

1 Assessment of attachment behaviour to human caregivers in wolf pups (*Canis lupus*
2 *lupus*).

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19 **Abstract**

20 Previous research suggested that 16-week old dog pups, but not wolf pups,
21 show attachment behaviour to a human caregiver. Attachment to a caregiver in dog
22 pups has been demonstrated by differential responding to a caregiver compared to
23 a stranger in the Ainsworth Strange Situation Test. We show here that 3-7 week old
24 wolf pups also show attachment-like behaviour to a human caregiver as measured
25 by preferential proximity seeking, preferential contact, and preferential greeting to
26 a human caregiver over a human stranger in a modified and counterbalanced
27 version of the Ainsworth Strange Situation Test. In addition, our results show that
28 preferential responding to a caregiver over a stranger is only apparent following
29 brief isolation. In initial episodes, wolf pups show no differentiation between the
30 caregiver and the stranger; however, following a 2-min separation, the pups show
31 proximity seeking, more contact, and more greeting to the caregiver than the
32 stranger. These results suggest intensive human socialization of a wolf can lead to
33 attachment-like responding to a human caregiver during the first two months of a
34 wolf pup's life.

35 **Keywords:** Attachment, wolves, *Canis lupus*, Strange Situation Test, domestication

36

37 **1. Introduction**

38 Attachment behaviour refers to any “affectional tie” that one individual, be it
39 human or non-human animal, displays towards another specific individual
40 (Ainsworth and Bell, 1970). According to Ainsworth and Bell (1970, p. 50) “The
41 behavioural hallmark of attachment is seeking to gain and to maintain a certain
42 degree of proximity to the object of attachment, which ranges from close physical
43 contact under some circumstances to inter-action or communication across some
44 distance under other circumstances.” To help explain the origins and function of
45 attachment behaviour, Bowlby and Ainsworth formulated a framework for
46 attachment that posited the attachment to a caregiver is critical for the survival of
47 infants of many species since caregiver proximity can function as protection against
48 predators (Bowlby, 1958; Bowlby, 1982; for a review see Bretherton, 1992 or
49 Kraemer, 1997). This perspective incorporated the findings from the primate
50 literature that highlighted the importance of mother care for the healthy
51 development of rhesus monkeys and the readiness with which infant monkeys will
52 form attachments even to inanimate mother surrogates (Harlow, 1971; Kraemer,
53 1997).

54 More recent attachment research has extended the attachment framework to
55 the dog human-caregiver relationship. Topál et al. (1998) were the first to adapt the
56 Ainsworth’s Strange Situation Test (SST; Ainsworth & Bell, 1970) to assess whether
57 adult pet dogs show attachment to their human owners. In the SST, the subject is
58 brought into a novel room. Then, in a series of brief episodes, the presence of the

59 caregiver and a stranger is systematically manipulated. A brief isolation episode also
60 occurs approximately halfway through the test, which typically leads to mild
61 distress. Observers then score the subject's response to the presence and absence of
62 the stranger and caregiver to assess attachment-related behaviours towards the
63 human caregiver. Topál et al. (1998) recorded the amount of physical contact
64 between the dog and owner and dog and stranger in addition to how often the dog
65 engaged in play, exploration, passive behaviour, or waiting at the door in the
66 owner's or stranger's absence. They found that the dog-owner relationship could be
67 described in terms of attachment between the dog and owner, as some dogs showed
68 the secure-base effect in which exploration increased in the presence of the owner
69 compared to the stranger. In addition, dogs were shown to span a variety of
70 attachment styles along the secure-insecure dimension, which is similar to human
71 child attachment classifications (Topál et al., 1998).

72 Topál et al. (2005) explored the possible effects of domestication on dogs'
73 formation of attachment to human caregivers by comparing the attachment
74 behaviour of 16-week old hand-reared wolves, hand-reared dogs, and
75 conventionally reared dogs (i.e. mother nursed in human homes) during an SST.
76 Dogs that were raised in human homes (conventionally reared or hand-reared)
77 showed greater responding to a human caregiver than a stranger, whereas hand-
78 reared wolf pups showed equal responding to the caregiver and stranger. The
79 authors of this study suggested that, through domestication, dogs might have
80 evolved "a capacity for attachment to humans that is functionally analogous to that

81 present in human infants” (Topál et al., 2005, pp. 1373), whereas wolf pups did not
82 appear to form this same attachment to their human caregiver.

83 However, recent research has brought to light the importance of socialization
84 procedures, and experimental methodology in behavioural comparisons between
85 dogs and wolves. For example, adult wolves, once thought to be incapable of
86 following human’s points, are now known to be as responsive to human gestures
87 and attentional state as pet dogs given equivalent rearing and testing conditions
88 (Gacsi et al., 2009; Udell et al., 2008). Thus the hypothesis that dogs display a unique
89 attachment mechanism to form attachments to humans, distinct from that displayed
90 by other mammals (e.g. Cairns, 1966; Harlow, 1971; Kraemer, 1997) warrants
91 further investigation.

92 Human infants start to use their mother as a secure base when exploring the
93 environment at the age of eight months; however, from the second year on, their
94 attachment behaviour becomes more flexible and they will be less dependent on the
95 presence of their mother when interacting with others (Bowlby, 1969). Perhaps
96 wolves may be more likely to show a caregiver preference in a novel situation at a
97 younger age than the 16 weeks tested by Topál et al (2005). It’s unclear whether a
98 wolf’s attachment to a human changes with age, but if wolves do form attachments
99 to a human caregiver, it may be most apparent at a younger age when the wolves
100 may require the presence of a caregiver to be comfortable and explore a novel
101 situation. Thus attachment in wolves may be most apparent when wolves are first

102 starting to emerge from the den around three weeks of age (Packard, Mech, & Ream,
103 1992).

104 In addition, it is also important to note that at the time of testing, the wolves
105 tested by Topál et al. (2005) were no longer living with their human caretaker, but
106 had been relocated to a private wolf farm between 2-4 months of age (see Virányi et
107 al., 2008). As a result, at the time of testing, interactions with their caretaker had
108 been reduced to half a day twice per week (Virányi et al., 2008). Reduced levels of
109 caretaker-wolf contact may have altered the attachment relationship during this
110 period, which may have contributed to the study's findings (Udell & Wynne, 2010).

111 The aim of the present study was to investigate whether human-raised wolf
112 pups, still experiencing around the clock interactions with their primary caregiver,
113 would show an attachment response to that caregiver on the SST. Recent research
114 with dogs in the SST has introduced a counterbalanced version of the SST
115 controlling for the order in which the owner and stranger entered and exited the
116 room (episode order; Palmer & Custance, 2008). While Palmer and Custance (2008)
117 confirmed that adult dogs show attachment behaviours towards their owners, it was
118 also found that episode order could significantly influence a dog's response towards
119 their owner. Rehn et al. (2013) further investigated order effects within the SST in
120 dogs by implementing a control condition in which two equally unfamiliar
121 individuals entered and exited the room as they would in the normal SST. Here, the
122 only difference between the two individuals was the order in which they entered
123 and exited the room. Rehn et al. found that dogs displayed attachment-like

124 behaviour to one of the unfamiliar people simply as a function of the order in which
125 the unfamiliar persons entered and exited. However, exploration was more
126 susceptible to this order effect than proximity-seeking behaviours such as initiating
127 contact.

128 In the present study, we therefore use a counterbalanced version of the SST
129 to test 10 human-reared wolf pups' attachment-like behaviour to a human
130 caregiver. Pups were tested three times, once each at 3, 5 and 7 weeks of age,
131 throughout which time the pups were receiving near 24-hour care from a human
132 caregiver.

133 **2. Methods**

134 *2.1 Subjects*

135 Ten wolf pups (*Canis lupus*) from two litters (one litter of four and one litter
136 of six) participated in the present experiments. They were removed from the den
137 when they were approximately 10 days of age and hand-reared according to the
138 procedures outlined in Klinghammer and Goodman (1987) by two human-
139 caregivers at Wolf Park in Battle Ground, IN (see Table 1 for subject information).
140 The hand-rearing procedure involved the presence of a human caregiver in an
141 indoor room for 24 hours a day with the pups for the first 1.5- 2 months of life, at
142 which point the caregivers were present for approximately 16 hours a day.
143 Caregivers were also responsible for bottle-feeding the pups every 4-6 hours until

144 the pups were able to eat solid foods. Testing procedures were approved by the
145 University of Florida Institutional Animal Care and Use Committee.

146 *2.2 General procedure*

147 Wolf pups were given a modified version of the Ainsworth Strange Situation
148 Test (detailed below) during their 3rd, 5th and 7th week of life (see Table 1 for
149 exact ages). At each age, a novel testing room and a novel stranger were used. The
150 caregiver remained the same across ages.

151 In total, nine subjects were tested during week 3, nine during week 5, and ten
152 during week 7. One subject was ill during week 3 and 5 and was only tested at 7
153 weeks of age. One additional subject's last two episodes from week 3 were excluded
154 due to an experimenter error in which the episode order was inverted for the last
155 two sessions.

156 Each novel testing room was an indoor space (approximately 18 m²) to which
157 the pups had never previously been exposed. In each testing room, two 2m-
158 diameter non-overlapping circles were marked on the floor with tape. The marked
159 circles were used to code proximity to the caregiver or stranger by having the
160 stranger and caregiver sit in the centre of each circle. Approximately six toys were
161 distributed between the two circles. Toys were not included for the testing at 5 and
162 7 weeks of age for litter two due to experimenter error.

163 *2.3 Modified Strange Situation Test*

164 The pup was brought to a novel room where the presence and absence of a
165 caregiver and stranger were manipulated over six episodes each lasting 2 minutes.
166 Each time the stranger and/or caregiver entered the room; they opened and closed
167 the door, slowly walked to the centre of one of the circles and sat down on the floor.
168 The circle the caregiver or stranger sat in was determined randomly prior to the
169 start of the test. During the episode, the pups were free to move about the room
170 without restriction. If the pup approached the caregiver or stranger in the circle and
171 initiated contact, the caregiver or stranger would pet the pup. If the pup initiated
172 play by bringing a toy to either the caregiver or stranger, the caregiver or stranger
173 could engage in play. The stranger and caregiver, however, were instructed not to
174 move outside their circle during an episode. To exit at the end of an episode, the
175 caregiver or stranger stood up, turned to the door, and slowly walked towards it.
176 Upon reaching the door, the caregiver or stranger said "goodbye" and exited.

177 To control for potential order effects, two counterbalanced sequences of the
178 entering and exiting of the stranger and caregiver were utilized. Table 2 outlines
179 these two episode orders and indicates whether the stranger alone, caregiver alone,
180 stranger and caregiver, or neither was in the room with the pup. For Episode Order
181 1, the caregiver sat alone in the room with the pup for the first episode. After 2 mins,
182 a stranger entered the room and sat in the adjacent circle to the caregiver for the
183 second episode. Next, the caregiver left the room, leaving the stranger and pup alone
184 in the room for Episode 3. For Episode 4, the strange left the room, leaving the pup

185 alone. In Episode 5 the stranger returned to the room. In the final episode, the
186 caregiver entered so the caregiver and stranger were present with the pup. Episode
187 Order 2 followed a similar pattern except that it counterbalanced Episode Order 1
188 (see table 2). The episode order assigned for each pup was pseudo-randomly
189 determined so that at each age, half of the pups were tested with each order. In
190 addition, the order each pup was tested with was changed across the three testing
191 weeks so that each pup was tested once with one episode order, and twice with the
192 other episode order.

193 *2.4 Behaviour coding*

194 During each episode, the pups' behaviour was recorded on video for
195 subsequent analysis. The behaviours scored, the behavioural definition, and
196 observer agreement scores are listed in Table 3. Briefly, during each episode we
197 scored the amount of time the pup spent in proximity (within the 2m circle) and
198 within physical contact of the caregiver and stranger. These two behaviours were
199 not mutually exclusive: a pup could be in contact while also in proximity. Both
200 proximity and contact behaviours were scored to assess the approach and
201 investigative behaviour (proximity) as well as close contact seeking (contact). Both
202 are important, as pups could prefer to approach and investigate one individual (high
203 proximity), but prefer not to be touched (low contact). This would indicate a fearful
204 curiosity, whereas a high proximity high contact would be more indicative of
205 comfort seeking. We also scored whether the pups greeted and followed the
206 caregiver and stranger when entering or exiting the room, with a zero indicating no

207 greeting or following, a one indicating a calm greeting or following, and a two
208 indicating an excited greeting or follow (see Table 3). Scored episode times were
209 approximately 2 min; however, due to minor variations in time taken for the human
210 to enter, sit down or exit across episodes, all behaviours are reported as a
211 proportion of the episode time, except for greeting and following which were rated
212 categorically. A second observer scored 37% of the videos. Percent agreement was
213 calculated for the continuous behaviours and Cohen's Kappa was calculated for the
214 categorical scale by comparing the two raters' scores on an episode-by-episode
215 basis. For the continuous behaviours, an agreement was defined as both observers
216 scoring within two seconds (or 5% of the scored time) of each other. Any larger
217 discrepancy was scored as a percent agreement by dividing the smaller scored time
218 by the larger.

219 *2.5 Statistical analyses*

220 Data were analysed using the statistical package R (R Core Team, 2012) and
221 plotted with the R package *ggplot2* (Wickham, 2009). Linear mixed effects models
222 using the *lme4* package (Bates, Maechler, Bolker & Walker, 2013) were used to
223 assess the effects of the presence and absence of the caregiver and stranger on the
224 subjects' behaviour. P-values for ANOVA tests and *t*-tests were generated from the
225 *LmerTest* package (Kuznetsova, Brockhoff & Christensen, 2013) using a
226 Satterthwaite approximation for the degrees of freedom. Each model included
227 random intercepts for the subject and litter variables. The subject term was nested
228 in the litter term.

229 3. Results

230 Linear mixed effects models were run for each scored behaviour to
231 investigate the effect of the episode and condition on that behaviour. To test
232 whether pups engaged in differential levels of a scored behaviour towards the
233 caregiver compared to the stranger, linear models included a dummy coded variable
234 to indicate whether the behaviour occurred towards/ in the presence of the
235 caregiver or the stranger.

236 *3.1 Proximity seeking to the caregiver and stranger*

237 Figure 1 shows the overall patterns of proximity to the caregiver and
238 stranger across episodes separated by age and episode order, and an overall
239 averaged summary across weeks. Overall, similar patterns of responding were
240 observed at each age of testing (3, 5, 7 weeks); however, differences appeared in
241 proximity to the stranger and caretaker across the episodes.

242 *3.1.1. Episodes 2 & 6:* During Episode 2 and Episode 6, both the caregiver and
243 stranger were present in the room. A linear mixed effect model was used to test
244 whether pups' proximity to a person was predicted by the familiarity of that person
245 (caregiver vs. stranger), the pups' age (3, 5 or 7 weeks), the episode order (Order 1
246 or Order 2), a 2-way interaction between the episode (2 vs. 6) and the person
247 (caregiver vs. stranger), and a 2-way interaction between the episode and age.
248 There was no interaction between age and episode ($F_{(1,98.95)} = 0.05$, $p = 0.82$), but
249 there was a significant interaction between the episode and proximity to the

250 caregiver and the stranger ($F_{(1,98.95)} = 12.60, p < 0.001$), indicating that preference
251 for the caregiver and stranger changed from Episode 2 to Episode 6.

252 We therefore explored the pups' preference between the caregiver and
253 stranger during Episode 2 and Episode 6. For Episode 2, there was no indication
254 that pups had different preferences for approaching the caregiver over the stranger
255 depending on the episode order (Person by episode order Interaction: $F_{(1, 41.22)} =$
256 $0.11, p = 0.74$). There was also no effect of age ($F_{(1, 44.02)} = 0.40, p = 0.53$), episode
257 order ($F_{(1, 47.75)} = 0.70, p = 0.41$), or difference between the caregiver and stranger
258 ($F_{(1, 41.22)} = 2.61, p = 0.11$). This contrasts the findings in Episode 6, which showed a
259 significant interaction between the episode order and the person the pup
260 approached ($F_{(1, 46.98)} = 7.77, p < 0.01$), indicating that pups' preference for the
261 caregiver depended on the episode order. When looking at each episode order
262 separately, there was a significant preference for proximity to the caregiver over the
263 stranger ($F_{(1, 24.99)} = 16.73, p < 0.0001$) in Episode Order 1. For Episode Order 2
264 where the caregiver was already present in Episode 5, there was no significant
265 difference in time spent with the caregiver and stranger in Episode 6 ($F_{(1, 20.99)} =$
266 $0.05, p = 0.82$). In both episode orders, there was again, no effect of age (Order 1: $F_{(1,$
267 $24.99)} = 0.05, p = 0.83$; Order 2: $F_{(1, 20.99)} = 0.60, p = 0.45$).

268 *3.1.2. Episodes 1, 3 & 5:* Given that there was no indication of an age effect
269 across Episodes 2 and 6, the data were averaged across age to provide a complete
270 within-subject data set. There was a significant effect of episode ($F_{(2, 50.98)} = 8.00, p <$
271 0.001) showing that pups spent more time in proximity to both the caregiver and

272 stranger in Episode 5 than Episode 1 ($t_{50.98} = 3.99$, $p < 0.001$). When proximity was
273 averaged across Episodes 1, 3 and 5, pups overall spent more time in proximity to
274 the stranger ($t_{50.99} = 2.07$, $p = 0.043$). When considering Episode 5 alone (the first
275 reunion following isolation), however, there was no difference in proximity between
276 the stranger and caregiver ($t_{6.83} = 0.989$, $p = 0.36$).

277 *3.2 Contact between the caregiver and stranger*

278 Figure 2 shows the proportion of each episode the pup made physical contact
279 (i.e. petting) with the caregiver and stranger across episodes for both episode
280 orders across all three weeks of testing. The overall patterns are similar to
281 proximity seeking, with contact changing as a function of the episode. Again, there
282 appears to be little effect of testing across ages.

283 *3.2.1 Episodes 2 & 6:* Overall, contact seeking showed an identical pattern to
284 proximity seeking. During Episode 2, pups showed no preference between the
285 caregiver and stranger ($F_{(1,48.98)} = 3.07$, $p = 0.09$). During Episode 6, however,
286 preference between the caregiver and stranger depended on the episode order
287 (Episode by person interaction: $F_{(1,46.98)} = 6.93$, $p = 0.01$). Pups in episode Order 1
288 significantly preferred the caregiver ($F_{(1,24.98)} = 20.79$, $p < 0.001$), whereas there was
289 no difference in time with the caregiver and stranger for Order 2 when pups were
290 already re-united with the caregiver in Episode 5 ($F_{(1,21)} = 0.05$, $p = 0.83$). Similar to
291 proximity, there was no effect of age ($F_{(1, 98.95)} = 0.11$, $p = 0.74$), or interaction
292 between age and episode ($F_{(1, 98.95)} = 0.05$, $p = 0.82$).

293 3.2.2. Episodes 1, 3 & 5: Pups overall showed the same pattern of contact
294 with the stranger and caregiver as they did for proximity. Pups overall showed
295 significantly more contact in Episode 5 than Episode 1 ($t_{50.97} = 4.44$, $p < 0.0001$) and
296 more contact with the stranger when averaged across episodes 1,3, and 5 than with
297 the caregiver ($t_{50.98} = 2.05$, $p = 0.046$). When looking at Episode 5 alone (the first
298 reunion following isolation), however, there was no significant difference in time
299 spent in contact with the caregiver or stranger ($t_{8.90} = 0.30$, $p = 0.77$).

300 3.3. *Greeting and following the caregiver and stranger*

301 Greetings were scored when the caregiver or stranger entered the room,
302 which occurred during Episodes 2, 5 and 6. Following was scored when the
303 caregiver or stranger exited a room, which occurred during Episodes 3 and 4. A
304 mean greeting and following score was computed for each pup across all ages.
305 Figure 3 shows the mean score for following and greeting both the stranger and
306 caregiver. Overall, pups were not more likely to follow the caregiver over the
307 stranger ($F_{(1, 9.00)} = 1.18$, $p = 0.31$). The pups, however, did show more excited
308 greetings to the caregiver than they did to the stranger ($F_{(1, 9.00)} = 6.40$, $p = 0.03$)
309 upon their return.

310 4. Discussion

311 Overall, the pups showed differential responding to the caregiver when
312 compared to the stranger. Pups were more likely to greet the caregiver with whines
313 and ears back upon reunion than they did the stranger. In addition, pups showed an

314 effect of reunion in Episode 6, seeking greater proximity and physical contact with
315 the caregiver than the stranger. However, this effect was only evident when pups
316 were tested with episode Order 1. This is likely due to the fact that the caregiver was
317 already present during the post-isolation Episode 5 in Order 2. This provided time
318 for the pups to engage in reunion behaviour during Episode 5, followed by increased
319 exploration of other environmental features, including the unfamiliar human, in
320 Episode 6. In fact, this is what is predicted when a secure attachment is present,
321 known as the secure base effect (Ainsworth & Bell, 1970). However in Order 1,
322 Episode 6 was the first reunion with the caregiver, which led to a highly significant
323 bias for the caregiver- an outcome also predicted in previous attachment literature
324 (Ainsworth & Bell, 1970).

325 We did not observe many differences in preference between the caregiver
326 and the stranger prior to isolation (Episode 4). Instead, pups were indifferent in
327 Episode 2 when given a choice between these two people, and in fact showed a
328 slight stranger preference for contact across Episodes 1, 3 and 5. However, after a
329 brief 2-minute isolation phase, the pups showed a strong caregiver preference in
330 Episode 6 if the caregiver returned, but the pups showed no preference for the
331 stranger if the stranger returned in Episode 6. Interestingly, however, we did not
332 observe any differences in proximity and contact between the stranger and
333 caregiver during Episode 5, the episode immediately after isolation. One possibility
334 is that because this comparison was across episode orders (Order 1 vs. Order 2) and
335 averaged across ages allowing order effects or minor age effects to potentially mask
336 an effect. Another explanation is that isolation in Episode 4 was sufficiently stressful

337 that it activated general proximity seeking to any available person, even if the only
338 available person was not a caretaker. Importantly, however, if the caregiver was
339 present in Episode 5, the pups showed indifference between the stranger and
340 caregiver in Episode 6. If the stranger was present in Episode 5, the pups showed a
341 dramatic caregiver preference in Episode 6 indicating that the effect of the presence
342 of the caregiver or stranger in Episode 5 was not equivalent. Only if the caregiver
343 was present in Episode 5, did the pups show the same pattern that was shown
344 before isolation in Episode 2. This suggests that despite the pups showing similar
345 time in contact and proximity with the caregiver and stranger in Episode 5, only
346 contact and proximity with the caregiver in Episode 5 functioned to return the pup
347 to baseline preferences.

348 The fact that caregiver preferences only occurred in Episode 6 exemplifies
349 Ainsworth and Bell's (1970) hypothesis that attachment behaviour is heightened in
350 situations perceived as threatening, which in the present case, was isolation. One
351 difference; however, in our findings from those observed with human infants and
352 dogs is that separation from the attachment figure alone was not sufficient to
353 activate attachment behaviours (e.g. Ainsworth & Bell, 1970, Palmer & Custance,
354 2005; Topál et al., 1998; Topál et al., 2005). In the present study, complete isolation
355 (Episode 4) was required to activate attachment behaviours. However, this could
356 have been a by-product of the rearing and socialization practices employed with the
357 pups used in this study. The pups were regularly introduced to novel humans and
358 environments (Klinghammer & Goodman, 1987), making it unsurprising that being
359 in a room with a novel human was not, in itself, a strange or stressful situation.

360 Thus, this study provides evidence that the behaviour demonstrated by
361 hand-raised wolf pups towards humans can be categorized as attachment
362 (Ainsworth and Bell, 1970) in some cases, given early socialization to humans
363 (Klinghammer and Goodman, 1987), with continued contact with the caregiver
364 through the time of testing, and the implementation of methodological controls for
365 known order effects (Palmer & Custance, 2008; Rehn et al., 2013). This of course
366 does not mean that all wolf pups will necessarily show strong attachment behaviour
367 towards humans (e.g., Topal et al., 2005), as early rearing history and differences in
368 caretaker behaviour are known to influence both the initial formation of the
369 attachment bond and the attachment style that develops between an individual and
370 their caretaker (Ainsworth and Bell, 1970). The fact that the wolves tested by Topál
371 et al. (2005) were older, less human dependent, and no longer living with their
372 human caretaker at the time of testing could have altered their attachment
373 behaviour.

374 At present, however, we cannot determine which if any of these factors
375 contributed to the differences between the present study and Topál et al (2005).
376 Age may be a significant factor (16 weeks vs. 3,5 & 7 weeks), however, differences in
377 the length of time spent with the pups on a daily basis, time spent overall during the
378 subject's lifetime, or other unknown rearing differences could have contributed to
379 the differences between the two studies. It is unknown what effect age may have on
380 attachment to humans. In our limited age range of testing, we saw no effect;
381 however, we may have observed an effect had testing been carried out until 16
382 weeks of age. Future studies are necessary to determine the typical developmental

383 stages of wolf attachment to humans and the rearing factors that may influence it.
384 Early socialization and life experiences may likely influence attachment test
385 performance for canids. In fact, many feral dogs actively avoid human contact in the
386 absence of early socialization (Ortolani, Vernooij & Coppinger, 2009). Future studies
387 on the development of attachment bonds in canids may carefully detail the ontogeny
388 of attachment formation and the conditions that lead to its development and
389 maintenance in later life allowing for further comparisons between wolves and
390 dogs.

391 Overall, the results show that wolf pups will form attachments to their
392 human caregivers. This is an important finding, as it suggests that capacity to form
393 attachments to humans is not itself a product of domestication. This suggests that
394 young non-domesticated canids can form attachments to humans. Our study also
395 suggests that the conditions under which attachment behaviour is displayed, for
396 example, that isolation is required to elicit attachment like responding, may differ
397 between wolves and what is seen with dogs. Another area for future investigation is
398 looking into the maintenance of attachment into adulthood. Although it's unclear
399 whether the differences in results from Topál et al. (2005) and the present one is
400 due to the age of the subjects at testing, it's possible that domestication influenced
401 how attachments are maintained throughout development and into adulthood.
402 When tested, most adult dogs typically show attachments to their owners; it's not
403 clear whether this would be the case for wolves or even all breeds of dogs. Thus, our
404 results highlight that the ability to form attachments to humans likely proceeded
405 domestication, but domestication may have changed the ease at which these

406 attachments could be formed, the conditions under which they are shown, and how
407 they are maintained as adults.

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412

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494

Subject	Sex	Litter	Age in days (Week 3)	Age in days (Week 5)	Age in days (Week 7)
Kanti	M	2	23	37	50
Bicho	M	2	23	36	50
Mowgli	M	2	25	35	53
Pigeon	F	2	24	37	51
Bigboy	M	2	25	36	51
Fiona	F	2	24	35	50
Dharma	F	1	23 ⁺	35	47
Devra ¹	F	1	NA	NA	47
Gordon	M	1	21	35	47
Tilly	F	1	22	35	47

495 Table 1. Subject information. Table gives sex and exact age at each testing week.

496 ¹Devra was unable to be tested at 3 and 5 weeks due to illness. ⁺Last two episodes

497 were excluded due to experimenter error.

498

499

Order	Episode 1	Episode 2	Episode 3	Episode 4	Episode 5	Episode 6
1	Caregiver	Caregiver + Stranger	Stranger	Isolation	Stranger Returns	Stranger + Caregiver
2	Stranger	Stranger + Caregiver	Caregiver	Isolation	Caregiver Returns	Caregiver+ Stranger

500

501 **Table 2.** Outline of the two episode orders. Each cell displays whether the

502 caregiver, the stranger or both were present in the testing room with the pup.

503

Behaviour	Definition	Per cent Agreement	Cohen's Kappa
Behaviours During an Episode			
Proximity seeking caregiver	Proportion of the episode in which the pup had at least 2 paws within the 2 m circle the caregiver/stranger was sitting in.	95.7%	
Proximity seeking stranger			

Contact caregiver	Proportion of the episode in which the pup engaged in physical contact with the caregiver/stranger (not mutually exclusive with proximity)	93.4%	
Contact stranger			

Behaviour Between Episodes			
Greeting Caregiver	A score from 0-2 on the type of greeting that occurred within 15 s of the caregiver/stranger entering the room and sitting: 0: "No greeting- did not approach" 1: "Calm Greeting-approached but did not display ears back or whining" 2: "Excited greeting- approached with ears back and whining"		0.71
Greeting Stranger			

Following Caregiver	A score from 0-2 on the type of following that occurred within 15 s of the caregiver/stranger exiting the room.	0.63
	<p>0: “No following”- did not approach leaving person or door</p>	
	<p>1: “Calm follow”-followed leaving person but did not try to follow through door, jump on door or</p>	
Following Stranger	whine	
	<p>2: “Excited follow”- followed person and tried to exit through door, jump at door or whined</p>	

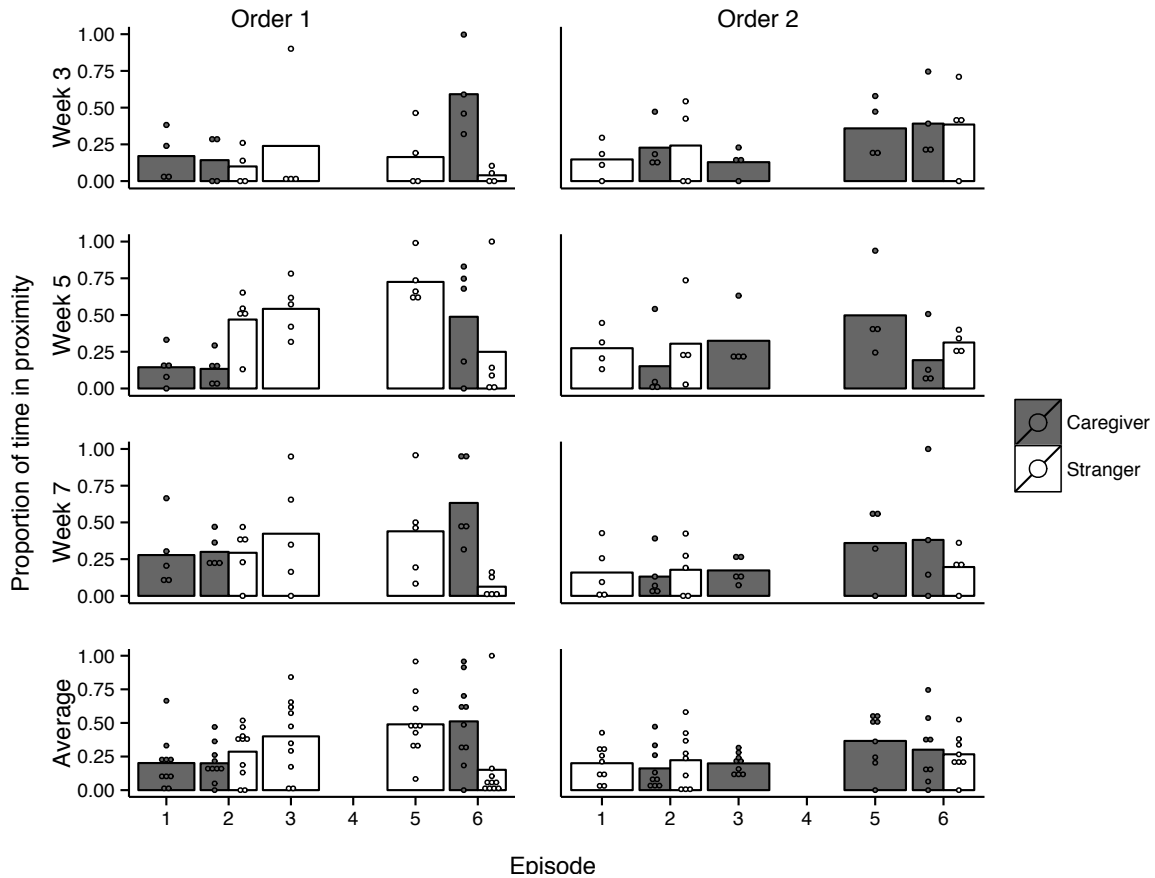
505

506 **Table 3.** Behavioural coding: definitions for each behaviour. For continuous
507 variables, per cent agreement is reported from 36% of the videos double coded (10
508 of 28 videos). For categorical variables, Cohen’s Kappa is reported for the 36% of
509 double coded videos.

510

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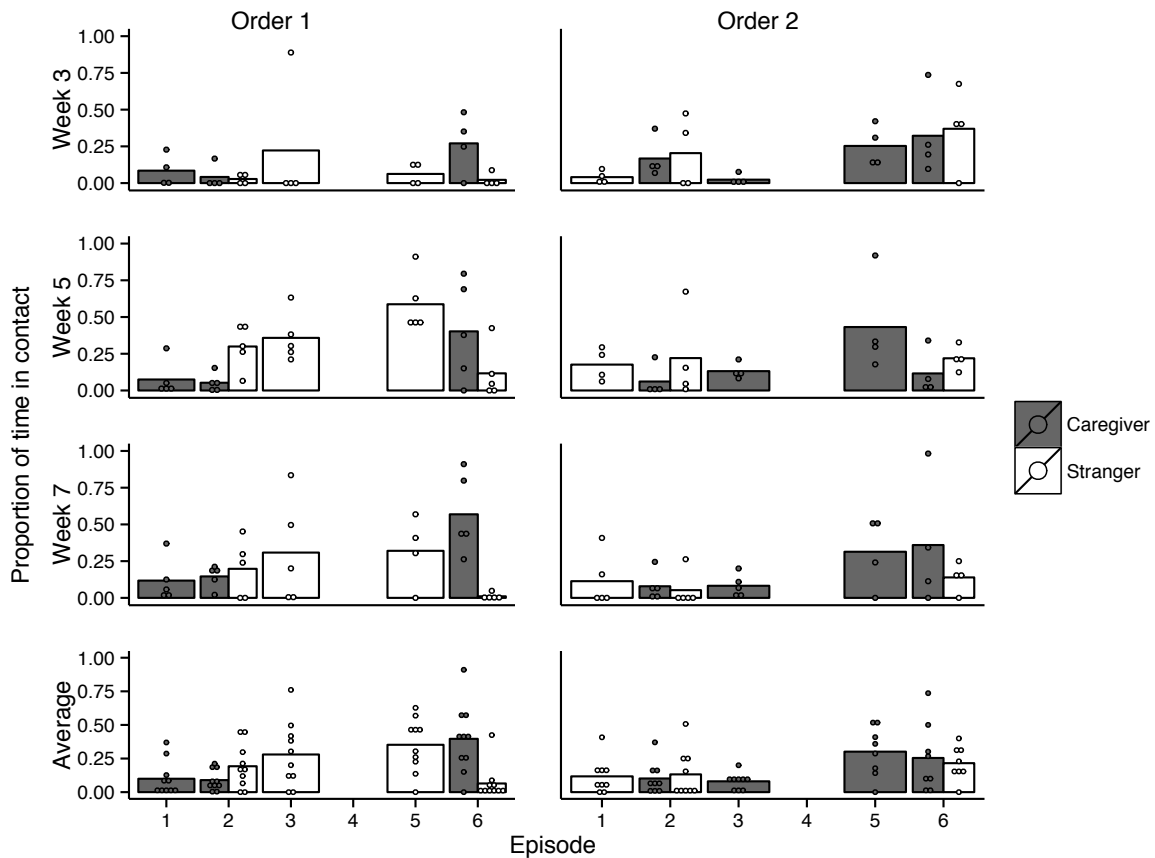
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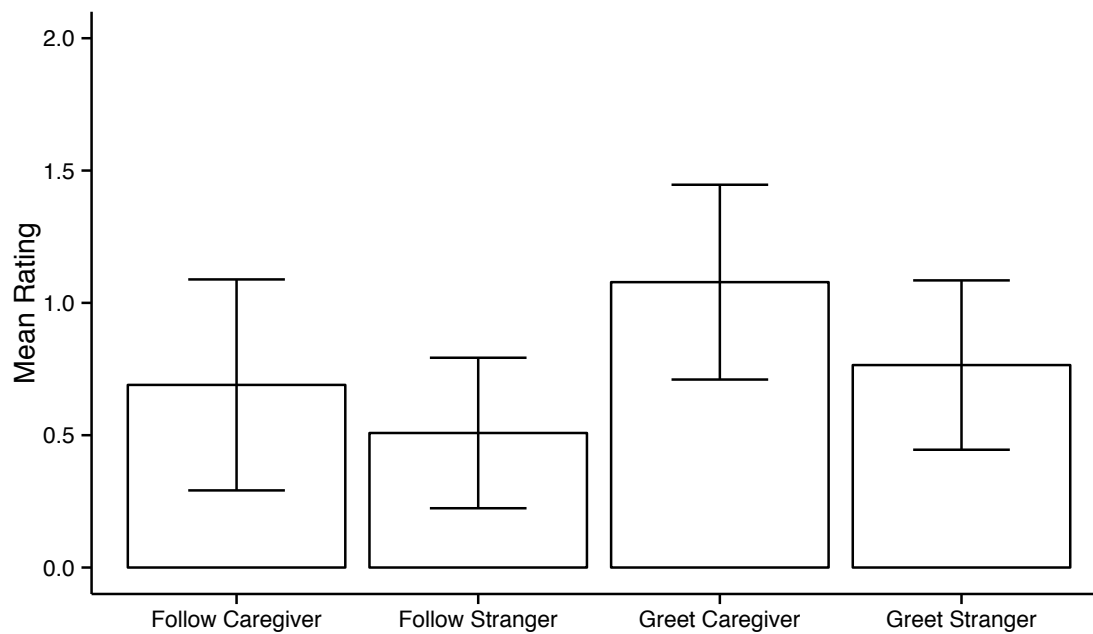
Episode

514 **Figure 1.** Proportion of time in each episode spent in proximity to the caregiver and
 515 stranger in the Ainsworth SST procedure. The left column represents subjects
 516 experiencing Episode Order 1, the right column subjects experiencing episode order
 517 2. Bars indicate the mean and dots show each data point. Each row shows the
 518 results for a different age and the final row shows the results averaged across all
 519 ages. Episodes 2 & 6 indicate proximity to both the caregiver and stranger as both
 520 were in the room (2 bars). Episodes 1, 3 and 5 indicate proximity to only the
 521 caregiver or stranger, as only one was present and the person present depended on
 522 the episode order. Episode 4 is blank, as neither the caregiver nor stranger was
 523 present (i.e. isolation). The Average row indicates each pups proportion in
 524 proximity averaged for the episode order in which they received twice.



525

526 **Figure 2.** Proportion of episode time spent in contact with the caregiver and
 527 stranger in the Ainsworth SST procedure. The left column represents subjects
 528 experiencing Episode Order 1, the right column subjects experiencing Episode
 529 Order 2. Bars indicate the mean and dots indicate each data point. Each row shows
 530 the results for a different age and the final row shows the results averaged across all
 531 ages. Episodes 2 & 6 indicate contact with both the caregiver and stranger as both
 532 were in the room (2 bars). Episodes 1, 3 and 5 indicate proximity to only the
 533 caregiver or stranger, as only one was present and the person present depended on
 534 the episode order. Episode 4 is blank, as neither the caregiver nor stranger was
 535 present (i.e. isolation). The Average row indicates each pups proportion in
 536 proximity averaged for the episode order in which they received twice.



537

538 **Figure 3.** Mean rating for following the caregiver and stranger as well as greeting
539 the caregiver and stranger. See Table 3 for the scoring system. Bars show the mean
540 score and error bars show the 95% confidence interval.