

1 Intrinsically ball retrieving wolf puppies reveal standing ancestral
2 variation for human-directed play behaviour

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7

8 **Abstract**

9 Domestication dramatically alters phenotypes. Standing variation among ancestral
10 populations often drives phenotypic change during domestication, but some changes are
11 caused by novel mutations. Dogs (*Canis familiaris*) engage in interspecific play with humans
12 and it has specifically been suggested that the ability to interpret social-communicative
13 behaviour expressed by humans is a novel dog-specific skill. Thus, wolves (*Canis lupus*) are
14 not expected to engage in interspecific play with a human based on social-communicative
15 cues. Here we report the observation of three eight week-old wolf puppies spontaneously
16 responding to social-communicative behaviours from a stranger by retrieving a ball. This
17 unexpected and novel observation has significant implications for our understanding and
18 expectations of the genetic foundations of dog behaviour. Importantly, our observations
19 indicate that behavioural responses to human social-communicative cues are not unique to
20 dogs. This suggests that, while probably rare, standing variation in the expression of human-
21 directed behaviour in ancestral populations could have been an important target for early
22 selective pressures exerted during dog domestication.

23

24 *Key words: Domestication, interspecific cooperation, dogs, wolves, playfulness, social-*
25 *communicative behaviour.*

26 **Introduction**

27 Domesticated animals express dramatic phenotypic alterations compared to their ancestral
28 species [1,2]. While phenotypic change can be attributed to novel mutations, a growing body
29 of evidence suggest that evolutionary change relies heavily upon standing genetic variation
30 [3,4]. Indeed, though few novel mutations with large effects account for some phenotypic
31 differences between domestic and ancestral populations [4,5], animal domestication was
32 likely initiated by selection on standing genetic variation within ancestral populations [4]. The
33 potential for domestic phenotypes to derive from existing variation has been well
34 demonstrated in the farm fox project [6,7], where strong selection regimes based on observed
35 variation in the behavioural trait tameness (i.e. reduced aggression and increased docility)
36 among pre-selection foxes brought about rapid occurrence of classic morphological
37 phenotypes associated with domestication. Knowing whether the basis for traits selected upon
38 during early domestication are variants from ancestral populations or unique mutations arising
39 during domestication is central to developing our understanding of the domestication process.
40 For instance, wild species expressing variation for the trait tameness are arguably more likely
41 to be successfully domesticated compared to species that do not [8]. Therefore, disentangling
42 whether phenotypic change in domesticates is caused by novel mutations or selection on
43 standing ancestral variation is important if we are to advance our understanding of the
44 domestication process and its generalities across species.

45

46 The dog (*Canis familiaris*), which was domesticated from the grey wolf (*Canis lupus*) at least
47 15,000 years ago [2], show extreme phenotypic variation as a species. Present day dogs are
48 bred for highly breed-specific requirements for behaviour and morphology [9,10], and while a
49 significant amount of the resulting variation is believed to originate from standing genetic
50 variation in ancestral populations [11], novel mutations have had a significant impact during

51 breed formation [4]. For instance, black coat colour [12,13], chondrodysplasia (foreshortened
52 limbs [5]) and brachycephaly (pathologically short muzzle [14]) are traits that have occurred
53 in modern dogs through novel mutations. An additional example comes from a genome wide
54 analysis of genetic difference between dogs and wolves, which identified dogs as having an
55 increased copy number of the amylase locus (*AMY2B*), which was argued to be a novel
56 adaptation to a starch-rich diet in early-domesticated dogs [15]. However, investigation of a
57 wider range of individuals revealed standing variation in amylase copy numbers in wolves,
58 thereby shifting the *AMY2B* example from being a novel mutation important in domestication,
59 to yet another example of selection upon standing variation as an essential substrate for
60 domestication [16]. This critical distinction has important implications for hypothesizing how
61 domestication could have taken place. Thus, the *AMY2B* example illustrates the importance of
62 including enough observations to detect existing variation among wolves and thereby
63 avoiding over-interpreting the uniqueness of traits expressed in dogs.

64
65 While much progress has been made in studying the morphological and physiological
66 differences between wolves and dogs, understanding the basis and origins of behavioural
67 variation have proven more elusive [17]. One behavioural skill that has been suggested to be
68 novel in dogs is interspecific social competence [18-20]. Specifically, it has been posited that,
69 unlike wolves, dogs possess unique skills to interpret human cues [18] and that these skills
70 might have arisen after the domestication process from the grey wolf had been initiated
71 [20,21]. The ability to interpret human social cues has received considerable interest from
72 researchers comparing behaviour in dogs and wolves. However, due to substantial differences
73 in testing procedures, environmental factors and interpretation of results, consensus among
74 these studies is lacking [22-26]. Consequently, whether wolves have the ability to interpret
75 human social cues, or whether this is a novel dog-trait, remains unresolved.

76

77 Here we focus upon human-directed play behaviour, which has been reported in some
78 domesticated species [27,28], including dogs [29-32]. Dogs can interpret human play cues
79 and adjust their behavioural repertoires when playing with a human instead of a conspecific
80 [31,33]. Within a domestication context, wherein animals have been selected for greater
81 tolerance of and interactions with humans, interspecific human-directed play behaviour
82 represents a highly relevant behaviour to address. However, to date only one study exists
83 comparing human-directed playfulness in a domesticated species and its ancestral proxy
84 species [34], and studies on human-directed play behaviour in wolves have never been
85 attempted.

86

87 Here we report on the spontaneous expression of human-directed play behaviour, in the form
88 of ball retrieving for a stranger, in eight week old, hand-raised wolves. Our observations
89 occurred during a test in which puppies, with no prior training, are vocally encouraged to
90 retrieve a ball and thus respond to social-communicative behaviours from a human they had
91 never met before. Based on the existing literature, we expected that human-directed play
92 behaviour is a novel trait that occurred during the domestication of dogs and that wolves
93 therefore would not respond to interspecific social-communicative behaviours or engage in
94 human-directed play with a stranger.

95

96

97 **Materials and methods**

98 *a) Study animals*

99 From 2014 to 2016 we hand-raised three litters of European grey wolves (N = 13) at Tovetorp
100 Zoological Research Station, Stockholm University, Sweden. The wolf litters from 2014,

101 three females and two males, and 2015, two males, were full siblings. The 2016 wolf litter
102 consisted of four males and two females and was not related to the wolf litters from 2014 and
103 2015. Hand-raising was initiated from the age of 10 days, before eye opening, for all litters.
104 By choosing a hand-raising set-up, we were able to minimize environmental bias, including
105 maternal effects, which is well-documented to affect the development of behavioural patterns
106 [35-37]. Wolves were raised within litters and extensively socialized, which included 24-hour
107 presence of human caregivers for the first eight weeks. All wolves were reared under
108 standardized conditions across all three years. Hand-rearing was initiated in identical indoor
109 rooms and at the age of five weeks the wolves were given access to smaller roofed outdoor
110 enclosures. After a habituation period of one week, the wolves were given additional access to
111 a larger fenced grass enclosure at six weeks of age. Thereafter the wolves had free access to
112 the indoor room and the two enclosures during the day and access to the indoor room and the
113 roofed enclosure during the night. Behavioural observations began at 10 days of age and
114 behavioural testing was initiated at 6 weeks of age. Hand-raising, testing procedures and
115 exposure to the new environments were standardized over all three years, which included the
116 implementation of rules to assure that rearing was standardized across all caregivers. This
117 included that wolves were never disciplined or trained. Wolves never met strangers until their
118 vaccination program was completed at eight weeks of age and, importantly, not until the
119 completion of the test in which the observations of this study were recorded. Behavioural
120 testing prior to eight weeks of age did not include other people than the caregivers.

121

122 *b) Behavioural sampling*

123 Wolves were tested in the Puppy Mental Assessment (PMA) at eight weeks of age. The PMA
124 is a standardized behavioural test battery developed by the Swedish Working Dog Association
125 based on the need to offer dog breeders a standardized test to describe puppy behaviour in

126 specific situations. The results from the PMA can serve as a tool for dog breeders to choose
127 suitable new owners for their puppies. As such, puppies are tested before they leave the
128 breeder at seven to nine weeks of age. The PMA consists of 42 standardized tests situations
129 covering behaviours in four main groups: 1) Social play with a stranger, here the puppy
130 assessor, 2) Object play and object interest 3) Social comfortableness and fearfulness and 4)
131 Interest in strangers, here the puppy assessor, including greeting. The puppy is tested in a
132 novel room or an enclosure. The PMA starts with the owner or familiar person (in this study
133 CHW or HT) placing the puppy in the middle of the test room, in which the puppy assessor is
134 already present (but neutral), and then leaves the room swiftly. The whole test takes
135 approximately 10-15 minutes. The subtest in which our observations occurred is related to
136 social play with a stranger. In this test the puppy assessor throws a tennis ball across the room
137 and calls the puppy back, encouraging it to retrieve the ball. Retrieving and cooperation is
138 measured by the puppy's willingness to return the ball to the puppy assessor and is scored on
139 a 1 to 5 scale (Table 1). The test is repeated three consecutive times.

140

141 *Ethical statement*

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

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151 **Results**

152 Three wolves, all from the 2016 litter, fully retrieved the ball at least two times and one of
153 those wolves fully retrieved the ball all three times (Score: 5). On one occasion one of the
154 wolves fully retrieving the ball two times also played with the ball, but ignored the puppy
155 assessors call (score: 3). One wolf from the 2014 litter and one from the 2016 litter showed
156 some interest in playing with the ball on at least one trial but aborted (Score: 2). Eight wolves
157 (four from the 2014 litter, both from the 2015 litter and two from the 2016 litter) showed no
158 interest in the ball on any of the three trials (Score: 1).

159

160

161 **Discussion**

162 Here we provide the first empirical evidence that a behaviour thought unique to dogs, namely
163 interspecific play with a human based on social-communicative cues, actually exists in
164 wolves. Our finding is surprising given that the ability to interpret social-communicative
165 behaviour expressed by humans has been suggested to be a novel dog trait [18-21].
166 Importantly, our results suggest that, while probably rare, standing variation in the expression
167 of human-directed behaviour, including play, in ancestral populations could have been an
168 important target for early selective pressures exerted during dog domestication.

169

170 Our novel observations of three wolf puppies retrieving a ball are highly relevant for the on-
171 going discussion on the effect domestication has on behaviour and further have significant
172 implications for our understanding and expectations about the genetic foundations of the
173 behaviours in modern day dogs. Specifically, in relation to current attempts to reveal the
174 genomic basis of behavioural changes during domestication [16,38,39], our observations
175 indicate signatures of selection for human-directed behaviour in dogs are likely to be weak

176 and prone to false positives [*sensu lato* 40,41]. This is because 1) we must now consider that
177 selection likely acted upon standing variation in interspecific social-communicative behaviour
178 in wolves, 2) this behaviour almost certainly has a polygenic genetic architecture, and 3)
179 samples sizes in recent genomic studies are small and therefore lacking sufficient power to
180 detect the resulting expected selection dynamics.

181

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

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300 **Tables**

301

302 **Table 1. Behavioural scoring.** *Cooperation in the three consecutive retrieving tests is measured on a scale from*

303 *1 to 5, where 1 is no cooperation and 5 is full cooperation.*

Score	Behaviour
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

304

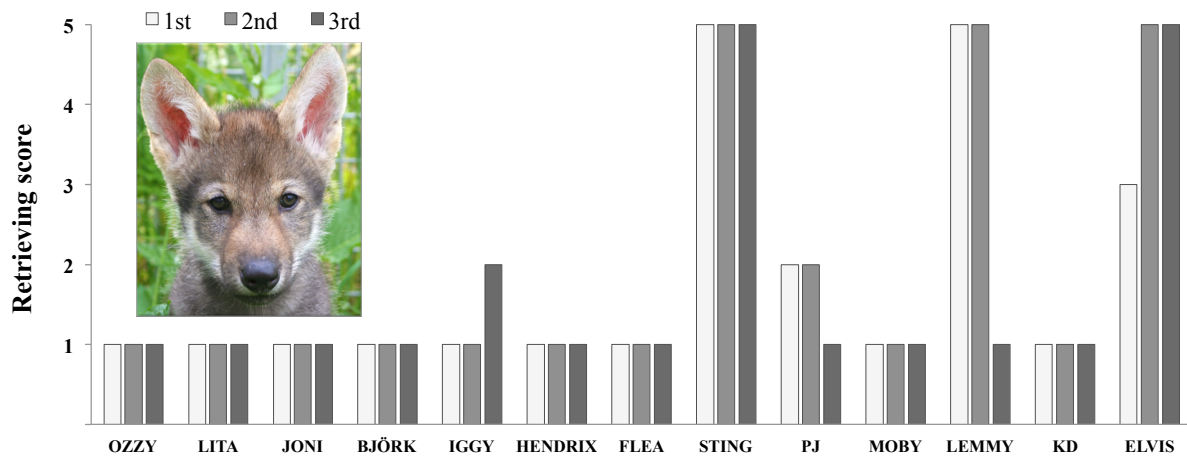
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308 **Figures**

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311

312 **Fig. 1. Behavioural scores.** Cooperation scores in the retrieving test 13 wolves on three consecutive trials
313 (shading from light to dark with the 1st trial being light, 2nd medium and 3rd dark). Behaviour is scores on a
314 scale from 1-5. Only scores 4 and 5 include partial or full retrieving, respectively. Photo credit: Christina
315 Hansen Wheat

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