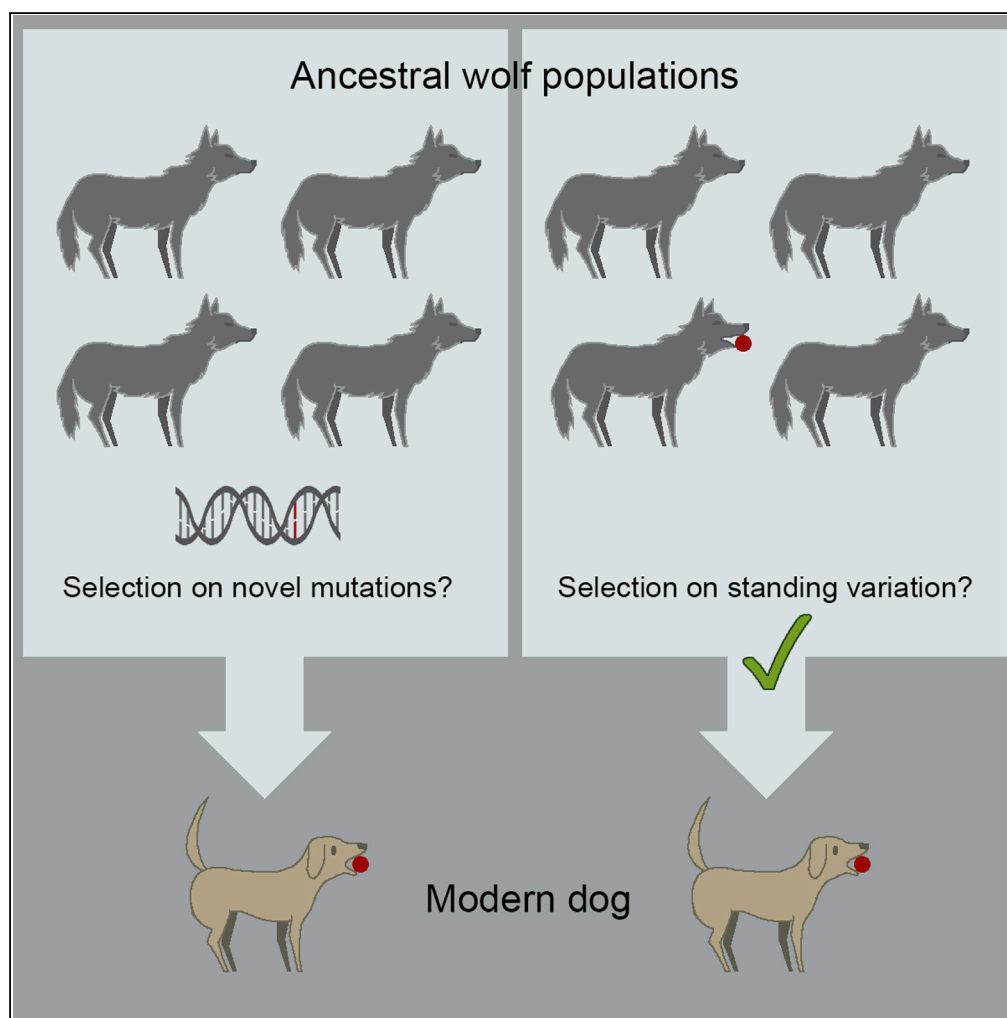


Article

Intrinsic Ball Retrieving in Wolf Puppies Suggests Standing Ancestral Variation for Human-Directed Play Behavior



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HIGHLIGHTS

Dogs, unlike wolves, are suggested to have the ability to interpret human cues

Yet, untrained wolf puppies will spontaneously retrieve a ball based on human cues

Standing variation for human-directed behavior in ancestral wolf populations

Human-directed behavior important during early selection in dog domestication

Article

Intrinsic Ball Retrieving in Wolf Puppies Suggests Standing Ancestral Variation for Human-Directed Play Behavior

Christina Hansen Wheat^{1,2,*} and Hans Temrin¹

SUMMARY

Domestication dramatically alters phenotypes across animal species. Standing variation among ancestral populations often drives phenotypic change during domestication, but some changes are caused by novel mutations. In dogs (*Canis familiaris*) specifically, it has been suggested that the ability to interpret social-communicative behavior expressed by humans originated post-domestication and this behavior is thus not expected to occur in wolves (*Canis lupus*). Here we report the observation of three 8-week-old wolf puppies spontaneously responding to social-communicative behaviors from an unfamiliar person by retrieving a ball. This behavioral expression in wolves has significant implications for our understanding and expectations of the genetic foundations of dog behavior. Importantly, our observations indicate that behavioral responses to human social-communicative cues are not unique to dogs. This suggests that, although probably rare, standing variation in the expression of human-directed behavior in ancestral populations could have been an important target for early selective pressures exerted during dog domestication.

INTRODUCTION

Domesticated animals express dramatic phenotypic alterations compared with their ancestral species (Darwin, 1868; Driscoll et al., 2009). Although phenotypic change can be attributed to novel mutations, a growing body of evidence suggests that evolutionary change relies heavily upon standing genetic variation (Barrett and Schluter, 2008; Larson et al., 2014). Indeed, although few novel mutations with large effects account for some phenotypic differences between domestic and ancestral populations (Parker et al., 2009; Larson et al., 2014), animal domestication was likely initiated by selection on standing genetic variation within ancestral populations (Larson et al., 2014). The potential for domestic phenotypes to derive from existing variation has been well demonstrated in the farm fox project (Belyaev et al., 1985; Trut et al., 2009), where strong selection regimes based on observed variation in the behavioral trait tameness (i.e., reduced aggression and increased docility) among pre-selection foxes brought about rapid occurrence of classic morphological phenotypes associated with domestication. Clarifying whether the basis for traits selected upon during early domestication are variants from ancestral populations is central to developing our knowledge of the domestication process. For instance, wild species expressing variation for the trait tameness are arguably more likely to be successfully domesticated compared with species that do not (Dobney and Larson, 2006). Therefore, disentangling whether phenotypic change in domesticates is caused by novel mutations or selection on standing ancestral variation is important if we are to advance our understanding of which traits had a fundamental role during initial stages of animal domestication.

The dog (*Canis familiaris*), which was domesticated from the gray wolf (*Canis lupus*) at least 15,000 years ago (Driscoll et al., 2009), shows extreme phenotypic variation as a species. Present-day dogs are bred for highly breed-specific requirements for behavior and morphology (Svartberg, 2006; Mehrkam and Wynne, 2014), and although a large amount of the resulting variation is believed to originate from standing genetic variation in ancestral populations (Ostrander and Wayne, 2005), novel mutations have had a significant impact during breed formation (Larson et al., 2014). For instance, black coat color (Candille et al., 2007; Anderson et al., 2009), chondrodysplasia (foreshortened limbs, Parker et al., 2009), and brachycephaly (pathologically short muzzle, Schoenebeck et al., 2012) are traits that have occurred in modern dogs through novel mutations. An additional example comes from a genome-wide analysis of genetic difference between dogs and wolves, identifying dogs as having an increased copy number of the amylase locus (*AMY2B*), which was argued to be a novel adaptation to a starch-rich diet in early-domesticated dogs (Axelsson et al., 2014). However, investigations of a wider range of individuals revealed standing

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Score	Behavior
1	The puppy shows no interest in the ball
2	The puppy plays with the ball on its own, but aborts
3	The puppy plays with the ball on its own, but ignores the puppy assessor's call
4	The puppy responds to the puppy assessor's call, initiates retrieving but releases the ball
5	The puppy responds to the puppy assessor's call and retrieves the ball to her

Table 1. Behavioral Scoring

Cooperation in the three consecutive retrieving tests is measured on a scale from 1 to 5, where 1 is no cooperation and 5 is full cooperation.

variation in amylase copy numbers in wolves, thereby shifting the *AMY2B* example from being a novel mutation important in domestication to yet another example of selection upon standing variation as an essential substrate for domestication (Freedman et al., 2014). This critical distinction has important implications for hypothesizing how dog domestication could have taken place. Thus, the *AMY2B* example illustrates the importance of continued research on ancestral species to better describe existing variation among wolves, thereby avoiding misclassification of traits expressed in dogs as novel.

Although much progress has been made in studying the morphological and physiological differences between wolves and dogs, understanding the basis and origins of behavioral variation has proven more elusive (Larson and Fuller, 2014). One behavioral skill that has been suggested to be novel in dogs compared with wolves is their interspecific social competence (Topál et al., 2009; Miklósi and Topál, 2013). Specifically, it has been posited that, unlike wolves, dogs possess unique skills to interpret human cues (Hare et al., 2002; Topál et al., 2009) and that these skills might have arisen after the domestication process from the gray wolf had been initiated (Hare et al., 2002; Hare and Tomasello, 2005; Miklósi and Topál, 2013). The ability to interpret human social cues has received considerable interest from researchers comparing behavior in dogs and wolves. However, owing to substantial differences in testing procedures, environmental factors, and interpretation of results, consensus among these studies is lacking (Hare et al., 2002; Miklósi et al., 2003; Gácsi et al., 2009; Udell et al., 2008, 2012). Consequently, whether wolves have the ability to interpret human social cues, or whether this is a novel trait in dogs, remains unresolved.

The ability to follow human gestures to access a food reward has been demonstrated in a range of both domesticated and non-domesticated species (Maros et al., 2008; von Bayern and Emery, 2009; Hall et al., 2011; Nawroth et al., 2013). However, these studies all include some degree of prior training and/or familiarity with the communicative person. We remove all these factors, including the food reward, by focusing upon human-directed play with an unfamiliar person as a behavior exemplifying human-animal cooperation and animals' ability to interpret human social cues. Human-directed play behavior has been reported in some domesticated species (Melotti et al., 2014; Mertens and Turner, 2015), including dogs (Horváth et al., 2008; Rooney and Bradshaw, 2002; Rooney et al., 2001; Tóth et al., 2008). Dogs can interpret human play cues and adjust their behavioral repertoire when playing with a human instead of a conspecific (Rooney et al., 2000, 2001). Within a domestication context, wherein animals have been selected for greater tolerance of and interactions with humans, interspecific human-directed play behavior represents a highly relevant behavior to address. However, to date only one study exists comparing human-directed playfulness in a domesticated species and its ancestral proxy species (Hansen Wheat et al., 2018), and studies on human-directed play behavior in wolves have never been attempted.

Here we report on the spontaneous expression of human-directed play behavior, in the form of ball retrieving for an unfamiliar person, in 8-week-old, hand-raised wolves. Our observations occurred during a standardized test in which wolves, with no prior training, are vocally encouraged to retrieve a ball and thus respond to social-communicative behaviors from a human they had never met before. Based on the existing literature (Hare et al., 2002; Topál et al., 2009; Hare and Tomasello, 2005; Miklósi and Topál, 2013), we expected that human-directed play behavior as a trait occurred after the initial domestication of dogs and that wolves therefore would not respond to interspecific social-communicative behaviors or engage in human-directed play with a stranger.

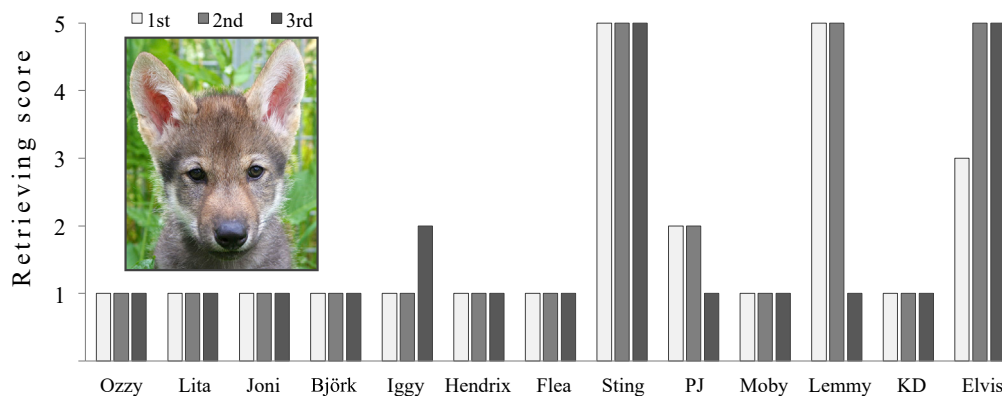


Figure 1. Behavioral Scores

Cooperation scores in the retrieving test for 13 wolves on three consecutive trials (shading from light to dark with the first trial being light, second medium, and third dark). Behavior is scored on a scale from 1 to 5. Only scores 4 and 5 include partial or full retrieving, respectively. Photo credit: Christina Hansen Wheat.

RESULTS

Our observations occurred during a subtest in a standardized test battery aimed at describing the behavior of puppies at the age of 7–9 weeks. Specifically, the subtest quantifies social play and cooperation with an unfamiliar person, the puppy assessor, who throws a tennis ball across the test room. The puppy is given the opportunity to chase after and play with the ball, before the puppy assessor encourages the puppy to cooperate with her and retrieve the ball to her. The subtest is repeated three consecutive times and cooperation is scored on a scale from 1 to 5, where 1 is no expressed interest in the ball and 5 is full cooperation/retrieving (Table 1). Thirteen hand-raised wolf puppies were subjected to this test at the age of 8 weeks. Wolves had not been trained and had only spent time with their caregivers prior to testing, i.e. at the time of testing it was the first time they interacted with an unfamiliar person.

Three wolves, all from the 2016 litter, fully retrieved the ball at least two times, and one of those wolves fully retrieved the ball all three times (Score: 5, Figure 1, Video S1). One of the wolves fully retrieving the ball two times also played with the ball in one of the trials, but ignored the puppy assessor’s call (score: 3, Video S2). One wolf from the 2014 litter and one from the 2016 litter showed some interest in playing with the ball on at least one trial but aborted (Score: 2). Eight wolves (four from the 2014 litter, both from the 2015 litter and two from the 2016 litter) showed no interest in the ball in any of the three trials (Score: 1, Video S3).

DISCUSSION

Here we provide the first empirical evidence that wolves, and not only dogs, express interspecific play with a human based on social-communicative cues. Our finding is surprising given that dogs’ ability to interpret social-communicative behavior expressed by humans has been suggested to be a novel trait occurring after domestication had been initiated (Hare et al., 2002; Hare and Tomasello, 2005; Topál et al., 2009; Miklósi and Topal, 2013). Importantly, our results suggest that, although probably rare, standing variation in the expression of human-directed behavior, including play, in ancestral populations could have been an important target for early selective pressures exerted during dog domestication.

Our observations of three wolf puppies retrieving a ball are highly relevant for the on-going discussion on how domestication affects behavior and further have significant implications for our understanding and expectations of the genetic foundations of behavior in modern-day dogs. Specifically, in relation to current attempts to reveal the genomic basis of behavioral changes during domestication (Pendleton et al., 2018; Freedman et al., 2014; Kukekova et al., 2018), our observations indicate that signatures of selection for human-directed behavior in dogs are likely to be weak and prone to false positives (*sensu lato* Messer and Petrov, 2013; Pritchard et al., 2010). This is because (1) we must now consider that selection likely acted upon standing variation in interspecific social-communicative behavior in wolves, (2) this behavior almost certainly has a polygenic genetic architecture, and (3) samples sizes in recent genomic studies are small and therefore lacking sufficient power to detect the expected selection dynamics.

In sum, we argue that, to answer questions about the evolutionary foundation of dog behavior, research attention should refocus away from solely conducting direct species comparisons and include studies upon whether or not specific behavioral variation inherently exists among wolves. Identifying such instances has important ramifications upon expectations of how dog domestication proceeded.

Limitations of the Study

We present results based on a limited number of wolves. However, because our results provide proof of concept by demonstrating the presence of a specific behavioral trait in wolves, the number of tested individuals is not crucial for the interpretation of our results.

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2019.100811>.

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AUTHOR CONTRIBUTIONS

C.H.W. and H.T. conceived the study and arranged the testing. C.H.W. wrote the manuscript with input from H.T. Both authors approved of the final version of this manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

- Anderson, T.M., vonHoldt, B.M., Candille, S.I., Musiani, M., Greco, C., Stahler, D.R., Smith, D.W., Padhukasahasram, B., Randi, E., Leonard, J.A., et al. (2009). Molecular and evolutionary history of melanism in North American gray wolves. *Science* 323, 1339–1343.
- Axelsson, E., Ratnakumar, A., Arendt, M.-L., Maqbool, K., Webster, M.T., Perloski, M., Liberg, O., Arnemo, J.M., Hedhammar, Å., and Lindblad-Toh, K. (2014). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495, 360–364.
- Barrett, R., and Schluter, D. (2008). Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23, 38–44.
- Belyaev, D.K., Plyusnina, I.Z., and Trut, L.N. (1985). Domestication in the silver fox (*Vulpes fulvus* Desm): changes in physiological boundaries of the sensitive period of primary socialization. *Appl. Anim. Behav. Sci.* 13, 359–370.
- Candille, S.I., Kaelin, C.B., Cattanaach, B.M., Yu, B., Thompson, D.A., Nix, M.A., Kerns, J.A., Schmutz, S.M., Millhauser, G.L., and Barsh, G.S. (2007). A defensin mutation causes black coat color in domestic dogs. *Science* 318, 1418–1423.
- Darwin, C. (1868). *The Variation of Animals and Plants under Domestication* (John Murray, Albermarle Street).
- Dobney, K., and Larson, G. (2006). Genetics and animal domestication: new windows on an elusive process. *J. Zool.* 269, 261–271.
- Driscoll, C.A., Macdonald, D.W., and O'Brien, S.J. (2009). From wild animals to domestic pets, an evolutionary view of domestication. *Proc. Natl. Acad. Sci. U S A* 106, 9971–9978.
- Freedman, A.H., Gronau, I., Schweizer, R.M., Vecchy, D.O.-D., Han, E., Silva, P.M., Galaverni, M., Fan, Z., Marx, P., Lorente-Galdos, B., et al. (2014). Genome sequencing highlights the dynamic early history of dogs L. Andersson. *PLoS Genet.* 10, e1004016.
- Gácsi, M., Gyoöri, B., Virányi, Z., Kubinyi, E., Range, F., Belény, B., and Miklósi, Á. (2009). Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills C. Allen. *PLoS One* 4, e6584.
- Hall, N.J., Udell, M.A.R., Dorey, N.R., Walsh, A.L., and Wynne, C.D.L. (2011). Megachiropteran bats (pteropus) utilize human referential stimuli to locate hidden food. *J. Comp. Psychol.* 125, 341–346.
- Hansen Wheat, C., Fitzpatrick, J., Tapper, I., and Temrin, H. (2018). Wolf (*Canis lupus*) hybrids highlight the importance of human-directed play behavior during domestication of dogs (*Canis familiaris*). *J. Comp. Psychol.* 32, 373–381.

- Hare, B., Brown, M., Williamson, C., and Tomasello, M. (2002). The domestication of social cognition in dogs. *Science* 298, 1634–1636.
- Hare, B., and Tomasello, M. (2005). Human-like social skills in dogs? *Trends Cogn. Sci.* 9, 439–444.
- Horváth, Z., Dóka, A., and Miklósi, Á. (2008). Affiliative and disciplinary behavior of human handlers during play with their dog affects cortisol concentrations in opposite directions. *Horm. Behav.* 54, 107–114.
- Kukekova, A.V., Johnson, J.L., Xiang, X., Feng, S., Liu, S., Rando, H.M., Kharlamova, A.V., Herbeck, Y., Serdyukova, N.A., Xiong, Z., et al. (2018). Red fox genome assembly identifies genomic regions associated with tame and aggressive behaviours. *Nat. Ecol. Evol.* 2, 1479–1491.
- Larson, G., and Fuller, D.Q. (2014). The evolution of animal domestication. *Annu. Rev. Ecol. Evol. Syst.* 45, 115–136.
- 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., et al. (2014). Current perspectives and the future of domestication studies. *Proc. Natl. Acad. Sci. U S A* 111, 6139–6146.
- Maros, K., Gácsi, M., and Miklósi, Á. (2008). Comprehension of human pointing gestures in horses (*Equus caballus*). *Anim. Cogn.* 11, 457–466.
- Mehrkam, L.R., and Wynne, C.D.L. (2014). Behavioral differences among breeds of domestic dogs (*Canis lupus familiaris*): current status of the science. *Appl. Anim. Behav. Sci.* 155, 12–27.
- Melotti, L., Bailoo, J., Murphy, E., Burman, O., and Wurbe, H. (2014). Play in rats: association across contexts and types, and analysis of structure. *Anim. Behav. Cogn.* 1, 489–501.
- Mertens, C., and Turner, D.C. (2015). Experimental analysis of human-cat interactions during first encounters. *Anthrozoös* 2, 83–97.
- Messer, P.W., and Petrov, D.A. (2013). Population genomics of rapid adaptation by soft selective sweeps. *Trends Ecol. Evol.* 28, 659–669.
- Miklósi, Á., and Topal, J. (2013). What does it take to become “best friends?” Evolutionary changes in canine social competence. *Trends Cogn. Sci.* 17, 287–294.
- Miklósi, Á., Kubinyi, E., Topál, J., Gácsi, M., Virányi, Z., and Csányi, V. (2003). A simple reason for a big difference. *Curr. Biol.* 13, 763–766.
- Nawroth, C., Ebersbach, M., and Borell von, E. (2013). Juvenile domestic pigs (*Sus scrofa domestica*) use human-given cues in an object choice task. *Anim. Cogn.* 17, 701–713.
- Ostrander, E.A., and Wayne, R.K. (2005). The canine genome. *Genome Res.* 15, 1706–1716.
- Parker, H.G., vonholdt, B.M., Quignon, P., Margulies, E.H., Shao, S., Mosher, D.S., Spady, T.C., Elkhahoun, A., Cargill, M., Jones, P.G., et al. (2009). An expressed fgf4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. *Science* 325, 995–998.
- Pendleton, A.L., Shen, F., Taravella, A.M., Emery, S., Veeramah, K.R., Boyko, A.R., and Kidd, J.M. (2018). Comparison of village dog and wolf genomes highlights the role of the neural crest in dog domestication. *BMC Biol.* 16, 64.
- Pritchard, J.K., Pickrell, J.K., and Coop, G. (2010). The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr. Biol.* 20, R208–R215.
- Rooney, N.J., and Bradshaw, J.W.S. (2002). An experimental study of the effects of play upon the dog-human relationship. *Appl. Anim. Behav. Sci.* 75, 161–176.
- Rooney, N.J., Bradshaw, J.W.S., and Robinson, I.H. (2000). A comparison of dog–dog and dog–human play behaviour. *Appl. Anim. Behav. Sci.* 66, 235–248.
- Rooney, N.J., Bradshaw, J.W.S., and Robinson, I.H. (2001). Do dogs respond to play signals given by humans? *Anim. Behav.* 61, 715–722.
- Schoenebeck, J.J., Hutchinson, S.A., Byers, A., Beale, H.C., Carrington, B., Faden, D.L., Rimbault, M., Decker, B., Kidd, J.M., Sood, R., and Boyko, A.R. (2012). Variation of BMP3 contributes to dog breed skull diversity. *T. Leeb. PLoS Genet.* 8, e1002849.
- Svartberg, K. (2006). Breed-typical behaviour in dogs—historical remnants or recent constructs? *Appl. Anim. Behav. Sci.* 96, 293–313.
- Topál, J., Gergely, G., Erdőhegyi, Á., Csibra, G., and Miklósi, Á. (2009). Differential sensitivity to human communication in dogs, wolves and human infants. *Science* 325, 1269–1272.
- Tóth, L., Gácsi, M., Topál, J., and Miklósi, Á. (2008). Playing styles and possible causative factors in dogs’ behaviour when playing with humans. *Appl. Anim. Behav. Sci.* 114, 473–484.
- Trut, L., Oskina, I., and Kharlamova, A. (2009). Animal evolution during domestication: the domesticated fox as a model. *Bioessays* 31, 349–360.
- Udell, M.A.R., Spencer, J.M., Dorey, N.R., and Wynne, C.D.L. (2012). Human-socialized wolves follow diverse human gestures... and they may not be alone. *Int. J. Comp. Psychol.* 25, 97–177.
- Udell, M.A.R., Dorey, N.R., and Wynne, C.D.L. (2008). Wolves outperform dogs in following human social cues. *Anim. Behav.* 76, 1767–1773.
- von Bayern, A.M.P., and Emery, N.J. (2009). Jackdaws respond to human attentional states and communicative cues in different contexts. *Curr. Biol.* 19, 602–606.

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Supplemental Information

Intrinsic Ball Retrieving in Wolf Puppies Suggests Standing Ancestral Variation for Human-Directed Play Behavior

Christina Hansen Wheat and Hans Temrin

Supplemental Information

Intrinsic ball retrieving in wolf puppies suggests standing ancestral variation for human-directed play behaviour

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Transparent Methods

a) Study animals

From 2014 to 2016 we hand-raised three litters of European grey wolves (N = 13) at Tovetorp Zoological Research Station, Stockholm University, Sweden. The wolf litters from 2014, three females and two males, and 2015, two males, were full siblings. The 2016 wolf litter consisted of four males and two females and was not related to the wolf litters from 2014 and 2015. Hand-raising was initiated from the age of 10 days, before eye opening, for all litters. By choosing a hand-raising set-up, we were able to minimize environmental bias, including maternal effects, which is well-documented to affect the development of behavioural patterns (Bray et al. 2017; Clark & Galef 1982; Wilsson & Sundgren 1998). Wolves were raised within litters and extensively socialized, which included 24-hour presence of human caregivers for the first eight weeks. All wolves were reared under standardized conditions across all three years. Hand-rearing was initiated in identical indoor rooms and at the age of five weeks the wolves were given access to smaller roofed outdoor enclosures. After a habituation period of one week, the wolves were given additional access to a larger fenced grass enclosure at six weeks of age. Thereafter the wolves had free access to the indoor room and the two enclosures during the day and access to the indoor room and the roofed enclosure during the night. Behavioural observations began at 10 days of age and behavioural testing was initiated at 6 weeks of age. Hand-raising, testing procedures and exposure to the new environments were standardized over all three years, which included the implementation of rules to assure that rearing was standardized across all caregivers. This included that wolves were never disciplined or trained. Both male and female hand-raisers were socializing the wolves across all three years. Wolves never met strangers until their vaccination program was completed at eight weeks of age and, importantly, not until the completion of the test in which the observations of this study were recorded. Behavioural testing prior to eight weeks of age did not include other people than the caregivers.

b) Behavioural sampling

Wolves were tested in the Puppy Mental Assessment (PMA) at eight weeks of age. The PMA is a standardized behavioural test battery developed by the Swedish Working Dog Association based on the need to offer dog breeders a standardized test to describe puppy behaviour in specific situations. The results from the PMA can serve as a tool for dog breeders to choose suitable new owners for their puppies. As such, puppies are tested before they leave the breeder at seven to nine weeks of age. The PMA consists of 42 standardized tests situations covering behaviours in four main groups: 1) Social play with a stranger, here the puppy assessor, 2) Object play and object interest 3) Social comfortableness and fearfulness and 4) Interest in

strangers, here the puppy assessor, including greeting. The puppy is tested in a novel room or an enclosure. The PMA starts with the owner or other familiar person (in this study CHW or HT) placing the puppy in the middle of the test room, in which the puppy assessor is already present (but neutral), and then leaving the room swiftly. The whole test takes approximately 10-15 minutes. The subtest in which our observations occurred is related to social play and cooperation with a stranger. In this test the puppy assessor throws a tennis ball across the room and gives the puppy the opportunity to chase after and play with the ball, before she encourages the puppy to cooperate with her by retrieving the ball. The test is repeated three consecutive times. For the purpose of standardization across years, the same unfamiliar female puppy assessor conducted the tests all three years. Unlike dogs, wolves do not seem to generalize familiarity with human hand-raisers to strangers (Zimen 1987; Lord 2013) and the use of an unfamiliar person, and not a caregiver, as the puppy assessor in the test further served to eliminate potential bias caused by familiarity between puppy assessor and wolf.

c) Behavioural scoring

Retrieving and cooperation was measured by the puppy's willingness to return the ball to the puppy assessor upon encouragement and was scored on a 1 to 5 scale (Table 1). Two trained puppy assessors scored the puppies' behaviour independently. The puppy assessor conducting the test scored the behaviour directly during the test and subsequently another puppy assessor, who had no prior knowledge of the puppies, scored the behaviour from video recordings of the test. The agreement between the two puppy assessors was 100%.

Ethical statement

Daily care and all experiments were performed in accordance with relevant guidelines and regulations under national Swedish Law. The experimental protocols in this study were approved by the Ethical Committee in Uppsala, Sweden (approval number: C72/14). Facilities and daily care routines were approved by the Swedish National Board of Agriculture (approval number: 5.2.18-12309/13). All wolves were born in animal parks and CITES certified with at least F2 status.

Supplemental references

Bray, E.E. et al., 2017. Characterizing Early Maternal Style in a Population of Guide Dogs. *Frontiers in Psychology*, 8, p.1218.

Clark, M.M. & Galef, B.G., Jr, 1982. Environmental Effects on the Ontogeny of Exploratory and Escape Behaviors of Mongolian Gerbils. *Developmental Psychobiology*, 15(2), pp.121–129.

Lord, K., 2013. A Comparison of the Sensory Development of Wolves (*Canis lupus lupus*) and Dogs (*Canis lupus familiaris*) D. Zeh, ed. *Ethology*, 119(2), pp.110–120.

Wilsson, E. & Sundgren, P.-E., 1998. Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. *Applied animal behaviour science*, 56, pp.245–254.

Zimen, E., 1987. *Ontogeny of approach and flight behavior towards humans in wolves, poodles and wolf-poodle hybrids* H. Frank, ed., Dr. W. Junk Publishers, Dordrecht.