Revisiting two hypotheses on the "domestication syndrome" in light of genomic data

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Domesticated mammals of many different species share a set of physical and physiological traits that are not displayed by any of their wild progenitors. This suite of traits, now termed the "domestication syndrome" (DS), has been a puzzle since Charles Darwin discovered it. Two general explanations of its basis have been proposed, which in principle, could also apply to other vertebrates, such as fish and birds, whose domesticated varieties show some of its elements. The two ideas are termed here, respectively, the thyroid hormone hypothesis or the THH, and the neural crest cell hypothesis, the NCCH. The two ideas make distinctly different genetic predictions. Here, the current relevant evidence from genomics is evaluated and it is concluded that the NCCH has more support. Nevertheless, one set of observations, from chickens, suggest a potentially important role of altered thyroid metabolism in domestication. In addition, recent studies indicate the possibility of additional genetic factors in domestication, affecting tameness and sociality, that may go beyond either hypothesis. The tasks that lie ahead to fully ascertain the genetic bases of the "domestication syndrome" and the behaviors that characterize mammalian domestication are discussed briefly.

Key words: animal domestication; "domestication syndrome"; Charles Darwin; comparative genomics; neoteny; neural crest cells; thyroid metabolism.

«Синдром одомашнивания» в свете геномных данных

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Доместицированные млекопитающие разных видов имеют общий набор физических и физиологических признаков, которых не было у их диких предков. Совокупность этих признаков, называемая «синдромом одомашнивания», остается загадкой со времен Чарльза Дарвина, открывшего этот феномен. В настоящее время существуют две общие гипотезы, объясняющие это явление, которые отчасти применимы и к другим позвоночным, например рыбам и птицам. Одну из этих гипотез мы называем гипотезой тиреоидных гормонов (THH), а другую – гипотезой клеток нервного гребня (NCCH). Каждая из гипотез приводит к совершенно разным выводам на уровне генетики. Основываясь на анализе последних данных геномных исследований, имеющих отношение к обсуждаемому вопросу, мы пришли к выводу, что более обоснованной выглядит гипотеза NCCH. Тем не менее ряд наблюдений, сделанных на курах, указывает на потенциально важную роль измененного метаболизма тиреоидных гормонов для процесса одомашнивания. Кроме того, недавние исследования указывают на возможность существования дополнительных факторов одомашнивания, оказывающих влияние на приручаемость и социальность и не учитываемых ни одной из рассматриваемых гипотез. Кратко обсуждаются задачи, направленные на выявление генетических основ «синдрома одомашнивания» и особенностей поведения, специфичных для процесса одомашнивания млекопитающих.

Ключевые слова: одомашнивание животных; «синдром одомашнивания»; Чарльз Дарвин; сравнительная геномика; неотения; клетки нервного гребня; метаболизм тиреоидных гормонов.

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The domestication of animals and, in particular, that of various mammalian species, was crucial for the development of human civilizations (Diamond, 1999; Larson et al., 2014; Francis, 2015). Involving more than 20 mammalian species, and a few bird and fish species, animal domestication commenced in different places on different continents at different times but took place primarily during the past 11–10,000 years, following the rise of agriculture (see Larson et al., 2014, Fig. 1). (The dog is one species, however, whose initial domestication took place considerably earlier, and perhaps twice, independently, probably more than 15,000 years ago (Frantz et al., 2016).)

Given the historical importance of the domestication of mammals, it is of great interest to understand both its historical roots and its biological basis. Although there are many specific questions about the histories of the different domestication events, the places and approximate dates for many species are increasingly well known (Larson et al., 2014; Francis, 2015). The biology underlying domestication, however, presents a major puzzle. Although the domestication of each species must have involved direct or indirect selection for docility (lack of fear) and tameness (ability to be handled by humans), the domesticated breeds of the different mammalian species all share a distinctive suite of physical and physiological traits, not seen in their wild progenitors. The suite of traits is neither universal amongst species nor amongst all breeds of a given species (Sanchez-Villagra et al., 2016). Nevertheless, it is sufficiently generic to be seen as a signature feature of mammalian domestication. The relationship of these traits to the initial selected traits of docility and tameness, however, is not readily apparent. The particular secondary morphological and physiological traits that mark the domesticated state include: floppy ears, smaller jaws, smaller teeth, pigmentation changes in the coats (toward white and brown spots), reductions in adrenocortical hormone titers, increased frequency of estrus cycles, reduction in brain size, and alterations in concentration of several brain neurotransmitters. (For a comprehensive tally of which domestication-specific traits appear in the different mammalian species that have been domesticated, see Figure 1 of Sanchez-Villagra et al., 2016.)

This condition has been dubbed the "domestication syndrome", abbreviated here as the DS. The term itself appears to have been first used in connection with a parallel set of observed commonalities amongst domesticated plants (Hammer, 1984) but was later applied to animals (Larson et al., 2014; Wilkins et al., 2014). (Some authors, however, prefer the term "domesticated phenotype" to avoid the implication of illness associated with the word "syndrome", e. g. (Leach, 2003).) That these traits are a product of domestication itself rather than a condition that developed independently and subsequently in each line is shown by their rapid appearance during the experimental domestication of foxes, rats and mink, which involved selection only for increased docility (Belyaev, 1974; reviewed in Trut, 1999). This shows that the genetic factors underlying tameness are linked in some way with the physical and physiological traits of the DS.

Darwin's discovery of the DS

The search for an explanation of the DS began with the man who discovered the phenomenon (although he did not name it): Charles Darwin. He had been trying to develop a theory of the nature of heredity since at least the early 1850s and in 1868 published his monumental work on heredity, *Variation of Animals and Plants under Domestication* (Darwin, 1868). Darwin was writing decades before there was an experimental science of genetics, or even any kind of theoretical framework for understanding biological inheritance, and he had to rely on the work of animal and plant breeders for the data he collected. In the course of compiling all the information for his monumental work on heredity, he noticed that domesticated breeds, regardless of species, tended to share a common set of visible traits, most of those listed above. (The physiological traits of the DS were discovered much later, however.)

There are two particularly puzzling aspects of the condition. First is the variety of the different traits of the DS, which share little immediate obvious connection with each other. Second is the fact that the initial selection in each instance of domestication was almost certainly for tameness, permitting humans to get close to the animals involved. (This pertains even to the evolution of dogs from wolves, where there may not have been deliberate taming by humans but a self-selection of individual animals who neither attacked nor fled from people around human settlements.) The other traits were apparently dragged along as consequences of the initial selection, through poorly understood connections. This phenomenon, in which selection for one trait brings in train one or more additional, unexpected traits, Darwin termed "unconscious selection" though perhaps "unintended co-selection" might be more apt.

Darwin's own explanation of the phenomenon was neither totally self-consistent nor complete. He wanted to ascribe these changes to the gentler "conditions of living" provided by the anthropogenic environment but he also realized that in many cases, the characteristics were or had become heredity, hence not solely a function of the anthropogenic environment. Furthermore, he could not explain why the particular traits seen, and not others, were the ones that appeared in association with the domesticated state. It is, of course, not puzzling that he himself could not answer the question in the 19th century, even approximately, given the general ignorance of Mendel's work, which would eventually provide the foundations of modern genetics. What is perhaps more surprising in retrospect is that 20th century genetics also failed to solve the problem. Some heroic and important large efforts were made, however, and a significant start was made with the work of the pioneering Soviet geneticist Dmitry Belyaev and his colleagues from the early 1960s onwards (Belyaev, 1974, 1979; reviewed in Trut et al., 2009), but the answer remained stubbornly elusive.

Today, in principle, comparative genomics should be able to provide the solution via comparisons of the genomes of domesticated animals with those of their respective wild progenitors. This work so far, however, has not yet produced a clear answer. There are two difficulties that impede a solution. One is that, often, a reference "wild" progenitor strain is not always known or still extant; there are ways around this, however (as discussed below). A second difficulty is that often a wealth of genetic differences is found in each comparison between domesticated breeds and their putative ancestral stock. The challenge is to isolate those that were crucial to the initial domestication from all those that may have arisen subsequently during domestication. In effect, there are many

"domestication genes", the majority probably being those that accrued well after the first events and amplified by the selective breeding that later ensued. Some, perhaps many, of those genetic changes might have involved genetic drift after various bottle-necks in breeding while numerous others would be consequences of selection for particular breed-specific characters. Still others may reflect the fact that domestication may mobilize transposable elements whose movements create more genetic changes (Glazko et al., 2014).

Altogether, the problem of identifying the genes involved in the initial events of domestication is not so much the equivalent of finding a single needle (a putative causative "domestication gene") in a haystack (the genome) as finding what is probably a set of specific needles (the initially selected domestication genes that trigger the DS) within one relatively small haystack (the total set of "domestication genes") that itself is dispersed throughout a much larger haystack (the genome). A further complication is that, from what is known today, nothing demands that the initiating domestication genes were always the same. In effect, there need not be a universal set of such genes; the genomic findings, reviewed below, confirm this. "Domestication" thus may consist of a set of conditions, or even be a continuum of states, underlain by a variety of different genetic changes (Vigne, 2011; Sanchez-Villagro et al., 2016).

Two hypotheses
To aid the process of identifying the relevant initial domestication genetic changes, however, it would help to have a hypothesis about the DS. In principle, that organizing idea could help focus the search. The hypothesis should be one that links genes and development since all the traits of the DS are initiated by events taking place during embryonic and fetal development. Indeed, two such ideas have been offered. The first focuses on thyroid hormones and the possibility of timing shifts in development (so called "heterochronic" changes) due to altered concentrations of these hormones in early development. Though not named by its proposer, I will call it the "thyroid hormone hypothesis" or THH. The second posits a crucial role of alterations in neural crest cell development in the early embryo in generating the phenotypic changes seen in the DS. It will be labeled here "the neural crest cell hypothesis" or NCCH.

The THH was proposed by Susan Crockford (Crockford, 2002). Its basic premise is that the DS is a reflection of "neoteny", a genetic shift leading to an extended juvenile developmental phase before sexual maturity is achieved. Neotenous features associated with domestication include floppy ears, smaller jaws, and certain behavioral traits signifying prolonged juvenility. Other features, however, such as the pigmentation changes and more frequent estrus cycles do not readily fit this description. Furthermore, it has been questioned how general even behavioral neoteny is in domestication, though it certainly exists in dogs (Price, 1999). If tameness itself, however, is seen as a neotenous trait – and younger animals are often less frightened and more readily handled than adults – then domestication as a whole might be seen as a form of neoteny.

Since thyroid hormones play key roles in regulating the rates of growth and maturation in animals, the hypothesis assigns a major role to the thyroid hormones. In this interpretation, domestication involved selection for genetic changes that regulate thyroid hormone concentrations or sensitivity to those hormones, triiodothyronine (T3) and its precursor tetraiodothyronine (T4). These hormones have long been known to affect postnatal and juvenile development but are now known to be produced during embryonic and fetal development as well. Since in postnatal development, their concentrations tend to be higher in juvenile stages than later, the genetic changes involved in the initial stages of domestication and the development of the DS would presumably have involved, under the assumption of neoteny in domestication, longer-lasting high thyroid hormone levels in post-natal development. There is some support for such correlations: bonobos, *Pan paniscus*, a putatively neotenous and "self-domesticated" species of chimpanzee (Hare et al., 2012) has significantly extended periods of thyroid hormone production compared to the related non-neotenous species, *Pan troglodytes* (Behringer et al., 2013).

Given the central role of altered thyroid metabolism in this hypothesis, the idea predicts that genetic changes in thyroid hormone concentrations, or sensitivities to them, underlie the DS. In principle, single gene changes or a very small number of genetic changes should be capable of producing such. Thus, though this is not stated in Crockford (2002), the genetic prediction of the THH is that *domesticated lines should show one or a small number of changes in genes involved in thyroid hormone metabolism, which are not seen in the presumed progenitor wild strains*. (Conceivably, the mutations could affect the development of the thyroid gland but that seems less likely since such mutations would be more likely to have strongly deleterious effects.) The THH, of course, does not exclude the possibility that domesticated animals will have many other, additional genetic differences from their wild forebears but posits that the number of genes needed to initiate domestication is small, even in the limiting case, single gene mutations.

The other hypothesis, the neural crest cell hypothesis or NCCH takes a different starting point: the fact that most of the affected features in the DS are linked through a shared cell type in their development, namely the neural crest cells of the early embryo. Wilkins et al. (2014) proposed that all the "phenes" (single phenotypic traits) of the DS might be explained by relatively small deficits of neural crest cells in their final locations – relative to the progenitor wild breeds – after these cells have migrated in early embryonic development from the dorsal side of the neural tube. In this view, selection for docility and tameness – presumably the initial step in domestication – entails selection for those properties produced as a consequence of mild neural crest cell deficiencies in development. They further suggested that docility in the early stages of domestication specifically reflected smaller adrenal glands (which derive in part from neural crest cells) producing lower concentrations of adrenocorticotropic hormones, leading to delayed and/or reduced "fight-or-flight" responses (Wilkins et al., 2014). (Domesticated rats and foxes, in fact, have smaller adrenal glands than their wild counterparts and produce lower concentrations of adrenocorticotropic hormones.) This is not the only conceivable pathway toward docility and tameness but it is reasonable and consistent with the evidence.

In contrast to the THH, the NCCH posits genetic complexity, indeed a polygenic basis for the DS. Although many genes are known that affect and are required for neural crest

cell formation, migration or correct cellular differentiation of the cells that form from them, there are no known single-gene mutations in any species in this set of genes that create the DS, although a number of single gene mutations in this group of genes generate some features of the DS, especially pigmentation changes and mild alterations in craniofacial features (Wilkins et al., 2014). While severe loss-of-function mutations in these genes tend to produce lethality or neurocristopathies, mild loss-of-function mutations should, in principle, be viable. In this explanation, the DS is a product of the *additive* (or perhaps synergistic) effects of partial loss-of-function mutations in several, perhaps many, neural crest cell genes in each domesticated line of animals. Hence, the prediction of the NCCH for genomic data is that *the genomes of domesticated animal lines will show a number of variant neural crest cell genes that are not seen in the ancestral or surrogate progenitor wild-strain genomes*.

When alternative hypotheses are proposed, it is often the case that both capture some aspect of reality. In this particular case, it is worth noting that while the study of thyroid hormone effects has been most extensively characterized in fetal development, thyroid hormones are also produced, though at low levels, in embryos and have long been suspected to have developmental effects in that stage. This has recently been confirmed in a study showing that inhibition of thyroid hormone receptor action, either by drug inhibition or knock-down of expression, strongly reduces neural crest cell migration (Bronchain et al., 2016). Thus, it might be possible to link the two hypotheses by postulating that the "domestication syndrome" reflects minor neural crest cell deficits as a consequence of *mild decreases* of thyroid hormone during embryonic development or partial loss-of-function mutations in the receptor(s). This suggestion, however, conflicts with the idea that the neotenous characteristics associated with domestication are most readily explained by *increased* duration and signaling of thyroid hormones (see above). Nevertheless, the connection between thyroid hormones and neural crest cells should be remembered in considering the possible developmental foundations of domestication.

Comparative genomic analysis: evaluating the two hypotheses

In principle, as noted, comparative genomic analysis should be able to test the two ideas since they make such different genetic predictions. To do these comparisons requires an appropriate reference genome, namely that of the putative wild stock from which the domesticate strain had been bred. Nevertheless, even if such an ancestral stock is unknown or presumed extinct, the situation is not hopeless if genomes can be recovered from preserved bones of the presumed ancestral type. Below, some of the more relevant and extensive studies, grouped by species, are reviewed. In the discussion, the term "neural crest genes" will refer to those genes active and required in early neural crest cell development or those activated distinctively in cell lineages derived from neural crest cells. In what follows, many interesting genomic facts will be omitted, to keep the focus on those genetic differences that bear specifically on the two hypotheses.

Horses. The first extensive horse genomics study involved the comparison of genomes from six present-day domesticated breeds with Przewalski's horse (a wild line of horses that is not the precursor of domesticated species but may be closely related to that stock) and those from two horse fossils from the late Pleistocene, approximately 43,000 and 16,000 years before present (BP), dates that well precede horse domestication (estimated at 5,500 years from the oldest agreed fossils of domesticated horses) (Schubert et al., 2014). Using several tests to detect which genes and genomic regions had undergone selection in the domesticated horse genomes, the authors concentrated on 125 target genes detected by these tests, which had already been implicated as contributing to physical or physiological features of domesticated horses. This set of genes largely excludes the key early developmental genes that are the focus of both the THH and the NCCH. Nevertheless, two neural crest cell genes, the *KIT* and *MC1R* genes, known to be expressed in pigment-generating tissues derived from neural crest cell genes, were found to show evidence of having undergone selection. Such selection, however, could well reflect later events in domestication, not those involved in generating the DS.

More informative comparisons, with respect to early events in horse domestication, were presented in a recent paper by Librado et al. (2017). They sequenced and analyzed the genomes of 14 horse skeletons from three locations in northern Asia, dating to between 4.1 and 2.3 thousand years ago. Using a new analytical method, LSD (Levels of exclusively Shared Differences), they detected evidence of selection for a variety of genes early in horse domestication. In particular, the analysis picked out a number of genes involved in neural system development, probably associated with cognitive and behavioral differences in domestication. Of special interest here, however, they also detected three neural crest cell genes, *TCOF1*, *KITL*, and *FGFR1*. These genes play roles in such properties as neural crest cell morphology, ear shape, cranial mesenchyme, and development of the mid-brain nucleus, the substantia nigra (the latter containing neural crest cell-derived dopaminergic neurons). The authors state, "Our findings thus support the neural crest hypothesis of animal domestication". No genes involved in thyroid metabolism were indicated as having been detected.

Cats. The principal cat genomic analysis to date used two living wild-cat species (one from Europe, one from the Middle East) as reference wild species against six domestic breeds (from different lineages and regions) and screened all protein-coding genes for signs of selection (specifically, a higher dn/ds ratio in the codons of genomes of the domesticated ones) after identifying regions of the genome in the domesticated species that looked genetically differentiated from those in the wild species. The authors found 13 genes that appear to be strong candidates for domestication genes by genetic criteria used to detect selection (Montague et al., 2014). None of these genes apparently has any known role in thyroid hormone metabolism. On the other hand, one genomic region that had high Fst, when pooled domesticated cat genomes were compared with those of wild cats, included the *TSHR* gene, the gene encoding the thyroid stimulating hormone receptor. This finding does not prove that this gene itself was selected, nor, if it was, that it was selected at an early stage in domestication but this observation is consistent with the predictions of the THH.

More strikingly, however, five of the 13 strong candidate genes can be considered neural crest cell genes: they are expressed in neural crest cells and are necessary for full neural crest cell function, almost certainly in cell migration. The first two are protocadherin genes, *PCDHA1* and *PCDHB4*, implicated in both neural crest cell migration and several brain functions involving synapse formation. Three others are *ARID3B*, *DCC*, and *PLEKHH1*, which are also required for neural crest cell migration. The last, *PLEKHH1*, also interacts with *MYC*, a transcriptional regulator within neural crest cells. In addition, this study also identified *KIT* as a domestication gene, specifically as the gene responsible for the "gloving" phenotype (white paws) in the Birman breed of domestic cats. As mentioned above, *KIT* has long been known as a neural crest cell gene involved with melanocyte pigmentation and has also been implicated as a domestication gene in both horses (as noted above) and pigs (Rubin et al., 2012). Altogether, these findings support the NCCH. As the authors say, "The genetic signals from this analysis fall in line with the predictions of the domestication syndrome hypothesis (50), which posits that the morphological and physiological traits modified by mammalian domestication are explained by direct and indirect consequences of mild neural crest cell deficits during embryonic development" (Montague et al., 2014).

Rabbits. An extensive comparative rabbit genome analysis compared the genomes of wild rabbits from 14 different locations in France and on the Iberian peninsula with six different domestic species (Carneiro et al., 2014). The analysis identified SNPs in all parts of the genome – not just coding regions – that were enriched in the domesticated species. This approach allows identification of putative regulatory control regions in addition to any changes that might be found in coding regions. Rabbit domestication has a much shorter history relative to cat and horse domestication, perhaps only 1,400 years, hence one might expect a comparatively weak selection signal. Despite that, a large number of differences between the wild and domesticated animals were found. Intriguingly, there was an approximately 30-fold greater number of SNPs associated with conserved non-coding elements (CNEs), these being putative regulatory regions, than with coding regions. Amongst the several protein-coding gene SNPs identified as associated with domestication, however, were found two well-characterized neural crest cell genes, *SOX2* and *PAX2*. No SNPs in genes involved in thyroid metabolism were reported.

Significantly, no sites were found to have gone to fixation in any of the domesticated breeds, either in CNEs or in exonic coding regions. The implication is that domestication in the rabbit has involved different combinations of genes operating in a quasi-additive polygenic fashion. This, indeed, is the authors' principal conclusion from their work (Carneiro et al., 2014) and is in line with perceptions from earlier work on domestication in both plants and animals that suggests that there are no single gene mutations that create the domesticated state (Larson et al., 2014). The further implication is that there can be multiple genetic routes toward domestication, even within the same species. This is also a feature of the NCCH though that hypothesis stresses specifically the number and variety of different neural crest cell genes that might be involved, with different sets possibly involved in different domesticated

mammalian lines. Not least, the fact that Belyaev's experimental domestication of silver foxes, rats and mink, required multiple successive generations, with selection for ever-tamer animals and the gradual onset of phenes of the DS is a strong argument for the polygenic basis of domestication.

Dogs. Two extensive comparative dog genomic studies, looking for genetic signs associated with domestication are of note. The first, by Axelsson et al. (2013), searched the dog genome for "candidate domestication regions", CDRs, those genomic segments likely to have been targets for selection. The comparisons were of genomes derived from 12 wolves from diverse geographical regions, and 60 dogs from 14 modern breeds. Altogether, the authors found 36 CDRs. The emphasis in analyzing these CDRs was on searching for genes known to be involved in features modified in domestication, hence neural system development and aspects of metabolism. Nineteen CDRs were found to be enriched in genes involved in neural development. A further 11 genes were identified as important in this respect from a search of the literature. One of these was CRYM, a T3-binding protein important in brain function (see their Table S9), a finding consistent with the THH. No neural crest genes were specifically identified as such in this study but the candidate gene approach, focusing on genes known to be involved in neural development and metabolism, would have militated against finding affected genes that are primarily involved in early development. By using modern dog breeds, many of the identified differences probably reflect genetic changes in the past 200–300 years, the period in which these breeds were created, not the earliest stages of dog domestication.

A more recent investigation, however, has focused on trying to identify genes involved in those early stages of dog domestication (Pendleton et al., 2017). It involved genomic comparisons between wolves and village dogs found from around the world. Village dogs, unlike modern dog breeds, are more likely to be closer genetically to the earliest stages of dog domestication than modern breeds are. Any significant differences with respect to wolf genomes would be candidates for domestication-related changes. This study, based on the genomes of 10 wolves and 43 village dogs, identified 37 CDRs, containing 172 genes in total. As with the findings of Axelsson et al. (2013), these included various genes affecting metabolism but also genes that had not been picked up in that earlier study. Amongst the latter were many affecting various developmental aspects, such as bone development, and within the set of developmental genes were a large number of neural crest cell genes. (For the full breakdown according to functional category of the genes associated with the CDRs, see their Table 2.) The neural crest-cell related genes included a number involved specifically in Wnt-, BMP- and FGF-signalling pathways. All were found in regions associated with selective sweeps and the inference is that many were themselves selected as part of the domestication process. (The list is given in Table, here.) The paucity of coding changes found in this study in the entire set of 172 genes in the CDRs suggests further that the changes selected were regulatory, not changes in coding sequences. Though not providing definitive proof, the results supply strong confirmation of the NCCH. In contrast, no genetic differences involving genes of thyroid metabolism were found in this study.

Genomic signatures of domestication relevant to the THH and NCCH

Animal type	Study	Genes identified supporting	
		THH	NCCH
Horses	Schubert et al., 2014	–	KIT, MC1R
	Librado et al., 2017	–	TCOF1, KITL, FGFR1
Cats	Montague et al., 2014	TSHR	PCDHA1, PCDHD4, ARID3B, DCC, PLEKHH1, KIT
Rabbits	Carneiro et al., 2014	–	SOX2, PAX2
Dogs	Axelsson et al., 2013	CRYM	–
	Pendleton et al., 2017		FGF13, WNT9b, WNT3, ZIC3, AXIN2, AXIN11, SMO, NOL11, SNX19, PRKCA, WF1KKN1

Current status of the two hypotheses, in light of the genomics data

The collective results of these genomic studies do not solve the genetic basis of the "domestication syndrome" but they are informative. The genetic differences showing signatures of domestication with respect to the THH and the NCCH are summarized in the Table. Two genetic differences in the combined data set are consistent with the THH but if the THH was truly the general explanation of domestication, one would predict a much stronger signal of thyroid hormone involvement to have been detected. In contrast, the genomic work provides much supports for the NCCH, as can be seen even with a glance at the Table.

Can one therefore, at this point, regard the THH as having effectively been ruled out? The answer is "no". After all, the majority of studies would probably have missed single nucleotide changes (SNPs) that might have affected regulatory changes in thyroid metabolism in domesticates. Indeed, the only analysis so far that has systematically looked for SNPs throughout the genome associated with domestication, that of Carneiro et al. (2014) on domesticated rabbits, found that such regions had been far more frequently selected in the course of domestication signatures than mutations in coding sequences. This is also an inference from the Pendleton et al. (2017) analysis of dog genomes, with its findings of multiple CDRs in the dog genome but few protein-coding changes. The importance of identifying regulatory genetic differences applies of course to the neural crest cell genes as well as those of thyroid metabolism. Because systematically identifying which genes are transcriptionally controlled by distant conserved non-coding elements, CNEs, is difficult, the road ahead may be a long one. In effect, the comparative genomic investigation of domestication and of the DS may still be at a relatively early stage.

Apart from taking into account possible oversights in the analyses, however, there is a finding that supports the idea of altered thyroid hormone metabolism in domestication. It has been shown that a mutation in the TSHR genes in chickens can alter photoperiod response, with reduced seasonality of reproduction, and more frequent egg production (Karlsson et al., 2015, 2016). The mutation is fixed in the domestic breed, the White Leghorn chicken, and not found (at least in high frequency) in the ancestral strain, Red Jungle Fowl. In effect, this mutation establishes one important feature of the domesticated state, increased reproduction not tied to seasons. Intriguingly, it is also tightly associated with lower aggressiveness, hence greater tameness, the defining feature of the domesticated state.

These findings, though involving just one gene in one bird species, may, of course, have relevance to the larger phenomenon of animal domestication. It will be fascinating to see if similar but engineered mutations in the TSHR have similar effects in mammals. One analysis on the genetic basis of altered seasonality in rabbits picked out a number of candidate genes but TSHR was not one of them (Carneiro et al., 2015). Nevertheless, if subsequent tests in mammals should indicate a link between TSHR and domestication, then it would be of interest to see how this, in turn, might connect with the growing body of evidence that supports the NCCH.

Taking stock and looking ahead

This is a good time to take stock of our understanding of animal domestication, in general, and the DS, in particular, and then to assess what now needs to be done. In thinking about the domestication of animals, it would be hard to overestimate the importance of the work of Dmitry Belyaev and his colleagues. That work has been the crucial foundation of modern thinking about animal domestication and much of the subsequent research on it. One can identify several key elements that have made it so. First, they established that it is possible to experimentally recapitulate the process of domestication and to do so in a relatively short number of generations. Second, they showed that simply selecting for tameness brings in train other well-known features of domestication, in effect that the DS is an intrinsic feature of the process, not an accidental by-product or later-developing concomitant. Third, Belyaev and company provided the first strong evidence that many genes must be involved, thus that the domesticated state truly has a polygenic basis. Fourth – although this aspect has not been explored in this article – they presented some good arguments and some initial evidence (in particular that concerning the Star mutation in foxes) that epigenetic changes may provide the initial steps in changes associated with domestication (reviewed in Trut et al., 2009). (This idea can be related, in turn, to Darwin's emphasis on the importance of the "conditions of living" in generating the domesticated state.) To the extent that epigenetic changes is part of the process, then something like the "Baldwin effect" or Waddington's "genetic assimilation" must also have subsequently kicked in, replacing epimutations with true mutations, since so much of the domestication genotype is based on hereditable mutations (Wilkins, 2011).

Settling the role of epimutations in domestication is surely one of the main challenges ahead.

This article has focused on the genetic sources of the DS itself and has examined recent genomic evidence bearing on two principal hypotheses, the THH and the NCCH. The tentative conclusion is that the NCCH has stronger support. Nevertheless, the recent work on the *TSHR* mutation in domesticated chickens, which affects both reproductive cycles and behavior, keeps alive the possibility that changes in thyroid metabolism have played an important part in the domestication of animals. It will be important to see if similar observations can be recapitulated experimentally in mammals. Furthermore, and most generally, it must be remembered that the comparative genomics work, despite its breadth and depth, is still at an early stage. The roles of *cis*-regulatory mutations, particularly, in CNE sequences has only begun to be examined; most of the comparative genomics work so far has concentrated on coding sequences, while both hypotheses about the DS implicitly place a strong emphasis on regulatory mutations. Hence, much yet remains to be learned about the genetics of domestication. It is to be hoped that definitive analysis of the genomes of domesticated foxes vs those of their farm-bred non-domesticated cousins will help illuminate the matter, since fox domestication was achieved rapidly and without deliberate selection for anything but tameness; this should reduce the number of potentially confounding genetic changes that have occurred in the long history of well-established domesticates. In addition, now that many neural crest cell genes have been provisionally connected to domestication, it will be of interest to see which of these genes is associated with particular phenes of the DS.

Apart from the desirability of new findings, there is also need for integration of a growing body of observations on the genetics of behavior, particularly of tameness and social interactions, with the existing ideas and findings about the DS. A crucial beginning in the genetic analysis of tameness was made in a large study carried out by Albert et al. (2009). They crossed the tame and aggressive lines of rats that had been selected and developed by Belyaev and his colleagues and did a QTL analysis to determine loci affecting tameness. They found two QTLs that significantly and directly affected tameness which were part of an epistatic network of five loci. One of the QTLs affected adrenal weight and would be a good candidate for containing a neural crest cell gene. More broadly, the work confirms the polygenic basis of tameness itself.

Whatever the neural crest cell gene inputs to the foundations of tameness, one would like to know the specific features of the CNS, and their immediate genetic sources, that convey tameness and more broadly sociality. For example, a recently published study reports that in selection over five generations for lack of fear in Red Jungle Fowl, the ancestor of the domestic chicken, there was concomitant selection for altered growth and reproductive properties (Belteky et al., 2016). Whether these connections reflect linkage of other genes to those directly selected or to pleiotropic effects is not yet clear.

Beyond tameness, there may be connections between genes involved in domestication and other aspects of sociality. A high proportion of genes that have been identified as risk factors in schizophrenia are associated with neural crest genes and the domestication syndrome (Benitez-Burraco et al., 2017).

A number of these genes are involved in language-use specifically, an aspect of sociality that is uniquely human. The possibility that these specific traits are connected to deeper roots in animal sociality certainly merits further investigation. In addition, several genes in dogs that are associated specifically with dog-human social interactions have been implicated in humans as risk factors in neurological diseases affecting social interactions (autism and schizophrenia) (Persson et al., 2016). Ultimately, these sorts of investigations may lead to new insights on a matter that is receiving more and more attention, the question of whether humans can be regarded as a "self-domesticated" species (Brüne, 2007; Francis, 2015; Theofanopolou et al., 2017).

Such matters, of course, go well beyond the phenomenon of the "domestication syndrome" as it has been defined but it is an intriguing thought that probing the genetic roots of animal domestication may help illuminate the underlying biology of features of human existence that we have long thought unique to our species.

Acknowledgments

Conflict of interest

The author declares no conflict of interest.

References

Albert F., Carlborg O., Plyushina I., Besnier F., Hedwig D., Lautenschläger S., Lorenz D., McIntosh J., Neumann C., Richter H., Zeising C., Kozhemyakina R., Shchepina O., Kratzsch J., Trut L., Teupser D., Thiery J., Schöneberg T., Andersson L., Pääbo S. Genetic architecture of tameness in a rat model of animal domestication. Genetics. 2009;182(2):541-554.

Axelsson E., Ratnakumar A., Arendt M.L., Maqbool K., Webster M.T., Perloski M., Liberg O., Arnemo J.M., Hedhammar A., Lindblad-Toh K. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature. 2013;495:360-364.

Behringer C., Deschner T., Murtagh R., Stevens J.M.G., Hohmann G. Age-related changes in thyroid hormone levels of bonobos and chimpanzees indicated heterochrony in development. J. Hum. Evol. 2013;66:83-88.

Belteky J., Agnavall B., Johnsson M., Wright D., Jensen P. Domestication and tameness: brain gene expression in red junglefowl selected for less fear of humans suggests effects on reproduction and immunology. R. Soc. Open Sci. 2016;3:160033.

Belyaev D. Domestication, plant and animal. Encyclopaedia Brittanica, Edition 15. Ed. H.H. Benton. Chicago: Encylopaedia Brittanica-Helen Hemingway Benton Publ., 1974.

Belyaev D. Destabilizing selection as a factor in domestication. J. Heredity. 1979;70:301-308.

Benitez-Burraco A., Di Pietro L., Barba M., Lattanzai W. Schizophrenia and human self-domestication: an evolutionary linguistics approach. Brain Behav. Evol. 2017. https://doi.org/10.1159/000468506.

Bronchain O.J., Chesneau A., Monsoro-Burq A.-H., Jolivet P., Pailland E., Scanlan T.S., Demeneix B.A., Sachs L.M., Pollet N. Implication of thyroid hormone signaling in neural crest cells migration: Evidence from thyroid hormone receptor beta knockdown and NH3 antagonist studies. Mol. Cell. Endocrinol. 2017;439:233-246. DOI 10.1016/j.mce.2016.09.007.

Brüne M. On human self-domestication, psychiatry and genetics. Philosophy, Ethics, and Humanities in Medicine. 2007;2:21-29.

Carneiro M., Rubin C.-J., di Palma F., Albert F.W., Alifoldi J. Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. Science. 2014;345:1074-1079.

Carneiro M., Piorno V., Rubin C.-J., Alves J.M., Ferrand N., Alves P.C., Andersson L. Candidate genes underlying heritable differences in reproductive seasonality between wild and domestic rabbits. Anim. Genetics. 2015;46:418-425.

Crockford S. Animal domestication and heterochronic speciation. Human Evolution Through Developmental Change. Eds. N. Minugh-Purvis, K.J. McNamara. Baltimore: Johns Hopkins Univ. Press, 2002;122-153.

Darwin C. The Variation of Animals and Plants under Domestication. London: John Murray, 1868.

Diamond J. Guns, Germs and Steel. London: Vintage Books, 1999.

Francis R.C. Domesticated: Evolution in a Man-Made World. W.W. Norton, New York, 2015.

Frantz L.A.F., Mullin V.E., Pionnier-Capitan M., Lebrasseur O., Ollivier M. Genomic and archaeological evidence suggests a dual origin of domestic dogs. Science. 2016;352:1228-1231.

Glazko V., Zybaylov B., Glazko T. Domestication and genome evolution. Int. J. Genet. Genom. 2014;2:47-56.

Hammer K. Das domestiationsyndrom. Kulturpflanz. 1984;32:11-34.

Hare B., Wobber V., Wrangham R. The self-domestication hypothesis: bonobo psychology evolved due to selection against aggression. Anim. Behav. 2012;83:573-585.

Karlsson A.-C., Svemer F., Eriksson J., Darras V.M., Andersson L., Jensen P. The effect of a mutation in the thyroid stimulating hormone receptor (TSHR) on development, behavior and TH levels in domesticated chickens. PLoS ONE. 2015. DOI 101371/journal.pone.0129040.

Karlsson A.-C., Fallahshahroudi A., Johnsen H., Hagenblad J., Wright D., Andersson L., Jensen P. A domestication-related mutation in the thyroid stimulating hormone receptor gene (*TSHR*) modulates photoperiodic response and reproduction in chickens. Gen. Comp. Endocrinol. 2016;228:69-78.

Larson G., Piperno D.R., Allaby R.G., Purugganan M.D., Andersson L., Arroyo-Kalin M., Barton L., Climer Vigueira C., Denham T., Dobney K., Doust A.N., Gepts P., Gilbert M.T.P., Gremillion K.J., Lucas L., Lukens L., Marshall F.B., Olsen K.M., Pires J.C., Richerson P.J., Rubio de Casas R., Sanjur O.I., Thomas M.G., Fuller D.Q. Current perspectives and the future of domestication studies. Proc. Natl. Acad. Sci. USA. 2014;111(17):6139-6146.

Leach H.M. Human domestication reconsidered. Curr. Anthrop. 2003;43:349-368.

Librado P., Gamba C., Gaunitz C., Der Sarkissian C., Pruvost M., Albrechtsen A., Fages A., Khan N., Schubert M., Jagannathan V., Serres A., Kuderna L.F.K., Povolotskaya I., Seguin-Orlando S., Lepetz S., Neuditschko M., Theves C., Alquaraishi S., Alfarhan A.H., Al-Rasheid K., Rieder S., Samashev Z., Francfort H.P., Beneccke N., Hofreiter M., Ludwig A., Keyser C., Marques-Bonet T., Ludes B., Crubezy E., Leeb T., Willerslev E., Orlando L. Ancient genomic changes associated with domestication of the horse. Science. 2017;356:442-445.

Montague M., Li G., Gandolfi B., Khan R., Aken B., Searle S.M.J., Minx P., Hillier L., Kolboldt D.C., Davis B.W., Driscoll C.A., Barr C., Blackistone K., Quilez J., Lorente-Galdos B., Marques-Bonet T., Alkan C., Thomas G.W.C., Hahn M.W., Menotti-Raymond M., O'Brien S.J., Wilson R.K., Lyons L.A., Murphy W.J., Warren W.C. Comparative analysis of the domestic cat genome reveals genetic signatures underlying feline biology and domestication. Proc. Natl. Acad. Sci. USA. 2014;111:17230-17236.

Pendleton A.L., Shen F., Taravella A.M., Emery S., Veeramah K.R., Boyko A.R., Kidd J.M. Selective sweep analysis using village dogs highlights the pivotal role of the neural crest in dog domestication. BioRxiv. 2017. dx.doi.org/10.1101/118794.

Persson M.E., Wright D., Roth L.S.V., Batakis P., Jensen P. Genomic regions associated with interspecific communication in dogs contain genes related to human social disorders. Sci. Rep. 2016;6:33439. DOI 10.1038/srep 33439.

Price E.O. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 1999;65:245-271.

Rubin C.-J., Megens H.-J., Martinez Barrio A., Maqbool K., Sayyab S., Schwochow D., Wang C., Carlborg Ö., Jern P., Jørgensen C.B., Archibald A.L., Fredholm M., Groenen M.A.M., Andersson L. Strong signatures of selection in the domestic pig genome. Proc. Natl. Acad. Sci. USA. 2012;48:19529-19536.

Sanchez-Villagra M.R., Geiger M., Schneider R.A. The taming of the neural crest: a developmental perspective on the origins of morphological co-variation in domesticated mammals. R. Soc. Open Sci. 2016;3:160107.

Schubert M., Jonsson H., Chang D., Sarkissian C.D., Ermini L., Ginolhac A., Albrechtsen A. Prehistoric genomes reveal the genetic foundation and cost of horse domestication. Proc. Natl. Acad. Sci. USA. 2014;111(52):e5661-e5669. DOI 10.1073/pnas.1416991111.

Theofanopolou C., Gastaldon S., O'Rourke R., Samuels B.D., Messner A., Martins P.T., Delogu F., Alamri S., Boeckx C. Comparative genomic evidence for self-domestication in *Homo sapiens*. BioRxiv. 2017. doi.org/10.1101/125799.

Trut L. Early canid domestication: the farm-fox experiment. Am. Scientist. 1999;87:160-168.

Trut L., Oskina I., Kharlamova A. Animal evolution during domestication: the domesticated fox as a model. BioEssays. 2009;31:349-360.

Vigne J.D. The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. Comptes Rendues Biologies. 2011;334(3):171-181.

Wilkins A.S. Epigenetic inheritance: Where does the field stand today? What do we still need to know? Transformations of Lamarckism: From Subtle Fluids to Molecular Biology. Eds. S.B. Gissis, E. Jablonka. Cambridge: MIT Press, 2011;389-393.

Wilkins A.S., Wrangham R., Fitch T. The "domestication syndrome" in mammals: a unified explanation based on neural crest cell behavior and genetics. Genetics. 2014;197:795-808.