

Correspondence

Selection for tameness has changed brain gene expression in silver foxes

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A long-term selection experiment for tameness in silver foxes offers a unique model to study transcriptome evolution associated with early canid domestication without confounding environmental effects. Our results suggest that dramatic behavioural and physiological changes caused by selection for tameness may be associated with only limited changes in brain transcriptome.

The first step in domestication of mammals was selection for tame individuals adapted to life with humans and to frequent handling. We previously reported that selection for tameness has changed brain gene expression in dogs (*Canis familiaris*) compared to wolves (*Canis lupus*) [1], but these changes could not be attributed solely to behavioural selection, as it was impossible to separate genetic changes from confounding environmental effects.

Farmed silver foxes (*Vulpes vulpes*) have been selected in Russia for over 40 generations for non-aggressive behaviour towards men [2–4], resulting in animals that are docile, friendly and as skilled as dogs in communicating with people [4,5]. The selected foxes also show developmental, morphological and neurochemical changes concordant with those observed in other domestic animals [4]. In 1996, a few of the selected (S) foxes were imported by the Norwegian University of Life Science, where they have been kept with non-selected (NS)

foxes in exactly the same farming conditions (Supplemental data).

Although selection was discontinued in the Norwegian farm and all animals were equally handled, foxes from the S and NS lines showed apparent differences in the way they reacted to human presence, and the offspring from crosses between the two lines showed intermediate behavioural responses (Figure 1). These results suggest that the differences in behaviour have an additive genetic basis.

We investigated gene expression for three brain regions in the two lines of farm foxes as well as in foxes living in the wild using cross-species hybridizations of pools of fox mRNA to human microarrays. Cross-species hybridizations are useful when the genome of the species under study is poorly known [6,7], and the method is sufficiently sensitive for identification of some genes with large expression differences [8]. In total, out of 29,750 clones investigated 3,091 showed evidence for an expression difference between wild foxes and S foxes, and 2,753 clones differed between wild and NS foxes (penalized F-ratio > 8.0, Figure 2). Most of the expression differences between the wild foxes and the two groups of farm foxes overlapped: 2,469 of the clones

differed between wild foxes and both farm fox lines (8% of the investigated clones).

Contrasting with the differences to the wild foxes, the S and NS groups showed mRNA expression differences in only 40 clones (penalized F-ratio > 8.0; less than two false positives would be expected by chance alone), representing 0.1% of the clones (Supplemental Table S1). Three of these clones were verified by real-time RT-PCR (Supplemental Table S2). Each of the three brain regions separately showed a limited expression divergence between S and NS: 21.5 ± 0.39 (summed over 40 clones \pm SE, \log_2 scale) in the amygdala; 23.5 ± 0.32 in the frontal lobe; and 23.0 ± 0.38 in the hypothalamus. Cross-species hybridization may have limited sensitivity as a result of less stringent hybridization conditions, and the method will bias the detection of expression differences towards abundant mRNA species with a conserved sequence. So, a significant proportion of the true expression differences between NS and S may have been overlooked. This, though, cannot explain the contrast of a large difference between wild and farm animals and a small difference between S and NS foxes, as the same sensitivity and bias apply to all

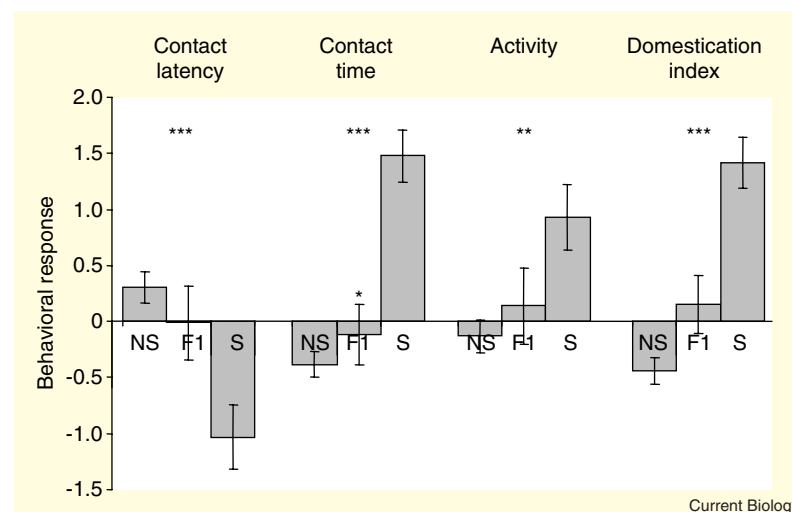


Figure 1. Behavioural response (mean \pm SE) in foxes selected for tameness (S), in non-selected (NS) foxes and in the F1 inter-cross.

Asterisks indicate a significant difference in the behavioural response between S and NS foxes. An asterisk by the F1 bar indicates a significant deviation of the F1 foxes from the average response in S and NS foxes (***, $p < 0.0001$; **, $p < 0.01$; *, $p < 0.05$). Behavioural responses have been standardized to zero mean and unit variance.

comparisons. Experimental variation in postmortem factors, age and health status at time of death could also have contributed to the expression divergence observed in the wild animals [9].

The widespread changes seen in the farmed foxes following selection for tameness have been interpreted as a model of what may have taken place during the domestication process for other mammals [4]. Our results suggest that the striking and widespread differences between *NS* and *S* animals are accompanied by limited amount of gene expression divergence compared to that between wild and farmed animals. As the *S* line had been founded by a small number of individuals, founder effects might have increased the differentiation between *NS* and *S* foxes; if this were the case, however, the real effect of behavioural selection would be even smaller than that reported here. As the *NS* and *S* foxes lived in identical environments, the observed differences most probably reflect the consequences of behavioural selection, with perhaps some contribution from founder effects, whereas the differences between farm and wild animals are likely to derive from both genetic (adaptation to captivity) and environmental differences. These combined results suggest that the dramatic behavioural and physiological changes caused by selection for tameness may be associated with only limited changes in the brain transcriptome. This contrasts with studies with *Drosophila melanogaster* [10] which have shown that selection for behavioural traits can result in much larger transcriptome changes. Further research will help to elucidate to what extent the observed expression divergence causes tameness in the foxes, or whether it reflects down-stream alterations produced by adaptive changes to the life with humans.

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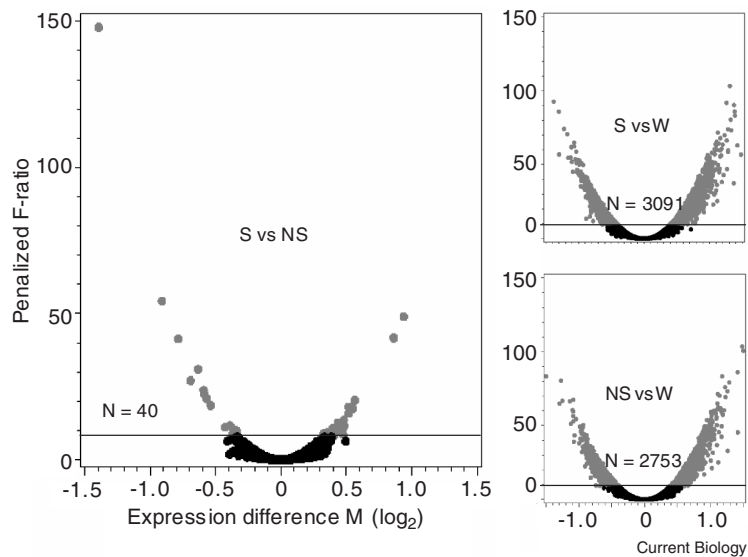


Figure 2. Differences in gene expression between pooled samples of farm foxes selected for tameness (*S*), non-selected farm foxes (*NS*) and wild foxes (*W*) in three regions of the brain.

For each of 29,750 human cDNA clones both the average expression difference (*M*) and the ranking statistic (penalized *F*-ratio) are shown for pair-wise comparisons. Forty clones with a penalized *F*-ratio above eight (marked in grey) showed a significant expression difference between selected and non-selected farm foxes (false discovery rate, *FDR* = 0.045).

samples from wild foxes. This work was supported by grants from the Swedish Research Foundation (621-2002-4258) and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (217-2004-2012), and a strategic grant from the Swedish University of Agricultural Sciences

Supplemental data

Supplemental data including Experimental Procedures are available at <http://www.current-biology.com/cgi/content/full/15/22/R915/DC1/>

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