FOX FARM EXPERIMENT: HUNTING FOR BEHAVIORAL GENES

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Specific strains of silver fox (*Vulpes vulpes*) with markedly different behavioral phenotypes have been
developed at the Institute of Cytology and Genetics of the Russian Academy of Sciences. Foxes bred for
docility demonstrate a friendly response to humans similar to that of domestic dogs. In contrast, foxes from
a strain selected for aggressive behavior are aggressive towards humans, and difficult to handle. Because
these genetically determined behavioral differences segregate in pedigrees of a single species they offer
an unprecedented opportunity to identify the loci and genes responsible. Although these fox strains have
been carefully studied for several decades, only recently has it become possible to consider a systematic
approach to identify the loci and molecular mechanisms controlling these behavioral phenotypes. The
recent development of modern tools for the dog genome has made analysis of the genetic basis of complex
phenotypes in canids feasible, and enabled the current project to map the loci responsible for behavioral
phenotypes in the silver fox.

During the first phase of this project we developed tools and resources for genetic mapping in foxes.
1) A meiotic linkage map of the fox genome was constructed with 320 microsatellite markers adapted from
the canine genome. The resulting sex-averaged map covers 16 fox autosomes and the X chromosome with
an average inter-marker distance of 7.5 cM. 2) Three-generation experimental fox pedigrees segregating
behaviors derived from the founder strains were developed by crossing tame and aggressive founders,
then backcrossing F1 progeny to the tame strain. 3) A rigorous system for measuring behavior as a truly
continuous variable has been implemented, utilizing Principal-components analysis. This newly refined
method of behavioral assignment has identified 50 binary scoreable observations that non-redundantly and
reliably distinguished behaviors in the reference populations and in experimental cross-breed pedigrees. The
first principal component (PC1) accounts for approximately 37% of the total variation in behavior among
foxes and is significantly related to the expected additive genetic distance between the populations.

Identification of loci and genes underlying fox behavioral phenotypes promises to yield insight into the
genetics of social behavior and its underlying molecular mechanisms, not only in foxes, but in other mammals,
including humans and contribute into our understanding of the animal domestication process as a whole.

Domesticated species are easily distinguishable
from their wild relatives by behavioral characteristics
that evolved during domestication. Their behavior
is usually characterized by reduced aggressiveness,
increased social tolerance among conspecifics, and
reduced sensitivity to environmental changes (for
review Sachser, 1998; Clutton-Brock, 1999; Price,
2002). Tameability, a unique ability to interact
with humans in a positive way is a distinctive
characteristic exhibited by domesticated species
(Price, 2002; Hare *et al*., 2002, 2005; Kaminski *et
al*., 2004; Topal *et al*., 2005). Although this unusual
suite of behaviors has long fascinated biologists,
the molecular forces driving development of
these complex behavioral phenotypes during
domestication remain unknown.

Comparative analysis of mitochondrial DNA and zooarchaeological records has revealed the ancestry, approximate time, and origin of domestication for nearly all domesticated species (Wayne *et al.*, 1997, 1999; Kijas *et al.*, 2001; Vilà *et al.*, 2001; Jansen *et al.*, 2002; Larson *et al.*, 2005). According to genetic and paleontologic studies, taming of the common domesticated species took place in pre-historic times with the dog the first animal to be domesticated, diverging from the gray wolf as long ago as over 15 thousand years ago (Tchernov, Valla, 1997; Davis, Valla, 1978; Nobis, 1979; Dayan, 1994; Leonard *et al.*, 2002; Sablin, Khlopachev, 2002; Savolainen *et al.*, 2002).

Differences between dogs and wolves in their social interactions with humans strongly suggest a genetic basis for these behavioral phenotypes. Understanding these genetic differences could shed light on genetic mechanisms implicated in human social behavior and provide candidate genes for autistic and other behavioral disorders. Unfortunately, the genetic analysis of these behaviors in dogs is hampered by the fact that most of the selection for tame behavior took place in prehistoric times and there is currently no significant set of populations segregating these behavioral differences. Broadly speaking, all dogs possess the domesticated phenotype, and all wolves posses the wild phenotype (i.e. the behaviors are genetically fixed within the respective populations) and therefore it is not practicable to produce informative pedigrees in which the critical phenotypes segregate.

More recently, however, another member of the *Canidae* has been domesticated. At the Institute of Cytology and Genetics (ICG) in Novosibirsk, Russia the process of animal domestication has been experimentally reconstructed and a strain of domesticated fox with behavioral patterns extremely similar to those of domestic dogs has been produced. (Belyaev 1969, 1979; Trut 1987, 1999, 2001; Trut *et al.*, 2004; Hare *et al.*, 2005; http://cbsu.tc.cornell.edu/ccgr/behaviour/index.html). Unlike modern dogs, the strain of domesticated foxes was created rapidly by selection focused only on specific behavioral traits. Because these genetically determined behavioral differences segregate in very large pedigrees of a single species, they offer an opportunity to map and identify the loci and genes responsible.

I. The fox model

The silver fox is taxonomically close to the dog but, although reared in captivity, had not been domesticated previously. Under standard farm conditions foxes normally exhibit distinct patterns of aggressive and fear-aggressive behavior towards humans. Starting in 1959 with a large farm population, ICG bred foxes separately for tame and aggressive behavior for 45 generations and created two fox strains characterized by different phenotypes (for review see Trut, 1999, 2001). Breeding foxes for tameness started with selection against fear and aggression towards humans, and continued by selection for contact-seeking behavior. Subsequently, only the tamest individuals in each generation were bred, while at the same time a deliberate effort was made to avoid inbreeding (Trut, 1999, 2001; Trut *et al.*, 2004; Kukekova *et al.*, 2004). Domesticated foxes communicate with humans in a positive manner and are eager to establish human contact by one month postnatal, whimpering to attract attention and sniffing and licking, like dogs. Play activity, which is normally only seen in infantile wild type foxes, persists into adulthood and these foxes actively seek out both other members of their own species and humans, in a playful, friendly and communicative manner.

The major selection criterion for selection of foxes for aggressive behavior was the critical distance between the experimenter and the caged fox (when the animal first demonstrates an aggressive reaction to the experimenter moving towards its cage), and the level of expression of the aggressive behavior. Foxes from the strain selected for aggressive behavior are, like wildtype foxes, aggressive towards humans, and difficult to handle (Trut, 1980a, b; 1999, 2001).

The tame and aggressive behavioral phenotypes have been well defined; a rigorous system for scoring behavioral phenotypes in these foxes has been established, and the heritability of these traits has been clearly and carefully demonstrated in studies including experimental cross-breeding of tame and aggressive animals controlled by cross-fostering of newborn pups and transplantation of embryos (Trut, 1980a, b, 2001).

Although selection was strictly limited to defined behavioral criteria, further developmental and behavioral differences emerged in tame

foxes. Opening of the eyelids and the external auditory canal was accelerated; the sensitive period for socialization persisted past 60 days of age (compared to less than 45 days in unselected foxes); and play activity (normally only seen in infantile wild type foxes) extended into adulthood (Belyaev *et al.*, 1984/1985; Plyusnina *et al.*, 1991; Trut, 2001). Significant differences in corticosteroid and neurotransmitter levels were also found between tame and control foxes (Oskina, Tinnikov, 1992; Popova *et al.*, 1997). Significant differences in hypothalamic-pituitary-adrenocortical (HPA) system reactivity in ontogenesis of foxes from tame and unselected populations were observed (Trut, Oskina, 1985; Oskina, 1996). Significantly lower density of serotonin 5-HT1A receptors was observed in the hypothalamus; and significantly higher levels of serotonin and tryptophan hydroxylase were detected in the midbrain and hypothalamus of domesticated strain of foxes (Popova *et al.*, 1997; Trut, 2001). These biochemical changes are particularly significant as similar abnormalities in neurotransmitter metabolism are reported in several human neuropsychiatric diseases (Nebigil *et al.*, 2001; Shekhar *et al.*, 2001) and elevated platelet serotonin has been observed in patients with autism (Gordon *et al.*, 1993; Cook, Leventhal, 1996).

Several *de novo* traits were detected in the fox population selected for tameness. In particular, coat color changes such as the appearance of a white spot on the head (Star phenotype) and loss of pigment in other areas began to appear in the eighth selected generation (Belyaev *et al.*, 1981). It is intriguing that white spotting, which appeared without direct selection or inbreeding in the tame fox population, is frequently observed as a distinctive difference between domesticated animals of several species (dogs, cats, cattle, horses, etc.) and their wild progenitors. Other morphological characteristics also arose in the same manner: floppy ears, rolled tails, shorter tails, and changes in skull shape; all of which resemble differences between domesticated dogs and wolves (Wayne *et al.*, 1999).

Because all foxes live under consistent conditions, have similar interactions with humans, and fox behavior is tested at precise time points using standard tests under constant conditions, the environmental factors that might influence behavior are held to a strict minimum. For at least 40 generations, well characterized tame and aggressive fox populations have been bred separately and selectively to exhibit distinctly different and precisely measurable behavioral traits. They have thus provided ideal founder populations for the newly developed crossbred and backcross populations, and an opportunity for mapping loci and genes responsible for these markedly different behavioral phenotypes.

II. Recent advances in canine genetics

The unique potential of canine models for studying and curing human inherited disorders has become well appreciated in the last decade, and led to striking progress in canine molecular genetics. Canine integrated genetic and RH maps, the Affymetrix GeneChip® Canine Genome Arrays (www.affymetrix.com/products/arrays/specific/canine.affx), a single nucleotide polymorphism (SNP) map containing more than 2.1 million SNPs, and two sequences of the canine genome (7.6x and 1.5x) (Kirkness *et al.*, 2003; http://www.ncbi.nlm.nih.gov/genome/guide/dog) have enabled rapid recent progress in mapping and identifying an increasing number of canine genes and single locus phenotypes (e.g. Acland *et al.*, 1998, 1999; Lin *et al.*, 1999; Jonasdottir *et al.*, 2000; Sidjanin *et al.*, 2002; Kijas *et al.*, 2002; van De Sluis *et al.*, 2002; Zhang *et al.*, 2002; Lingaas *et al.*, 2003; Mellville *et al.*, 2005; Kukekova *et al.*, 2006; Zangerl *et al.*, 2006), and more complex traits (Chase *et al.*, 2002, 2004, 2005a, b; Lowe *et al.*, 2003; Lohi *et al.*, 2005). The physiology, disease presentation and clinical phenotype of dogs often mimic human diseases closely (Ostrander, Kruglyak, 2000) and offer critical models for the study and treatment of corresponding human diseases (Priat *et al.*, 1998). A particular compelling example is provided by a canine disease directly equivalent to human Leber Congenital Amaurosis (LCA) arising from RPE65 gene mutations. Gene therapy has successfully restored vision in canine LCA and opened prospects for treatment of this childhood blindness in humans (Acland *et al.*, 2001, 2005).

The dog has recently emerged as an excellent model for analyzing the genetic architecture of complex phenotypes (Lark *et al.*, 2006). QTLs affecting size, sexual size dimorphism, bilateral asymmetry of hip joints, and other parameters of mammalian skeletal morphology have been

identified in dogs and provide new insight into mammalian skeletal development and function (Chase *et al.*, 2002, 2004, 2005a, b; Carrier *et al.*, 2005; Lark *et al*., 2006). Strong similarities in the complex genetic background of Addison's disease in humans and dogs establish the dog as an important natural model of corresponding human disease: 2 loci that regulate late onset Addison's disease have been identified in Portuguese Water Dogs pedigrees (Chase *et al*., 2006).

The close evolutionary relationship between the fox and the dog (Wayne, 2001), the well established cytogenetic homology between these species (Yang *et al*., 1999, 2000) and the high level of conservation of genomic DNA sequence of the two species (Kukekova *et al*., 2004), allow tools developed for canine molecular genetics to be used to study the genetics of simple and complex traits in foxes.

III. Molecular genetics of tame and aggressive behavior in silver foxes

Behavioral differences between tame and aggressive fox strains are likely controlled by more than one gene located on different chromosomes. Identification of these genes and loci requires a systematic approach and well developed tools. To undertake a genetic analysis of these complex behavioral phenotypes in foxes the following resources and data are required:

1. A meiotic linkage map of the silver fox genome that will provide a comprehensive set of polymorphic markers distributed across the whole genome and a relative marker order, with known distances, along each individual fox chromosome.

2. Three-generation experimental pedigrees, derived from crosses between tame and aggressive fox populations, are needed that yield informative litters segregating the behaviors of the founders.

3. The behavior phenotype of each animal from the informative generation needs to be characterized by an objective quantitative system.

III.1. Construction of a meiotic linkage map of the fox genome

We applied a comparative genomic approach to build the first meiotic linkage map of the silver fox (*Vulpes vulpes*) (Kukekova *et al*.,

2007). Development of the map was facilitated by established homologies between the canine and vulpine cytogenic maps. The dog karyotype comprises 78 mostly acrocentric chromosomes, that of the red fox 34 metacentric chromosomes (and 0-8 B chromosomes) and the correspondence between chromosomal arms of the two species has been previously established (Yang *et al*., 1999, 2000; Graphodatsky *et al*., 2000, 2001). The canine genome has now been sequenced (Lindblad-Toh *et al*., 2005) and the high level of genomic sequence identity between dog and fox allows canine microsatellite markers to be adapted for genotyping and meiotic mapping in foxes (Kukekova *et al*., 2004, 2005).

III.1.a. Markers and pedigrees selected for map construction. We selected 320 microsatellite markers, anchored in the 7.6X sequence of the canine genome, for construction of the fox meiotic linkage map. Markers were selected to 1) robustly amplify fox DNA, 2) have adequate polymorphism information content (PIC) for use in fox reference pedigrees; and 3) cover the anticipated fox linkage groups completely and uniformly as predicted by comparison of the integrated canine genome map (Breen *et al*., 2001) with the canine/fox comparative cytogenetic map (Yang *et al*., 1999, 2000).

Thirty-seven 3-generation silver fox pedigrees, developed and maintained at the experimental farm of the ICG, were selected for genotyping and map construction. 34 of these fox pedigrees were built by breeding foxes from tame and aggressive strains and then backcrossing the F1 progeny to the tame strain. In all, 286 individuals from thirty-seven pedigrees were used for map construction.

III.1.b. Genotyping strategy. Fox samples were genotyped at the Mammalian Genotyping Service of Marshfield Laboratories (Madison, WI) using the Marshfield canine microsatellite marker set supplemented with an extra 151 microsatellite markers genotyped at Cornell University.

320 non-overlapping markers from both sets were polymorphic and worked robustly on fox DNA. On average, 195 informative meiosis per marker were observed. Allele number varied among loci with a mean of 5.4 alleles per marker. The average marker PIC value was 0.5.

III.1.c. Map construction. Two-point recombination fractions and LOD scores were calculated for all marker pairs. Markers were assigned to

linkage groups if linked to at least one other marker in the group with a recombination fraction ≤ 0.25 and a LOD ≥ 4.0. A sex-averaged, framework map was then constructed for each fox chromosome. Of the 320 markers tested, 199 were assigned to unique locations on the framework map with a confidence of 1000 : 1. 28 additional markers were uniquely placed with a confidence of 99 : 1, and the most likely order was determined for the rest of the markers at a confidence of 99 : 1. The number of markers mapped to each fox chromosome ranged from 6 to 35. The length of fox chromosomes, assessed as the map distance between the most distant markers of each linkage group, ranged from 33.5 to 119.4 cM. The average spacing among markers mapped to unique locations was 6.8 cM. Five inter-marker intervals equal or larger than 20 cM remain to be filled. Alignment of fox linkage groups with homologous regions of the integrated canine genome maps and sequence, together with comparative cytogenetic maps of the fox and dog genomes allowed us to assign fox linkage groups to fox chromosomes. As an example, the linkage map of fox chromosome 1 is presented in Figure 1 (Kukekova *et al.*, 2007); a complete set of fox chromosomal maps can be obtained from the website: <http://cbsu.tc.cornell.edu/ccgr/behaviour/ Integrated_Map.htm>. Fox chromosomes are designated using the nomenclature established for the fox karyotype (Wipf, Scackeford, 1942; Graphodatsky, Radjably, 1981; Makinen, 1985).

III.2. Construction of 3-generation experimental fox pedigrees segregating behavioral phenotypes. To produce litters informative for behavior, experimental backcross pedigrees have been developed. Backcross-to-tame pedigrees were produced by crossing tame and aggressive founders, then backcrossing F1 progeny to the tame strain. Backcross-to-aggressive pedigrees were constructed by crossing tame and aggressive foxes, and then backcrossing F1 progeny to the aggressive strain.

III.3. Measuring fox behavior. As the fox colony continues to be maintained on the same farm, environmental influences on the fox behavioral phenotypes are minimized and fox behaviors are evaluated under consistent, standardized conditions. Foxes are bred once per year in January–February and pups are born in March-May. Littermates are housed together until they are 2.5 months old. At

this age, each pup is moved to its own cage. To ensure that tameness results from genetic selection, foxes are not trained and are only allowed brief «time dosage» contacts with human beings.

Semiquantitative measures of tame and aggressive fox phenotypes were developed at ICG to drive the selective breeding program (Trut, 1980a, b, 1999; Vasilieva, Trut, 1990). Although the original farm fox population showed a continuous variation in behavior, the phenotypes in the selected tame and aggressive populations diverged rapidly. For this reason, two systems for scoring fox behavior, one for the tame and another for the aggressive populations were developed to separately measure variation in «tameness» and «aggressiveness». (A detailed description of the ICG scoring system is in Trut, 1980b; Kukekova *et al.*, 2005). Assignment of behavioral phenotypes in experimental pedigrees clearly demonstrated that the traditional scoring system has limited resolution for measuring fox behavior in the F1 and backcross generations. Behavioral patterns characteristic of the founder populations become fragmented or reshuffled in crossbred offspring. Therefore, to map the genetic loci underlying these behavioral variations, we developed a unified, high resolution, objective, quantitative method for behavioral evaluation of fox populations. Our analytic method dissects the inherited components of fox behavior into independent underlying traits that distinguish between the reference populations (Tame, Aggressive, F1) and resegregate in the backcross generations (Acland *et al.*, 2004; Kukekova *et al.*, 2008).

Behavior of foxes from parental strains and experimental pedigrees is tested in a precisely defined protocol. Foxes are tested in their home cages by the same observer at a standard time period during the day, in five steps (Step 1 «approach» – observer approaches the fox cage; Step 2 «stay» – observer stays near closed cage; Step 3 «door» – observer is near open cage but does not initiate tactile contact; Step 4 «touch» – tactile contact; Step 5 «end» – observer stays near closed cage). The length of each step (except step one) is 1 minute. Behavior of each fox is tested at least twice by the same observer and all tests are videotaped to preserve a permanent record.

A comprehensive list of 319 primary, objective behavioral observations was initially identified
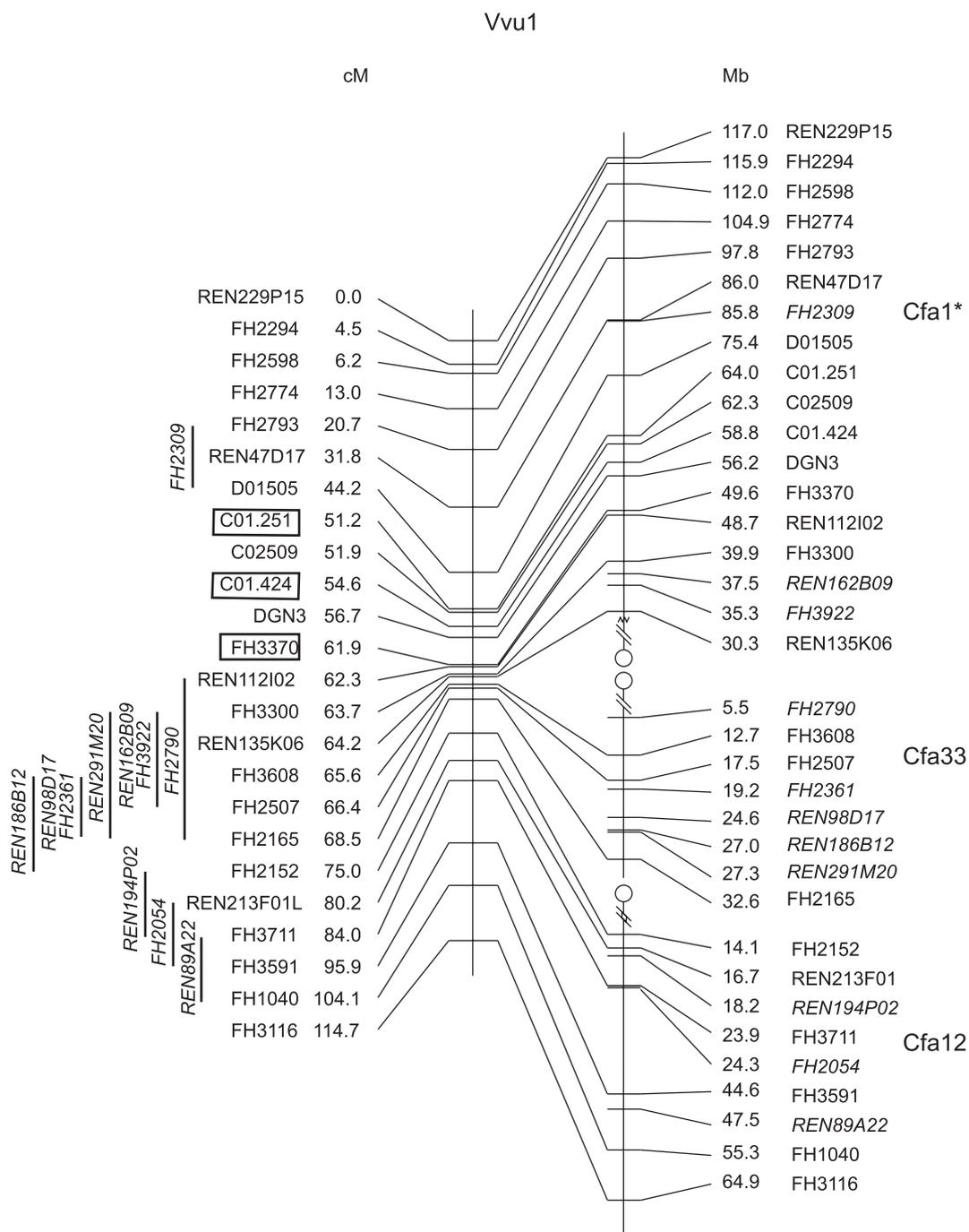
Vvu1

Fig. 1. Integrated meiotic linkage map of the silver fox (*Vulpes vulpes*) chromosome 1 (VVU1). Fox chromosome 1 is presented on the left side of the panel, and aligned with the corresponding segments of 7.6X sequence of the dog genome. Lines connect markers mapped onto the fox meiotic linkage map and identified in the assembly of the dog genome. Dog chromosome 1, which is homologous to more when one fox chromosome is marked by asterisks. (from Kukekova *et al*., 2007).

by ethological survey of video records of fox behavior in the standard ICG test (Kukekova *et al.*, 2008). Subjective assessments (e.g. «Fox is afraid») were avoided. The traits selected were physically observable events recording specific fox actions, location in the cage and time spent there, body postures, positions of particular parts of the body, and sounds. Examples, each answerable «yes or no», are: «Wagging tail?»; «Ears pricked?»; «Leaning forward against the cage door?». Traits which were recorded in more than one test step (for example, «Fox touches front door with nose») were treated as independent traits. The validity of identified traits was tested in a preliminary set of 95 foxes including randomly selected animals from each of the tame, aggressive, F1, and backcross populations. Behavior of 95 foxes at 5.5–6 month age was scored from video records using the 319 trait set. Statistical analysis, including Principal-components (PC) analysis was used to identify the independent, resegregating traits underlying the phenotypic variation expressed in these multiple correlated observations (Kukekova *et al.*, 2008).

From the original set of 319 primary traits, the 50 most reliable traits were retained for measuring behavior in Tame, Aggressive, F1 (Tame × Aggressive progeny) and Backcross-to-tame (F1 × Tame progeny) animals. These measurements on the four populations constituted a matrix for PC analysis, within which the F1 and Backcross-to-tame populations could be compared with the Tame and Aggressive populations.

Definition of Behavioral PC1. Principal-component analysis of 50 videotaped behavioral observations, from a combined population of 431 foxes (45 tame, 293 backcross-to-tame, 45 F1 and 48 aggressive), identified the first principal component (PC1). Each fox is scored for each trait as 1 if the trait was observed and 0 if the trait was not seen. PC1 for each fox is a linear sum of each such trait score multiplied by the corresponding loading. Table 1 defines the significant traits and loadings for PC1, a continuous quantitative measure that defines the major component of fox behavior from aggressive (negative) to tame (positive); traits with the highest loadings typify the principal component. PC1 accounts for 37 % of the total variation and is highly significantly related to the expected additive genetic distance between the populations (Figure 2).

Figure 2 presents a scatter graph comparing PC1 value for the four populations. The additive genetic distance between the populations is graphed on the X axis in arbitrary units against the individual fox PC1 value on Y axis. The PC1 values of the F1 population is distributed with a median about half way between the tame and aggressive populations and the backcross-to-tame population is distributed with a median about half way between the F1 and tame populations. Thus, the behavioral variation represented by PC1 is heritable and in the F1 and the backcross-to-tame populations the broad range of variation in PC1 values is consistent with the polygenic character typical of a quantitative trait.

IV. Approaches for QTL mapping

To analyze genetic mechanisms that regulate behavioral differences between the tame and aggressive fox strains, we test the association of a particular behavioral phenotype with particular molecular markers to identify genetic loci contributing to the phenotype. QTL analysis will proceed in 2 stages:

1. We first adopt the simplifying hypothesis that the selection involved in creating the aggressive
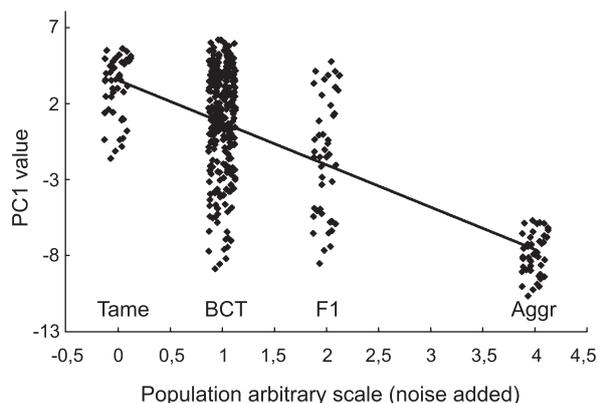
Fig. 2. First principal component of behavior (PC1, Y-axis) for foxes in 4 populations (tame, aggressive, F1 and backcross-to-tame). PC1 is a continuous quantitative variable – extreme negative values represent the most aggressive behavior, and extreme positive values the most tame. For display purposes only (to allow each point to be visualized), a small amount of X-axis noise has been added to each point. The line through the data plots the regression of PC1 against an arbitrary additive genetic scale (tame = 0, BC = 1, F1 = 2, aggressive = 4).

Loadings of selected most significant traits for the PC1

Test step	PC1 loading	Trait description
Step 3	–0.1718	Fox is not on the floor of zone 2
Step 4	–0.1613	Pinned ears
Step 4	–0.1598	Aggressive sounds
Step 3	–0.1417	Fox spends at least 40 sec in zones 5–6
Step 4	–0.1395	Attack alert
Step 2	–0.1334	Fox spends at least 40 sec in zones 3–4–5–6
Step 3	–0.116	Fox immediately moves back to zone 5 or 3–5–6
Step 2	–0.1078	Moving back for at least one zone during first 15 sec
Step 4	–0.1035	Attack
Step 5	–0.0986	Fox spends at least 40 sec in zones 5–6
Step 4	0.1545	Allows to touch head
Step 4	0.1575	Moved to the zone 2
Step 4	0.1607	Allows to touch nose
Step 3	0.1617	Fox sniffing front door
Step 5	0.1654	Fox spends at least 40 sec in zones 1–2–3–4
Step 5	0.1654	Come to the zone 1–2
Step 3	0.1657	Fox tries to poke hand with nose
Step 4	0.171	Tame ears
Step 5	0.1736	Fox is in the zone 2 during first 5 sec
Step 5	0.1764	Fox touches front door with nose
Step 5	0.1768	Fox spends at least 30 sec in zone 1–2
Step 3	0.1803	Fox comes to hand and sniffs hand
Step 3	0.1868	Fox comes into zones 1–2

Notes. Traits recorded in more than one test step were treated as independent traits. Zones 1–2 are located in the front of the fox cage (zone 2 is the closest to experimenter), zones 5–6 on the back of the cage, zones 3–4 are in the middle.

and tame founder populations would have fixed alleles responsible for the behavioral phenotypes in these populations. If so, then any such alleles with major effects should resegregate in the backcross generation, and be most readily detected using a backcross analysis. Backcross analysis will be performed using QTLExpress (Seaton *et al.*, 2002).

2. Because a) initial analysis suggested that the segregating traits might have significant non additive components, b) the broad variation in both the F1 and BC1 populations suggested possible interactive effects, and c) concerns that the hypothesis of fixation in the founder population might not hold, we next undertake association analysis, using the "lm" function of the statistical program R (R Development Core Team, 2005). This method performs a linear regression of the trait value onto allele count for each marker allele, taking the assumption that all amplicons of the same length represent the same allele. This assumption will lead to false negative results but cannot produce false positives.

Once behavioral QTLs are identified we will fine map the QTL intervals using linkage and linkage disequilibrium (LD) mapping approaches using microsatellite and SNP markers. A combination of linkage and LD mapping exploits both observed informative meioses in our experimental pedigrees and historical recombinations (unobserved meioses) which took place in the fox population during and before the almost 50 years of selection for tame

behavior and will lead to the further reduction of the QTL intervals.

When QTL intervals in foxes are well defined, the putative gene content of the minimal LD regions will be identified by examination of the corresponding regions in the dog and human genome sequences. A high order of conservation was observed between homologous regions of the fox and dog genomes by comparison of the fox meiotic linkage map and the sequence of the dog genome. To confirm that the same genes are present in the fox LD regions, and establish gene order for the fox QTL interval, gene-specific polymorphic markers will be designed based on canine sequence, genotyped in fox three-generation pedigrees and placed on the fox meiotic map. If positional candidate genes in the dog and human genomes can be identified, these genes will be screened for sequence alterations in foxes.

V. Conclusion

Until recent, it was practical to study genetic basis of complex behavioral phenotypes only in a limited set of animal species (Flint *et al*., 2005; Kendler, Greenspan, 2006). The model choice was predetermined in large by availability of the information on the genome, well-defined behavioral phenotypes, and an opportunity for experimental breeding. New genomic initiative made available sequences of species representing different mammalian groups (http://www.broad.mit.edu/mammals/) and therefore opened an opportunity to apply genetic analysis for the species that previously have not been considered as conventional genetic models. Further more, the information on the genomes of well studied animals now can be used for genetic studies of closely related species including well established models for behaviors, whose analysis was hampered by the luck of major genetic tools (Scott, Fuller, 1965; Trut, 1999; Williamson *et al*., 2003; Suomi, 2006).

There is a large and largely unexploited resource of animal models intermediate between humans and rodents in behavioral complexity. Such models can offer advantages in mapping potential compared to human pedigrees, and serve as a bridge between humans and rodents for molecular studies of behavior. All too often, human families segregating complex behavioral phenotypes offer only limited power for linkage studies, which may also be hampered by heterogeneity between and within families. Expanding genetic research to a spectrum of animal models, learning from each what it can uniquely provide opens an opportunity to test correspondence of the molecular mechanisms underlying analogous behaviors in different mammalian species and to obtain more robust insights into human behavioral genetics.

Here we described approaches for studying genetics of complex behavioral phenotypes in specific strains of silver foxes. Although these fox strains have been carefully studied for several decades, it is only recently has become possible to consider a systematic approach for mapping the loci and genes involved. The close evolutionary relationship between the fox and the dog (Wayne *et al*., 1997) and the strong genomic conservation of the two species allow the use of canine genomic tools for genetic studies in foxes.

Using 320 microsatellite markers selected from the canine genome we constructed the first meiotic linkage map of the fox that covers 16 autosomes and X chromosome with an average inter-marker distance of 7.5 cM. The map was aligned and compared to the dog genome and correspondence between homologous chromosomal regions of two species has been confirmed (Kukekova *et al*., 2007). The fox map provides a critical resource for genetic studies in foxes and it is sufficient for an initial genome wide scan of fox experimental pedigrees segregating tame and aggressive behavioral phenotypes.

To characterize behaviors of offspring in newly established fox experimental pedigrees we developed a new approach for behavioral assignment. This approach allows us to convert fox behavior into quantitative phenotypes, which distinguish between founder populations and determinate range of behaviors encountered in F1 and backcross generations. PC1, described here, is stable and heritable, and represents a valid and reliable measure of fox behavior, suitable for quantitative genetic experiments including quantitative trait loci (QTL) mapping (Kukekova *et al*., 2008).

All progeny from the fox experimental pedigrees will be genotyped with markers equally spaced across the whole genome and their behaviors will be measured and assigned using PC analysis. To identify locations of the genetic loci responsible

for the tame and aggressive behaviors in foxes we must associate a particular behavioral phenotype (PC1) with particular molecular markers for each individual fox from the pedigree. Once these behavioral loci are mapped, position of fox QTLs regions will be identified in the canine genome and fine mapping of fox QTLs will be pursued.

The range of behaviors exhibited in these fox strains has significant parallels to that of normal and disordered patterns of human sociability. Identification of the genetic loci that controls behavioral difference between these fox strains may provide new candidate genes for evaluation in human neurological and behavioral disorders. Further more, understanding genetic mechanisms controlling tame and aggressive phenotypes in foxes promises to yield insights into the genetics of social behavior and its underlying molecular mechanisms, not only in foxes, but in other mammals, and shed light on molecular mechanism acting during animal domestication process.

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