From wolf to dog: Behavioural evolution during domestication

Christina Hansen Wheat

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Abstract

Biologists since Darwin have recognized that domestication, where species are selected to live in human-controlled environments, exerts strong selection on organisms and dramatically impacts their evolutionary trajectories. Across domesticated mammal species, characteristic morphological, physiological and behavioural changes occur simultaneously, as correlated traits, a phenomenon known as the *domestication syndrome*. Key behavioural alterations are connected with the domestication syndrome, in which domesticated animals express decreased aggression and fearfulness alongside increased sociability and playfulness compared to their wild counterparts. To investigate various aspects of the behavioural implications of domestication, we used the dog (*Canis familiaris*) and its extant ancestor, the grey wolf (*Canis lupus*), as our study species. Since we currently lack quantitative confirmation that correlated changes in behaviours follow domestication, we evaluated correlations among sociability, aggression, fearfulness and playfulness in more than 90,000 dogs in ***Paper I***. Contrary to expectations, we found weak support for behavioural correlations in modern dog breeds, but observed exaggerated effect sizes of correlations in ancient breeds. We suggest that while selection on suites of behaviour have been relevant during early dog domestication, a recent shift in selection pressures in modern dog breeds affects the expression of domestication-related behaviours independently. In ***Paper II*** we therefore contrasted the expression of sociability, aggression, fearfulness and playfulness during domestication in wolf hybrids and dogs, and found that while wolf hybrids were less playful and overall more fearful than dogs, they were not less social or more aggressive than dogs. Our results suggest that behavioral alterations during domestication do not necessarily occur in concert as predicted by the domestication syndrome and point to an important, but previously overlooked, role of selection on playfulness during the domestication of dogs. Finally, while it has been established that behavioural responses in adult domesticated animals are altered compared to ancestral species, we know little about when such species differences occur. We therefore conducted two studies addressing the effects of domestication on behavioural ontogeny. First, we examined the ontogeny of sociability, playfulness, aggression and fearfulness in wolves and dogs in ***Paper III*** and found that while wolves became less social and less playful than dogs at 12 and 16 weeks of age, we found no species differences in the development of fear. Our results suggest that the alteration of behaviours in the domestication syndrome do not develop simultaneously, and that species differences in fear might not occur until later in ontogeny. Then, in ***Paper IV*** we present the first extended examination of the development of fear behaviour in wolves and dogs throughout their first 26 weeks of life. We found that while dogs, but not wolves, expressed decreased fear towards novelty with age, this did not result in a species difference in fear response until 26 weeks of age. Our results suggest that differences in fear expression between wolves and dogs occur late during juvenile development and are caused by a loss of sensitivity towards novelty with age in dogs. Together, the four papers in this thesis highlight the need for a re-evaluation of the behaviours hypothesized to be shaped by domestication.

Keywords: *Domestication syndrome, behavioural evolution, behaviorual ontogeny, correlated traits, sociability, playfulness, aggression, fear, Canis familiaris, Canis lupus, artificial selection.*

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Department of Zoology

Stockholm University, 106 91 Stockholm

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Christina Hansen Wheat

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“All his life he tried to be a good person. Many times, however, he failed. For after all, he was only human. He wasn’t a dog.”

– Charles M. Schulz

- LIST OF PAPERS -

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I **Wheat C. Hansen**, Fitzpatrick J. & Temrin H. Behaviours of the domestication syndrome are decoupled in modern dog breeds. *Submitted manuscript, reviewed and in revision for Nature Communications*

II **Wheat C. Hansen**, Fitzpatrick J., Tapper I. & Temrin H. (2018). Wolf (*Canis lupus*) hybrids highlight the importance of human-directed play behaviour during domestication of dogs (*Canis familiaris*). *Journal of Comparative Psychology, in press*

III **Wheat C. Hansen**, Berner P., Larsson I., Tapper I. & Temrin H. Key behaviours in the domestication syndrome do not develop simultaneously in wolves and dogs. *Manuscript*

IV **Wheat C. Hansen**, Van der Bijl W. & Temrin H. Dogs, but not wolves, lose their sensitivity towards novelty with age. *Manuscript*.

Candidate contributions to thesis articles*

	I	II	III	IV
Conceived the study	Significant	Significant	Substantial	Substantial
Designed the study	Substantial	Substantial	Substantial	Substantial
Collected the data	NA	NA	Significant	Substantial
Analysed the data	Substantial	Substantial	Significant	Significant
Manuscript preparation	Substantial	Substantial	Substantial	Substantial

* ***Contribution Explanation.*** *Minor: contributed in some way, but contribution was limited. Significant: provided a significant contribution to the work. Substantial: took the lead role and performed the majority of the work.*

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INTRODUCTION

Domestication is a process in which species are selected to live in human-controlled environments (Price 2002). The evolutionary trajectories of numerous animal and plant species has dramatically impacted by domestication, and the process thus provides an ideal framework for studying evolutionary responses to selection (Driscoll et al. 2009). In some of his most influential work, Darwin (1859; 1868) used domestication as a powerful exemplification of how various traits can be modified by selection. There are numerous examples of how artificial human-induced selection pressures exerted during domestication affects the same traits across a wide range of species (Darwin 1868; Brown et al. 2009; Trut et al. 2009; Wilkins et al. 2014). Specifically, compared to their wild counterparts domesticated animals typically express repeated patterns of altered physiology, morphology and behaviour, a phenomenon known as the "domestication syndrome" (Darwin 1868; Hammer 1984). For example, domesticated mammals commonly express increased tameness, reduced brain size, depigmentation, floppy ears, curly tails and changes in hormonal profiles (Driscoll et al. 2009; Sánchez-Villagra et al. 2016, Figure 1). The repeated occurrence of suites of traits across a wide range of domesticated species seems unlikely to be caused by unique mutations, but rather to be a result of correlated traits driven by altered selection pressures during domestication (Trut 1999; Trut et al. 2009).

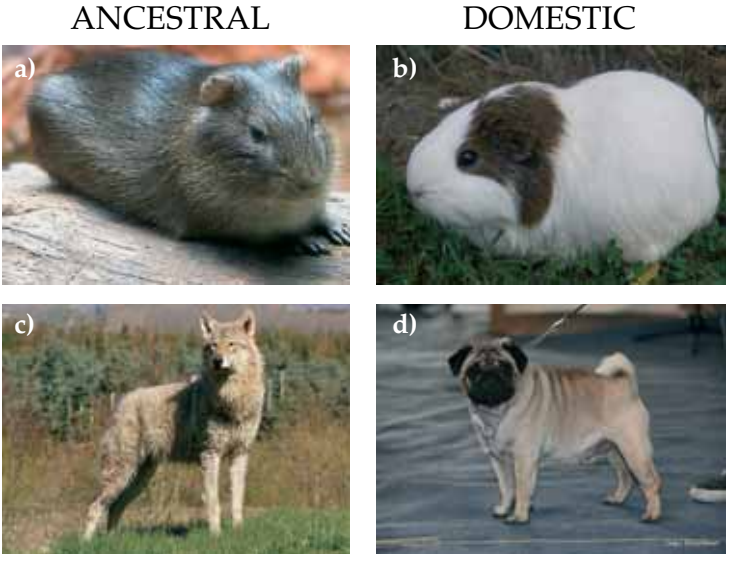

Figure 1. Phenotypic alterations following domestication. *White pigmentation in b) the domesticated guinea pig (Cavia porcellus), a trait not seen in the ancestral species a) the wild guinea pig (Cavia aperea). Curly tail and floppy ears in d) the domestic dog (Canis familiaris, Pug) compared to the ancestral species c) the grey wolf (Canis lupus).*

Perhaps the most well-known demonstration of the domestication syndrome is the long-term selection experiments on silver foxes (*Vulpes vulpes*), initiated in 1959 by the Russian geneticist Dimitri K. Belyaev (*1917-1985*, (Belyaev et al. 1985; Trut 1999; Spady and Ostrander 2007)). Belyaev wanted to investigate the genetics behind the evolutionary process of domestication, and believed that the striking morphological similarities seen across a wide range of domesticated animal species are the result of correlated traits. Specifically, he hypothesized that these correlations between traits appear as a by-product from selection for specific behavioural traits (Trut et al. 2004; Spady and Ostrander 2007). This main hypothesis was based on Belyaev's observation that while silver foxes held in small cages at fur farms generally expressed strong aggressive and fearful behaviour towards humans, a small proportion of the foxes expressed lowered levels of fear and aggression (Morey 2010). This led Belyaev to hypothesize that variation in the behavioural trait tameness, i.e. the social adaptation or sociability towards humans, was the main precursor initiating animal domestication (Trut et al. 2009).

Belyaev successfully demonstrated that by subjecting silver foxes to strict selection pressures on tameness alone, morphological changes, such as white pigmentation and floppy ears, appeared after only eight to ten generations (Trut 1998; 1999; Gulevich et al. 2004). The selection on tameness also had a strong behavioural impact and resulted in foxes expressing decreased fear and aggression alongside increased sociability and playfulness after only a few generations (Trut et al. 2004; Kukekova et al. 2006). These behavioural alterations were subsequently suggested to have a shared physiological causation, in which alteration of the hypothalamic-pituitary-adrenal axis results in adrenal hypofunction and reduced secretion of stress hormones (Trut et al. 2004; 2009). The studies on silver foxes thereby strongly suggest that the domestication syndrome is a product of correlated traits. Later studies have supported that morphological features of the domestication syndrome follows selection on tameness, for instance in rats (*Rattus norvegicus*, (Albert et al. 2008)) wild house mice (*Mus musculus domesticus*, (Geiger et al. 2018)).

The work on silver foxes illustrates a central assumption of the domestication syndrome hypothesis, namely that selection for increased tameness was characterized primarily by reduced fearfulness and aggression (Belyaev et al. 1985; Popova et al. 1991; Trut et al. 2006; Hare et al. 2012; Wilkins et al. 2014). Moreover, domesticated animals typically express increased sociability compared to wild species (guinea pigs, Künzl and Sachser 1999; Trut et al. 2006; 2009). The simultaneous alterations of aggression, fearfulness and sociability, generally emerge from the literature as the most prominent behavioural changes during domestication. Additionally, a fourth behavioral trait, playfulness, is often connected to the

domestication syndrome (Burghardt 1984; 2005; canids, Kukekova et al. 2006; rats, Himmler et al. 2013). The increased expression of playfulness seen in adult domesticated animals is hypothesized to be an indirect result of paedomorphism, the retention of juvenile traits into adulthood, which represents another typical sign of domestication (Goodwin et al. 1997; Kukekova et al. 2006; Dobney and Larson 2006; Himmler et al. 2013). Playfulness is therefore thought to be a by-product of domestication, and its role in the domestication process itself remains unclear.

Despite the recognized role of behavioral alterations during the initial stages of animal domestication, the lack of archaeological records complicates our understanding of how domestication has affected behaviour on a temporal scale. Thus, we know little about the gradual, and assumed simultaneous, change in the expression of the behavioral traits in the domestication syndrome. Though it is currently unclear which role pleiotropic effects (Price and Langen 1992) and/or a shared underlying physiological mechanisms have in simultaneous expression of various traits in domesticated animals
(Crockford 2002; Gulevich et al. 2004; Trut et al. 2004; 2009; Wilkins et al. 2014), the concept of correlated changes is now commonly used as a standard paradigm for studying domestication (Trut 1998; Trut et al. 2009). However, while the correlation of behaviours in the domestication syndrome are analogous to more commonly studied behavioural syndromes (Sih et al. 2004a), the alteration of sociability, playfulness, aggression and fearfulness have traditionally been studied separately or pairwise (Feddersen-Petersen 1991; Blanchard et al. 1994; Künzl and Sachser 1999; Plyusnina et al. 2011; Himmler et al. 2013), and not in concert as a syndrome (Sih et al. 2004a,b). Consequently, whether the simultaneous alterations of these key behavioural traits are consistently expressed throughout the course of domestication remains unclear.

Abundant evidence suggests that several ontogenetic processes have been modified during domestication. Altered developmental rates, a phenomenon known as heterochrony (Goodwin et al. 1997; Price 1999; Dobney and Larson 2006), are commonly observed in domesticated animals and is expressed as accelerated and/or delayed onsets of various ontogenetic stages. For instance, compared to ancestral species, domesticated animals express earlier sexual maturation and the retention of juvenile traits into adulthood (Coppinger et al. 1987; Morey 1994; Price 1999; Crockford 2002). Specifically for behavioural ontogeny, it has been demonstrated that juvenile foxes from domesticated strains develop fearful behaviour three weeks later, at 60-65 days of age, compared to foxes from non-domesticated strains, at 40-45 days of age (Belyaev et al. 1985; Plyusnina et al. 1991; Trut et al. 2004). Thus, these findings indicate that the observed behavioural differences between ancestral and domesticated

animals, e.g. the reduced fear towards novelty seen in domesticated animals (Zeder 2015), arise already during early stages of development.

However, while abundant evidence suggests that quantifiable differences in behavioural expression exist between adult wild and domestic species (Künzl and Sachser 1999; Trut et al. 2006; 2009; Himmler et al. 2013; Moretti et al. 2015), it is not well resolved when these species differences occur. Specifically, no study has collectively and quantitatively examined the ontogeny of the four key behavioural traits in the domestication syndrome and thus it remains unclear whether the attenuated expression of sociability, playfulness, aggression and fearfulness develop simultaneous in young domesticated animals. Adding to that, and particularly exemplified by wolves and dogs, is the controversy surrounding the question of when during ontogeny species differences occur in the expression of fear (Scott and Marston 1950; Zimen 1987; Lord 2013; Morrow et al. 2015; Marshall-Pescini et al. 2017), and studies investigating the continued development of fear are lacking on a general scale. Thus, investigations into the trajectory of key behaviours over extended time periods during early life are needed to further understand how and when domestication driven changes in behaviour develop.

STUDY SPECIES

In the Mesolithic period humans lived in nomadic hunter-gatherer societies with a geographical overlap with wolf populations, and it was during this period, at least 15,000 years ago, the first dogs (*Canis familiaris*) were domesticated from grey wolves (*Canis lupus*) (Coppinger and Coppinger 2001; Price 2002; Miklósi 2007; Driscoll et al. 2009; Morey 2010). Though there is an ongoing debate of the dog's geographical origin (Savolainen et al. 2002; Wayne and vonHoldt 2012), evolutionary age (Vila et al. 1997; Klütsch and Savolainen 2001; Ovodov et al. 2011; Wang et al. 2013) and how it was domesticated (Coppinger and Coppinger 2001; Miklósi 2007), the domestication of the dog is unique in multiple ways. Not only was the dog the first animal to ever be domesticated, but it is also the only large carnivore, and the only canid, to ever be domesticated (Clutton-Brock 1992; Wayne and vonHoldt 2012).

The approximately 400 registered present day dog breeds (Lindblad-Toh et al. 2005) are divided in two major groups, *ancient* and *modern* breeds, based on their established divergence from the wolf (vonHoldt et al. 2010; Parker et al. 2017). Ancient breeds are a small group of dog breeds originating more than 500 years ago. This breed group is characterized by a detectable genetic admixture with wolves and represents an early stage of dog domestication

(vonHoldt et al. 2010). Modern breeds represent the vast majority of present day dog breeds, which are highly divergent from both ancient breeds and wolves, with no detectable wolf admixture. Modern breeds originated from recent stringent breeding efforts, dating only 200 years back (Lindblad-Toh et al. 2005; vonHoldt et al. 2010), creating strong and highly breed-specific selection pressures (Coppinger and Coppinger 2001; Svartberg 2006; Careau et al. 2010; Zapata et al. 2016). This strong directional selection for desired traits drives the striking morphological differences among dog breeds (Ostrander et al. 2000; Spady and Ostrander 2008; Zapata et al. 2016), and emphasizes the dramatic alteration from wolf to dog. While there is an on-going debate as to whether strong selection for highly specific behaviours to fulfill specialized functions, such as herding, guarding or hunting, cause quantifiable behavioural differences between dog breeds (Scott and Fuller 1965; Coppinger and Coppinger 2001; Svartberg 2006; Careau et al. 2010; Lord et al. 2013; Starling et al. 2013; Mehrkam and Wynne 2014; Zapata et al. 2016), it is clear that we should expect substantial behavioural differences between wolves and dogs. Domestication has profoundly altered factors with a high potential impact on behaviour from wolf to dog, including physiology, reproduction, foraging and social structure (Goodwin et al. 1997; Axelsson et al. 2014; Range et al. 2015).

Together, the presence of both wolves and dogs and the unique history of dog domestication, with strong directional selection exerted on different dog breeds, make wolf-dog comparisons ideal model systems to address questions on behavioural evolution.

AIMS OF THE THESIS

The overall aim of this thesis is to investigate the behavioural implications of domestication using a wolf-dog system. Specifically, we focused on four key behavioural traits believed to be affected by domestication; sociability, playfulness, aggression and fearfulness. Across the four chapters the specific aims are:

- To investigate if specific behaviours in the domestication syndrome are correlated, as has been suggested, and if such correlations persist over time (***Paper I***)

- To investigate if sociability, playfulness, aggression and fearfulness are expressed simultaneously as predicted by the domestication syndrome hypothesis when contrasting dogs with ancestral representatives (***Paper II and III***).

- To establish when during ontogeny behavioural differences occur between dogs and wolves (***Paper III and IV***)

- To identify important behavioural differences between dogs and ancestral representatives that might have prominent roles in domestication (***Paper I, II, III and IV***)

METHODS

All four thesis chapters are based on behavioural comparisons within a domestication setting.

For both ***Paper I and II*** we focused on behavioural quantifications of the key traits in the domestication syndrome, i.e. sociability, playfulness, aggression and fearfulness. To do this we extracted behavioural data from the Swedish Kennel Club's database on the performance of dogs in the Dog Mentality Assessment test (DMA). The DMA is a widely used test in Sweden, developed to assess various behavioural traits, including sociability, playfulness, fearfulness and aggression in dogs (Svartberg and Forkman 2002), and is a great tool to quantitatively assess both intra- and interbreed differences. The DMA consists of a total of 10 test situations covering 33 different assessments in which dogs are rated by a describer on an intensity scale from 1 – 5, were 1 is the lowest intensity level and 5 is the highest. The dog goes through the test accompanied by a familiar handler (usually the owner) and a test leader (a stranger) interacts with the dog and/or instructs the handler throughout the test. Compared to frequently used owner-based questionnaires (e.g. C-BARQ, (Duffy et al. 2008)), the DMA provides an excellent alternative to estimate behavioural parameters in dogs, as behavioural reactions are quantified under standardized testing conditions using certified, non-owner observers. The test thereby provides a more neutral assessments of behaviours and avoids potential bias that familiarity with a human might affect a dog's willingness to engage in social interactions (Mitchell and Thompson 1990), by evaluating behavioural responses when dogs interact with a stranger (the test leader). The use of a stranger provides the comparable basis to engage in social interactions for all dogs during the DMA.

Paper I

In ***paper I*** we tested the hypothesis that altered selection pressures during domestication have created a syndrome in which direction-specific correlations between sociability, playfulness, aggression and fearfulness are stable over time. We used behavioural data collected from more than 90,000 dogs over a 16-year period. A total of 78 registered breeds were represented in our data set, and these breeds were divided into ancient (N = 7) and modern breeds (N = 71). To investigate the temporal stability of behavioural correlations, we first generated separate correlation matrices for ancient and modern breeds among all behavioural traits using Spearman rank correlation, and based our subsequent analyses on the standardized effect sizes (Zr) of these correlations. Then, based on recent cladogram generated using an identity-by-state distance matrix and a neighbour-joining tree algorithm of 161 dog breeds (Parker et al. 2017), we build a phylogenetically informed linear model that compared *Zr* values between ancient and modern breeds while accounting for the divergence difference between each breed and the grey wolf. To validate our results we i) conservatively compared *Zr* values between ancient and modern breeds using a Mann Whitney Wilcoxon test, and ii) re-ran our analyses with a subsampling approach repeated 1,000 times to account for difference in within-breed sampling effort between ancient and modern dog breeds and out rule the possibility that the reduced sampling effort among ancient breeds could have inflated the effect sizes. The results from these conservative analyses were consistent with the results obtained using the phylogenetically informed linear model.

Paper II

In ***Paper II*** we tested the hypothesis that domestication has caused simultaneous behavioural alterations in dogs, where the expression of sociability and playfulness is increased and the expression of aggression and fearfulness is decreased. For this purpose we used wolf hybrids as a proxy of an ancestral state in the early domestication process of the dog. Hybrids express intermediate behaviour compared to the original species (Delmore and Irwin 2014) and wolf hybrids can therefore help us understand the basic behavioural differences between wolves and dogs. We contrasted the behavioural responses of wolf hybrids with the responses of dog breeds found in their pedigrees; Alaskan malamutes, German Shepherds and Siberian Huskies (Figure 2). We also used behavioural scores from Czechoslovakian Wolfdogs, a now registered dog breed that originated in Czechoslovakia in 1955 as a hybrid cross between a German shepherd and a Carpathian wolf (Smetanová et al. 2015). The last wolf introgression in the breeding lines was in 1983, and present day Czechoslovakian Wolfdogs dogs thus have diluted wolf component and thereby serves as a good control group for our dog and wolf hybrid comparison.

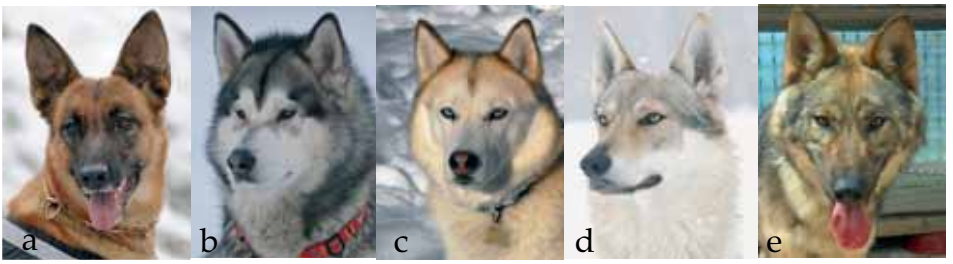

Figure 2. Dog breeds and wolf hybrids. *Dogs represented by a) German shepherd, b) Alaskan malamute and c) Siberian husky, and hybrids, with varying degree of wolf introgression, represented by d) Czechoslovakian wolfdog and e) wolf hybrid.*

We evaluated and contrasted behaviors among wolf hybrids and dogs using the dog mentality assessment test (DMA) for which data has been collected over a 17-year period. To address overall differences between dogs and hybrids, we divided our data set into a dog group containing Alaskan Malamutes (N = 27), German Shepherds (N = 7,495) and Siberian Huskies (N = 18) and a hybrid group containing Czechoslovakian Wolfdogs (N = 8) and wolf hybrids (N = 9). Our analyses consisted of two approaches i) contrasting dogs and hybrids to detect behavioural differences related to wolf component by running unpaired Mann-Whitney-Wilcoxon, and ii) identify potential breed-specific differences by looking at the three dog breeds and the two hybrid breeds separately by using Kruskal-Wallis tests with post hoc Dunn's test. We implemented a resampling approach in order to account for the strong over-representation of German shepherds. Our final results were based on bootstrapped distributions to obtain estimated means, p-values and 95% confidence intervals.

The second half of this thesis, ***Paper III and IV***, is based on behavioural data collected on three litters of European grey wolves (N = 13) and two litters of Alaskan huskies (N = 12), hand-raised and extensively socialized at Tovetorps Zoological Research Station of Stockholm University in the years 2014 – 2016. Puppies were raised in litters under similar conditions from the age of 10 days, i.e. prior to eye opening, with 24-hour presence of human caregivers for the first two months to ensure successful socialization (ref). Using this set-up we avoided environmental bias, including maternal effects, which is well-documented to affect behavioural development (Clark and Galef 1982; Wilsson and Sundgren 1998; Bray et al. 2017). Care taking, testing and gradual exposure to the new environments, such as larger enclosures, were standardized over all three years and all five litters were exposed to the same novelty at the same age. Data from behavioural observations and testing were collected continuously until 26 weeks of age.

Paper III

In ***Paper III*** we investigated if the key behavioural traits in the domestication syndrome develop simultaneously in dogs and wolves leading to quantifiable differences in the simultaneous expression of sociability, playfulness, aggression and fearfulness during the first 16 weeks of life. For this purposed we subjected wolves and dogs to a standardized behavioural test for puppies, the Puppy Mental Assessment (PMA), at eight, 12 and 16 weeks of age. The PMA is a behavioural test developed by the Swedish Working Dog Association normally applied to puppies seven to nine weeks of age, and can serve as a tool for dog breeders to find suitable new owners for their puppies. The PMA consists of 42 standardized tests situations covering behaviours in four main groups: 1) Social play with a stranger, 2) Object play and object interest, 3) Social comfortableness and fearfulness, and 4) Interest in strangers, including greeting. An educated puppy assessor scores the puppy's behavioural response in each test situation on an intensity scale from 1 to 5. For this study we had a clear *a priori* hypothesis and therefore we focused on eight PMA subtests specifically quantifying sociability, playfulness, fearfulness and aggression. To investigate the development of behaviours in wolves and dogs, we implemented a repeated measures approach. We fitted linear mixed models for each of the behaviours, with the behavioural score as the dependent variable and species, age and their interaction as fixed effects. We then ran anovas on the best-fitted model and post-hoc tests on both species and/or age when appropriate.

Paper IV

In ***Paper IV*** we investigated the ontogeny of fear of novelty in wolves and dogs to establish when and if species differences in fear expression occurs during the juvenile period. For this purpose we subjected wolves and dogs to repeated novel object tests on a monthly basis at six, 10, 14, 18, 22 and 26 weeks of age (Figure 3).

Figure 3. Novel object tests*. a) Wolf 10 weeks and b) dog 18 weeks*

To avoid habituation a new novel object was used at each age. We measured the latency to approach and the latency to make contact with the novel object, as well as time spent investigating, manipulating and looking at the novel object. We also measured behaviours not related to the novel object, including active and passive behaviour. For latency measures, we used the time delay to approach the novel object within the distance of 1 meter, and the time delay to make contact with the novel object after it had been approached. For all other behaviours, we used ratios of duration spent on the behaviour/total test time. To investigate if the development of fear differed in wolves and dogs we fitted linear mixed models for each behavior to test for species effects. For behaviours where there was an interaction between species and age, we additionally fitted a model where age was a discrete variable, and used that to perform post-hoc tests for species differences at each age.

MAJOR FINDINGS AND DISCUSSION

Paper I

The domestication syndrome predicts direction-specific correlations between sociability, playfulness, aggression and fearfulness, which should be difficult to decouple due to a shared physiological origin (Sih et al. 2004a). However, in ***Paper I*** we found that while modern dog breeds exhibited significant correlations between 13 out of 17 behavioural trait combinations the effect sizes in 11 out of 13 correlations were considered small (<0.1, Figure 4), with low biological predictive value. Only two correlations had significant effect sizes in modern breeds, namely the correlations between sociability and playfulness (Zr = 0.268) and between fearfulness and aggression in a persistent threatening situation (Zr = 0.194). In ancient dog breeds we detected seven significant correlations between behavioural traits within the domestication syndrome, all of which had significant effect sizes (>0.1, Figure 4). An analysis considering phylogeny confirmed that ancient breeds showed a significant exaggeration of effect sizes in seven behavioural trait correlations and a statistical trend suggesting an exaggerated effect size in an additional three behavioural correlations when compared to modern breeds (Figure 4).

The exaggerated effects sizes found in ancient breeds suggest that selection exerted on this breed group acts simultaneously on suites of behaviours, thereby supporting the domestication syndrome hypothesis. However, we found that modern dog breeds only expressed generally weak behavioural correlations. The contrasting patterns in the strength of behavioural correlations found between modern and ancient dog breeds suggest that suites of

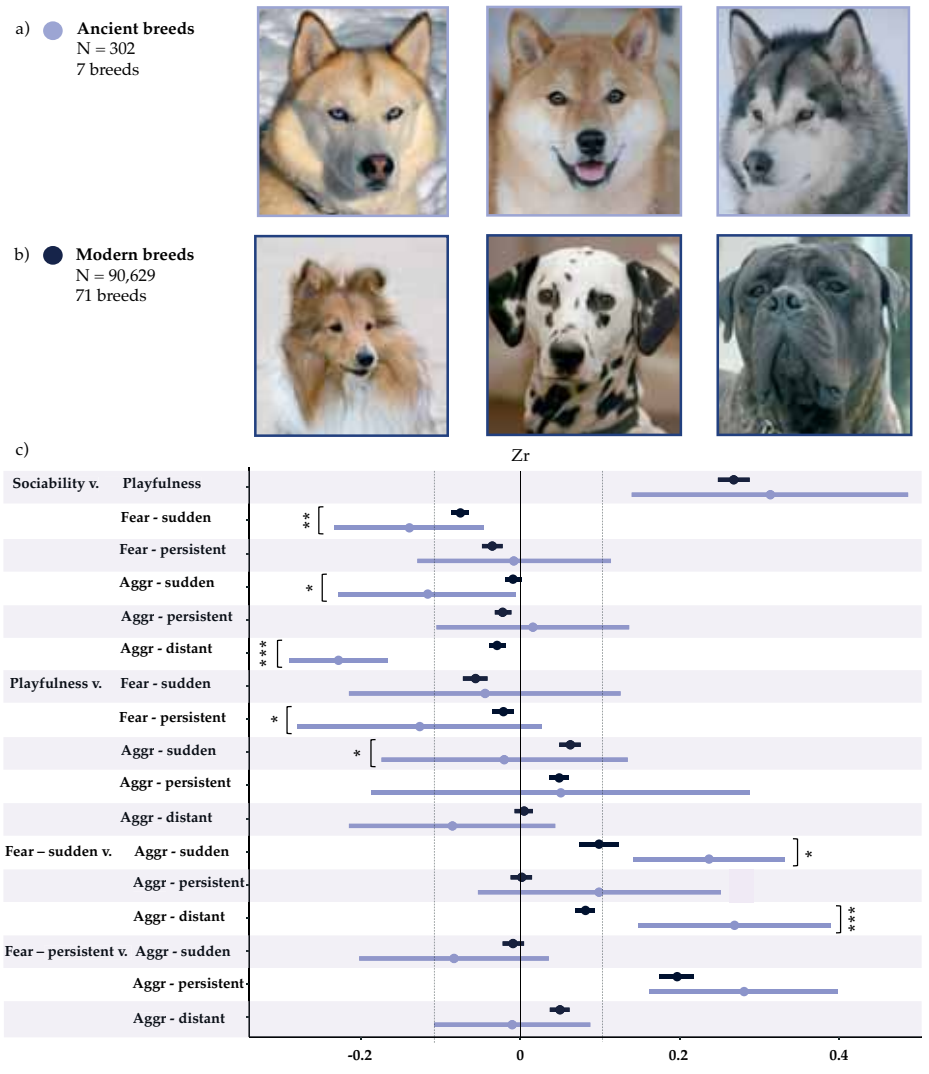

Figure 4. Contrasts of behavioural correlations between ancient and modern dog breeds*. a) Examples of ancient breeds in the study: Siberian husky, Shiba and Alaskan malamute. b) Examples of modern breeds in the study: Shetland Sheepdog, Dalmatian and Bullmastiff. c) Based on a meta-analytic approach we obtained effect sizes (Zr) for correlations between all behavioural combinations; Sociability, playfulness, two measurements of fearfulness (fear, in a sudden and persistent threatening situation), and three measurements of aggression (aggr, in a distant, sudden and persistent threatening situation). Mean Zr values and 95% CI's are given. Zr values <+/- 0.1 are considered small (dotted lines). Significant differences between ancient and modern breeds based on phylogenetic informed analyses are marked with brackets and to indicate p-values * (< 0.05), ** (<0.01) and *** (<0.001)*

correlated behaviours implicated in the domestication syndrome hypothesis can be decoupled, thereby questioning the stability of the domestication syndrome. However, it is noteworthy that the correlation between sociability and playfulness and between fearfulness and aggression in a persistent threatening situation had significant effect sizes in both ancient and modern breeds, thereby emphasizing their biological relevance and temporal persistence. Overall, the general discrepancy in the behavioural correlations between ancient and modern breeds indicates that behaviours were decoupled due to different selection pressures at different times during dog domestication, and we suggest that a recent shift in selection pressures in modern dog breeds affect the expression of sociability, playfulness, aggression and fearfulness independently. Thus, the results in ***Paper I*** do not support a recent suggestion that a single underlying physiological mechanism is the origin of the myriad of changes in behaviours, physiology and morphology observed in domesticated animals (see Wilkins et al. 2014). Instead, we suggest that multiple independent factors act simultaneously to shape the behavioural changes observed in the domestication syndrome, thus potentially making traits available for individual selection.

Paper II

Based on the domestication syndrome hypothesis, it is expected that domesticated animals express increased sociability and playfulness long side decreased aggression and fearfulness compared to their ancestral species. In ***Paper II*** we found, in accordance with our predictions, that hybrids were less playful than dogs ($W = 316.69$, $df = 1$, $p < 0.01$, Figure 5) and more fearful than dogs in a startle situation ($W = 281.31$, $df = 1$, $p < 0.01$, Figure 5) and in a persistent threatening situation ($W = 468.81$, $df = 1$, $p < 0.01$, Figure 5). Notably, Czechoslovakian Wolfdogs, the other hybrid tested in this study, displayed intermediate scores between dogs and wolf hybrids in playfulness, fear towards a startle stimuli and fear in the ghost test (Figure X). However, contrary to our predictions, we found no differences in fearfulness towards sound ($W = 274.7$, $df = 1$, $p = 0.543$), sociability ($W = 432.03$, $df = 1$, $p = 0.291$) or aggression ($W = 501.76$, $df = 1$, $p = 0.781$) between dogs and hybrids.

The results in ***Paper II*** challenge the idea presented in the domestication syndrome hypothesis, that sociability, playfulness, fearfulness and aggression undergo simultaneous alteration during dog domestication. Specifically, while we found that wolf hybrids were less playful and overall more fearful than dogs, we did not detect any differences in sociability or aggression between wolf hybrids and dog breeds. We find it particularly surprising that hybrids were not more aggressive than dogs, since aggression has been broadly suggested to be a key component in the domestication process (Hare et al. 2012). Instead, the retained

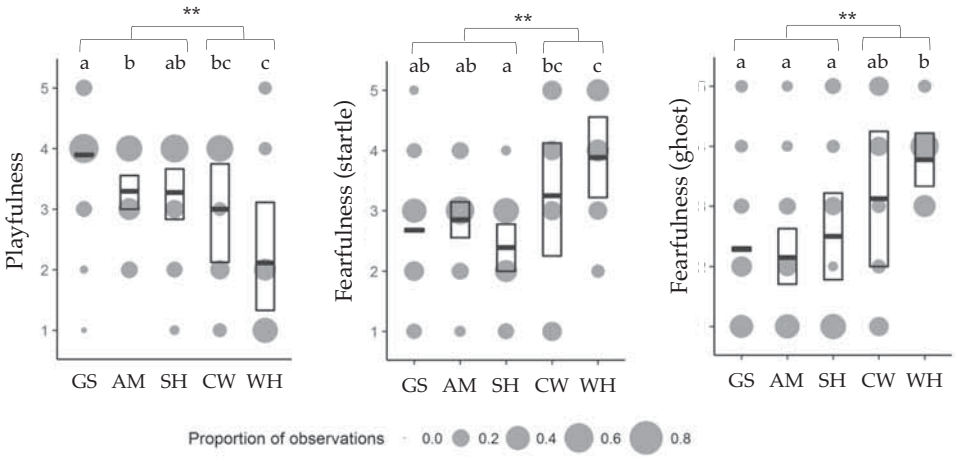

Figure 5. Distribution of behavioral scores. *Graphic representation of the distributions of behavioral scores (1-5) obtained in the behavioral categories Playfulness, Fearfulness (startle) and Fearfulness (ghost) for 7,495 German shepherds (GS), 27 Alaskan malamutes (AM), 18 Siberian huskies (SH), 8 Czechoslovakian wolfdogs (CW) and 9 wolf hybrids (WH). Boxes indicate lower and upper bootstrapped 95% confidence intervals, the bold bar indicates the mean and the grey circles indicate the proportion of observations of behavioral scores from 1-5 with in a behavioral category. ** or ns indicates significant and non-significant differences, respectively, between dogs and hybrids based on pairwise Mann Whitney Wilcox tests. For the five breed comparisons, breeds not connected with the same letter a, b or c) are significantly different from each other.*

difference in playfulness between dogs and wolf hybrids suggests that human-directed play behavior may have been selected for as an individual behavioral trait during the domestication of dogs. This indicates that play behavior had a greater importance in the domestication process of dogs than previously thought, thereby questioning the existing view that playfulness in adult domesticated animals is merely a by-product of domestication. Given that dog and human experience positive physiological rewards after dog-human play interactions (Odendaal and Meintjes 2003; Horváth et al. 2008; Romero et al. 2014; Horowitz and Hecht 2016) and that dogs can interpret and respond to play cues communicated by humans (Rooney et al. 2000), we suggest social play between dog and human may have helped form and maintain strong social bonds during the course of domestication.

Paper III

The alteration of aggression, fearfulness, sociability and playfulness are expected to occur simultaneously, as part of the domestication syndrome (Trut 1998; Künzl and Sachser 1999; Hare et al. 2012; Himmler et al. 2013). However, in ***Paper III*** we found only weak support for a simultaneous change in these behaviours during ontogeny in both wolves and dogs. Specifically, while we found that sociability ($F = 4.71$, $p = 0.014$, Figure 6) and four measures of

playfulness (Chasing ball: F = 4.13, p = 0.023, Figure 6; Retrieving ball: W = 3.29, p = 0.047; Playing with ball: W = 6.00, p = 0.005; Tug of war: W = 3.36, p = 0.044) develop differently in wolves and dogs, the development of fear of novelty (Stranger: W = 2.22, p = 0.120; Six objects: W = 1.76, p = 0.186, Figure 6) follow similar trajectories in both species. Furthermore, there was no variation in a measurement of resource defense (aggression) across age in wolves and dogs. Additionally, previous work in canids (Scott and Marston 1950; Wooply and Ginsburg 1967; Fentress 1967; Fox 1972; Zimen 1987; Lord 2013) suggests that behavioural differences caused by domestication manifest before eight weeks of age. However, we found that species differences did not occur until 12 and 16 weeks of age.

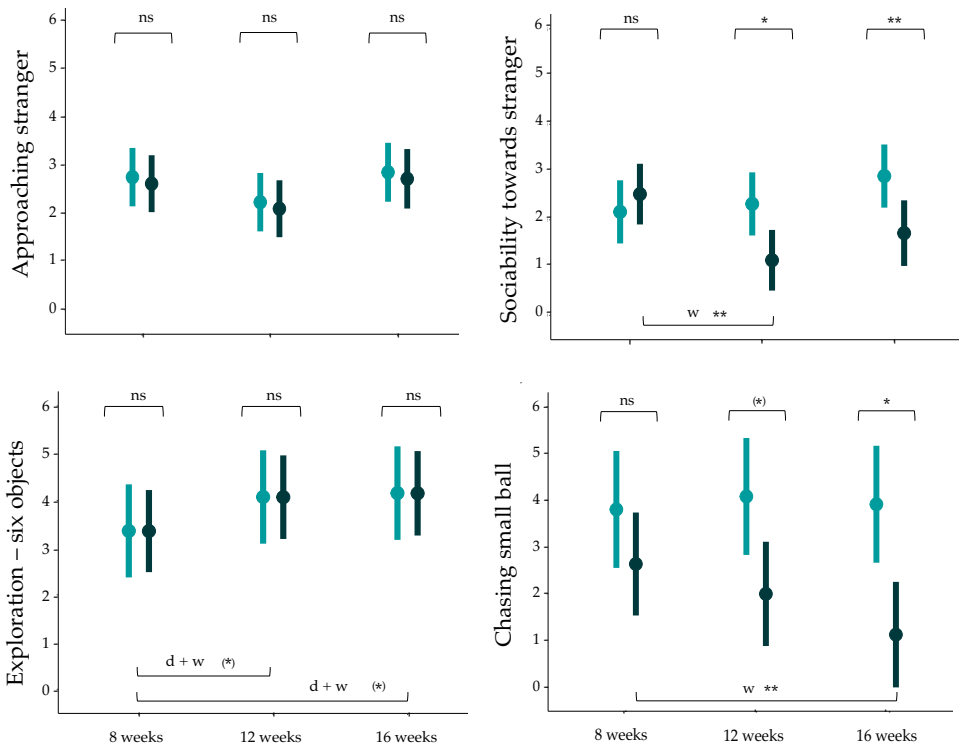

Figure 6. Age and species differences. *Fitted plots of mean and 95% confidence intervals for wolves (dark green) and dogs (light green) based on best suited mixed model for repeated measures for the behaviours Approaching stranger, Sociability, Exploration – six objects and Chasing small ball)during the test at 8, 12 and 16 weeks of age. The three upper brackets indicate species differences with ns (non-significant), (*) (<0.1) and *(<0.05). Lower brackets indicates age differences within wolves (w) and dogs (d) with *(<0.05) and **(<0.01).*

Together, the results in ***Paper III*** demonstrate that key behaviours in the domestication syndrome are not subject to a simultaneous developmental pace as differences in the expression of sociability, playfulness, aggression and fearfulness between wolves and dogs do not follow similar trajectories. Furthermore, our results challenge the idea that behavioural differences between wolves and dogs arise early in ontogeny, supporting the suggestion that the degree of flexibility in developmental processes might be much more extensive than previously assumed both across and within species (Scott 1962; MacDonald 1983; Miklósi 2007; Stamps and Groothuis 2010; Morrow et al. 2015).

We found that altered expression of sociability and playfulness between wolves and dogs occurred at 12 and 16 weeks of age, which indicates that selection on these behavioural traits have been of key importance during domestication. It is surprising that we found no species differences in the expression of fear across age, as previous studies on wolves and dogs have specifically suggested that the ontogeny of fear has been altered by domestication (Belyaev et al. 1985; Plyusnina et al. 1991; Coppinger and Coppinger 2001). However, since adult wolves express increased fear towards novelty compared to dogs (Moretti et al. 2015), our results might suggest that this species difference occurs later in ontogeny than previously assumed. Thus, the findings in ***Paper III*** suggest a more complex relationship between the behaviours in the domestication syndrome during development, with sociability, playfulness, aggression and fearfulness following unsynchronized trajectories leading to a lack of simultaneous behavioural alterations during early development in wolves and dogs.

Paper IV

Adult wolves express increased fear towards novelty compared to dogs (Moretti et al. 2015). Though it has been suggested that altered developmental rates have delayed the onset of fear behaviour in dogs, and that wolves and dogs differ in their expression of fear already during early ontogeny (Scott and Fuller 1965; Fox 1972; Zimen 1987; Coppinger and Coppinger 2001; Lord 2013), it remains unclear when during ontogeny this species difference occurs (Zimen 1987; Lord 2013; Marshall-Pescini et al. 2017). By testing puppies repeatedly from the age of six weeks, we demonstrate in ***Paper IV*** that a species differences in fear of novelty occurs for the first time at the age of 26 weeks, with dogs decreasing their latency to approach a novel object compared to wolves ($t = -3.131$, $df = 18.666$, $p = 0.006$, $p_{adjusted} = 0.034$, Figure 7). Notably, this species difference did not coincide with altered developmental patterns in making contact to or interacting with the novel object in dogs and wolves.

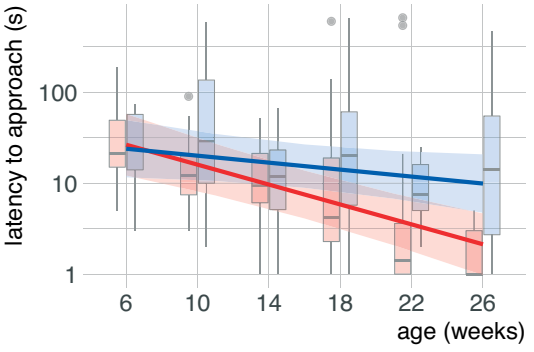

Figure 7. Latency comparison. *Boxplots show latency to approach a novel object, comparing dogs and wolves across age. Overlaid are the fits and confidence intervals from the best model, selected by AIC. Boxes indicate the quartiles, and the whiskers reach maximally 1.5 times the interquartile range. Values beyond that are shown as points. Note that panels a and b make use of a log(x) scale, and panels e, f and g use log(x + 1).*

Decreased expression of fear is considered a key behavioural alteration in domesticated animals, and evidence suggests that domestication drives altered developmental rates delaying the initial onset of fear response during early ontogeny (Belyaev et al. 1985; Plyusnina et al. 1991). Interestingly, we found no evidence in support of a delayed onset of fear response in dogs compared to wolves during early stages of development, thereby challenging the existing literature on the subject (Scott and Fuller 1965; Fox 1972; Zimen 1987; Coppinger and Coppinger 2001; Lord 2013). Specifically, we found no differences in fear of novelty, measured as the latency to approach a novel object, in wolves and dogs until 26 weeks of age, much later than expected. Importantly, this species difference was not driven by wolves becoming more fearful with age, but because dogs decreased their time to approach the novel object with age. Together, the results in ***Paper IV*** suggest that the continued behavioural trajectory of fear in wolves and dogs is not affected by the initial onset of fear response, and that a possible delay in onset of fear in dogs does not explain species differences occurring later in ontogeny. Instead we suggest that species differences in fear expression are caused by dogs, unlike wolves, losing their sensitivity towards novelty with age.

INTEGRATIVE CONCLUSION

Together the four papers in this thesis highlight some fundamental issues with the way we currently view the behavioural effects of domestication.

In ***Paper I*** we investigated the strength of correlations between sociability, playfulness, aggression and fearfulness in dogs. In this paper we found that correlations between behavioural traits in the domestication syndrome are weak in modern dog breeds, but exaggerated in ancient dog breeds, thus suggesting that while selection on suites of behaviours

has occured during early dog domestication, the behaviours in the domestication syndrome can be decoupled on a temporal scale. These findings suggest that behaviours might not be expressed simultaneously as predicted by the domestication syndrome hypothesis. In ***Paper II and III*** we specifically investigated the expression of behaviours in the domestication syndrome. In these two papers we show that alteration of sociability, playfulness, aggression and fearfulness is neither expressed simultaneously when comparing wolf hybrids to dogs nor do the behaviours undergo simultaneous development in juvenile wolves and dogs. Together, ***Paper I, II and III*** suggest that the behavioural correlations in the domestication syndrome are not stable over time.

Our finding in ***Paper II*** that wolf hybrids are overall more fearful than dogs supports a previous study reporting that adult wolves are more fearful towards novelty than dogs (Moretti et al. 2015). While there is a general consensus that the development of fear response is delayed in dogs compared to wolves (Scott and Fuller 1965; Fox 1972; Zimen 1987; Coppinger and Coppinger 2001; Lord 2013), a recent study found no difference in fear towards novelty in six and eight weeks old wolves and dogs (Marshall-Pescini et al. 2017). Our findings that wolves and dogs did not differ in their fear towards strangers or novel objects during the first 16 weeks of life in ***Paper III*** thus add support to the notion that domestication driven differences in the expression of fear might not be established until later in ontogeny. We subsequently tested when species differences in fear occur during ontogeny in wolves and dogs in ***Paper IV*** using repeated novel object test from six to 26 weeks of age. Our finding that a species difference in latency to approach a novel object did not occur until 26 weeks of age, does not support the traditional view of fear development in wolves and dogs, and represents the first indication of when a quantifiable difference in fear towards novelty arises in wolves and dogs. Thus, together ***Paper II, III and IV*** demonstrate that domestication does affect the expression of fear, but that wolves and dogs do not differ in their fear responses until the age of 26 weeks, much later than previously thought.

Finally, in ***Paper II*** we demonstrate that increased human-directed playfulness might have been a key component in dog domestication, which challenges the current view that playfulness in adult domesticated animals has no causal influence on the domestication itself. Our findings that the correlation between human-directed playfulness and sociability express evolutionary stability during dog domestication in ***Paper I***, supports the idea that playfulness had a significant role in domestication. Increased playfulness expressed by adult domesticated animals is generally believed to be the result of paedomorphism, i.e. retention of juvenile traits into adulthood (Goodwin et al. 1997; Dobney and Larson 2006; Kukekova et al. 2006; Himmler et al. 2013). However, to confirm that increased playfulness in domesticated animals is caused

by paedomorphism, the ontogenetic stage when differences between wild and domesticated animals occur must be identified (Pongrácz et al. 2010). Thus, by detecting a shift in the expression of play behaviour in wolves and dogs at 12 and 16 weeks of age, with wolves becoming less playful or dogs becoming more playful, in ***Paper III,*** we provide evidence supporting the hypothesis that increased playful behaviour in dogs is caused by paedomorphism. While wolves did not differ in playful behaviour from dogs at eight weeks, they did express large variation. Specifically, three eight weeks old wolves scored similarly to the most playful dogs in retrieving a small ball for a stranger (i.e. human-directed playfulness). Such a variation can target a behavioural trait for selection, and thus ***Paper I, II and III*** together demonstrate that playfulness could have been an important component in the domestication of dogs.

DIRECTIONS FOR THE FUTURE

This thesis presents results on the impact of domestication on behaviour that deviates from the currently available literature. Thus, in combination the four chapters emphasize the need for a re-evaluation of the behaviours hypothesized to be shaped by domestication, and further in-depth investigations will likely yield significant insight into of the behavioural implications of domestication.

Paper I, II and III all demonstrated that the behaviours in the domestication syndrome are not necessarily expressed simultaneously. Domesticated animals are continuously subjected to strong, highly species-specific and human-induced directional selection, and together with their highly varied domestication history (e.g. cats, rabbits, horses (Irving-Pease et al. 2018; Ottoni et al. 2017; Gaunitz et al. 2018)) it might be erroneous to assume that a synonymous domestication syndrome applies to all domesticated animals. However, current contemporary cases in which humans and populations of wild animals overlap and interact (e.g. dingos, coyotes and even modern wolf populations (Newsome et al. 2017)) could prove a valuable source for evaluating behavioural changes related to domestication in real time. Furthermore, ***Paper I*** showed that correlations in the domestication syndrome exhibited context specificity. To formally evaluate the extent of such specificity, a crucial next step would be to quantitatively evaluate if correlations between not only behaviours, but also morphology and physiology, are present not just in dogs, but also in other domesticated species.

Paper III and IV both showed that the development of behavioural differences in wolves and dogs might be more complex than previously assumed and our findings in these two papers

highlights the need for further research on how domestication affects behavioural ontogeny. An important next step in this field would therefore include investigations following the trajectory of key behaviours for an extended period of time. Additionally, developmental pace appears to be breed-specific in dogs (Scott 1962; Scott and Fuller 1965; Udell et al. 2010; Morrow et al. 2015), with variation across breeds in important ontogenetic phases such as the sensitive period (Scott and Fuller 1965; Morrow et al. 2015). The degree of paedomorphism varies across dog breeds and this could potentially affect the ontogeny of behaviour across breeds (Udell et al. 2010; Morrow et al. 2015). Thus, future studies should include investigations across various dog breeds to fully understand the impacts of domestication on behavioural development. Furthermore, the results in ***paper III and IV*** provides a previously undocumented variation in behavioural responses in wolves. Studies comparing wolves and dogs are restricted by limited samples sizes and the variation demonstrated in this thesis, highlights the need for replications of such studies to verify findings.

Finally, the highly varied patterns of correlations involving fear and aggression in ***Paper I*** call for stricter definitions of broad behavioural terms. This is supported by the context specificity of fearfulness and playfulness expressed by wolf hybrids in ***Paper II*** and wolves and dogs in ***Paper III***, respectively**.** Future studies would therefore benefit from identifying which subtypes of key behaviours have been affected by domestication.

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Figure 3
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Figure 4
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SVENSK SAMMANFATTNING/SWEDISH RESUMÉ

Domesticering är en process där arter har selekterats för att leva i miljöer kontrollerade av människan. Den har dramatiskt påverkat de evolutionära vägar som växter och djur tagit, och det finns många exempel på hur selektionstryck från människan påverkat samma karaktärer hos ett brett antal arter under domesticeringen. Mer specifikt visar domesticerade djur, jämfört med sina vilda motsvarigheter, upprepade likartade mönster av förändrad fysiologi, morfologi och beteende. Ett fenomen känt som ”domesticeringssyndromet”. Den här upprepade förekomsten av en uppsättning karaktärer hos ett brett antal domesticerade arter tros vara ett resultat av att vissa karaktärer korrelerar med varandra, på grund av fysiologiska kopplingar, och därmed har de utvecklats tillsammans under domesticeringen.

I denna avhandling fokuserar jag på de beteendemässiga konsekvenserna av domesticeringen. Domesticerade djur uttrycker typiska samtidiga förändringar av beteenden, där aggression och rädsla minskar och socialitet och lekfullhet ökar, jämfört med de vilda arterna. Även om det antas att förändringen av dessa fyra viktiga beteendeegenskaper uttrycks tillsammans, har det aldrig formellt testats om aggression, rädsla, socialitet och lekfullhet är korrelerade som förväntat från hypotesen om domesticeringssyndromet. Jag testade därför förhållandet mellan alla de fyra beteenden som ansetts vara påverkade av domesticering i ***Kapitel I***, där jag korrelerade uttryck för aggressivitet, rädsla, socialitet och lekfullhet hos över 90.000 hundar.

I ***Kapitel II*** testade jag hypotesen att domesticering har orsakat samtidiga beteendeförändringar hos hundar. För detta ändamål använde vi varghybrider som en företrädare (”proxy”) för det ursprungliga tillståndet i hundens tidiga domesticeringsprocess, och kontrasterade varghybridernas beteenden med hundarnas beteenden. Jag följde upp den här studien i ***Kapitel III*** genom att undersöka om de viktigaste beteendemässiga egenskaperna i domesticeringssyndromet utvecklas samtidigt hos hundar och vargar, och om detta leder till kvantifierbara skillnader i samtidiga uttryck av socialitet, lekfullhet, aggression och rädsla under deras första 16 veckor efter födseln. För detta ändamål testade jag vargar och hundar i ett standardiserat beteendestest för valpar vid åtta, 12 och 16 veckors ålder.

Slutligen undersökte jag i ***Kapitel IV*** utvecklingen av rädsla för helt nya föremål (”novel objects”) hos vargar och hundar för att fastställa när och om artskillnader i rädsla tar sig uttryck under den juvenila tiden. För detta ändamål testade jag vargar och hundar i upprepade tester mot nya föremål varje månad vid sex, 10, 14, 18, 22 och 26 veckors ålder.

I denna avhandling visar jag att de beteendemässiga egenskaperna i domesticeringssyndromet inte förekommer i stabila korrelationer under domesticeringen, och att aggression, rädsla, socialitet och lekfullhet kan selekteras för oberoende hos dagens moderna hundraser. Resultaten ger också ett svagt stöd för att det sker en samtidig förändring av aggression, rädsla, socialitet och lekfullhet under hundens domesticering. Dessutom har jag gett en första inblick i när och varför artskillnader i uttryck av rädsla uppträder under ontogenin mellan vargar och hundar.

Sammanfattningsvis erbjuder denna avhandling nya och delvis annorlunda resultat om domesticeringens inverkan på beteenden än vad tidigare studier visat. Tillsammans betonar de fyra kapitlen behovet av en omvärdering av de beteenden som antagits vara ett uttryck för domesticeringen, och därmed visar de på behovet av ytterligare djupgående undersökningar av vad domesticeringen inneburit för förändringen av beteenden.

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I moved to Stockholm five years ago to start this PhD. I had a lot of hopes and expectations for what doing a PhD would entail, but I had never imagined that everything else following from the decision to accept this PhD position would end up becoming the journey of a lifetime.

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The foundation for this thesis was laid in Copenhagen, Denmark. I was fortunate enough to be born into a family that was curious and striving for knowledge about the world we live in, and I think the seed for my future career as a biologist was planted quite early on…my dad probably later regretted to ever have taken me to the Natural History Museum in Copenhagen, because I insisted on going every weekend for years after that. When I began my biology studies at University of Copenhagen, I quickly steered in the direction of animal behaviour and got to know Torben, my future master supervisor, and he is the reason I ended up in Sweden. He took me under his wing and, without me knowing, slowly (but firmly) pushed me in the direction that was right for me. Because of him, I ended up working with swift foxes in Colorado, an experience that ended up having a major consequences for the future direction of my personal and professional life, and he was also the one who encouraged me to apply for the PhD position that lead to this thesis. Throughout this crazy journey, often taking me far away from home, I have had nothing but support from my parents, Lilian and Kenneth, and my two trusted friends, Iben and Pia. Thank you all so much.

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