

Animal evolution during domestication: the domesticated fox as a model

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We review the evolution of domestic animals, emphasizing the effect of the earliest steps of domestication on its course. Using the first domesticated species, the dog (*Canis familiaris*), for illustration, we describe the evolutionary peculiarities during the historical domestication, such as the high level and wide range of diversity. We suggest that the process of earliest domestication via unconscious and later conscious selection of human-defined behavioral traits may accelerate phenotypic variations. The review is based on the results of a long-term experiment designed to reproduce early mammalian domestication in the silver fox (*Vulpes vulpes*) selected for tameability or amenability to domestication. We describe changes in behavior, morphology and physiology that appeared in the fox during its selection for tameability, which were similar to those observed in the domestic dog. Based on the data of the fox experiment and survey of relevant data, we discuss the developmental, genetic and possible molecular genetic mechanisms underlying these changes. We ascribe the causative role in evolutionary transformation of domestic animals to the selection for behavior and to the neurospecific regulatory genes it affects.

Keywords: animal domestication; behavior; developmental rates; evolution; morphological diversity; regulatory system; selection

Domestication in evolutionary terms – from Darwin to the present day

It is well known that Darwin has focused much attention on domestication as the process during which striking variation arises. Although believing that the range of changes in any direction may be different, he admitted that the “tendency to general variability is unlimited”⁽¹⁾. He repeatedly raised the question why domestic animals are so variable. In his analysis of the causes of variation under domestication, Darwin thought the changes to be exclusively due to environmental influences. He maintained that the state of the parent organism during

fertilization or embryonic development has profound effects on the characteristics of the offspring. Darwin emphasized that the constitution of the organism will largely determine the kind of changes induced by the environment, and acknowledged the occurrence of peculiarities as being due to unknown laws acting on individual constitution.

Besides environmental influences on variation, Darwin noted the effects of crosses and inbreeding known in his time from the experience of animal breeders. Relying on their observations, Darwin presented vivid examples of the results of breeding selection apparent in a comparatively short time. However, he also assigned a great role to unconscious selection acting on animals for thousands of years. Because all our domestic animals have been first exposed to domestication at very remote periods, it is unknown when animals started to change and at what rates. Darwin maintained that animals continued to be variable for long periods after their early domestication, suggesting that the early domesticates were even more variable than the now existing ones, and proposed that the capacity to become more variable under domestication is common to all species.

Darwin also raised the problem of similarity between the changes observed in different domestic animals. He regarded certain features shared by many domestic species as the result of their domestication.

Darwin's observations and conclusions on variation under domestication hold true today. The evolutionary peculiarities of domestication, such as the wide phenotypic diversity of domesticates, remain puzzling. Indeed, the variation range of certain traits within a domestic species occasionally exceeds that within whole families or even orders.^(2–5) Morphological variation in dogs, our earliest domesticates, is most illustrative in this respect (Fig. 1).

According to conventional genetic theory, rare (10^{-5} – 10^{-6} per gene per generation) random mutations are the most common mechanisms of phenotypic changes.⁽⁶⁾ The divergence of the dog from the wolf might have happened some 12,000–15,000 years ago,^(3,7,8) which is a short span of time on the evolutionary scale. Nevertheless, variability has accumulated at immense rates which are incommensurate with random mutations. Therefore, the nature and sources of the variation under domestication are intriguing.

Another hallmark feature of variation under domestication is its similar pattern in different domestic mammalian

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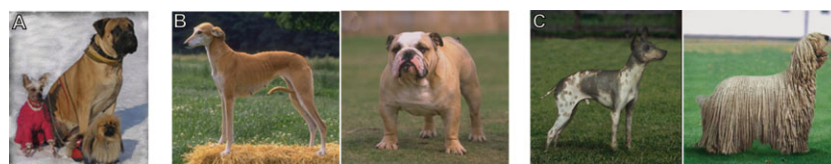


Figure 1. Variability range in dogs for A: body size (Chinese crested, Pekingese and Bullmastiff). B: body shape (Greyhound and English bulldog). C: coat types (hairless terrier and Komondor).

species.^(9,10) When subjected to domestication, animals whose evolutionary pathways did not cross, started to evolve in the same direction. They all lost the species-specific wild-type behavioral response to humans. The activity of their reproductive system became enhanced and relatively uncoupled from the environmental photoperiod, and they all, unlike their wild ancestors, acquired the capacity to breed in any season and more often than once a year.^(10,11) In contrast, the activity of the hypothalamic–pituitary–adrenal (HPA) axis, the key hormonal regulator of adaptation to stress, became attenuated in the very few domesticates studied in this respect.^(12–14) The same morphological changes, first in terms of overall body size and its proportions and also color, length, and texture of the coat, appeared in many domesticates.^(2,3–5,7,8,11) Some of these attributes (white spotting, floppy ears, curly tails) have been aptly called the morphological markers of domestication (Fig. 2). It seems unlikely that these similar trends of morphological and physiological transformation of different domestic animals depend on homology-independent mutations of structural homologous genes. The Russian evolutionary biologist Belyaev has suggested more than 50 years ago that domestication might involve other mechanisms that contribute to phenotypic variation, mainly changes in the activity of genes regulating development.^(2,15,16)

During evolution the same phenotypic changes can be generated through different developmental pathways underlain by different genes. However, there may be developmental processes underlain by key genes with many regulatory functions which under certain recurring selective conditions may most likely be targeted by selection. According to Belyaev, the crucial selective factor during early domestication was the new social environment, i.e., the first encounter of a wild species with humans. This extremely stressful setting rendered the behavior – tolerance, docility toward humans and the correlated stress resistance – the main target of selection. In his opinion, the genes that control behavioral variation play a key regulatory role during development. Belyaev therefore suggested that behavioral variation was the causative variation during domestication. According to his line of thought, the relationship between behavioral variation and transformation of domestic animals would become more intelligible when domestication would be traced from the beginning, i.e., when this process would be reproduced experimentally. Such a sophisticated model was set up using silver foxes (*Vulpes vulpes*) at the Institute of Cytology and Genetics, Novosibirsk, Russia, about 50 years ago.^(16–19) Belyaev was the initiator of this experiment. There were two reasons why silver foxes were given preference for experimental domestication. One was the close taxonomic relation-

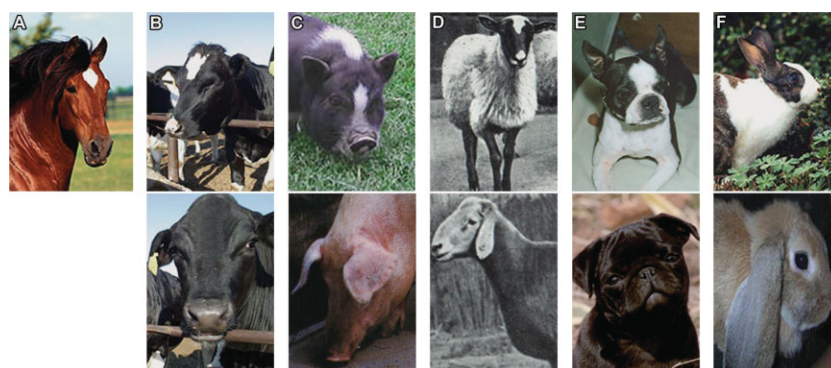


Figure 2. Representatives of different families and orders show the most specific morphological markers of domestication, white spotting on the head (top row) and floppy ears (bottom row): A: Horse (*Equus caballus*): Order *Perissodactyla*, family *Equidae*, breed Russian heavy draught horse. B: Cow (*Bos taurus*): Order *Artiodactyla*, family *Bovidae*, breed Aberdeen-Angus (top), banteng (bottom). C: Pig (*Sus scrofa domestica*): Order *Artiodactyla*, family *Suidae*, breed Vietnamese hybrid (top), Landrace (bottom). D: Sheep (*Ovis*): Order *Artiodactyla*, family *Bovidae*, breed Romanov (top), balbas (bottom). E: Dog (*Canis familiaris*): Order *Carnivora*, family *Canidae*, breed Boston terrier (top), pug (bottom). F: Rabbit (*Oryctolagus cuniculus*): Order *Lagomorpha*, family *Leporidae*, breed Holland white-black (top), German ram (bottom).

ship between the fox and the dog; the other was that cage breeding of foxes had already been started at the beginning of the 20th century. Thus, by the onset of the experiment, captive foxes had already been subjected to rigorous selection for adaptation to the new social environment. This considerably facilitated the long life experiment and reduced its duration.

Development of the experimental model of domestication

Although silver foxes had been farm-bred for about 50 years before our experiment, they retained the standard morphological phenotype, seasonal pattern of breeding specific to the foxes of natural populations, and the wild-type aggressive (growing and biting) or avoidance response to humans.⁽²⁰⁾ Figures 3A–C shows the polymorphism of the expression of these behavioral responses in foxes of large farm-bred populations. Altogether several thousands foxes were tested at fur farms. In the behavioral test, the experimenter approached the home cage, tried to open it and monitored the expression of the response. This observational test was highly reproducible. About 10% of farm foxes displayed the wild-type responses very weakly or not at all (Fig. 3C). Of these foxes, 100 females and 30 males were chosen from different farm-bred populations as the initial parental generation for selection for tolerance towards humans or docility, then for tameability. The fox population was outbreeding.⁽²⁰⁾

The rationale of the experiment was that subsection of an experimental fox population to selection for a single behavioral trait, tameability, is sufficient for domestication to occur, and the experimental design followed this rationale.^(20,21) The researcher tested the tameability during the time-dosed contacts with pups on the basis of their responses to hand feeding and to attempts to touch or pet them. All the pups underwent time-dosed contacts for 2.0–2.5 months. Those pups that continued to show aggressive-avoidance responses despite the contacts were discarded from the experimental population. The pressure of selection was very rigorous: less than 10% of the most tame individuals of every generation were used as parents of the next generation.^(17–21) As a result of such rigorous selection, the offspring exhibiting the aggressive and fear avoidance responses were eliminated from the experimental population in just two to three generations of selection.

In the fourth selected generation, there appeared pups that responded to humans by dog-like tail wagging. The offspring of the next selected generations displayed more and more dog-like behavior. In the sixth generation, there appeared pups that eagerly sought contacts with humans, not only wagging, but also whining, whimpering, and licking in a dog-like manner. Such foxes were assigned to the "elite of domestication" (Fig. 3 DEF). Elite pups constituted only 1.8% (4 of the total 213 foxes) in the sixth generation of selection, 17.9% (66 of 379) in the tenth and 35% (503 of 1438) in the twentieth generation. In the thirtieth generation, the proportion of elite pups was as high as 49%, (804 of 1641).⁽¹⁹⁾ By 2005/2006 almost all the foxes of the domesticated population



Figure 3. Behavior of foxes of the farm-bred populations (top row) and of the population selected for tameability (bottom row): A: A fox showing the aggressive response to humans; B: A fox showing the avoidance response to humans; C: A fox weakly expressing the wild-type aggressive-avoidance response to humans; D–F: Domesticated foxes referred to the behavioral elite. F: from left to right: Prof. K.G. Lark (University of Utah, Salt Lake City), Prof. L.N. Trut (Institute of Cytology and Genetics, Novosibirsk), Prof. G.M. Acland (Cornell University, Ithaca) with domestic foxes.

were assigned to the behavioral elite. Quantitative differences between the behavior of elite foxes are now estimated using video records of behavioral tests. From these records, fifty most informative behavioral parameters (for example frequency of wagging, specific vocalizations, posture of body, and its communicative parts such as tail, ears, and others) were analyzed by the principal component analysis (PCA).^(22,23) This statistical method identifies the quantitative phenotypic variation in tameness as linear combinations of correlated parameters or principal components (PCs).⁽²⁴⁾ These new integrated independent traits are genetically analyzable phenotypes.^(25,26)

Throughout the experiment, altogether 10,500 foxes were used as parents. All in all, about 50,000 offspring were obtained and tested for their amenability to domestication.⁽¹⁹⁾ The result of this directional selection is impressive: a unique domestic fox behaving very similar to another species, the domestic dog, has been developed through methodically applied selection.

Multifaceted results

The rigorous selection of the silver fox solely for tameability brought about correlated changes in certain features of behavior, physiology, and morphology.

Thus, the rise of the dog-like ability of foxes to “read” human social cues, for example gestures and glances, was a noteworthy change in their behavior. Domestic foxes became more skillful than farm-bred foxes in using human cues for coping with the man-made environment not only during brief experimental tests and selection procedures, but also during daily routine care.⁽²⁷⁾

The most important physiological consequence of fox domestication is the transformation of the seasonal reproductive pattern. The mating season of foxes in nature as well as at fur farms occurs only once a year during increasing of daylight (January to March). In some genealogical groups of foxes that were more advanced with regard to tameness, sexual activity and mating were observed outside the limits of the breeding season (Table 1),^(20,28) which is normally strongly restricted by natural selection.⁽¹¹⁾ Moreover, a few vixens have even mated twice a year. The total number of animals exhibiting changes in their reproductive pattern was small throughout the years of the experiment. However, their emergence among the tame foxes is of crucial importance. Reproduction seasonality is under strong selective constraint.⁽¹¹⁾ Variations in the mating dates of animals within the mating season are mainly due to environmental factors. As a consequence, direct selection for mating timing during the season is ineffective and does not change the limits of the breeding period. However, the data in Table 1 suggest that the capacity to breed at any time throughout the year, which is

characteristic of domestic animals, might have occurred as a correlated consequence of selection for tameability.

The consequences of selection for behavior are thought-provoking (Fig. 4). In the foxes of the domesticated population, several morphological traits that mirror dog morphological features began to appear at different frequencies (Table 2). The changes in standard coat color pattern appeared earlier than the other changes, namely in the 8th–10th selected generation. Specific piebald Star spotting (Figs. 4F and 5A,B) and brown mottling on the background of standard silver-black color (Fig. 5C) are the most typical patterns. The Star spotting is determined by the incompletely dominant autosomal Star mutation.⁽²⁹⁾ The gene control of brown mottling in foxes is still unclear. In dogs, it is controlled by one of the mutations at the Agouti locus. According to cultural-historical evidence, similar changes in coat color occurred in early dogs.⁽⁸⁾

In the domesticated foxes, morphological aberrations such as floppy ears and curly tails occurred in addition to changes in standard coat color. These morphological traits are also characteristic of many domesticates, particularly dogs (Figs. 4F–J). At the more advanced steps of selection, changes in the parameters of the skeletal system began to arise. They included shortened legs, tail, snout, upper jaw, and widened skull (Fig. 4).

Some of the phenotypic changes appeared not only in the domesticated foxes, but also in those foxes of the farm-bred populations that were not subjected to selection for tameability (Table 2). The above observations suggest a relation between selection for tameability and the appearance of a subset of phenotypic changes characteristic of domestic animals. The appearance of some phenotypic changes in the foxes of the nonselected populations is not at variance with this suggestion. These populations have been bred in captivity for about a hundred years, during which they have been inevitably subjected to selection for adaptation to captivity or amenability to domestication. The analysis of this subset in terms of traditional quantitative genetics of correlated variability is difficult.⁽³⁰⁾ In fact, strong selection pressure on any quantitative trait, especially on those having adaptive significance, breaks down the genetic system kept in balance by preceding evolution and, as a consequence, inevitably leads to the appearance of deviants from the phenotypic means whose reproductive capacity is reduced. Certainly, the correlated responses of any population to any selection depend on the structure of its genetic pool, and therefore they are, in a sense, unique, and irreproducible. However, domestication of different populations of one species, different species or even orders is, as well as selection of foxes for tameability, consistently associated with the same morphological, and physiological changes. This remarkable parallelism can be hardly regarded as usual correlated responses to selection for any quantitative trait. In

Table 1. The time course of mating in the tame foxes showing extraseasonal sexual activity. The circles next to the order number of males indicate their matings in the years when they mated outside the breeding season. White circles, sterile mating; black circles, fertile mating.

Male	10 Oct	10 Nov	15 Nov	20 Nov	25 Nov	30 Nov	10 Dec	15 Dec	25 Dec	30 Dec	10 Jan	20 Jan	30 Jan	10 Feb	20 Feb	28 Feb	10 Mar	30 Mar	5 Apr	30 Apr	5 May	10 May	15 May
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Mating season

addition, the reproductive performance of all animals under domestication improved, in contrast to what happened in the case of the correlated responses in terms of traditional quantitative genetics.⁽³⁰⁾

The inbreeding coefficients of the population were estimated on the basis of its reproductive performance^(19,20) and of a set of randomly chosen polymorphic microsatellite markers⁽³¹⁾ at different stages of selection; the coefficient values were not smaller than 0.02 and never greater than 0.07 in both the domesticated and farm populations. These estimates indicate that the probability of appearance of changes due to stochastic fixation of the alleles pre-existing in the initial population and controlling each and every observed phenotypic change was very low.

Finally, it is unlikely that the changes in the domesticated foxes were a result of randomly arisen new mutations. Thus, in the same litter of phenotypically standard parents and even in the same offspring of parents assigned to the tame elite, several different changes in the standard phenotype appeared (Fig. 5A). This is incompatible with the mutational nature of those changes. The results of the genetic analysis of morphological changes are also incompatible with the view that each phenotypic alteration is due to a single

independent gene, since the offspring of parents with one or another morphological alteration showed, contrary to expectation, quite different morphological changes. Only the Star depigmentation phenotype showed an independent genetic basis. The results of genetic analysis of the other phenotypic changes demonstrated that a common genetic basis may underlie the set of different morphological aberrations. All this strongly suggests that the phenotypic variation within the domesticated fox population may result from changes in the regulation of development by the key genes caused by selection for tameability.^(18–20)

Association between phenotypic changes and developmental rate

The association between the newly generated traits and changes in the developmental rates of domestic foxes is noteworthy. Certain behavioral and morphological changes are correlates of delayed development of normal traits. The emotional expression of positive responses to human, widened skulls, shortened snouts, floppy ears, curly tails are all juvenile traits that certain domesticated individuals

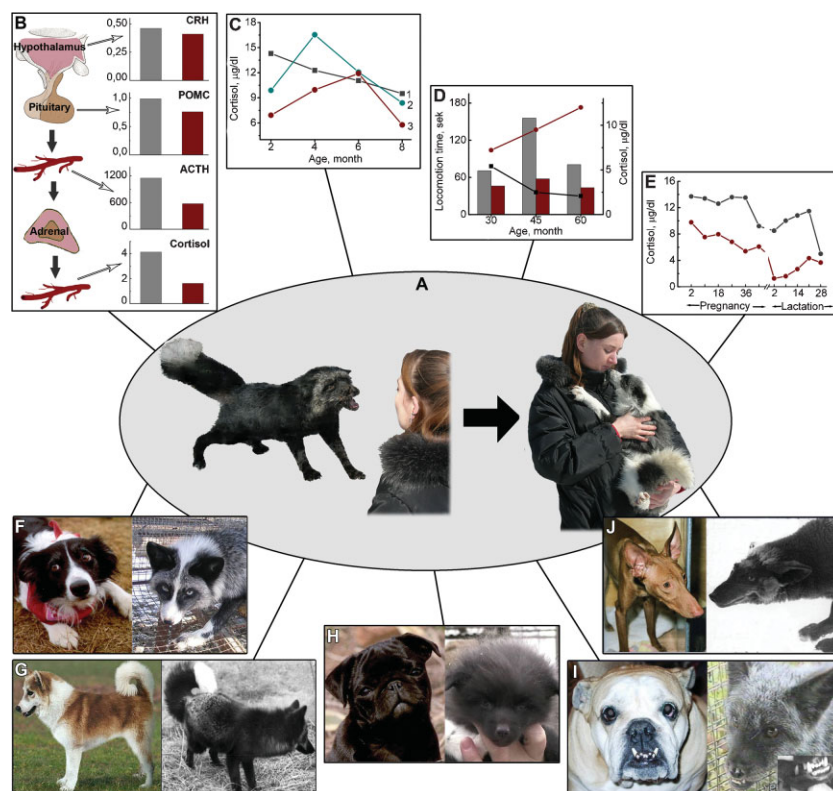


Figure 4. A schematic representation of the results of the fox domestication experiment. A: Transformation of fox behavior resulting from selection for tameability. Left: a fox of the farm-bred population not selected for behavior. Foxes of this population show the typical aggressive response to humans. Right: a fox of the experimental domesticated population. The dog-like behavior of foxes of this population is the result of many years of selection for tameability. B–E: Activity of the HPA axis in farm-bred and domesticated foxes. Farm-bred foxes are shown in grey, the domesticated in red. B: Hypothalamic CRF (CRFM RNA/18SmRNA) and pituitary *POMC* (*POMC*mRNA/18SmRNA) gene expression, as well as ACTH (pg/ml) and cortisol (μ g/dl) levels in farm-bred and domesticated foxes. C: Age-related changes in plasma cortisol level in farm-bred and domesticated foxes: 1) farm-bred foxes exhibiting aggressive responses to humans, 2, 3) foxes of the domesticated population with low (2) and high (3) domestication scores. D: Total time of locomotion, an indicator of exploratory behavior, and plasma cortisol levels in farm-bred and domesticated foxes at the age of 1–2 months: locomotion is plotted on the graph; plasma cortisol levels are represented as bars. E: Plasma cortisol in silver foxes during pregnancy and lactation. F–J: Dog-like morphological changes arisen in foxes of the domesticated population: F: Similarity of coat depigmentation between dogs and foxes: Left, border collie; Right, tame fox. G: Tail carriage, curly tail: Left, Islandsk Farehund; Right, tame fox. H: Ears are floppy and face skull is widened in some pups of tame foxes: Left, pug; Right, tame fox pup. I: Long jaw (elongation of the lower jaw), characteristic of the English bulldog, occurs among tame foxes: Left, English bulldog; Right, tame fox. J: Elongation of face skull in certain dog breeds and tame foxes: Left, Pharaoh hound, Right, tame fox.

retain to adulthood.^(3,32,33) Delay in developmental rates was observed as early as during embryonic morphogenesis. As already noted, the specifically located Star depigmented spots are the specific markers of domestication (Figs. 2 and 5). It was shown that retardation of the development (proliferation and migration from the neural crest) of the embryonic precursors of melanocytes, or primary melanoblasts, is the mechanism underlying depigmentation.⁽³⁴⁾ This retardation leads to the absence of melanocytes from specific areas of the coat and, hence, to its depigmentation.

The shifts in the timing of development brought about by selection of foxes for tameability have a neotenic-like tendency; the development of individual somatic traits is decelerated, while sexual maturation is accelerated. Higher

levels of sexual hormones in the plasma and increased gonadal weight during the prepubertal period are indicators of earlier sexual maturation of tame foxes. Judging by these

Table 2. Frequencies of phenotypic changes newly arisen in fox populations.

Traits	Frequency in populations	
	Domesticated	Farm-bred
Star	1.24 10^{-1}	0.71 10^{-2}
Brown mottling	0.45 10^{-2}	0.9 10^{-3}
Floppy ears	0.23 10^{-2}	0.17 10^{-3}
Shortened tails	0.14 10^{-2}	0.2 10^{-3}
Curly tails	0.94 10^{-1}	0.8 10^{-2}

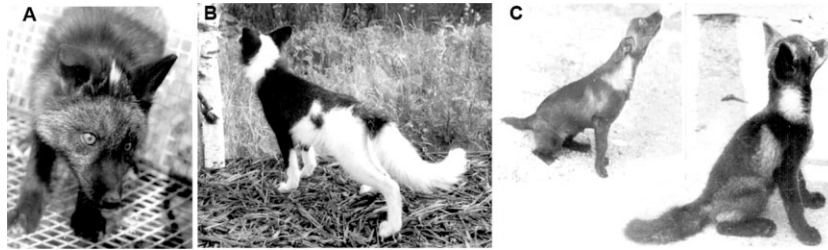


Figure 5. Specific markers of domestication. A: Two different phenotypic changes in an *Ss* heterozygote: the Star depigmentation and a floppy right ear. B: Homozygote for the *Star* mutation (*SS*). C: Phenotypic similarity between brown mottling in fox and dog.

parameters, tame foxes reach sexual maturity, on average, one month earlier than their farm-bred counterparts.⁽³⁵⁾ Many researchers regard the neotenic tendency of development as a mechanism of evolutionary transformation of domestic animals. However, the causes of the emergence of this tendency during domestication remain unclear.^(8,32,33,36) Our fox experiment demonstrated that neotenic shifts in developmental rate may arise as a correlated consequence of selection for tameability, i.e., social adaptation to humans. The point is that there is a sensitive socialization period in mammals when they explore their environment and get used to particular social factors.^(37,38) This period starts early with the functioning of the sense organs and the formation of locomotion, and enables the animal to learn about its surroundings and to form attachments. The maturation of the neurophysiological substrate of the fear response hampers socialization. Increasingly fearful pups start to avoid the impact of social factors, thus limiting their further socialization. It was found that selection of foxes for tameability slowed down the development of the fear response and shifted it to later dates. In foxes that were not selected for tameability, the exploratory activity (estimated on the basis of locomotion in an unknown setting) became sharply reduced by the age of 45 days or so when fear was first manifested.⁽³⁹⁾ This reduction was lacking in the tame foxes until the age of four months.⁽¹⁹⁾ Quite plausibly, retardation of the development of the neurophysiological substrate of fear is just one of the numerous manifestations of the regulatory effects of genes that affect developmental rates and are targeted by selection for domestication. It cannot be excluded that these genes perform a broader function by regulating the developmental rates of other traits.

Neuroendocrine changes under domestication

Which putative genes affecting the developmental rate may possibly be involved in selection for domestication? We assumed that some of these genes might perhaps control the

glucocorticoid (GC) status in animals. This assumption is based on the close association between social behavior and the formation of the GC status; the reduction in exploratory activity, which, as noted above, sets an end to the sensitive socialization period, was found to be correlated with a rise in plasma cortisol levels during early postnatal development.⁽⁴⁰⁾ In the tame pups, whose socialization period was longer, this rise occurred later (Fig. 4D). Not only the rate of social development, but also of the embryonic precursors of melanocytes were found to be associated with the GC status.^(41,42) These observations suggested that GCs may function as coordinators of the parameters characterizing the timing of development. Evidence exists that during embryogenesis GCs may have an inhibitory effect on cell proliferation and thereby promote cell transition to differentiation, which is the next step of early development.^(43–46)

Only few studies were concerned with the function of glucocorticoids under domestication. It has been reported that domestication of grey rats is associated with a decrease in the weight of adrenals, changes in their morphology and alterations in the glucocorticoid synthesis under basal conditions and stress or under the effect of exogenous adrenocorticotrophic hormone (ACTH).^(12,13) Comparison of domestic guinea pigs with their wild ancestors revealed a decrease in the stress response of the HPA axis in domesticates.⁽¹⁴⁾ It is of importance that the function of the HPA axis decreased in foxes with advancing selection for tameness. We started the comparative analysis of the HPA axis from generation 10 of selection for tame behavior. In this generation, the levels of plasma glucocorticoids in the tame foxes were significantly lower in all seasons of the year compared with the farm-bred foxes.⁽⁴⁷⁾ The hormone levels decreased with advancing selection. Basal cortisol levels in the blood of the domesticated foxes in generation 20 were almost two-fold lower than of the non-domesticated foxes, and stress-induced levels were about 30% lower. In generation 45, basal and stress-induced blood cortisol levels in foxes of the tame population were already three- and five-fold lower than in the farm-bred foxes.⁽⁴⁸⁾ These data suggest that genes controlling plasma glucocorticoids were possibly the targets during selection for tameability.

In the foxes selected for domestication, the activity of the HPA axis is reduced at all levels (Figs. 4B–E). This includes the total GCs pool, the adrenal GC production *in vitro*, the basal ACTH level in plasma, and the adrenal response to stress.^(49,50) The expression of the pro-opiomelanocortin (POMC) gene, whose protein product includes ACTH and β -endorphin in the pituitary, was also reduced, and the expression of corticotropin-releasing hormone (CRH), the releasing factor for ACTH, tended to decrease in the hypothalamus.⁽⁵¹⁾ It is also important that in the tame foxes the total GC pool in the blood was considerably decreased during pregnancy and lactation.^(52,53) As a consequence, the entire embryonic and early postnatal development of the tame offspring proceeded on the background of lower maternal GCs. Given the multifarious effects of GCs on development,⁽⁵⁴⁾ the impact of their changes on developmental processes is evident. They are directly associated with the expression of the GC receptor (GCR) gene, whose promoter region has a complex structure. The multiple and tissue-specific promoters provide fine regulation of changes in GCR gene expression in different early environments.^(55,56) Most importantly, the GCR gene expression in the hippocampus, a brain structure modulating the regulation of behavior and activity of the HPA axis and in the frontal cortex, is higher in the domestic animals.⁽⁵⁷⁾ Thus, the effects of selection for tame behavior on the HPA axis are manifest at all levels, ranging from phenotypic parameters to the expression of the CRH, POMC, and GCR genes.

Domestication also affects the developmental neurotransmitter systems. The role of neurotransmitters in the regulation of development has been discussed⁽⁵⁸⁾ and recently reevaluated.⁽⁵⁹⁾ The serotonin system of the brain deserves special attention with reference to domestication. Its contribution to the inhibition of aggressiveness in animals, including foxes, has recently been discussed in Bioessays.⁽⁶⁰⁾ The studies of the brain's serotonin system showed that the domesticated foxes differed from the farm-bred by higher levels of serotonin and its main metabolite 5-hydroxyindol acetic acid in a number of brain structures. Differences in the activities of monoamine oxidase, the principal enzyme in serotonin degradation, and of tryptophan hydroxylase, the key enzyme in serotonin synthesis, were also demonstrated. It was shown that the activity of tryptophan hydroxylase was higher in the tame than in the non-tame foxes. The higher activities of this key enzyme and the higher levels of serotonin in the brains of domesticated foxes agree well with the data concerning the inhibitory influence of serotonin on a number of aggressive types of behavior.^(49,60) Thus, it is important to emphasize the role of serotonin in development. Previous⁽⁵⁸⁾ and recent⁽⁶¹⁾ publications have shown that serotonin acts as a multifarious signal molecule that is important during development and capable of eliciting a cascade of gene activations.

Destabilizing selection as a possible accelerator of evolutionary transformation of domestic animals

Surveying the observations made during the course of experimental fox domestication, Belyaev found no satisfactory explanation within the traditional genetic framework and came to the idea of destabilizing selection as a factor of domestication.⁽¹⁶⁾ As destabilizing selection Belyaev designated selection causing destabilization of regulatory systems controlling development and, hence, destabilization of the morphological and physiological organization stabilized by previous natural selection.⁽⁶²⁾ Under domestication, selection for particular behavioral features accomplishes the destabilizing function. Belyaev regarded the selection for just these features as a major accelerator of the evolutionary transformation of domestic animals. It appears likely that destabilizing selection can affect animals at any organizational level, whose populations experience selection pressure on behavioral traits. Illustrative examples of multifaceted consequences of selection for behavior^(63,64) were reported even in *Drosophila*.^(65,66,67) The evolutionary role of selection for behavior would increase greatly in the case of highly organized mammals, because their phenotypic manifestation of behavioral traits involves much more regulatory neurotransmitters and neurohormonal relationships.⁽⁶²⁾

The evolutionary function of destabilizing selection is the reverse to that of stabilizing selection.⁽⁶⁸⁾ Stabilizing selection acts always in an environment with which a species has coped well; this leads to stabilization of development to form a phenotype that is optimal under this condition. Stabilizing selection acts by suppressing the effects of mutations that disrupt development and alter the normal phenotype, or even by eliminating them. It maintains variability within the optimum range of the evolutionary established norm, but it does not produce variability beyond the phenotype of the species. Analysis of the effects of selection for behavior on development and variability in our fox model demonstrated that it sharply destabilized the correlated developmental systems and led to an increase in phenotypic variation. What mechanisms can be involved in this process? And what is the nature of the changes in the specific genes that are induced by destabilizing selection?

It appears plausible that the phenotypic novelties in the experimental fox population could be due to changes in gene activity, largely in its epigenetic modification. Hormonal changes under domestication may result in either gene silencing or gene activation. In this context, the above-mentioned Star phenotype (Fig. 5), which appears repeatedly at high frequencies (10^{-1} – 10^{-2}) in the population of tame foxes and which is determined by the incompletely dominant S gene, is of importance.⁽²⁹⁾ Segregation analysis of

offspring from crosses between Ss heterozygotes for this gene demonstrated a shortage of SS homozygotes. This shortage cannot be accounted for by selective embryonic, zygotic, and gametic mortality. Some foxes that were proven to be SS homozygotes have the heterozygous phenotype (Ss) and the inheritance pattern characteristic of heterozygotes. It was assumed that one of the two homologous mutant Star alleles became functionally inactive. Also, cases of birth of homozygotes in crosses between a heterozygous and a standard parent have been documented (unpublished data). This suggests the reverse process of activation of a wild-type "silent" allele in rare heterozygotes for the Star gene. A similar phenomenon of heritable gene inactivation/reactivation has been described for the kinked tail character in the mouse controlled by the dominant Axin-fused gene, the phenotypic expression of which is extremely variable, to the extent that some of its carriers have a normal phenotype.⁽⁶⁹⁾ Subsequent studies showed that the absence of the kinked tail phenotype correlates with the level of DNA methylation in a retro-transposon within AxinFu.⁽⁷⁰⁾ Moreover, the epigenetic modification of the mutant kinked allele of both maternal and paternal derivation passes meiosis, i.e., it does not undergo reprogramming during gametogenesis.⁽⁷⁰⁾

In the mouse, another example of inheritance of epigenetic modification in the Agouti locus, which controls wild-type coat color, has been described. The A^{vy} allele bears a transposon insertion. The number of viable yellow phenotypes was related to the methylation pattern of this insertion.^(71–74) The Agouti locus is implicated to be involved in brown mottling in the dog. This prompts to study the Agouti locus at the molecular level in the domesticated fox to reveal whether the locus possess structural components that can act as effective carriers of methylation marks.

Methylation of the DNA promoter regions is implicated as a mechanism of the epigenetic alteration in gene expression; methylation is related to the transcriptional silencing of regulated genes, while hypomethylation is related to their transcriptional activity. As to the Star mutation in foxes, it is unknown whether its activity changes at the transcriptional level or at subsequent steps of gene product conversion. It may be speculated that the selection-related genetic changes in the regulatory neurohormonal system of the domesticated foxes could induce changes in the expression of many other downstream genes, thereby causing phenotypic destabilization. However, only limited changes in gene expression in fox brain have been shown so far.^(51,75) As indicated above, these changes involve also genes of the HPA axis.^(48,51) This suggests that behavioral as well as morphological and physiological changes in the domestic foxes may depend on changes in the expression of a very small number of brain genes with many regulatory downstream effects.

It is of interest that the expression profiles of the brain-specific genes showed remarkable differences between

domestic dogs and their closest wild relatives, wolves (*Canis lupus*) and coyotes (*Canis latrans*).⁽⁷⁶⁾ In dogs, the most prominent changes in gene expression occurred in the hypothalamus, which is a biologically important, evolutionary conserved brain structure as well as a modulator of behavioral and neuroendocrine responses to environmental agents. Comparisons of the differences in gene expression between wolves and coyotes, which have been separated for 2 Myr BP,⁽⁷⁷⁾ have demonstrated that the differences are minimal and that the expression profiles of the hypothalamic genes in the wild canids are conserved. This is in striking contrast with the variable expression of these genes in dogs and with the differences between dogs and wild canids. It could not be ruled out that selection for tame behavior during 12,000–15,000 years of dog domestication accelerated divergence of gene expression in the brain, as postulated by the authors.⁽⁷⁶⁾

Molecular genetic implications for phenotypic changes under domestication

As yet, little is known about the correlation between the expression levels of specific genes and phenotypic variability in particular traits. However, some data indirectly indicate that this relation does exist. For example, it is known that coding regions abound with presumably noncoding simple sequence repeats (SSRs). It is curious that half of these repeats cluster in regions containing one or several genes playing a key role in development.⁽⁷⁸⁾ This suggests that they are important for the regulation of expression of these key developmental genes. Their altered regulation may be implicated as the major source of changes in many genes under their control. Thus, it has been shown that variation in the number of tandem repeats in two such genes, Runx-2 and Alx-4, may be related to the variation in skeletal traits in dogs.⁽⁷⁹⁾ Variation rates in the number of tandem repeats in genes for neurotransmitters and their receptors may be involved in determining variations in behavior.⁽⁸⁰⁾ The variation in the number of repeats can exceed by far that of point mutations. This suggested that variation in the length of tandem repeats may be a predominant source of rapid morphological evolution and that the repeats associated with the developmental genes increase the pace of evolution.⁽⁷⁹⁾

In addition to SSRs, phenotypic variation may be influenced by canid-specific small interspersed nuclear elements (SINEs).⁽⁸¹⁾ Both seem to be often present at positions influencing gene expression.^(82,83) Bsp repeats similar to canine SINEs have been detected in the fox genome, in which they may potentially perform regulatory functions.^(84,85) The presence of SSR, SINE, or Bsp elements in the genes of the hormonal and neurotransmitter systems may have important consequences. Changes in their

expression may be implicated in changes in the expression of many genes under their control.

The molecular genetic mechanisms underlying the behavioral and morphological traits of the now existing dog breeds may not, however, reflect the genetic nature of the early changes brought about by domestication. The domestic fox is therefore a unique model of the genetic events of early domestication. At the IC&G fur farm, foxes are methodically selected not only for domestication, i.e., elimination of the aggressive genes, but also in the opposite direction—for retention and enhancement of the expression of aggressive behavior. As a result, a population of aggressive foxes has also been set up. This population, together with the domesticated one, serves as valuable resource for genetic studies of early domestication.

It should be noted again that an intriguing consequence of domestication—historical of the dog and experimental of the silver fox—were the newly arisen morphological variations. This is amazing with respect to the traits of the skeletal system that define body size and conformation. We are now raising the important evolutionary question of whether behavioral and morphological traits are integrated at the genomic level, i.e., whether there exist loci which co-regulate morphological-behavioral traits. We have started research in this direction in collaboration with the University of Utah (Prof. Lark) and the Cornell University (Prof. Ackland and Dr. Kukekova). The PCA approach was used for genetic dissection not only of complex behavioral traits as above but also of skeletal traits.^(86,87) This approach revealed sets of correlated parameters (PCs) which are structuring variability. Differences between tame, aggressive, and control populations were expressed in terms of the PCs. The PCs behaved as individual phenotypes in the segregating generations from the Tame Aggressive cross and the subsequent backcrosses, as we reported.^(22,23,86) Thus, these phenotypes were appropriate for molecular genetic analysis. Search for the loci for behavior and morphology, along with their common loci, is of current interest, because co-segregation of some of their PCs suggests that they may share certain loci. Construction of the meiotic map of the genome of the fox was followed by genetic mapping of the loci for the PCs,⁽⁸⁸⁾ which will be used to further study the co-regulation of morphology and behavior.

Conclusions

We proceeded on the assumption that regulatory changes in gene activity may generate the remarkable level of diversity and its similar patterns among domestic animals. These regulatory changes were presumably caused by selection of animals for specific behavior, namely tameability as a

marker of tolerance and successful adaptation to the human social environment. The experimental model of domestication, as a kind of forced evolution, was developed by systematically applying selection for tameability on silver foxes.

The founders of the selected population were chosen from large farm-bred populations. They represented the genetic variants that had reproductive success and showed the weakest wild-type aggressive-avoidance response to humans. Such foxes had undoubtedly been under natural and artificial selection for tameability through all the years of cage breeding. However, this selection did not affect the standard morphological and physiological phenotype of the foxes in all populations we studied. We succeeded in rapidly setting up a unique population of domestic foxes following the effect of rigorous selection pressure for tame behavior. The behavioral patterns of these foxes are dog-like. These foxes also have a range of morphological and physiological traits attributable to domestic animals. The fox domestication experiment demonstrated that this selection affected the genes controlling the neurohormonal status. Some of the genes responsible for the association between tameability and hormonal and transmitter levels might have been brought together and become fixed by 8–10 selected generations. Their fixation might have modified the activity of many other downstream genes, thereby destabilizing and shifting timing of development, and uncovering some of the phenotypically hidden potentialities of the genome. This means that the interactions between genetic variants altered during selection might have produced new patterns of gene expression and new phenotypes.

Different molecular mechanisms are implied in the phenotypic expression of non-expressing genes and their epigenetic modification. They include non-coding sequences and methylation–demethylation of the regulatory regions of genes. Whatever the molecular mechanisms of changes in gene activity under domestication, the results suggest that these changes could be produced by selection for tameability.

In the light of the results of the fox domestication experiment, the similar patterns of behavioral as well as morphological and physiological transformation in foxes, dogs and other domesticates are suggested to be the result of selection for tameability. This selection is thought to be the causative and universal mechanism for evolutionary transformation of domestic animals.

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