

1 **Dogs, but not wolves, lose their sensitivity towards novelty** 2 **with age**

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4 Christina Hansen Wheat, Wouter van der Bijl & Hans Temrin

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6 *Department of Zoology, Stockholm University, SE-10691 Stockholm, Sweden*

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8

9 **Abstract**

10 Selection on behavioural traits holds a prominent role in the domestication of
11 animals. Specifically, a reduction of the fear response is considered a key component,
12 with domesticated animals expressing lower levels of fear towards novelty than their
13 wild counterparts. Previous work has suggested that this is caused by a delay in the
14 onset of fearful behaviour during early ontogeny in domesticated canids. However,
15 it remains unclear how the developmental timing of initial fear expression affects
16 fearfulness later in development. Here we present the first extended examination of
17 the development of fear behaviour in wolves and dogs, using repeated novel object
18 tests between six and 26 weeks of age. Contrary to expectations, fear of novelty did
19 not change in wolves with age, but dogs expressed decreased latency to approach a
20 novel object with age, resulting in a species difference at the end of the measured
21 period. Our results thereby suggest that differences in fear of novelty between
22 wolves and dogs are not caused by a domestication driven shift in the first onset of
23 fear response. Instead we suggest that differences in fear expression between wolves
24 and dogs are caused by a loss of sensitivity towards novelty with age in dogs.

25

26 **Key words:** *Fear, domestication, sensitive period, ontogeny, novel object test, Canis*

27

28 **Introduction**

29 Abundant evidence demonstrates how domesticated plants and animals express
30 dramatically altered phenotypes compared to their wild counterparts (1). For
31 animals, it is now clear that selection on behavioural traits alone had a prominent
32 role in domestication (2-4). Specifically, it has been demonstrated how selection
33 upon decreased fearfulness and aggression can lead to the myriad of morphological
34 and physiological alterations observed in domesticated animals (2,5). In wild
35 populations, fear is a key behaviour, as a timely and proper response to novelty, e.g.
36 flight response versus exploration, can have direct and large fitness consequences
37 (6,7). Appropriate fear responses are formed and modified throughout ontogeny,
38 during which juvenile animals gradually combine individual experience and social
39 information, thereby developing the ability to discriminate between threatening and
40 neutral stimuli (7-9). Domesticated animals express reduced reactivity towards novel
41 stimuli (10) and it is thus likely that altered selection pressures caused by
42 domestication have modified the ontogeny of fear related behaviours.

43

44 Ontogeny has been modified in several ways during domestication. Compared to
45 ancestral species, domesticated animals express altered developmental rates, a
46 phenomenon known as heterochrony (11-13), which have resulted in accelerated
47 and/or delayed onsets of various ontogenetic stages, such as earlier sexual
48 maturation and the retention of juvenile traits into adulthood (12,14-16).
49 Heterochrony has been suggested to affect behavioural ontogeny in domesticated
50 animals by prolonging the sensitive period (2,17-19), an important period during
51 behavioural development in which the juvenile animal is particularly sensitive to
52 imprint on and form social bonds with conspecifics (8,20-22). During the sensitive
53 period juvenile animals show increased exploratory behaviour, as they readily
54 approach novel stimuli and thereby learn about and socialize with their environment

55 (23). Importantly, in the context of the ontogeny of fear, the end of the sensitive
56 period manifests by progressive fear-related avoidance behaviour expressed as
57 increased fear and decreased exploration of novelty (2,21). A shift in the sensitive
58 period caused by domestication has been demonstrated in a long-term selection
59 study, in which juvenile foxes (*Vulpes vulpes*) from domesticated strains expressed a
60 delayed onset of fearful response, at 60-65 days of age, compared to the onset in non-
61 domesticated fox kits, at 40-45 days of age (2,5). While these findings suggest that the
62 basis for the quantifiable difference in fear between domesticated and non-
63 domesticated animals might arise already during early ontogeny, no study has
64 investigated the continued ontogenetic trajectory of fear behaviour after the initial
65 onset of the fearful response.

66

67 The domestic dog (*Canis familiaris*) is an excellent study species when addressing
68 questions about how domestication has affected the ontogeny of behaviour.
69 Domestication of the dog from the grey wolf (*Canis lupus*) occurred at least 15,000
70 years ago (1), making the dog the first domesticated species and with the ancestral
71 species extant, the opportunities for comparisons are ideal (24). Studies of
72 behavioural ontogeny in dogs have focused on the sensitive period, and the fear of
73 novelty is reported to manifest and continually increase in the dog puppy from eight
74 weeks of age onward (8,21,25,26). In wolves, consensus on when fear behaviour is
75 established is lacking, with the onset of fearful response reported to occur as varied
76 as four to eight weeks of age across studies (25,27-31). However, the majority of
77 these wolf studies were conducted over a short period of time and/or focused on
78 isolated individuals or single litters, limiting our ability to generalize from these
79 findings. Additionally, a recent study found that juvenile wolves explored novel
80 objects more than dogs at both six and eight weeks of age (32), thereby suggesting
81 that that wolves might not express fear towards novelty at an earlier age than dogs.

82 Thus, while adult wolves (33) and wolf-dog hybrids (34) are more fearful of novelty
83 than dogs, when this difference is established during ontogeny is not well resolved.
84 Therefore, extended studies of the continued development of fear after the onset of
85 fearful response is needed to further understand domestication driven changes in
86 behavioural ontogeny in canids.

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88 To investigate if behavioural differences in fear towards novelty differ between
89 wolves and dogs during the first six months of life, we here examine their
90 behavioural development by using repeated novel object tests. The novel object test
91 is an established method to quantify fear and exploration of novelty and has been
92 used on numerous species (32,33,35-37) We tested three litters of wolves (N = 13)
93 and two litters of dogs (N = 12), hand-raised under similar conditions at six, 10, 14,
94 18, 22 and 26 weeks of age, i.e. before sexual maturity, and varied the context by
95 using a new novel object in each of the six tests. Our overall goal was to test the
96 hypothesis that the sensitive period is prolonged in dogs, resulting in a delayed
97 onset of fearful response in dogs compared to wolves. Based on studies reporting
98 delayed onset of fear behaviour in domestic compared to ancestral species (2,38),
99 including dogs and wolves (22,31), we expected wolves to be more fearful compared
100 to dogs already at six weeks or, alternatively, at ten weeks of age. Furthermore, we
101 wished to investigate how different timing of the onset of fearful response affects the
102 continued behavioural development of fear in wolves and dogs. We predicted that
103 domestication has lowered the sensitivity to novelty in dogs (33,39), and dogs
104 therefore would express decreased fear towards the novel object compared to
105 wolves throughout the testing period.

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109 **Methods**

110 *a) Study animals*

111 During 2014 – 2016 two litters of Alaskan huskies (N = 12) and three litters of
112 European grey wolves (N = 13) were hand-raised and extensively socialized under
113 similar conditions from the age of 10 days at Stockholm University's Zoological
114 Research Station, Tovetorp. This set-up was chosen to minimize environmental bias,
115 including maternal effects, which is well-documented to affect the development of
116 behavioural patterns (40-42). Puppies were raised within litters and socialization
117 involved 24-hour presence of human caregivers for the first two months. From two
118 months of age, caregiver presence was decreased with a few hours a day until three
119 months of age and then further decreased during every other night at four months of
120 age. At six months of age, caregivers spent four to six hours with the puppies a day.
121 All wolf and dog litters were kept separate, but reared under standardized
122 conditions. From the age of 10 days to five weeks, puppies were reared in identical
123 indoor rooms and here after given access to smaller roofed outdoor enclosures. After
124 a week of habituation to the roofed outdoor enclosure, puppies were given access to
125 a larger fenced grass enclosure at six weeks of age. Hereafter the puppies had free
126 access to all three enclosures during the day and access to the indoor room and the
127 roofed enclosure during the night. When the puppies were three months old they
128 were moved to large outdoor enclosures (2,000 square meters), in which they
129 remained for the rest of the study period. We started behavioural observations at 10
130 days of age and behavioural testing was initiated at 6 weeks of age. Testing
131 procedures and exposure to the new environments were standardized over all three
132 years. As required by Swedish law, all hand-raisers were ethically certified and
133 trained to handle animals. Furthermore, rules were implemented to assure that
134 rearing was standardized across all caregivers. This included that puppies were
135 never disciplined, trained or forced to have contact with their caregivers. From the

136 age of eight weeks, puppies were gradually exposed to strangers through the fence
137 with the support of one or more human caregivers.

138

139 The dog litter from 2014 consisted of five males and one female and the 2015 litter of
140 three males and three females. The two dog litters were unrelated. The wolf litters
141 from 2014, three females and two males, and 2015, two males, were full siblings. The
142 third wolf litter from 2016 consisted of four males and two females and was
143 unrelated to the wolf litters from 2014 and 2015.

144

145 *b) Behavioural sampling*

146 To ensure that the puppies' senses were fully developed by the time of the first test
147 we conducted the first novel object test at six weeks of age (31). Novel object tests
148 were hereafter performed on a monthly basis at 10, 14, 18, 22 and 26 weeks of age.
149 The puppies were tested in a familiar room (4x4 meters) in which a novel object was
150 present, placed opposite of where the puppy would enter the room, approximately
151 four meters away. Puppies were lead into the room by a caregiver, who quickly left
152 the room and closed the door. The duration of a trial was 10 minutes and trials were
153 always monitored by CHW. Some trials (n = 11) were stopped prematurely because
154 the novel object was destroyed. All test were filmed with two mounted GoPro
155 cameras (model 3-4, GoPro Inc.) on opposite sides of the room.

156

157 *c) Novel objects*

158 A different novel object was used at each age in order to avoid habituation. Objects
159 were handled as little as possible and always with freshly washed hands to avoid
160 food smells transferring to the objects and possibly affecting the puppy's behaviour
161 towards the object. Novel objects were chosen based on previous studies with
162 wolves and dogs (Moretti et al. 2015, Marshall-Pescini et al. 2017) and varied on

163 several scales, such as shape, sound and movement, and included at six weeks: a
164 rolled up mattress, 10 weeks: a wheelbarrow (up-side down), 14 weeks: a mirror
165 mounted to the wall, 18 weeks: a stuffed wolverine toy, 22 weeks: a moving
166 mechanical dog and 24 weeks: a moving sheet (attached to a string).

167

168 *d) Behavioural scoring*

169 Behavioural scoring was carried out using the software BORIS v. 5.1.3. (43) based on
170 an ethogram (Table 1). Behaviours were logged both as frequencies and durations
171 (i.e. state behaviours). Similar to previous studies (Moretti et al., 2015), latency to
172 approach the novel object was measured as the duration from test start to the time
173 the puppy came within 1 meters distance of the novel object (Table S1). Latency to
174 make contact with the novel object was measured as the duration to make physical
175 contact with the novel object for the first time after the latency to approach (Table
176 S1). Behaviours were scored in a non-overlapping way, with prioritization of
177 behaviours related to the novel object, i.e. if the puppy was looking at the novel
178 object while walking around the test room this was scored as *looking at novel object*.

179

180 **Table 1. Ethogram.** Behaviours scored during novel object tests. Behaviours were scored in a non-overlapping
181 way, with prioritization of behaviours related to the novel object. Latency times were measured regardless of the
182 behaviour performed.

181	Behaviour	Code	Description
182	Active behaviour	A	Moving around in, or interacting with, the test room with no attention to the novel object
183	Investigating novel object	I	Sniffing novel object or looking novel object form less than 1 meter
184	Latency to approach novel object	-	Time delay to approach the novel object with less than 1 meter
185	Latency make contact with novel object	-	Time delay to physically touch the novel object (sniffing)
186	Looking at novel object	L	Looking at novel object from a distance of more than 1 meter
187	Manipulating novel object	M	Pawing, nosing, scratching, biting, carrying, standing on novel object
188	Passive behaviour	P	Standing, sitting or lying passively with no attention to the novel object or the test room, including by the door

189 Because there were cases where the total duration of the test was less than 10
190 minutes, the total test duration was included for use in further analysis (Table S2 and
191 S3). In cases where a puppy did not approach the novel object, the trial duration was
192 used as a measure of latency and in cases where the puppy did not make contact
193 with the novel object this was recorded as a missing value. Inter-rater reliability was
194 calculated using Cohen's kappa and was considered good with a value of 87.4%.

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196

197 *e) Statistical methods*

198 We tested for species effects in each behaviour by fitting linear mixed models, with
199 either latency or the time spent on a behaviour as the dependent variable. The fixed
200 effects of interest were species, age, their interaction and sex. Additionally, for the
201 models of time spent, we controlled for variation in the duration of each trial by
202 including duration as a covariate. To account for the repeated measures of
203 individuals and the non-independence of individuals from the same litter, we
204 included random intercepts for both factors. The full model in lme4 syntax: $y \sim$
205 $\text{species} * \text{age} + \text{sex} + \text{duration} + (1|\text{individual}) + (1|\text{litter})$. Models were then
206 reduced by backwards model selection using AIC (cut-off $\Delta\text{AIC} > 2$, Table S4),
207 where the parameters for species, duration and the random effects were always
208 maintained. Both latencies were \log_{10} transformed, and the time spent looking,
209 investigating and manipulating the novel object were log transformed after adding
210 1, in order to fulfil the assumption of normality in the model residuals. We centred
211 the age variable to aid interpretation of the species effect in case of an interaction.
212 When the interaction was retained in the model, we additionally fitted a model
213 where age was a discrete variable, and used that to perform post-hoc tests for species
214 differences at each age (Table S5). All p-values were obtained using Satterthwaite's

215 approximation of denominator degrees of freedom. Post-hoc p-values were
216 corrected for multiple comparisons using the Holm method.

217

218 All statistical analyses were performed in R (v3.4.3, R Core Team 2016), with mixed
219 effects models fitted using *lme4* v. 1.1-15 (44), Satterwaithe's approximation from
220 *lmerTest* v. 2.0-36 (45) and post-hoc testing using *emmeans* v. 1.1.2 (46).

221

222 *d) Ethical note*

223 Daily care and testing were performed in accordance with ethical legislation under
224 Swedish Law. This study was approved by the Ethical Committee in Uppsala
225 (C72/14), and our facilities and daily care routines were approved by the Swedish
226 Board of Agriculture (5.2.18-12309/13).

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228

229 **Results**

230 *Latency measures*

231 We found that wolves and dogs developed differently in latency to approach the
232 novel object within 1 meter, where dogs expressed a larger reduction in latency with
233 age compared to wolves ($t = 2.35$, $df = 120.046$, $p = 0.02$, Table 2, Figure 1a and 2).
234 Dogs significantly decreased their latency with time, while wolves did not (see table
235 S6 for slopes per species), resulting in dogs expressing significantly lowered latency
236 to approach at 26 weeks compared to wolves ($t = -3.131$, $df = 18.666$, $p = 0.006$,
237 $p_{\text{adjusted}} = 0.034$, Table S5). At younger ages we failed to detect significant differences
238 in latency to approach the novel object between dogs and wolves (Table S5). For the
239 latency to make contact with the novel object, we found no differences in wolves and
240 dogs ($t = 1.931$, $df = 2.16$, $p = 0.186$, Table 2, Figure 1b and 2), neither did we find
241 evidence of sex differences in either species.

242

Table 2. Model summary. Results for the best fitted model of repeated measures, with dogs as the reference, on 1) Latency to approach the novel object, 2) Latency to make contact with the novel object, 3) Looking at novel object (NO), 4) Investigating novel object, 5) Manipulating novel object, 6) Active behaviour and 7) Passive behaviour. Estimate, standard error, degrees of freedom, *t*-value and *p*-value are given. Significant *p*-values are marked in bold italic.

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	<i>Behaviour</i>	<i>Term</i>	<i>Estimate</i>	<i>Std. error</i>	<i>df</i>	<i>t</i>	<i>p</i>
245	Latency, approach	<i>(Intercept)</i>	0.875	0.133	2.240	6.576	0.017
		<i>species</i>	0.312	0.180	2.568	1.737	0.196
246		<i>age</i>	-0.055	0.011	118.419	-5.067	<0.0001
		<i>species:age</i>	0.036	0.015	120.046	2.350	0.020
247	Latency, contact	<i>(Intercept)</i>	0.253	0.094	1.821	2.704	0.126
		<i>species</i>	0.246	0.129	2.164	1.913	0.186
248	Looking at NO	<i>(Intercept)</i>	2.136	0.827	94.332	2.581	0.011
249		<i>species</i>	0.037	0.354	2.939	0.104	0.924
		<i>age</i>	0.121	0.021	117.899	5.848	<0.0001
250		<i>duration</i>	0.001	0.001	124.417	0.426	0.671
		<i>species:age</i>	-0.064	0.031	120.667	-2.058	0.042
251	Invetsigating NO	<i>(Intercept)</i>	0.416	0.717	126.889	0.580	0.563
		<i>species</i>	0.141	0.234	3.493	0.601	0.585
252		<i>age</i>	-0.119	0.019	138.727	-6.384	<0.0001
		<i>duration</i>	0.004	0.001	140.535	3.834	0.0002
253	Manipulating NO	<i>(Intercept)</i>	2.812	1.078	90.449	2.610	0.011
254		<i>species</i>	0.494	0.494	2.915	1.000	0.393
		<i>duration</i>	-0.001	0.002	141.262	-0.786	0.433
255	Active behavior	<i>(Intercept)</i>	-145.265	57.693	97.323	-2.518	0.013
256		<i>species</i>	102.697	24.105	2.977	4.260	0.024
		<i>age</i>	2.587	1.176	122.299	2.200	0.030
257		<i>duration</i>	0.598	0.090	127.554	6.644	<0.0001
258	Passive behavior	<i>(Intercept)</i>	152.645	54.263	25.688	2.813	0.009
		<i>species</i>	-91.478	39.977	3.003	-2.288	0.106
259		<i>age</i>	-4.106	0.962	121.459	-4.268	<0.0001
	<i>duration</i>	0.149	0.074	127.572	2.029	0.045	

260

261 *Behaviours related to the novel object*

262 We found that wolves and dogs developed differently in looking at the novel object
 263 from a distance ($t = -2.058$, $df = 120.667$, $p = 0.042$, Table 2, Figure 1e), but no such
 264 differences were detected in the post hoc tests (Table S5). While both wolves and
 265 dogs increased their time spent looking at the novel object from a distance with age
 266 ($t = 5.848$, $df = 117.899$, $p < 0.001$, Table 2, Figure 1e), dogs expressed a stronger effect
 267 of age than wolves (Figure 1e, Table S6).

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Figure 1. Dog – wolf comparisons. Boxplots shows behavioral scores during a novel object test, 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 $\log(x + 1)$.

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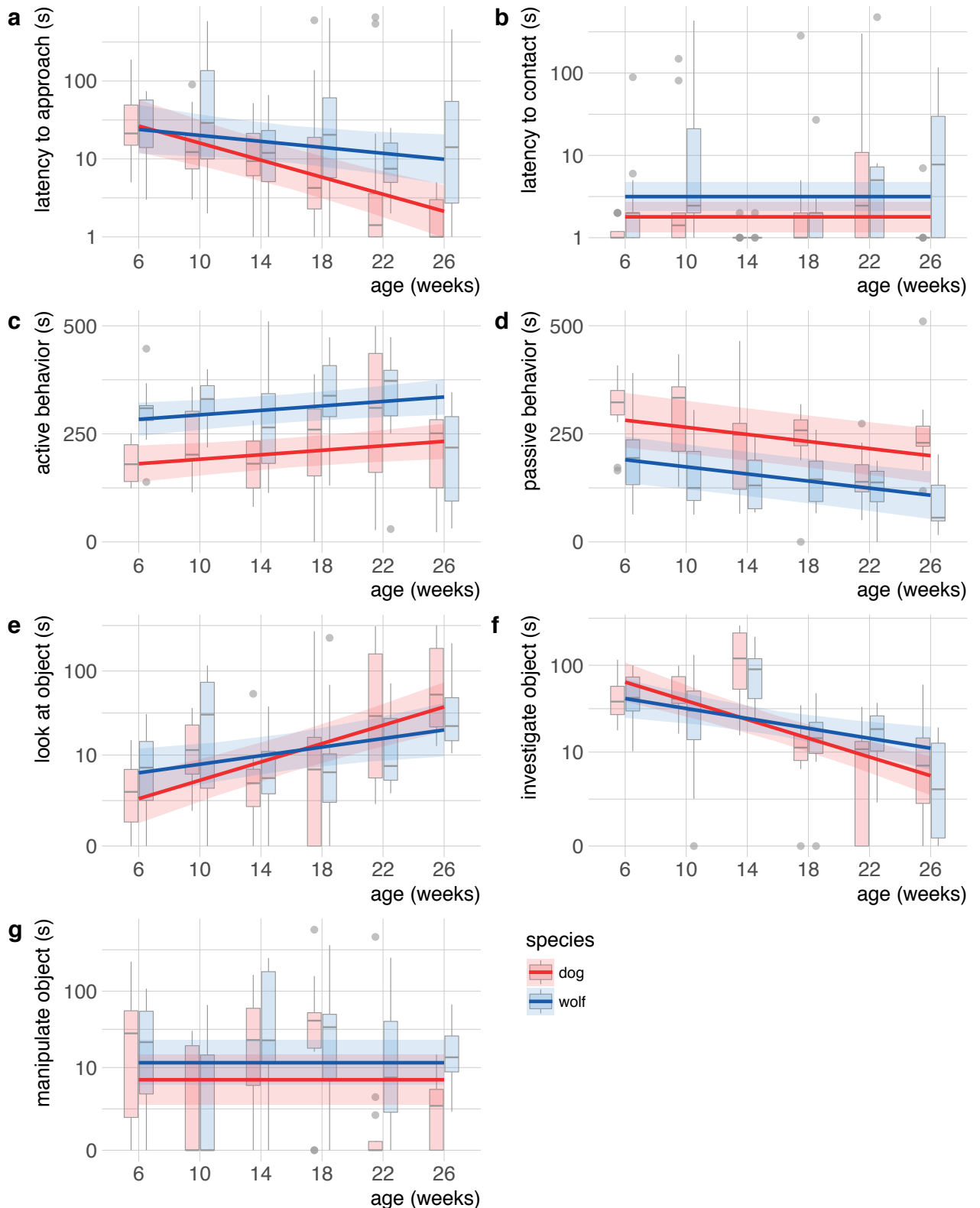
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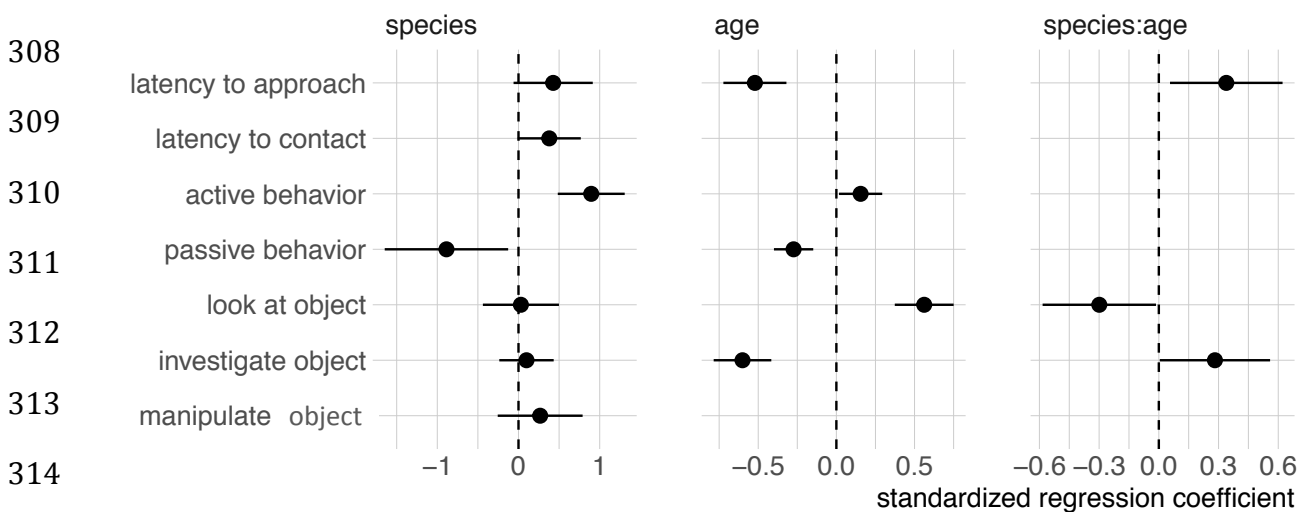


295 Wolves and dogs also showed different developmental trajectories for the time spent
 296 investigating the novel object ($t = 1.994$, $df = 139.315$, $p = 0.048$, Table 2, Figure 1f and
 297 2). Post-hoc tests revealed that wolves investigated the novel object for longer at 22
 298 weeks than dogs ($t = -2.831$, $df = 28.029$, $p = 0.008$, $p_{\text{adjusted}} = 0.051$, Figure 1f, Table
 299 S5). The significant interaction between species and age in investigating the novel
 300 object again consisted of stronger effect of age in dogs than in wolves (Figure 1f,
 301 Table S6), but with an overall decrease with age in both species ($t = -6.384$, $df =$
 302 138.727 , $p < 0.001$, Table 2, Figure 1f). We found that wolves and dogs developed
 303 similarly in time spent manipulating the novel object (Table 2, Figure 1g). We found
 304 no evidence of sex differences.

305

306 **Figure 2. Standardized regression coefficients.** Standardized regression coefficients, for the best model for
 each behavior, selected by AIC. Ranges indicate confidence intervals, computed using the likelihood profile.
 307 Missing estimates indicate that the term was not included in the best model.

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317 *Behaviours not related to the novel object*

318 We found that both species increased time spent on active behaviour with age ($t =$
 319 2.2 , $df = 122.362$, $p = 0.03$), with wolves expressing higher levels of activity than dogs
 320 ($t = 4.26$, $df = 2.977$, $p = 0.024$, Table 2, Figure 1c and 2, Table S5). Passive behaviour
 321 decreased with age in both wolves and dogs ($t = -4.268$, $df = 121.140$, $p < 0.001$, Table

322 2, Figure 1d), and while dogs appeared more passive than wolves the species
323 differences was not significant. We found no evidence of sex differences.

324

325

326 **Discussion**

327 Decreased expression of fear is considered a key behavioural alteration in
328 domesticated animals, and evidence suggests that domestication drives altered
329 developmental rates delaying the initial onset of fear response (2). However, while
330 previous studies in wolves and dogs have focused exclusively on the timing of the
331 initial onset of fear (23,30,31), it has been unclear if and how a developmental shift
332 during early ontogeny, caused by domestication, affects the continued development
333 and expression of fear in either species. Here we present the first extended
334 examination of the development of fear behaviour within the juvenile period in
335 wolves and dogs. Contrary to expectation, we found no evidence in support of a
336 delayed onset of fear response in dogs compared to wolves during early stages of
337 development. Instead we found that dogs strongly reduced their fear response to a
338 novel object in the period between six and 26 weeks of age. Critically, wolves did not
339 differ in their fear response towards novelty over time, and the detected species
340 difference was caused solely by a progressive reduced fear response in dogs.
341 Furthermore, dogs and wolves did on average not differ in their interaction with the
342 novel object. Together our results suggest that species differences in fear of novelty
343 are not caused by a domestication driven shift in the first onset of fear response.
344 Instead, we suggest that a loss of sensitivity towards novelty with increasing age in
345 dogs causes the difference in fear expression towards novelty in wolves and dogs.

346

347 Fearfulness has previously been quantified by the latency to approach and explore
348 novelty, and novel stimuli such as objects, arenas and people have been used to

349 detect the timing of the initial onset of fear response in both wolves and dogs
350 (2,8,21,23,25,31) However, while there is a general expectation that domestication has
351 caused a delay in the sensitive period in dogs, resulting in later onset of fear
352 behaviour in dogs compared to wolves (8,22,30,31,47), we detected no species
353 differences in fear expression during early development. This finding is in
354 agreement with a recent study comparing exploration of novelty in six and eight
355 weeks old wolves and dogs, which found no species differences in the latency to
356 make contact with a novel object (32). Yet, it has been reported that adult wolves
357 express increased latency to make contact to a novel object compared to dogs (33),
358 thereby indicating that species differences in fear expression might arise later in
359 development than previously thought. Thus, our finding that a species difference in
360 latency to approach a novel object occurred at 26 weeks of age represents the first
361 indication of when a quantifiable difference in fear towards novelty arises in wolves
362 and dogs. We do, however, caution against an overly strong confidence in the exact
363 timing of species differences occurring at 26 weeks. It is possible that the difference
364 emerges in the weeks prior, but that the current sample size is insufficient for
365 detection. However, it is clear that a difference between species progressively
366 develops towards the later end of the time period measured here, and that we have
367 captured the transition from equal fear towards novelty to a clear species difference.
368 Importantly, the species difference in fear towards novelty did not occur because
369 wolves became more fearful with age, as expected, but rather because dogs
370 decreased their time to approach the novel object. Specifically, we detected no
371 increased expression of fear towards novelty in neither wolves nor dogs throughout
372 the study period, and notably wolves did not change their latency to approach the
373 novel object throughout with age. This indicates that the development of fear
374 response in wolves and dogs follows different trajectories, with dogs, but not
375 wolves, losing their sensitivity towards novelty with age.

376 The species difference we found in the latency to approach the novel object is not
377 clearly reflected in differences in interaction with the same novel object. While fear
378 of novelty was expressed immediately, through a delayed approach, once the novel
379 object was approached this initial fearfulness appears to no longer affect behavioural
380 responses, and thus wolves and dogs did not differ in their latency to make contact
381 with or interact with the novel object. While the latency to approach the novel object
382 and the time spent being active and passive while in the test room showed consistent
383 linear development over time in both wolves and dogs, the pattern in looking at,
384 investigating and manipulating the novel object appeared variable across trials. This
385 variability was most likely caused by the different novel objects that were used in the
386 study, i.e. behaviours that are more closely related to the object itself show more
387 variability across tests. For example, the stuffed wolverine toy clearly provided more
388 incentive for manipulation than the upside-down wheelbarrow. It is possible that
389 this increased variance may have prohibited detection of additional species
390 differences in behavioural measures directly related to the novel object, such as
391 increased exploration and manipulation of novel objects as reported in both juvenile
392 and adult wolves compared to dogs (Moretti et al., 2015, Marshall-Pescini et al. 2017)
393
394 Wolves develop physically faster than dogs (48), and it has been suggested that
395 wolves express increased activity at an earlier age than dogs due to this difference in
396 developmental pace of motor patterns (32,48). However, while we do find a species
397 difference in how much time is spent on active behaviour during tests, this species
398 difference is consistent across age and not restricted to early ontogeny alone. This
399 indicates that wolves, on a general scale, are more active than dogs. While it cannot
400 be ruled out that active behaviour is affected by the presence of a novel object, it is a
401 less likely explanation for our findings, as we measured behaviours in a non-
402 overlapping way with priority of behaviours related to the novel object. Thus, the

403 measurement of activity does not include looking at, manipulating or approaching
404 the novel object, but only time spent on active behaviour with no attention to the
405 novel object. Instead the higher activity in wolves might reflect an increased
406 reactivity of being separated from littermates and being confined in the test room
407 compared to dogs.

408

409 Domestication has caused a general acceleration of sexual maturity in animals (12),
410 and earlier sexual maturation in dogs (11,14) could explain the steeper behavioural
411 change observed in dogs compared to wolves across some of the behaviours related
412 to the novel object in our study. However, while reproduction in wild living wolf
413 packs is restricted to the breeding couple, it is currently unclear if the lack of sexual
414 activity in non-reproducing pack members is caused by delayed sexual maturity,
415 behavioural suppression or restricted access to nutrition (49-51). Nevertheless, it has
416 been demonstrated that captive wolves removed from social constraints sexually
417 mature as early as nine months of age (50). Thus, it is unclear if we should expect
418 behavioural ontogeny to be affected by a shift in developmental pace caused by
419 earlier sexual maturity when comparing wolves and dogs living in captive, non-
420 reproductive groups. Our study was conducted before sexual maturity occurred in
421 either wolves or dogs and as we found no effect of sex on the expression of
422 behaviour, we suggest that the steeper development of some behaviours in dogs are
423 instead related to the loss of sensitivity towards novelty.

424

425 In conclusion, our study shows that wolves and dogs do not differ in their fear
426 towards novelty before late in the juvenile phase. Importantly, the species difference
427 does not occur because wolves become more fearful with age, but because dogs
428 become less fearful with age. These findings have general implications for our
429 interpretation of how domestication has shaped behavioural ontogeny. We suggest

430 that future studies quantify fear related behaviour on a long-term scale to increase
431 our understanding of the behavioural trajectory of fear through different
432 developmental stages.

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