UNCOVERING THE GENETIC BASIS FOR TAMENESS –
A RESEARCH STRATEGY

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D.K. Belyaev's experiments into the role of selection for tameness in animal domestication have provided the research community with unique animal models to study the genetic basis for tameness. Recently, a collaboration was initiated between researchers in Novosibirsk, Uppsala and Leipzig to study one of Belyaev's legacies – two lines of rats selected for increased and decreased tame behavior towards humans, respectively. Initial results confirmed large differences in the rats' behavioral response to humans as well as several associated behavioral and physiological differences. Research is under way to identify genomic regions associated with these differences. Once found, these can serve as a starting point to identify genetic variants causing tameness in this model system of early animal domestication.

Introduction

Dmitry K. Belyaev's groundbreaking studies into artificial selection for tameness and its role in animal domestication have rightly become famous among both scientific and public audiences (Trut, 1999; Price, 2002; Dobney, Larson, 2006; Spady, Ostrander, 2007). The selection lines he initiated from various mammalian species are unique in their scope and vision, providing a resource for researchers for more than four decades (Belyaev, 1969, 1979). His work is continued to this day at the Institute of Cytology and Genetics in Novosibirsk, under supervision of Lyudmila Trut, Irina Plyusnina and their colleagues. The two most well-known experiments involve silver foxes (*Vulpes vulpes*) and wild-derived gray rats (*Rattus norvegicus*) (Belyaev, Borodin, 1982; Trut, 1999). In both species, two lines of animals were created by artificial selection for increased tameness and for increased defensive aggression towards humans, respectively. Today, the animals differ dramatically in their response to humans. Tame foxes show dog-like skills in using human communication cues (Hare *et al*., 2005), while tame rats are completely tolerant of human handling. Aggressive foxes and rats, by contrast, attack and flee from humans when possible. While the behavior and many aspects of the physiology of both groups of animals have been extensively studied (Naumenko *et al*., 1989; Plyusnina, Oskina, 1997; Trut, 1999; Popova *et al*., 2005), the genetic basis of the behavioral differences remains unknown.

Over the last decade, genetic research has made advances that allow probing the animals' genomes for causative variants. Research has begun into the genetic basis of tameness in the fox lines (Kukekova *et al*., 2007). In 2005, a collaboration between researchers in Novosibirsk, Uppsala and Leipzig has been initiated to investigate the genetic differences between the tame and the aggressive rat line. In what follows, I will briefly introduce the joint efforts of our research groups, point out some first important findings, and provide a sketch of our plans towards uncovering the genetic basis for tameness.

History of the Project

To make full use of genetic technologies at the Max Planck Institute for Evolutionary Anthropology, daughter colonies of both rat lines were established in Leipzig. In late 2005, 15 tame and 15 aggressive rats arrived in our facilities, and were soon mated to become the ancestors of populations still thriving today. The founders were all unrelated individuals of the 64th generation of selection, and comprised ten females and 5

males, respectively. The rats are kept in dedicated rooms using more than 200 cages modified to allow maintenance with minimal handling. Care is taken to treat the two lines as identical as possible. Especially, tame rats are not handled more often than aggressive rats, and neither line is subjected to stressful treatment, permitting testing of animals naïve to human handling. To allow the animals to adapt to their new environment, experiments began only in the second generation of animals born in Leipzig.

First Results

We began our research effort by conducting a thorough screen for phenotypic differences between the two lines (Albert *et al*., 2008). Three main findings emerged.

First, and crucially, the behavioral differences observed in Novosibirsk persisted in the rats' new environment. Interactions between different laboratory environments and behavioral differences in rodents have been demonstrated in spite of rigorous standardization of testing and maintenance procedures (Crabbe *et al*., 1999) and can be substantial, especially for some measures of anxiety and agonistic behavior (Wahlsten *et al*., 2006). Despite small differences in results between tests performed in Novosibirsk and Leipzig (e.g., a difference in the number of nose-pokes in a hole-board test (Plyusnina, 2004) was not found in Leipzig), differences in the rats' reactions to humans were very robust. We developed a test designed to measure a rat's level of tameness/aggression, closely following the paradigm used during selection of the two lines. Tame rats tolerated being touched and handled by a human experimenter, whereas aggressive rats attacked, screamed loudly and fled from the experimenter's hand (Albert *et al*., 2008). Further, we replicated earlier findings of lower anxiety-associated behaviors in tame rats in an open-field (Plyusnina, Oskina, 1997) and a light-dark-test (Plyusnina, 2004), as well as lower startle responses to acoustic stimuli (Popova *et al*., 2000). We also confirmed earlier findings of lower serum corticosterone levels (Plyusnina, Oskina, 1997), smaller adrenal glands and larger spleens (Plyusnina, Oskina, 1997; Oskina *et al*., 2003; Prasolova *et al*., 2004) in tame rats, indicating differences in the rats' response to stress.

Second, and equally crucial for future genetic studies, we found that in a cross-fostering experiment where pups are being reared by a mother from the other line, all cross-fostered animals behaved similarly to those reared by their own mothers (Albert et al., 2008). This rules out an influence of postnatal maternal effects on the behavioral difference seen between the lines. Researchers have frequently found that postnatal effects can dramatically influence the behavior of rodents, as well as their response to stress (Meaney, 2001). One study even showed that maternal effects are mediated by epigenetic modifications at the promoter of the glucocorticoid receptor gene, which are in turn induced by maternal nursing behavior (Weaver *et al*., 2004). By contrast, our results indicate that the difference in tameness between the rat lines is probably due to allelic variation between the lines that exerts its effects independently of postnatal maternal effects.

Third, we found several unexpected physiological differences between the lines, such as larger kidneys and higher blood levels of free amino acids in the tame rats, as well as higher levels of taurine in tame rats' cerebral cortices (Albert *et al*., 2008). These differences have not been described before, and cannot readily be explained as being causally connected to tameness, low anxiety or a low stress response. It will be interesting to study whether these differences have a common genetic basis with tameness, or whether they are consequences of random genetic drift in the two lines of rats.

A Strategy to Search for Genes Contributing to Tameness

Phenotypic variation for most traits in mammals is continuous and quantitative, rather than falling into discrete classes as is seen for some, usually rare, disease traits. Such continuous traits are often influenced by a number of genetic loci termed quantitative trait loci (QTL). It seems reasonable to assume that the behavioral differences between the rat lines follow such a quantitative pattern, probably influenced by several QTL.

The genetic basis of quantitative traits has been intensively studied (Flint, 2003), and the methods to detect QTL are now well developed (Doerge, 2002). At their heart, they all attempt to detect associations between the genotypic states of

genomic markers and the phenotype of interest. If such a case is detected, it is likely that the marker will be physically close to some causative genetic variant. For this approach to be successful, the marker and the causative allele need to be located close to each other on a chromosome, i.e. be genetically linked (a state also termed as being in «linkage disequilibrium»). Lines divergently selected for a trait of interest (such as the tame and the aggressive rats) are perfectly suited for identifying QTLs by means of experiments involving crosses between the two lines (Lynch, Walsh, 1998). Direct comparison of the genomes of the two lines is unlikely to unambiguously identify causative variants, because presumably a number of neutral differences will have randomly accumulated during the course of selection. Rather, crossing the lines and intercrossing the resulting F1 progeny randomly reshuffles the genomes of the parental lines by recombination (Fig. 1). All animals in the
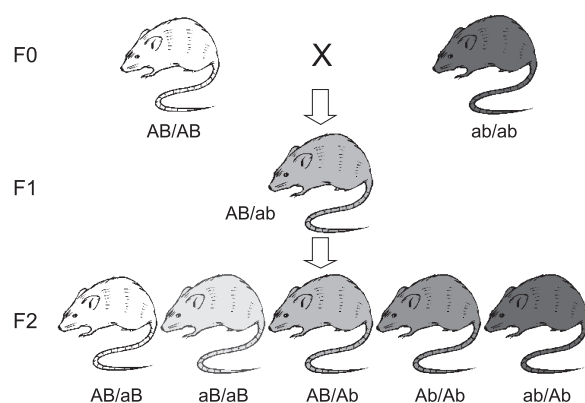

Fig. 1. Illustration of crossing scheme for QTL mapping in tame and aggressive rats. Phenotypic differences are illustrated as differences in shading. Genotypes at two unlinked marker loci A and B are shown. Capital letters indicate an origin of the allele in the aggressive line, lower-case letters indicate an origin in the tame line. Crossing of tame and aggressive animals (the «F0» generation) results in an F1 generation which is intercrossed to form the F2 generation. Often, the F1 will be intermediate in phenotype, whereas the F2 usually spans (and sometimes exceeds) the entire range of phenotypes between the two parental lines. A large number of F2 animals are phenotyped and genotyped, and statistical tests performed to examine the degree of association between alleles and the phenotype of interest. In this example, marker B is linked with tameness, whereas marker A shows no evidence of linkage.

resulting F2 generation can then be phenotyped (i.e., measured for the trait of interest) and genotyped at genetic markers distributed across the genome. QTL mapping proceeds by tracing each allele at every genetic marker back to its grandparental origin. At each marker, individuals are grouped according to their genotypes – individuals carrying two alleles from the tame line form one class, those with two from the aggressive line another class, and heterozygotes form a third class. Finally, the phenotypic measures of the individuals in the three classes are compared. If homozygous «tame» individuals differ significantly from homozygous «aggressive» ones, this is evidence for a QTL in the vicinity of that genetic marker. The heterozygotes provide a measure of genetic dominance at the given locus.

We are currently pursuing a strategy similar to that outlined above. We have crossed the tame and the aggressive rats, and have intercrossed the F1 generation (Fig. 1). Currently, we are obtaining phenotypic and genotypic data from the F2 animals. Our goal is to collect data from several hundreds of F2 individuals, which should give enough statistical power to detect even variants of moderate effect (Lynch, Walsh, 1998). This approach should allow us to uncover the genetic architecture of tameness. How many loci contribute to it? What are their effect sizes? Where on the genome are these loci? As pointed out before, the tame and the aggressive rats differ in many more respects than just their response to humans. In principle, any phenotype that can be measured in the F2 animals can also be mapped. We are hence attempting to collect as many phenotypic measures as possible, ranging from behavioral to physiological parameters. If QTL can be detected for several of these, it will be interesting to find out whether they collocate in the genome. If this turns out to be the case, this might indicate that the same alleles influence them either directly or indirectly.

One particularly interesting trait involves a pattern of coat color variation. Domestic animal species as diverse as dogs, cattle and chicken have long been observed to frequently display white color variants not found in their wild ancestors (Trut, 1999). During the course of selection, white ventral spots have appeared in both rat lines, but have gone to higher frequency in the tame line (Trut *et al.*, 2000). Similarly, in the fox line selected for

tameness, white color variants appeared as part of a suite of traits reminiscent of those found in domestic animals, without having deliberately been selected for (Trut, 1999). The cooccurrence of tameness and white coat color in two independent experiments suggests that coat color variation such as white spotting may be caused by genes that also influence tameness (Trut, 1999). If this is indeed the case, we should be able to find collocating QTL for tameness and for white spotting.

Prospects

Once QTLs are identified, identifying the underlying genes remains a formidable challenge (Flint *et al.*, 2005). However, various methods have been used to narrow QTL regions. Options available in the rats include the creation of an advanced intercross line (Darvasi, Soller, 1995), backcrossing of F2 individuals carrying recombinations in the QTL to the parental lines (Marklund *et al.*, 1999), or taking into account linkage disequilibrium patterns in the parental populations (Goddard, Meuwissen, 2005). These and other approaches might allow us to ultimately describe the allelic variants that contribute to the marked behavioral difference between the two rat lines.

The domestication of animals marked a turning point in human prehistory (Diamond, 2002). Genetic research has revealed the number, regions of origin and timing of many domestication events (Dobney, Larson, 2006) and several genes contributing to phenotypic variation in domestic animals have been found (Andersson, Georges, 2004). However, hardly anything is presently known about the genetic basis of one trait that all domestic animals share – tameness. It is my hope that our continuation of D.K. Belyaev's work can ultimately yield plausible and testable hypotheses on the processes by which humans turned wild animals into today's domestic animal species.

Acknowledgements

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