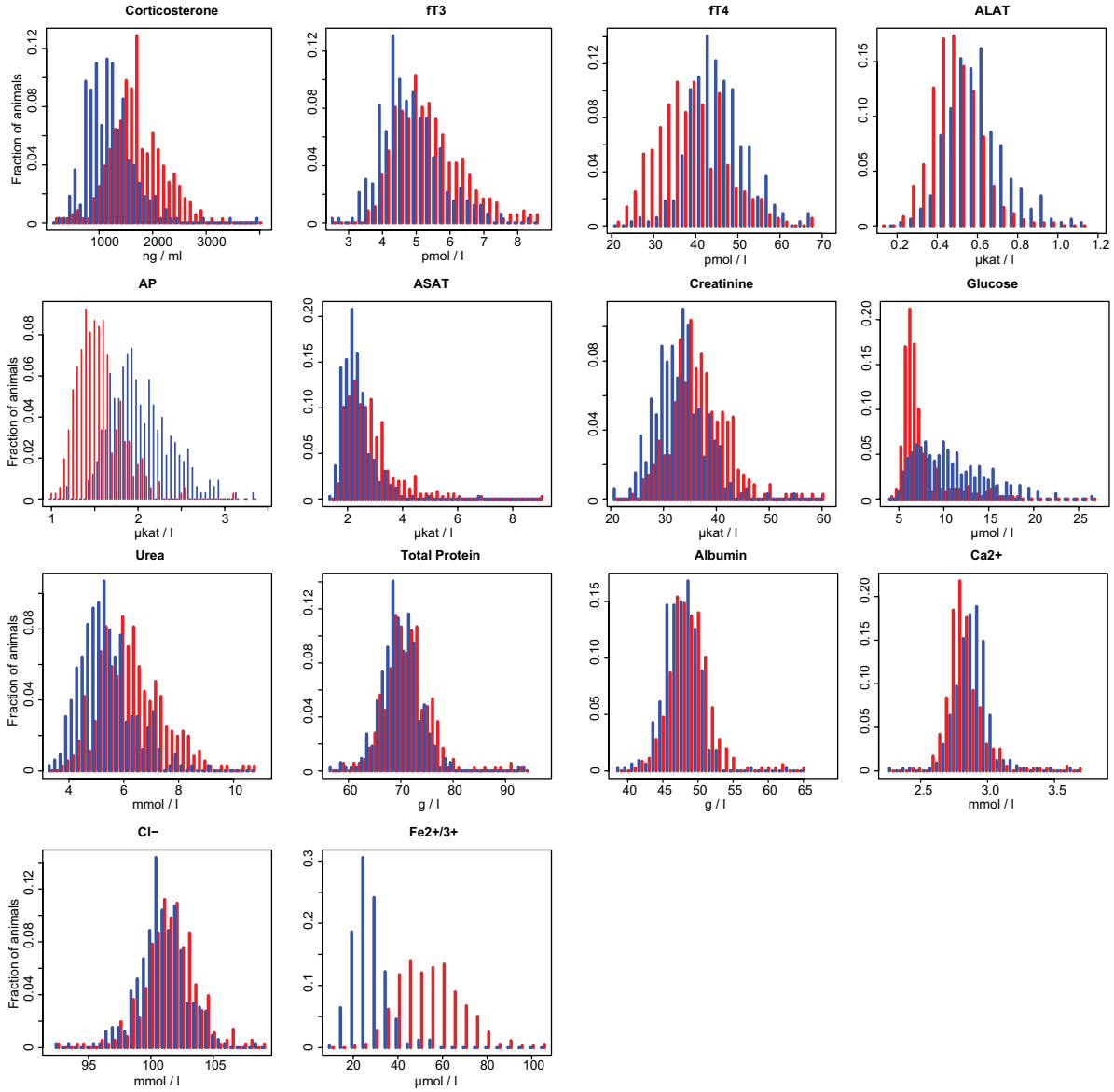

FIGURE S9.—Phenotype distributions for serum traits. Red: female F2 rats, blue: male F2 rats

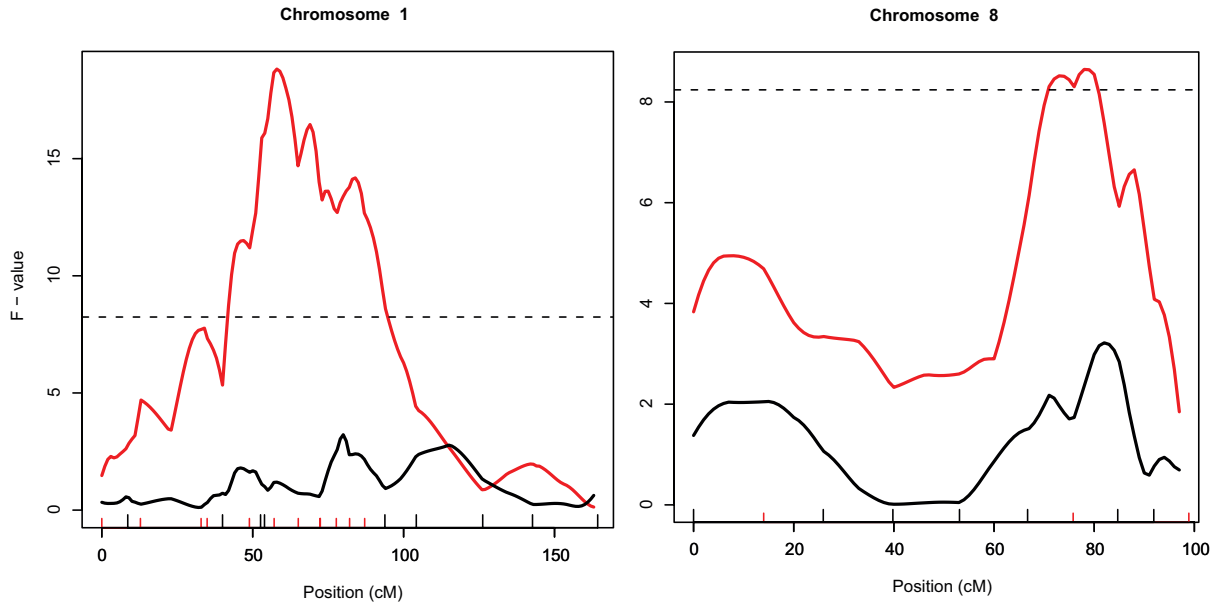

FIGURE S10.—QTLs for tameness do not influence white coat spotting. Red line: tameness. Black line: spotting. Black upward tickmarks: microsatellite marker positions. Red tickmarks: SNP marker positions

TABLE S1

Phenotype modelling

Phenotype group	Fixed effects	Random effects	Covariates	Adjusted effects	Effects included in QTL model
Glove test*	sex, trial	litter, observer	-	litter, trial, observer	sex
Open field & light-dark tests	sex	litter	-	litter	sex
Startle response	sex	litter	body weight	litter	sex, body weight
Organ weights	sex	litter	body weight	litter	sex, body weight
Body weight	sex	litter	age	litter	sex, age
Serum phenotypes	sex	litter	age§	litter, batch	sex, age§

To account for repeated observations of the same rat, trial and observer were modeled as being nested within individual rats. § age was included for traits for which a significant correlation (Pearson's product momentum correlation, $p < 0.05$) between trait and age was found (ALAT, ASAT, AP, creatinine, urea, chloride, iron, protein, albumin and fT4, but not for glucose, calcium, fT3 and corticosterone).

TABLE S2

Genetic markers used in QTL mapping

Table S2 is available as an Excel file at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

TABLE S3

Sex differences

Trait	p-value	females		males		Unit
Open Field Test		mean	s.e.m.[1]	mean	s.e.m.[1]	
time spent in center	0.4	3.46	0.2	3.43	0.2	%
time spent in corner	0.06	0.65	0.01	0.62	0.01	%
time spent moving	< 0.0001	41	0.5	36	0.6	%
time spent rearing	< 0.0001	23	0.7	17	0.6	s
locomotion speed	< 0.0001	29	0.4	25	0.4	cm / s
fecal boli	0.6	2.1	0.1	2.2	0.1	count
Light - Dark Test						
time spent in light compartment	0.6	26	0.9	26	1.1	%
time spent moving	< 0.0001	52	0.5	48	0.6	%
time spent rearing	< 0.0001	22	0.6	18	0.6	s
locomotion speed in light compartment	< 0.0001	22	0.5	19	0.6	cm / s
fecal boli	0.01	1.9	0.1	2.4	0.1	count
Startle Response Test						
startle response	0.01	76	9.9	72	16.4	g
Anatomy						
body weight	< 0.0001	111	1.3	222	2.2	g
adrenal gland weight	< 0.0001	42	0.4	40	0.4	mg
heart weight	< 0.0001	0.33	0.008	0.38	0.009	g
kidney weight	< 0.0001	0.04	0.004	0.07	0.007	g
lung weight	< 0.0001	0.67	0.01	0.64	0.01	g
spleen weight	< 0.0001	0.16	0.004	0.15	0.005	g
Serum traits						
Hormones						
corticosterone	< 0.0001	1650	26	1192	25	ng / ml
fT3	< 0.0001	5.3	0.05	4.8	0.05	pmol / l
fT4	< 0.0001	38.5	0.4	45.3	0.4	pmol / l
Enzymes						
ALAT	< 0.0001	0.49	0.006	0.6	0.008	µkat / l
AP	< 0.0001	1.5	0.01	2	0.02	µkat / l
ASAT	< 0.0001	2.7	0.05	2.3	0.03	µkat / l
Substrates						
creatinine	< 0.0001	36	0.3	33	0.3	µkat / l
glucose	< 0.0001	7.00	0.1	10.5	0.2	µmol / l

urea	< 0.0001	6.2	0.06	5.3	0.05	mmol / l
Serum protein						
total protein	0.014	71	0.2	70	0.2	g / l
albumin	0.0003	48.3	0.2	47.5	0.1	g / l
Electrolytes						
Ca^{2+}	< 0.0001	2.82	0.008	2.89	0.007	mmol / l
Cl^-	< 0.0001	102.5	0.3	101.3	0.2	mmol / l
$Fe^{2+/3+}$	< 0.0001	57	0.7	30	0.4	µmol / l

[1] standard error of the mean

TABLE S4

Correlations between phenotypes

Table S4 is available as an Excel file at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

TABLE S5

Suggestive autosomal QTLs

Trait	unit for genetic effects	Chromosome	peak position (cM)	1 LOD support interval (cM)	F-value	additive effect	dominance effect	% residual phenotypic variance explained	females?	males?
Glove Test										
Tameness (PC1)	-	3	109	93 - 117	5.4	0.12	0.73	1.4	-	-
Tameness (PC1)	-	10	42	11 - 69	5.4	0.4	-0.08	1.4	suggestive	-
Tameness (PC1)	-	12	5	2 - 9	5.4	0.13	-0.69	1.4	-	-
Tameness (PC1)	-	19	61	45 - 62	5	0.14	-0.75	1.3	-	-
PC2	-	14	5	0 - 25	6	0.32	-0.12	1.7	-	suggestive
PC2	-	11	49	36 - 54	4.8	-0.1	0.46	1.4	-	-
PC3	-	19	15	7 - 25	6	0.2	-0.26	1.7	-	-
PC3	-	10	59	41 - 67	5.1	-0.16	0.39	1.5	-	-
PC3	-	17	0	0 - 16	4.9	0.2	0.26	1.4	suggestive	-
approach	count	3	52	46 - 63	6.8	0.04	-0.15	1.9	suggestive	-
attack	count	8	72	67 - 90	6.6	-0.42	-0.19	1.8	-	-
attack	count	12	6	3 - 10	5.9	-0.18	0.68	1.6	-	-
attack	count	17	15	4 - 24	5.8	0.05	0.83	1.6	-	-
escape	count	10	94	89 - 94	7.8	-0.03	0.52	2.2	-	-
escape	count	18	24	16 - 38	6	-0.09	0.33	1.7	-	-
flight	count	10	49	36 - 71	6.8	-0.33	-0.16	1.9	suggestive	-
flight	count	9	27	16 - 41	5.7	-0.26	0.75	1.6	-	-
move & leave	count	7	1	0 - 5	6.3	0.01	0.38	1.8	-	-
move & leave	count	15	25	21 - 46	6	0.09	-0.36	1.7	-	-
move & leave	count	19	1	0 - 15	5.3	-0.19	-0.15	1.5	-	-
move & leave	count	11	43	33 - 52	5	0.03	-0.36	1.4	-	-
squeak	count	8	97	96 - 97	7.5	-0.02	-5	2.1	-	suggestive
squeak	count	17	1	0 - 11	6.8	1.1	1.5	1.9	-	-
squeak	count	3	63	51 - 72	5.5	-1.2	-0.5	1.6	-	-
boxing	s	6	15	6 - 26	7.3	-0.5	-0.3	2	-	-
boxing	s	1	78	72 - 102	7.1	-0.4	-0.5	2	-	-
boxing	s	19	54	42 - 62	6.8	-0.2	1.1	1.9	suggestive	-

screaming	s	8	72	63 - 82	8.6	-0.73	-0.46	2.3	-	-
screaming	s	19	60	42 - 62	5.4	0.14	1.1	1.4	suggestive	-
screaming	s	10	15	6 - 60	5.3	-0.65	0.17	1.4	-	-
screaming	s	12	8	2 - 23	5.1	-0.4	0.76	1.4	-	-
freezing	s	10	84	68 - 91	6.2	2	-0.7	1.7	-	suggestive
tolerate touch	s	18	30	23 - 39	7.2	0.35	-0.22	1.9	significant	-
tolerate touch	s	6	86	73 - 93	6.6	1.05	-1.09	1.8	-	-
tolerate touch	s	8	0	0 - 17	5.5	0.46	-0.21	1.5	-	-
tolerate touch	s	8	80	67 - 92	5.2	0.78	0.99	1.5	-	significant
tolerate handling	s	19	17	0 - 27	7	0.79	-0.17	2	-	-
tolerate handling	s	14	11	0 - 27	5.6	0.83	0.25	1.6	-	-
tolerate handling	s	6	61	51 - 80	5.3	0.68	-0.17	1.5	-	suggestive
tolerate handling	s	13	52	32 - 52	5.2	0.46	1.2	1.5	-	-
tolerate handling	s	11	49	40 - 54	5	-0.22	1.3	1.4	-	-
Open field										
time spent in center	%	8	27	18 - 56	7.8	0.0095	-0.22	2.2	-	suggestive
time spent in center	%	10	71	43 - 81	5.9	0.0079	0.0046	1.7	-	-
time spent moving	%	2	103	96 - 118	6.3	0.011	0.028	1.8	-	-
time spent moving	%	8	94	85 - 97	6.3	0.024	0.004	1.8	-	-
time spent moving	%	1	70	62 - 87	6.1	0.021	0.012	1.8	-	-
rearing	s	8	93	85 - 97	4.9	2.3	-0.2	1.4	-	-
locomotion speed	cm / s	2	86	83 - 95	6.9	0.5	2.7	2	-	suggestive
locomotion speed	cm / s	13	52	48 - 52	5.1	-1.1	-2.4	1.5	-	-
fecal boli	count	2	63	36 - 79	7.6	0.5	-0.2	2.2	suggestive	-
fecal boli	count	9	0	0 - 10	6.6	-0.5	-0.04	1.9	-	suggestive
fecal boli	count	10	80	68 - 91	6.6	0.6	-0.3	1.9	-	-
fecal boli	count	17	18	6 - 25	6.3	0.4	0.6	1.8	-	-

Light Dark Test										
time spent in light	%	6	38	27 - 54	7.6	0.044	-0.013	2.2	-	suggestive
time spent in light	%	19	53	43 - 62	7	-0.028	-0.097	2	significant	-
time spent in light	%	18	0	0 - 23	6	0.035	-0.025	1.7	-	suggestive
time spent in light	%	2	82	72 - 86	5.9	0.0009	0.064	1.7	-	-
time spent moving	%	3	0	0 - 2	7.1	0.015	0.036	2	-	-
time spent moving	%	7	35	18 - 73	6.2	-0.023	-0.027	1.8	suggestive	-
time spent moving	%	2	66	58 - 77	6	0.012	0.029	1.7	-	-
rearing	s	2	76	62 - 82	6	0.9	4	1.7	-	-
locomotion speed in light compartment	cm / s	7	24	7 - 52	5.4	2.2	-3.3	1.5	-	suggestive
locomotion speed in light compartment	cm / s	18	0	0 - 14	5.3	-0.3	2.5	1.5	-	-
fecal boli	count	18	21	2 - 25	8.3	0.5	0.3	2.4	suggestive	suggestive
fecal boli	count	4	58	52 - 71	6.5	0.5	-0.06	1.9	-	-
fecal boli	count	13	19	8 - 28	5	-0.3	-0.5	1.4	-	-
Startle response										
startle response	g	2	47	26 - 57	7.6	30	-81	2.1	-	-
startle response	g	4	73	63 - 86	6	-32	-64	1.6	-	significant
startle response	g	8	26	17 - 38	5.7	43	-32	1.6	-	-
startle response	g	13	15	3 - 22	5.2	50	23	1.4	-	-
Anatomy										
adrenal gland	mg	11	42	34 - 54	7.1	1.4	-1.9	1.8	suggestive	-
adrenal gland	mg	2	48	37 - 56	6.1	0.6	2.1	1.6	-	-

adrenal gland	mg	6	65	47 - 82	6.2	-1.4	1.3	1.6	-	-
adrenal gland	mg	8	64	42 - 79	6.1	1.5	0.4	1.6	-	suggestive
adrenal gland	mg	16	18	15 - 26	5.6	1.4	1.5	1.4	-	-
adrenal gland	mg	12	11	6 - 25	5	-1.1	1.3	1.3	-	-
body weight	g	18	16	0 - 22	6.8	6.3	-3.8	1.8	suggestive	-
heart	g	18	20	6 - 26	5.4	0.03	-0.005	1.5	-	-
kidney	g	3	54	31 - 66	6.9	0.02	0.01	2	-	-
kidney	g	7	13	0 - 25	5.9	0.02	0.02	1.8	-	-
kidney	g	8	88	85 - 97	5.9	0.02	0.01	1.7	-	-
kidney	g	20	26	11 - 42	4.3	0.003	0.007	1.3	-	suggestive
lung	g	10	53	35 - 67	5.8	0.02	-0.1	1.7	-	-
lung	g	6	35	19 - 43	5.2	-0.007	0.06	1.5	-	-
spleen	g	20	21	10 - 31	6.9	0.007	0.019	1.8	suggestive	-
spleen	g	3	63	51 - 75	6.6	-0.015	0.014	1.8	-	-
spleen	g	18	17	11 - 24	5.4	0.014	-0.005	1.5	-	suggestive
testis	g	10	92	86 - 94	6.4	0.06	0.26	3.9	n/a	n/a
testis	g	17	21	5 - 25	7.2	0.09	-0.09	4.3	n/a	n/a
spotting	-	6	48	39 - 56	5.8	0.06	-0.14	1.8	-	suggestive
Serum traits										
albumin	g / l	3	5	0 - 22	7.5	0.47	-0.66	2.3	significant	-
albumin	g / l	4	62	52 - 85	5.4	-0.46	-0.17	1.6	-	-
AP	µkat / l	10	52	8 - 72	5.8	-0.06	0.15	1.7	-	-
AP	µkat / l	7	92	78 - 103	5.3	0.02	-0.11	1.6	-	-
creatinine	µkat / l	15	23	15 - 28	6.9	-0.97	-0.67	2.1	-	-
creatinine	µkat / l	19	51	30 - 62	5	-1.03	-1.10	1.5	-	-
corticostero ne	ng / ml	5	18	1 - 30	6	-23.8	201	1.9	significant	-
corticostero ne	ng / ml	6	88	64 - 94	6	-110	29	1.9	-	-
fT4	pmol / l	5	94	76 - 109	8.5	-2.2	0.4	2.6	-	suggestive
fT4	pmol / l	14	34	3 - 47	7.1	-1.8	-0.5	2.2	suggestive	-
fT4	pmol / l	13	2	0 - 22	5.3	1.3	-1	1.6	-	-
ALAT	µkat / l	5	67	57 - 77	7.3	-0.015	-0.045	2.3	-	-
ALAT	µkat / l	12	21	10 - 42	5.8	0.026	-0.024	1.8	-	-
ASAT	µkat / l	8	87	81 - 92	7.8	-0.14	0.25	2.4	suggestive	-
glucose	µmol / l	2	49	37 - 57	6.6	0.67	-0.09	2	-	-
urea	mmol / l	7	106	94 - 106	5.8	-0.06	-0.37	1.8	-	-
total protein	g / l	3	117	112 - 17	6.3	0.75	0.38	2	-	significant

total protein	g / l	11	15	7 - 19	6	0.28	-1.2	1.9	suggestive	-
total protein	g / l	7	64	50 - 103	5.8	0.6	0.65	1.8	-	-
total protein	g / l	8	2	0 - 36	5.6	-0.72	-0.86	1.7	-	-
total protein	g / l	6	89	66 - 94	5.2	-0.64	-0.56	1.6	-	-
Cl-	mmol / l	2	144	134 - 147	6.3	-0.7	0.67	1.9	suggestive	-
Cl-	mmol / l	10	46	10 - 71	5.4	-0.51	-0.27	1.7	-	-
Fe2+/3+	µmol / l	2	96	87 - 117	7	-2.4	-0.1	2.2	suggestive	-
Fe2+/3+	µmol / l	18	22	0 - 26	5.4	-0.02	1.8	1.7	-	-

Shown are QTLs with chromosome-wide (but not genome-wide) significance

TABLE S6

X-Chromosomal QTLs

Trait	Peak (cM)	d.f.[1]	LR[2]	p value	additive effect	% variance[3]
urea	47	2	13.9	0.00096	-0.34	2.2

[1] degrees of freedom; [2] likelihood ratio; [3] residual phenotypic variance explained after accounting for fixed effects

TABLE S7

Sex-specific QTLs identified at genome-wide significance

Trait	sex	chr[1]	peak[2]	1 LOD c.i.[2, 3]	F	additive effect	dominance	% variance[4]
adrenal gland	females	5	48	44 – 58	9.5	-2.7 mg	-1.3 mg	5.3
corticosterone	females	12	0	0 - 2	8.5	12 ng / ml	280 ng / ml	4.9

Only significant QTLs that do not overlap with QTLs found in the sex-combined analyses are shown. See Table 3 and Table S5 for sex-specific QTLs overlapping QTLs found in the sex-combined analyses. [1] chromosome; [2] cM; [3] confidence interval; [4] residual phenotypic variance explained after accounting for fixed effects;