
THEORETICAL PAPERS
AND REVIEWS

An Experiment on Fox Domestication and Debatable Issues of Evolution of the Dog

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Abstract—This paper is a review of the results of the authors obtained in a long-term experiment on fox domestication. Debatable issues of dog evolution are discussed in light of these results. It is demonstrated that genetic physiological mechanisms of the behavior transformation during selection and the nature of the arising phenotypic changes are associated with retarded development of corresponding ontogenetic processes. As a result of this retardation, the adult animals retain juvenile traits of behavior and morphology (the phenomenon of neoteny). The role of hormonal changes caused by domestication in the evolutionary origin of neoteny is discussed.

INTRODUCTION

Although the appearance of dogs is a very ancient event in the human history, the issue of the evolution of their ancestors is still under debate. What caused the transformation of the wolf into the dog? When and where was the dog domesticated? What directed evolution of this species? Which factors determined the genetic loss of wild reactions in dog's behavior and the formation of strong devotion of dogs to humans? What made dogs so diverse although, as has been established, they all have originated from the standard and uniform wolves? Why their evolutionary rate has dramatically increased in the process of domestication? These key issues have been already addressed by Darwin [1], but even today they are still under debate [2–9]. The hypothetical significance of mutation and founder effect in domestication has been often discussed [6–9]. Evolutionists admit the existence of rapid, saltatory genetic transformations [10]. But even in view of this, 10 000–15 000 years that have elapsed since the appearance of first dogs [2, 7, 9, 11] seem to be too short an interval to accumulate all mutational changes that are needed to create the diversity of the extant breeds.

The role of temporal parameters of development in rapid evolutionary change, typical for domestic animals, is widely recognized [12, 13]. Neoteny, which is the process of retardation of development of somatic characters so that the animals retain juvenile traits at maturity, is regarded as a possible mechanism underlying evolutionary change during domestication [6, 14, 15]. The phenomenon of neoteny has been discovered and described for invertebrates whose larvae acquired the ability for sexual reproduction [12]. The term *neoteny* is used to describe temporal shifts related to dissociated rates of development of reproductive and somatic traits. However, this term is widely used in evolutionary literature as a synonym of *pedomorphosis*, which also

refers to delayed rates of somatic development accompanying accelerated sexual maturation.

Many adult dogs in fact are similar to puppies in their behavior and morphology. Moreover, the breed differentiation in the dog is believed to be possibly based on neotenic processes so that some morphological or physiological traits delayed at a particular developmental stage may become characteristics of the breed [6, 14, 15]. The issue on the appearance of neotenic processes is of principal importance. Since variability of developmental rates has an important role in evolution, a mechanism must exist that would protect this variability from direct selection pressure. However, in some cases, this variability hidden from direct selection may be subjected to indirect selection aimed at other traits.

D.K. Belyaev [3, 4] has paid particular attention to the role of developmental processes and their regulation in the evolution of domestic animals. He believed that the main processes affecting ontogenetic systems and principally determining the progression of domestication occurred at its very beginning, at the time of the first stage of establishing relationships between the animal and human as the novel environmental factor. Regardless of the scenario of this early period, the progression of the process was largely determined by selection on behavior of the animals and their ability for adaptation to and coexistence with humans. During this earliest stage of domestication, natural selection preserved its dominating role. The man was only a factor that shifted the direction of selection to behavior and ability of the dog to exist in the new, anthropogenic environment. It was only gradually that natural selection surrendered its role to artificial one. At first—probably, since very ancient times—humans practiced unconscious, unsystematic artificial selection, which gradually gave way to directed systematic selective breeding. The exact time of this is hard to determine,

but one thing is certain: target artificial selection opened a new era in the evolution of domestic animals. These issues were thoroughly examined by Kislovskii in his work "The Problem of Controlling Evolution of Domestic Animals" [16].

Ascribing major evolutionary significance to the earliest stage of domestication and selection (first natural, and then artificial unconscious), which has been operating for thousands of years, D.K. Belyaev maintained that its consequences could be reproduced in a very short evolutionary time interval of extremely strong directional selection. This selection for the ability to adapt to a new social factor, humans, primarily transformed the animal behavior towards domestication. However, as it involved key loci of gene networks [17] or functionally coordinated gene groups regulating development, it could destabilize ontogeny and its temporal parameters.

The aim of the present study is evaluating possible roles of each of the disputed factors in the evolution of the domestic dog on the basis of the evidence accumulated during a long-term domestication experiment with silver foxes *Vulpes vulpes*. This experiment started, as is generally known, at the initiative of D.K. Belyaev in the early years of Siberian Division of the Russian Academy of Sciences, has been ever since in progress at the Institute of Cytology and Genetics.

ONTOGENETIC MECHANISMS OF TRANSFORMATION OF FOX BEHAVIOR

The prerequisite for setting up a selection experiment was the genetic character of polymorphism for the expression of defensive reactions to humans, which had been found in farm populations of silver foxes [18–20]. In some animals, the number of which constituted less than 10% of the total number, the expression of these reactions was weak bordering on zero. In the early 1960s, these animals (100 females and 30 males) were selected from various farm populations to be the parents of the first generation of the experimental population. The main task at this stage of selection was eliminating defensive reactions to humans. In order to reveal variability in the expression of these reactions more completely, the animals in the selected population were subjected to more intensive contacts with humans than in usual practice. During these contacts, the pups were subjected to a number of tests: the experimenter attempted to hand feed, stroke or handle them. This type of human–animal communication continued for the first three to four months of life of the animals. As a result, the emotionally negative defensive reactions to humans in these foxes weakened, disappeared or, in some of the animals, emotionally positive reactions were formed. The foxes that retained aggressive–fearful reactions to humans in spite of the 3-month period of human contacts with them, were eliminated by selection from the population as soon as in 2 to 3 generations [18–20]. In generation 4 of selection, the first pups

appeared that did not form aggressive–fearful reactions to humans as a result of positive contacts with them. On the contrary, these pups demonstrated emotionally positive response to humans: when the experimenter approached them, they whined and wagged their tails anticipating a positive contact. The task of further selection was enhancing the expression of this response to selectively form in the foxes the type of behavior similar to that of the domestic dog. Throughout the experiment, about 10 000 animals was used that produced more than 50 000 pups. All pups that survived to the age of 7–8 months were tested for their response to human contact. Behavioral and autonomous components of this response were evaluated in different situations: the experimenter (1) approaches the cage; (2) stands by the cage; (3) opens the cage; (4) touches the animal; and (5) closes the cage. Selection was based primarily on the quantitative measurements of this response [19, 20]. Figure 1 illustrates the result of selection. The animals shown in this figure were the domestication elite. Their behavior is similar to that of the domestic dog: they do not escape humans but actively seek contact with them. Seeing a human even at a distance, they whine, yelp, and wag their tail anticipating a contact; during the contact, they try to lick the experimenter's face and hands. Some of these foxes, being out of cage, follow the experimenter like a dog (Fig. 1). The first pups assigned to domestication elite category appeared in generation 6 of selection. Note that this behavior was expressed in the early development of these pups (at the age of about three weeks), i.e., it was formed without any specific contacts with the experimenter. Because of this, such regular contacts were not conducted in further experiment. The contacts of animals with humans were restricted to those with the maintenance staff. Table 1 shows the dynamics of changes in the proportion of the domestication elite at some stages of selection. In generation 6, when they appeared, they constituted only 1.8%; in generation F_{10} , 17.8%; in generation F_{20} , already 35%; and in generation F_{30} , the elite pups constituted nearly half of the progeny (49%). At present almost 70% of the progeny are assigned to the domestication elite. Note that selection pressure was extreme: only 3% of males and no more than 8 to 10% of females were used as the parents of the next generation [20].

Thus, using strong systematic selection for behavior, a unique population of domesticated foxes was created that has a complex of behavioral reactions characteristic of the domestic dog rather than the fox as a species. This is a key formative consequence of selection for domestication.

What underlies the genetic transformation of fox behavior produced by selection? Which physiological mechanisms transformed the aggressive and fearful animals into domesticated ones, i.e., more adapted to the new social environment and to humans? The sensitive period of such adaptation (or primary socialization) is known to begin in the early postnatal development by functional maturation of sensory systems and locomo-



Fig. 1. The behavior of an animal from the domesticated population.

tion. Owing to that, the animals can perceive the environment and respond to it. The development of the fearful response is regarded as a factor that substantially complicates (if not altogether blocks) the processes of their exploration of the social environment and adaptation to it, which is true for all animals regardless of the level of their social organization [21–23]. In dogs, the fearful response manifests mainly by the age of 4 to 6 months (in some of them, by 8 to 10 months), whereas in wolf pups this response is formed by 1.5 months of age [22, 24]. It is noteworthy that such differences in

Table 1. The number and proportion of elite-behavior progeny at different stages of selection

Year of study (generation of selection)	Number of progeny scored	Out of them, elite animals	
		number	proportion, %
1965 (F ₆)	213	4	1.8
1970 (F ₁₀)	370	66	17.8
1980 (F ₂₀)	1438	503	35.0
1990 (F ₃₀)	1641	804	49.0
2002 (F ₄₂)	902	642	71.2

the time of formation of fearful response in the early postnatal development are also observed in domesticated and non-domesticated foxes, which means that selection for tame behavior involves systems controlling the rate of development of this response. Normally, in fox pups from the non-selected population, the fearful response is formed on average by the age of 45 days, similarly to wolf pups. Our experiments have shown that this age is characterized by a reduction in exploratory activity in a novel environment (Fig. 2). By contrast, in domesticated fox pups from generations 28–30 of selection (1988–1990), this does not happen even at the age of 3 months. We did not estimate parameters of exploratory behavior either in domesticated pups or in pups from the unselected population at the beginning of selection. However, a comparison of these parameters in domesticated pups from different generations of selection indicates that their changes result from selection. For instance, the total time of motor activity tested in novel environment in domesticated representatives of generations 17–18 (in 1977–1978) at the age of 65–70 days was 82 ± 9 s, and in representatives of generations 28–30, 175 ± 5 s (1991). The high parameters of exploratory activity in pups from generations 28–30 did not decrease by the age of 3 to 4 months being equal to 188 ± 6 s in 3-month-old and 180 ± 8 s, in 4-month-old pups. Due to these changes in the rates of behavior development, the sensitive period and effectiveness of social adaptation increase.

Genetic effects on fear response are currently examined at the molecular level. Experiments with rats have shown that variation in the expression of this response tested in a wide range of conditions is a direct effect of a QTL (quantitative trait locus) located on chromosome 5 [25]. Detection and localization in foxes of such QTLs whose expression would correlate with fearful response seems to be possible in near future (Kukekova *et al.*, in press). Some data indicate that regulation of the expression of these alleles involves adrenocortical hormones. The fact is that the age of 45 days in fox pups from unselected populations is characterized not only by the appearance of fearful response in ontogeny but also by a sharp increase of glucocorticoids in the peripheral blood (Fig. 2). In pups of the same age from the domesticated population, neither fearful response appeared and exploratory activity is diminished nor glucocorticoid content is increased. Glucocorticoids may be involved in the determination of development rates and changes of these rates during domestication.

HYPOTHALAMUS–HYPOPHYSIS-ADRENAL SYSTEM AND BRAIN NEUROTRANSMITTERS DURING SELECTION OF FOXES FOR DOMESTICATION

Together with the genetic transformation of fox behavior, selection significantly decreased the functional activity of the pituitary–adrenal system (PA). Selection for domestication decreases the total gluco-

corticoid level in blood, in vitro production of adrenal hormones, the basal blood level of adrenocorticotropic hormone (ACTH), and the adrenal stress response (Fig. 3) [26]. Owing to these changes, the fetal development of the domesticated fox pups occurs in the background of reduced levels of maternal corticosteroids (Fig. 4). The level of these hormones decreases even more sharply in the early days of lactation. These hormonal changes during pregnancy and lactation may result in diverse maternal prenatal and early postnatal effects. However, we did not find such effects on the formation of behavior in our experiments conducted in the late 1970s and dealing with cross-upbringing of domesticated pups by nondomesticated mothers and vice versa as well as cross-transplantation of blastocytes [18, 20]. We did not analyze the glucocorticoid levels in the mothers used in this experiment. A special study of the background level of glucocorticoids in pregnant and lactating females (Fig. 4) was conducted in 1990 on the foxes that were advanced by selection after blastocyst transplants [18, 20] for more than ten generations. We can thus assume that in the 1970s, the foxes did not show such substantial hormonal changes as were detected in the 1990s. The expression of these changes was shown to strongly correlate with the degree of domestication, i.e., selective transformation of behavior [27]. In any case, glucocorticoids play an important role in development. It is known that the action of glucocorticoids is mediated through glucocorticoid receptors, which are widely represented in various tissues of both adult animals and embryos [28, 29]. Multiple tissue-specific forms of glucocorticoid receptors exist whose expression can differentially change in different organs and tissues; that is, glucocorticoid may differently affect different developing systems [30]. An important regulatory property of glucocorticoids is their mediation of demethylation and thus of transcription activity of the genes [31]. Some authors believe that glucocorticoids inhibit proliferation in ontogeny thus mediating cell differentiation [32, 33]. Glucocorticoids may function as coordinators of temporal developmental parameters.

Systems of neurotransmitters, whose activity is also changed by selection for domestication, may also play a significant role in the set of regulatory mechanisms that ensure strictly coordinated process of development. Domesticated foxes have an altered activity of the serotonin, noradrenalin and dopamine systems in the specific brain sections involved in the regulation of the selected behavioral traits [34, 35]. Note that neurotransmitters intensely interact with glucocorticoids in their regulatory effects on behavior and functional formation of the HPA axis [36]. Glucocorticoids increase serotonin synthesis and release through glucocorticoids receptors in the midbrain raphe nuclei. This results in the enhancement of the extracellular mediator levels in the structures involved in the regulation of fear (limbic frontal brain sections including dorsal hippocampus, amygdala, and cortex) and in alteration of the expres-

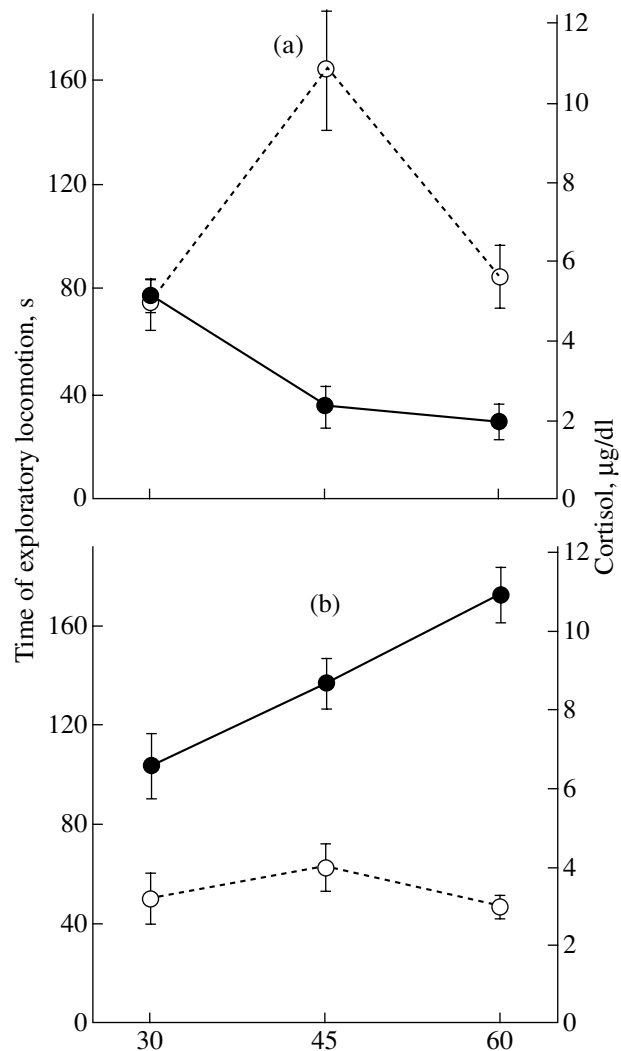


Fig. 2. The time of exploratory locomotion (solid line) and the level of blood cortisol (dashed line) in (a) unselected and (b) selected foxes.

sion of this reaction [37]. They are known to take part in the onset and regulation of cell division at the earliest stages of embryogenesis [38]. Therefore, changes in the activity of the HPA axis and neurotransmitter systems by experimental domestication seem to shift temporal parameters of ontogenetic processes.

PHENOTYPIC CHANGES OF DOMESTICATED FOXES AND TEMPORAL PARAMETERS OF DEVELOPMENT

The genetic transformation of behavior occurring in foxes during selection was accompanied by the appearance in them of morphological changes at different frequencies (10^{-1} – 10^{-4}). The most characteristic of them are presented in Figs. 5–8. First of all (in generations 8–10), color changes appeared. Yellowing-brown mottling (Fig. 5) and specifically localized depigmentation areas (piebaldness) (Fig. 6) appeared on the standard

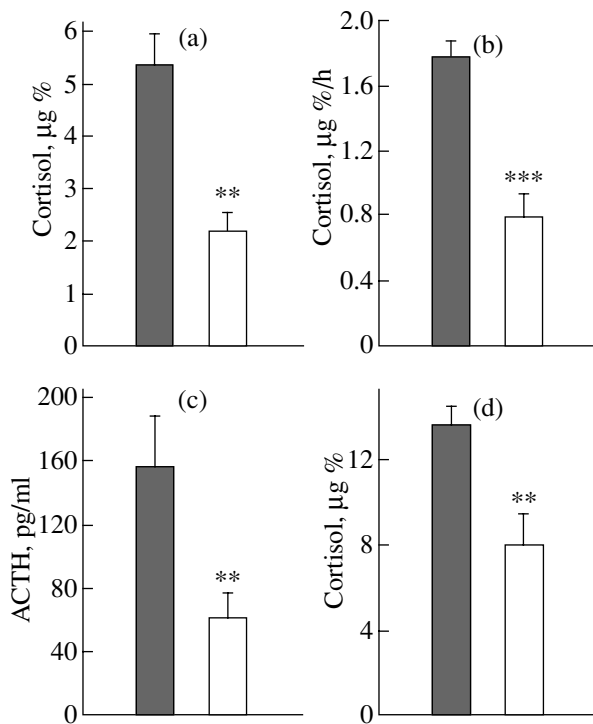


Fig. 3. Parameters of pituitary–adrenal axis in unselected (solid columns) and selected (open columns) foxes. (a) basal blood level of cortisol; (b) in vitro production of cortisol by adrenal glands; (c) basal blood level of ACTH; (d) stress response of the cortisol blood level. Significance of differences between selected and unselected animals: ** $P < 0.01$, *** $P < 0.001$.

silver-black fur coat. According to the historical data, the same changes in color were observed during the early history of the dog [2, 5, 7]. This may indicate the involvement of the common genes in the regulation of such seemingly different biological traits as specific behaviors and fur and skin pigmentation. Judging by the changes in coat color in domesticated animals, selection for specific domestication traits involves loci *Agouti* and *Extinction* as well as depigmentation loci. The *Agouti* and *Extinction* loci are known to be involved in neuroendocrinal physiology [39, 40]. In mice, *Extinction* codes for the α -melanocyte-stimulating hormone receptor, while *Agouti* encodes the A protein, which acts as an antagonist of the former binding to its receptor. The A protein can act as antagonist in other hormone receptor interactions, for example in those with ACTH. Interestingly, the receptors of the melanocyte-stimulating hormone involved in the regulation of melanin synthesis are located not only within melanocyte. This hormone has other receptors including that expressed exclusively in the brain tissue and occurring at high concentrations in hippocampus and hypothalamus, that is., in the structures regulating exploratory and emotional behavior [39].

An important biological role in development is played by genes controlling piebaldness, which is widely spread in domestic and laboratory animals. This

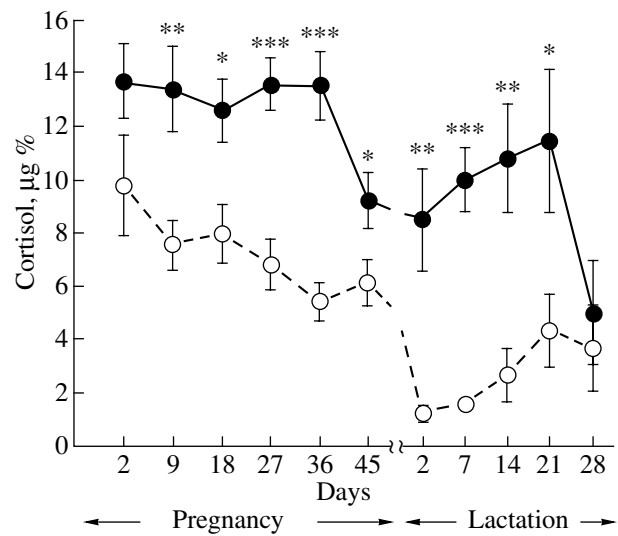


Fig. 4. The blood level of cortisol in unselected (solid line) and selected (dashed line) female foxes at the period of pregnancy and lactation. Significance of differences between selected and unselected animals: ** $P < 0.01$, *** $P < 0.001$.

color type appears in domestic foxes at high frequency. It has been recently shown that murine mutation *White spotting* is a mutation of the *C-kit* gene encoding receptor tyrosine kinase. This kinase and its ligand, the stem cell factor, which is a product of another depigmentation gene (gene *Steel*), play a significant role in the processes of hemopoiesis, gametogenesis, and pigmentation [41, 42]. In other words, our findings and literature data seem to indicate that pigmentation loci belong to the genetic systems involved in the regulation of behavior and development.

In addition to changes in standard coat color, domesticated foxes exhibited other morphological deviations (Figs. 7–9), which are similar to morphological traits of domestic dogs. At the later stages of the experiments, some phenotypic changes appeared also in the population unselected for behavior. Note that such populations, which are reproduced by humans for about a century, are also subject to selection (both natural and artificial) for behavior. The intensity of this selection is clearly incomparable to that in the experimental populations. The frequency of morphological changes in the former is also lower by an order of magnitude. However, it is of principal importance that they are phenotypically similar to phenotypic changes of domesticated foxes.

Taken together, the data obtained by studying the dynamics of appearance of the new morphological traits indicated that the probability of their generation by inbreeding and homozygotization of the mutations present in the population is too low. The main part of the domesticated population was reproduced by outbreeding. The values of the coefficient of inbreeding at different selection stages estimated from the effective

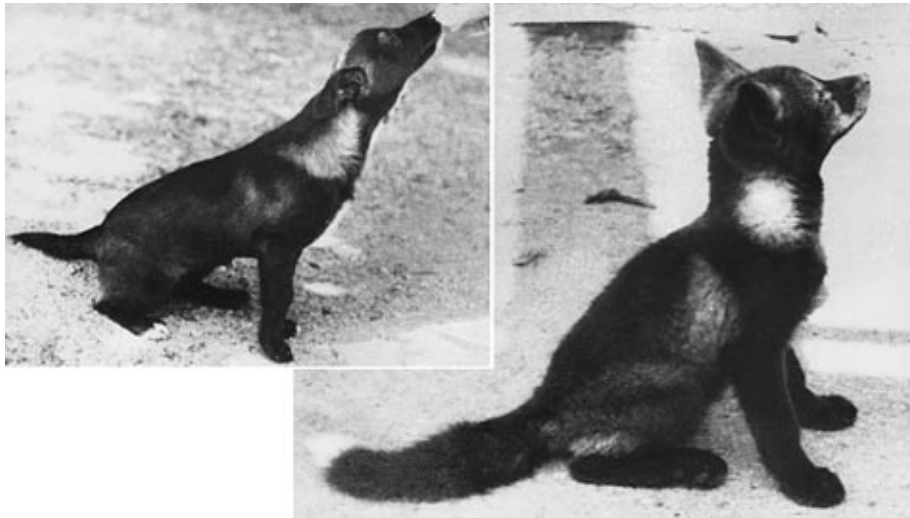


Fig. 5. Specifically localized yellow-brown spots (mottling) in a dog (left) and domesticated fox (right).



Fig. 6. Specifically localized depigmented spots (piebaldness) in a domesticated fox.

population size [43] ranged from 0.022 to 0.07 (Table 2). In 2003, the coefficient of inbreeding of the domesticated population was estimated using a set of 30 random polymorphic microsatellite markers (Kukekova *et al.*, in press). Its value (0.03) was within the limits calculated earlier. An additional argument against inbreeding as a factor increasing the frequency of morphological alterations is the fact that some lines of tame animals were purposefully maintained by inbreeding. However, the aberration frequency in these lines did not increase as compared to the whole domesticated population.

The appearance of morphophysiological diversity in the domesticated population does not seem to be caused by random new mutations. Various deviations from the standard phenotype occasionally occur within a litter of

phenotypically standard parents, which are typically assigned to domestication elite, or even in one individual from the progeny of these parents (Fig. 9). The probability of such events from the mutation viewpoint is negligibly small. Moreover, some pedigrees have many aberrant animals in different generations though the founders of these pedigrees were standard in phenotype [44]. The results of breeding analysis of morphological changes are difficult to ascribe to mutation: parents having a particular morphological aberration unexpectedly produced progeny with aberrations completely different from the parental one. Collectively, these data as well as high rates of morphological alterations and the fact that these alterations in foxes mirror the corresponding traits in dogs [45], suggest that their appearance in the domesticated population is a result of sys-



Fig. 7. Floppy-eared 3.5-month-old fox pup from the domesticated population.



Fig. 8. Curly tail characteristic of dogs and domesticated foxes.



Fig. 9. The manifestation of two morphological alterations—*Star* (white spot on the head) and floppy ears—in one same animal from the domesticated population.

temic regulatory changes rather than single random mutations. In this connection, it is noteworthy that some alterations appearing *de novo* in domesticated foxes are morphological correlates of retarded development. For instance, such morphological novelty as floppy ears, which is typical for dogs and many other domestic animals, is a juvenile trait preserved in older animals. In the early postnatal period, all fox pups have floppy ears. In pups from the farm populations, ears get up at the age of two to three weeks, and in pups from the domesticated population, at the age of three to four weeks. However, in some animals ears remain floppy for three to four months, and occasionally, for the whole life. Even some alterations of coat color are caused by a delay in the corresponding ontogenetic processes. For instance, as noted above, one of the first correlated responses to selection for behavior was the appearance of specifically localized *Star* depigmentation. In the *Star* carriers, the development of embryonic melanocyte precursors, primary melanoblasts, is delayed: they migrate from the neural crest (the embryonic structure of their origin) and proliferate more slowly. The first melanoblasts in fox embryo epidermis appear by day 28 of development in the norm, and by day 30, in the *Star* foxes. Consequently, in the latter melanoblasts reach potentially depigmented areas too late and do not enter hair follicles by the permissive time, which results in the absence of melanocytes in these areas [46].

Alterations of craniological measurements determining skull shape belong to a special group of morphological changes appearing in foxes during domestication and related to changes in rates of growth and development. In some individuals, these measurements sharply deviated from the norm leading to visibly altered skull shape (Fig. 10). Comparative analysis of craniological measurements in the domesticated and control populations showed that these alterations are particularly pronounced in males. The alterations are manifested as shortened and widened face skulls and reduced width and height of brain skulls in domesticated animals. Analysis of within-skull allometry showed that behavior selection shifts both the time of appearance of the structures and their growth rates. In addition, domesticated males become smaller with respect to all measurements, which diminishes sexual polymorphism that exists in unselected population [47]. Note again that the alterations in skull shape are similar to those occurring in dogs at the early stages of their domestication [2, 7, 9, 14]. The discussion of their nature focuses on selection for reduction in the total body size and in maturation rates [7–9]. During early domestication of the wolf, direct (natural or artificial) selection for total body size reduction in fact occurred. However, in the domestication experiments with foxes, animal behavior was a sole criterion of selection, whereas total body size was a selectively neutral trait. Comparative analysis of total body size of foxes, conducted at some selection stages (F_{15} – F_{17} and F_{25} – F_{26}) did not reveal correlated body size changes. Moreover,

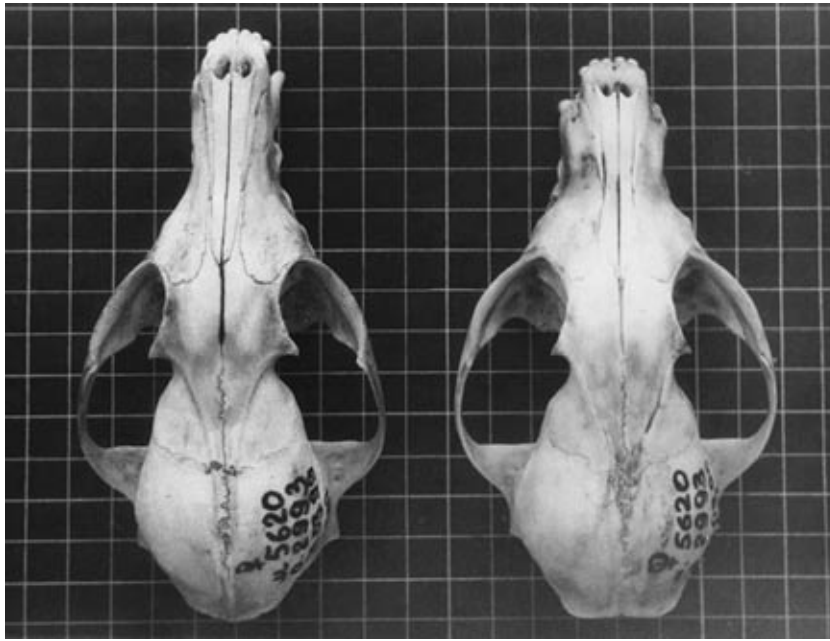


Fig. 10. Skulls of female foxes from the domesticated population: norm (left) and shortened and widened face skull (right).

a trend for an increase in total body size was recorded at these selection stages in the tame males. At the present stage of selection (that is, more than 40 generations after its start), the tame males became significantly larger ($P < 0.001$). However, the decrease in craniological proportions and alteration of face skull shape are more pronounced exactly in males.

As to the effect of direct selection for sexual maturation rate on the appearance of these alterations, the effectiveness of this selection is dubious. It is well known that all reproductive traits (time of maturation, seasonality and multiplicity of reproduction, litter size, etc.) are rigidly determined by stabilizing selection and

have very low additive variability. However, the trait alterations occurred in foxes as correlated response to selection for behavior. The boundaries of the reproduction season broadened; at particular selection stages, two matings per year were recorded in some females though the fox is normally a monoesthrus species (Fig. 11). Note that in tame foxes, the rate of maturation is somewhat increased. If the time of maturation is estimated by a rise in the level of sex hormones in the prepubertal period, in tame females and males sexual maturation occurs on average a month earlier [48, 49].

Thus, the craniological alterations found in foxes selected for domestication are unlikely to be caused by

Table 2. Population coefficients of inbreeding (F_t)* at different stages of fox selection

Year of study (generation of selection)	Number of progeny	Number of their mothers	Number of their fathers	F_t
1960–1961 (F_1)	170	34	11	0.022
1965 (F_5)	213	42	16	0.042
1970 (F_{10})	370	66	29	0.051
1975 (F_{15})	1063	170	79	0.028
1980 (F_{20})	1438	285	120	0.024
1985 (F_{25})	2410	410	137	0.023
1990 (F_{30})	1766	303	108	0.036
1995 (F_{35})	1512	333	93	0.040
2000 (F_{40})	994	200	66	0.072

* $F_t = 1 - (1 - 1/2N)^t$, where t is the generation of selection and N is the number of parents in the t th generation [43].

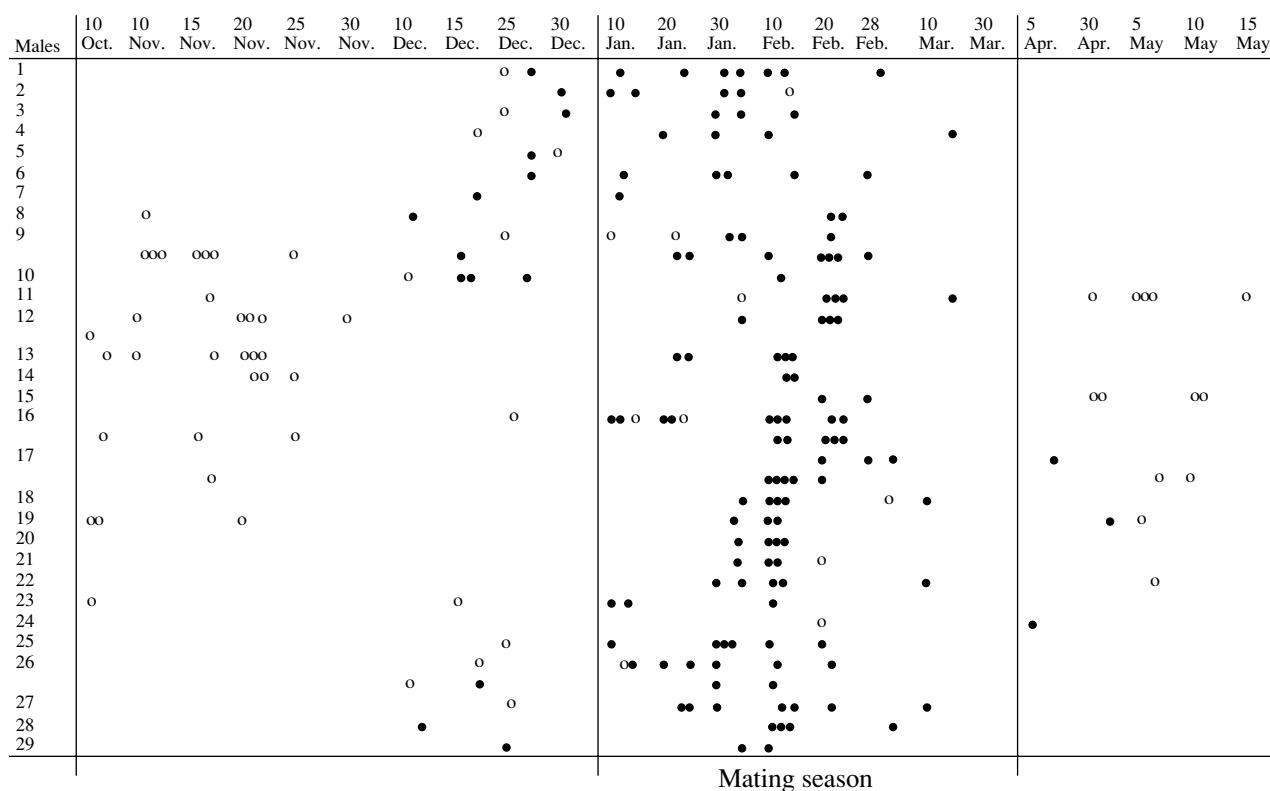


Fig. 11. Time of mating of tame foxes with out-season mating activity. Dots in the rows corresponding to the ordinal numbers of the males, show the dynamics of their mating in successive days of the same season. For males 9, 12, 16, 17, and 26, the second row corresponds to the next reproductive season. Open and solid dots show sterile and fertile matings, respectively.

direct selection for small body size and early maturation, which are discussed in literature. In our experiment, the changes in body size, reproduction parameters, and craniological traits occurred as a correlated response to selection for domestication.

IMPLICATIONS OF THE RESULTS OF THE EXPERIMENT TO THE HISTORICAL PROCESS OF DOG DOMESTICATION

We will probably never completely reconstruct the evolutionary pathway of the domestic dog. We can only with a certain degree of likelihood envisage this pathway and factors that have directed it. Did the results of the long-term extensive experiment on fox domestication increase this probability? Can we regard it as a reconstruction of the historical process? Apparently, today the conditions of domestication reproduction do not have even a remote similarity to the beginning of the historical period. However, the aim of the experiment was to reproduce what we thought was the key factor of early domestication: strong behavior selection. The same evolutionary situation of selection for specific behavioral features promoting adaptation to the new factor, humans, was characteristic at the early domestication stages not only for the dog but also for all animals without exclusion, irrespective of their systematic affiliation, time, and place of their domestication.

Which debatable issues of evolution of the domestic dog are clarified by the data accumulated in our experiment? This is primarily the issue on the mechanisms of transformation of the behavior towards domestication. Undoubtedly, selection played a decisive role but on which genetic systems forming more tame behavior it was targeted? The viewpoint of Hammer [5] is very popular. This author believes that selection acted on genetic systems that reduced sensitivity of systems of perception. For instance, “underreception” of the visual or acoustic analyzer weakens exploratory behavior, stress reactivity, and fear response. In Hammer’s view, a weak response to many environmental factors forms more calm and tame behavior. By contrast, our data show that tame foxes are characterized by earlier development of reaction to sounds, eyes of pups open earlier, and they have higher exploratory activity in a foreign environment, which cannot testify to weakened functional state of sensory systems upon selection for domestication [50, 51]. Our data showed that genetic reorganization of fox behavior toward tameness resulted from a later postnatal development of fear response to alien stimuli. This response is the upper limit of the sensitive period of socialization. In other words, selection for domestication involves primarily genetic systems affecting rates of behavioral development rather than on the sensitivity of analyzer systems.

The time of historical domestication is still under debate. When the first domestic dogs appeared? How long lasted the process of their behavioral transformation and when their morphological transformation started? According to the archeological evidence, first dogs appeared about 10 000–15 000 years ago. However, recently this age of the domestic dog was revised on the basis of molecular genetic data. The control region of mitochondrial DNA, which is highly polymorphic in wolves and dogs, was sequenced first [52]. Vila *et al.* [52] examined this region in 162 wolves from 27 world populations and in 140 domestic dogs of 67 breeds. Comparing the sequence divergence between dogs and wolves, on the one hand, and between wolves and coyotes diverged about 1 Myr ago, on the other, the authors suggested that the dog appeared about 135 000 years ago. However, they supposed that morphological changes in domestic dogs started much later: 10 000–15 000 years ago.

The experiment on fox domestication showed that extreme selection pressure on genetic systems of behavior results in a rapid transformation of behavior towards domestication accompanied by morphological and physiological changes. These results do not agree with the suggestion that the dog did not morphologically change for a long time [43] but conform to the classical view that the increase of the primary diversity occurred explosively at the earliest domestication stage [2, 7, 9].

A very recent revision of divergence of a longer mtDNA region in a far larger sample of dogs collected globally [11] confirms the former estimates of dog's evolutionary age indicating that about 15 000 years elapsed since the appearance of first dogs [11, 53].

Our experiments suggest that the accumulation of new mutations or homozygotization of mutations by inbreeding already present in the population did not play a decisive role in the original generation of diversity. This was discussed above in section "Phenotypic Changes [...]" Phenotypic changes that have occurred during the evolution of the dog are most likely caused by alteration of a small number of genes, but genes having systemic regulatory effects [13, 54]. Such genes occupy the highest level in the hierarchy of genetic networks regulating development; they integrate development into a whole process.

As in domestic dogs, many phenotypic changes in domesticated foxes result from temporal shifts of certain ontogenetic processes. Shifts of temporal parameters of development in tame foxes and dogs exhibit neotenic (pedomorphic) features: a trend for faster sexual maturation together with retarded formation of some somatic traits. As already mentioned, the discussion of the evolutionary nature of this phenomenon in dogs has been focused on direct selection for increased rates of sexual maturation and reduced body size [7, 9, 14, 15]. Our data suggest that the neotenic processes may be a correlated response to selection for tameness, which

involves genetic systems participating in the regulation of temporal parameters of development. As to the accelerated sexual maturation in foxes from the domesticated population, it can also be regarded as a correlated response to such selection.

Thus, taken together, the results of the experiment on fox domestication suggest that the similar behavioral and morphophysiological transformation in dogs and foxes as well as similar changes in temporal parameters of development result from the same genetic changes caused by the same selection vector.

In other words, this experiment has shown that many features of the evolutionary pathway of the dog that this species passed in the process of historical domestication can be reproduced in tens of generations of extremely strong selection pressure directed on specific behavioral traits promoting domestication. This selection acts as a key and universal mechanism of evolutionary transformation of animals during their historical domestication.

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