

Manuscript Number: Applan-D-16-248R2

Title: Intraspecific relationships in adult domestic dogs (*Canis familiaris*) living in the same household: a comparison of the relationship with the mother and an unrelated older female dog

Article Type: Research Paper

Keywords: Ainsworth Strange Situation Test; Attachment; Behaviour; Bond; Dog; Mother.

Corresponding Author: Dr. chiara mariti, DVM, Ph.D., Dipl. ECAWBM (AWSEL)

Corresponding Author's Institution: università di pisa

First Author: chiara mariti, DVM, Ph.D., Dipl. ECAWBM (AWSEL)

Order of Authors: chiara mariti, DVM, Ph.D., Dipl. ECAWBM (AWSEL); beatrice carlone; emilia votta; eva ricci; Claudio Sighieri, full professor; angelo gazzano, dvm, PhD, Dipl. ECAWBM (BM)

Abstract: There is scientific evidence that adult dogs establish attachment bonds towards human beings. Attachment as behavioural system exists in the puppy-mother relationship, but adult dogs tested with the Ainsworth Strange Situation Test (ASST) have been found to show a preference for the stranger over a conspecific living in the same household.

In the current study, 50 adult dogs were tested with an intraspecific version of the ASST where the role of the presumed attachment figure was played by an older female dog living in the same household, 18 being their own mother and 32 being an unrelated older dog. The two groups did not show remarkable differences when compared one to the other. However, the within-group analysis revealed that dogs tested with an household older female dog other than the mother showed a preference for the human stranger, who had a higher ameliorative effect than the companion dog. Dogs tested with their mother instead displayed both social and non-social behaviours in a very similar manner when in the company of the stranger or of the mother after being reunited with them. Considering the peculiar appeal that human beings have to dogs and the differences observed in the current study, it can be concluded that adult dogs showed a stronger bond for the mother. Future research may clarify if this depends on the maternal care and/or on the time spent with the mother since birth.

Comments:

I am pleased to inform you that this manuscript may now be acceptable following a satisfactory minor revision. The enclosed reviews explain the topics you need to consider and revise. I look forward to seeing your revised manuscript.

Yours sincerely,
Per Jensen
Editor-in-chief

Reviewer #1: The new version of the manuscript shows a very careful revision done by Authors. I am thankful for the detailed answers and explanations. I am glad to recommend the manuscript for acceptance - Authors made great efforts to make their introduction and conclusions clearer, and they performed a much more appropriate analysis at this time.

WE WISH TO THANK YOU REVIEWER #1 FOR THE POSITIVE COMMENTS AND FEEDBACKS.

Reviewer #2: The manuscript has been greatly improved. The current title "Intraspecific relationships in adult domestic dogs (*Canis familiaris*) living in the same household: a comparison of the relationship with the mother and an unrelated older female dog" summarizes relevant information to the reader, who can make a more appropriate choice about whether the manuscript is of interest or not. The Introduction has been shortened and it focuses on relevant information. The M&M, the Results and the Discussion are greatly improved too and, in general, easier to read than in the previous draft. However, I find that some parts are still difficult to easily read and understand. For example, in M&M, l.125, the Author presents "the first step of the statistical analysis" when a first step of the statistical analysis had been already introduced in the previous paragraph. I understand what the Author wants to say, i.e. that what explained in the first paragraph is a preliminary statistical analysis to make sure that there were not difference between groups; but at first I thought that the second paragraph was giving more information about what was summarized in the first. I think that, in the second paragraph of M&M, it may help to have an introductory part like "Once we studied the potential differences in factors between the two groups, we proceeded to analyze the differences between and within groups for the factor "test with the mother"... or something similar. In the Discussion I found the paragraph in ll.167-171 difficult to read; could it be rewarded for clarity? Maybe these minor changes can be also done during the editing process, since I found the manuscript almost ready to be accepted for publication.

WE WISH TO THANK ALSO REVIEWER #2. WE MODIFIED THE MANUSCRIPT ACCORDING TO HIS/HER SUGGESTIONS.

1 **Highlights**

- 2 ~~• Research on intraspecific attachment bonds in dogs is scarce.~~
- 3 • Eighteen adult dogs were tested with the mother and 329 with an older ~~cohabitant~~ female
- 4 dog
- 5 • A human stranger had a higher ameliorative effect than the unrelated cohabitant dog
- 6 • Dogs behaved similarly towards/in the company of their mother and of the stranger
- 7 • Adult dogs show a stronger bond for the mother than for another cohabitant dog

1 **Intraspecific relationships in adult domestic dogs (*Canis familiaris*) living in the same**
2 **household: a comparison of the relationship with the mother and an unrelated older female**
3 **dog**

4 Chiara Mariti*^{1,2}, Beatrice Carlone¹, Emilia Votta¹, Eva Ricci¹, Claudio Sighieri^{1,2}, Angelo
5 Gazzano^{1,2}

6 ¹ Dipartimento di Scienze Veterinarie – Università di Pisa, viale delle Piagge 2 – 56124 Pisa, Italy

7 ² ETOVET – Group of Research in Veterinary Ethology and Physiology, Pisa, Italy

8 *Corresponding author: chiara.mariti@unipi.it

9

10 **ABSTRACT**

11 There is scientific evidence that adult dogs establish attachment bonds towards human beings.
12 Attachment as behavioural system exists in the puppy-mother relationship, but adult dogs tested
13 with the Ainsworth Strange Situation Test (ASST) have been found to show a preference for the
14 stranger over a conspecific living in the same household.

15 In the current study, 50 adult dogs were tested with an intraspecific version of the ASST where the
16 role of the presumed attachment figure was played by an older female dog living in the same
17 household, 18 being their own mother and 32 being an unrelated older dog. The two groups did not
18 show remarkable differences when compared one to the other. However, the within-group analysis
19 revealed that dogs tested with an household older female dog other than the mother showed a
20 preference for the human stranger, who had a higher ameliorative effect than the companion dog.

21 Dogs tested with their mother instead displayed both social and non-social behaviours in a very
22 similar manner when in the company of the stranger or of the mother after being reunited with
23 them.

24 Considering the peculiar appeal that human beings have to dogs and the differences observed in the
25 current study, it can be concluded that adult dogs showed a stronger bond for the mother. Future

26 research may clarify if this depends on the maternal care and/or on the time spent with the mother
27 since birth.

28

29 **Keywords:** Ainsworth Strange Situation Test; Attachment; Behaviour; Bond; Dog; Mother.

30

31 **INTRODUCTION**

32 Attachment theory regards the propensity to make intimate emotional bonds to particular
33 individuals (Bowlby, 1988). Initially studied in the 1960s and 1970s primarily in the context of
34 children and parents, since the 1980's there has been an explosion of research examining attachment
35 processes beyond the parent-child dyad (Cassidy et al., 2013), which has supported Bowlby's belief
36 that attachment is a process that characterizes humans “from the cradle to the grave” (Bowlby,
37 1979).

38 Bowlby's theory of attachment is indeed largely connected to animal ethology (Bowlby, 1982; van
39 der Horst et al., 2007). Bowlby's postulated an inborn behavioural system that emerged as an
40 adaptation over the course of mammalian evolution. The presence of an attachment system is
41 therefore genetically determined, but the individual to which the attachment bond is formed
42 depends on experience (Bowlby, 1982). Maternal care takes on great importance for the
43 establishment of the bond (Bowlby, 1982), and a major role is played by the caregiver acting as a
44 secure base and safe haven (Bowlby, 1982; Ainsworth, 1989). However, secure base use and
45 support demand are present across the span of adulthood (Waters and Cummings, 2000). The
46 attachment bond is usually maintained between adult children and their parents (see e.g. Carpenter,
47 2001), and in adult humans other attachment bonds can be created, e.g. between partners, friends,
48 and intimates (Ainsworth, 1989).

49 There is scientific evidence that adult dogs establish attachment bonds towards human beings (for a
50 review see Payne et al., 2016). Adult dogs can use their owner as a secure base (Mariti et al., 2013),

51 and they can form new interspecific attachment bonds even after the breaking of previous ones
52 (Gácsi et al., 2001).

53 The presence of an attachment bond to conspecifics have instead received scant attention from
54 researchers. Studies on separation from conspecifics (Pettijohn et al., 1977; Tuber et al., 1996;
55 Walker et al., 2014) seem to point to a difference in the nature of the social relationships of dogs
56 with humans and with conspecifics. Attachment as behavioural system exists in puppy-mother
57 relationship (Prato-Previde et al., 2009), but there is no evidence of it in intraspecific relationships
58 between adult dogs living in the same household (Mariti et al., 2014). It can be hypothesized that
59 the attachment bond of puppies towards their mother is maintained, provided the possibility to live
60 together also in adulthood.

61 The aim of this study was to test this hypothesis, through the analysis of the behaviour of adult dogs
62 in the Ainsworth Strange Situation Test where the role of the presumed attachment figure was
63 played by an older female dog living in the same household, being their own mother or an unrelated
64 older female dog.

65

66 **MATERIALS AND METHODS**

67 Subjects

68 Fifty dyads of dogs were involved in this study. Dyads were divided into two groups: the mother-
69 offspring (MO) and the older female dogs (OF) group. The two groups were matched as much as
70 possible for factors which may have affected results, such as female/male ratio, age of the tested
71 dogs, age of the other dog, breed, number of dogs in the household, time spent together, and
72 environment in which they lived. More details are provided below and in the results section.

73 In the MO sub-sample each dyad was formed by the tested dog and his/her own mother living in the
74 same household since birth. Eighteen dogs (10 females and 8 males; 4 mixed-breed, 2 Labrador

75 Retrievers, 2 German Shepherd Dogs, 2 Border Collies, 2 Bearded Collies, 2 Welsh Corgi
76 Pembrokes, 1 English Springer Spaniel, 1 Australian Kelpie, 1 Flat Coated Retriever, and 1 Boxer;
77 mean age = 37.8 ± 13.7 months old) took part at the study as tested subjects. Each of these 18 dogs
78 were tested with his/her own mother (mean age = 75.1 ± 27.3 months old) as the presumed
79 attachment figure. The mean time spent living together with the mother was 37.8 ± 13.7 months.
80 The number of dogs living in the same household varied from 2-7, the mean being 3.7 ± 1.8
81 dogs/household.

82 In the OF sub-sample, the dyads consisted in dogs living in the same household but not related by
83 blood. Dogs had been adopted around 8-10 weeks of age; before that age puppies had lived with
84 their mother and littermates in a home environment and, after adoption, they had been living in the
85 same household until the testing time. Thirty-two dogs (18 females and 14 males; 8 mixed-breed, 7
86 Labrador Retrievers, 2 German Shepherd Dogs, 2 Border Collies, 2 English Setters, 2 Belgian
87 Shepherd Dogs, 1 Scotch Collie, 1 Flat Coated Retriever, 1 Bernese Mountain Dog, 1 English
88 Springer Spaniel, 1 Australian Kelpie, 1 Pug, 1 Beagle, 1 Shetland Sheepdog, and 1 Toy Poodle;
89 mean age = 46.7 ± 32.3 months old) participated in the study as tested subjects. Each dog was tested
90 with an unrelated older female dog living in the same household (mean age = 70.7 ± 30.5 months
91 old) playing the role of the presumed attachment figure. The time spent living together with the
92 other dog was 38.7 ± 25.0 months. The number of dogs living in the same household varied from 2-
93 8, the mean being 3.6 ± 1.7 dogs/household.

94 Each dog was tested in one condition only, but owners could participate with more than one dog. As
95 a result, part of the MO sub-sample (10 out of 18 dogs) was living in the same household of part of
96 the OF sub-sample (15 out of 39 dogs). This allowed the researchers to match as much as possible
97 the living environment of the two groups.

98 None of the female dogs were in estrus, nor were they pregnant at or around the time of testing.

99 The inclusion criteria for tested dogs were the same used in a previous paper about intraspecific
100 attachment in adult dogs (Mariti et al., 2014).

101 Dog owners were all volunteers recruited by personal contacts. The stranger (Stranger 1) was
102 played by five 25-35 year old women, unfamiliar to all the dogs; each of them performed the
103 stranger for some dogs of both the MO and OF groups. A second person (Stranger 2) helped
104 Stranger 1 for the entrance and exit of the dog acting as the presumed attachment figure.

105 The involvement of an unknown dog playing the role of the stranger was avoided for ethical
106 reasons, due to the high risk of intraspecific aggression.

107 This study did not require approval of an Ethical committee because it was observational in nature.

108

109 Experimental setting and procedure

110 The procedure was the same described in Mariti et al. (2014). The procedure was as faithful as
111 possible to the Ainsworth Strange Situation Test (Ainsworth and Bell, 1970). Seven 2-min episodes
112 were carried out in an experimental room, unfamiliar to all dogs. As recommended in the ASST, the
113 procedure included two separations from the presumed attachment figure (episode 3, dog with
114 stranger; episode 5, dog in complete isolation; and episode 6, dog with stranger) and two reunions
115 with him/her (episode 4 and 7). A more detailed description of the procedure is reported in table 1.

116 Dogs were videotaped throughout the test and their behaviour was analysed using a continuous
117 sampling method in order to measure the duration (in seconds) of dogs' social and non-social
118 behaviours. The complete list of analysed behaviours is reported in Mariti et al. (2014).

119

120 Statistical analysis

121 In order to exclude the possible impact of factors other than being tested with the mother, a
122 statistical analysis was performed on factors assessed through a quantitative measure: X^2 test ($p <$

123 0.05) for the female/male ratio and t test ($p < 0.05$) for age of the tested dog, age of the other dog,
124 time spent in the same household, and number of dogs in the household.

125 Once we studied the potential differences in factors between the two groups, we proceeded to
126 analyze the differences between and within groups for the factor "test with the mother". The first
127 step of the statistical analysis consisted in comparing the MO and the OF groups for all the episodes
128 using a U-Mann Whitney test (multiple comparison corrections were performed using the
129 Benjamini-Hochberg procedure).

130 The second step was a within-group comparison for both MO and OF groups. The Kruskal-Wallis
131 test was applied to each behaviour in all the seven episodes, and behaviours resulted statistically
132 different ($p < 0.05$) with that test were re-analysed with the Wilcoxon paired-sample test. Due to the
133 results of the first analysis and to previous literature (Mariti et al., 2013, 2014), such analysis was
134 performed on episodes 5 (isolation), 6 (reunion with the stranger) and 7 (reunion with the presumed
135 attachment figure: mother or older female dog). A Bonferroni correction for multiple comparisons
136 was applied, considering the three possible combinations: episode 5 versus 6, episode 5 versus 7,
137 and episode 6 versus 7. The significance level was therefore $\alpha = 0.0167$.

138

139 **RESULTS**

140 The statistical analysis revealed no difference between the two groups for any of the analysed
141 factors: female/male ratio ($X^2 = 0.062$, $p = 0.803$), age of the tested dog ($t = -1.110$, $p = 0.272$), age
142 of the other dog ($t = 0.508$, $p = 0.614$), time spent in the same household ($t = -0.141$, $p = 0.889$), and
143 number of dogs in the household ($t = 0.196$, $p = 0.846$).

144 The behaviour of dogs belonging to the two groups was then compared. The first statistical analysis
145 revealed no significant differences between MO and OF for episodes 1, 2, 3, 4, and 6. In episodes 5,
146 dogs of the OF group spent more time close to the door compared to dogs tested with their own
147 mother (range: 26-120 versus 9-120; median: 93.50 versus 77.00; $U = 177.00$; $p = 0.025$). In

148 episode 7, dogs of the MO group spent more time oriented to the door (range: 37-111 versus 11-
149 111; median: 77.00 versus 60.23; $U = 168.00$; $p = 0.015$).

150 The second step of the statistical analysis gave the results summarized in table 2 and 3. In the tables,
151 only analysed behaviours for which a comparison led to any statistically significant differences are
152 reported.

153 Figures 1 shows, as example, the results concerning the duration of behaviours against the door in
154 both groups in the three analysed episodes.

155

156 **DISCUSSION**

157 The analysis of dogs' behaviour during the ASST clearly showed that, when isolated in an
158 unfamiliar room, dogs of both groups displayed behaviours indicative of distress and attempt to
159 regain proximity (e.g. behaviours against the door, staying close to the door, and vocalisations) for
160 longer time compared to their display in the presence of a social stimulus. However, behaviours
161 indicative of distress (i.e. behaviours against the door and staying close to the door) were observed
162 for longer time in the presence of the older female dog than in the presence of the human stranger,
163 whilst such difference did not emerge for the group tested with the canine mother. In addition, the
164 comparison between episode 6 (reunion with the stranger) and episode 7 (reunion with the
165 presumed attachment figure, i.e. the mother or the older female dog), resulted in different findings
166 depending on the group: dogs looked more at the mother than at the stranger, but dogs displayed
167 more affiliative behaviours (following and seeking attention) to the stranger than to the unrelated
168 older female dog.

169 In the MO group it was found that the longer proximity far from the door towards the stranger was
170 likely due to the fact that the stranger had to stay seated, whilst the mother dog was free to move, so
171 she could stay close to the door together with the tested dog. In the OF group, proximity at first
172 glance could seem to be higher towards the older female dog, but the longer total proximity was

173 actually due to the higher time spent close to the door by both the tested and the older female dog.
174 In fact, proximity far from the door did not differ between episodes 6 and 7.

175 The results of the OF group are in line with findings previously described by Mariti et al. (2014),
176 where dogs, after separation, showed a preference for a stranger over another dog living in the same
177 household. However, from the current study it emerged that the characteristics of the relationship
178 between the two dogs involved in the Ainsworth Strange Situation Test affected dogs' behaviour
179 during the procedure. More in detail, the ameliorative effect of the mother dog was comparable to
180 the ameliorative effect of a human stranger (no difference between episodes 6 and 7, except longer
181 visual orientation to the mother); whilst dogs preferred the human stranger (longer following and
182 seeking attention behaviours) in case they were tested with an older female dog other than the
183 mother.

184 Contrary to what expected from previous literature on children-mother and dog-human bond, dogs
185 of neither group showed a preference for the presumed attachment figure over the stranger. Mariti et
186 al. (2014) explained this appeal of dogs to humans as a result of the special relationship linking
187 these species, which has strong peculiarities both in the phylogenesis and in the ontogenesis of the
188 domestic dog. Mariti et al. (2014) also hypothesized that intraspecific relationships in adult dogs
189 may have very different features: e.g. owners may represent the main figure of attachment for their
190 dogs, while cohabitant dogs may be secondary figures; or adult dogs, among them, may establish a
191 relationship that is more similar to that of peers, as found in human children towards siblings (e.g.
192 see Trouple-Cremel and Zauche-Gaudron, 2006) or between human partners (e.g. see Feeny, 1999).

193 The findings of the current study suggest that, given the possibility to keep it into adulthood, adult
194 offspring-mother relationship in dogs differ from the relationship between an adult domestic dog
195 and an unrelated older female dog. Interestingly, such difference is not due to sharing everyday life,
196 as whole our sample was composed by dogs living in the same household with the presumed canine
197 attachment figure since a long time. The relationship with the mother seems to have characteristics

198 that predispose it to be somehow maintained. In fact, 2 years old dogs are able to recognise their
199 mother even if they have been separated from them from 8-12 weeks after birth (Hepper, 1994).
200 The stronger bond with the mother is probably maintained from puppyhood (Prato-Previde et al.,
201 2009), and it is likely due to the relevance of maternal care in domestic dogs' social relationships,
202 as suggested by Guardini et al. (2016) and Foyer et al. (2016). However, also the possibility to
203 socialize and interact in the very first weeks of life may be responsible of it. Future studies may test
204 dogs living in the same households since birth without being linked by a mother-offspring
205 relationship, in order to clarify which are the effects of maternal care and of the familiarity in the
206 first weeks of life.

207 As this study was carried out using two independent groups of dogs, individual differences may be
208 regarded as responsible of the observed differences. Some measures were taken in order to reduce
209 that risk. For instance, an early weaning (Mogi et al., 2011), an early separation from littermates
210 (Pierantoni et al., 2011), and a disruption of the bond with owners (Prato-Previde and Valsecchi,
211 2007) are all factors known to affect the development of social and emotional behaviours of dogs.
212 For this reason, dogs included in this study were born at home or acquired at 2 months of age, living
213 with the mother before acquisition and with the same owner later on. In addition, the two groups
214 were as matched as possible for the age of tested dogs, the age of mother/older female dogs, the
215 time spent in the same household, the number of dogs living in the same household, and the
216 environment in which tested dogs were living. The fact that MO and OF dogs provided the same
217 response to the novel environment and to the human stranger at the first meeting (episode 1, 2 and
218 3) allowed us to consider the two groups basically equivalent for their "base" behaviour, and to
219 attribute the statistically significant differences to the kind of relationship that tested dogs have with
220 the other dog.

221 In this study, the gender of tested dogs was not considered as a factor possibly affecting the results,
222 based on previous findings of Tuber et al. (1996) and Mariti et al. (2014). It is desirable that future

223 studies will investigate the possible impact of the gender of both the tested dog and the dog playing
224 the role of the presumed attachment figure. However, the gender is not *per se* a variable related to
225 the care provided by adult dogs to younger ones (Pal, 2005).

226 Summarizing, the presence of a human stranger had a stronger ameliorative effect when compared
227 to the presence of an older female dog living in the same household, but the ameliorative effect was
228 almost identical when the stranger was compared to the canine mother. Considering the peculiar
229 appeal that human beings have to dogs (Mariti et al., 2014), these findings suggest that the bond of
230 adult dogs towards conspecifics sharing their daily life is stronger in case the conspecific is their
231 own mother rather than an unrelated older female dog. However, such bond does not fit all the
232 characteristics of an attachment bond as intended in a child-caregiver or in a dog-human bond
233 (Mariti et al., 2013).

234

235 **CONCLUSIONS**

236 Dogs are linked to conspecifics living in the same household by a bond that does not completely
237 correspond to the attachment bond in human children towards caregivers, nor to the attachment
238 bond in adult dogs towards their owners. However, the bond of adult dogs towards their own
239 mothers, when living together, seems to be stronger than the bond towards unrelated older female
240 conspecifics living together but not being the mother.

241 Future research should investigate more in depth this kind of bond, its features and its connection
242 with canine behaviour and social life.

243

244 **Acknowledgments**

245 The authors wish to thank all the owners and their dogs who participated at the study. A special
246 thank to: Teresa Ruocco, Michele Lazzerini, Valeria Migliaccio, Rosita Galli, Filippo Alganon,
247 Lisa Porquier, Virginia Orlandi, Laura Vassallo, Giusy Mazzalupi, Silvia Costamagna, and Lucia

248 Casini. Preliminary data of this study have been presented in abstract form at the Canine Science
249 Forum in Padua, Italy, 28th June-1st July 2016.

250 The idea for the article was conceived by C. Mariti and A. Gazzano. The experiments were
251 designed by C. Mariti, C. Sighieri and A. Gazzano. The experiments were performed by C. Mariti,
252 E. Votta, E. Ricci and B. Carlone. The data were analyzed by C. Mariti. The article was written by
253 C. Mariti and B. Carlone. All authors have approved the final article.

254

255 **Ethical considerations**

256 This research was an observational study involving owned dogs, thus it did not require the approval
257 by an ethical committee. Owner informed consent and authorization to video record were obtained
258 before testing each dog.

259

260 **Conflict of interest**

261 We have read and understood this journal's policy on declaration of interests and declare that we
262 have no competing interests.

263

264 **REFERENCES**

265 Ainsworth, M. S. (1989). Attachments beyond infancy. *American Psychologist*, 44, 709–716.

266 Ainsworth, M.D., Bell, S.M., 1970. Attachment, exploration and separation- illustrated by
267 behaviour of one year olds in a strange situation. *Child Dev.* 41, 49-67.

268 Bowlby, J., 1979. The making and breaking of affectional bonds. Tavistock, London, UK, p. 129.

269 Bowlby, J., 1988. A secure base. Basic Books, New York, pp. 120–121.

270 Bowlby, J., 1982. Attachment and loss, vol. 1, Attachment. 2nd edition. Hogarth Press/Chatto &
271 Windus, London.

272 Carpenter, D.B., 2001. Attachment bonds between adult daughters and their older mothers:
273 associations with contemporary caregiving. *J. Gerontol. B. Psychol. Sci. Soc. Sci* 56, 257-P266.

274 Cassidy, J., Jones, J.D., Shaver, P.R., 2013. Contributions of attachment theory and research: a
275 framework for future research, translation, and policy. *Dev Psychopathol.* 25, 1415–1434.

276 Feeny, J.A., 1999. Adult romantic attachment and couple relationships. In: Cassidy, J., Shavers, P.R.
277 (Eds.), *Handbook of Attachment: Theory, Research, and Clinical Applications*. Guilford, New
278 York, pp. 355-377.

279 Foyer, P., Wilsson, E., Jensen, P., 2016. Levels of maternal care in dogs affect adult offspring
280 temperament. *Sci. Rep.* 6, 19253.

281 Gácsi, M., Topál, J., Miklósi, A., Dóka, A., Csányi, V., 2001. Attachment behavior of adult dogs
282 (*Canis familiaris*) living at rescue centers: forming new bonds. *J. Comp. Psych.* 115, 423-431.

283 Guardini, G., Mariti, C., Bowen, J., Fatjò, J., Ruzzante, S., Martorell, A., Sighieri, C., Gazzano, A.,
284 2016. Influence of morning maternal care on the behavioural responses of 8-week-old Beagle
285 puppies to new environmental and social stimuli. *Appl. Anim. Behav. Sc.* 181, 137-144.

286 Hepper, P., 1994. Long-term retention of kinship recognition established during infancy in the
287 domestic dog. *Appl. Anim. Behav. Sc.* 33, 3-14.

288 Mariti, C., Ricci, E., Zilocchi, M., Gazzano, A., 2013. Owners as a secure base for their dogs.
289 *Behaviour* 150, 1275–1294.

290 Mariti, C., Carlone, B., Ricci, E., Sighieri, C., Gazzano, A., 2014. Intraspecific attachment in adult
291 domestic dogs (*Canis familiaris*): preliminary results. *Appl. Anim. Behav. Sci.* 152: 64-72.

292 Mogi, K., Nagasawa, M., Kikusui, T., 2011. Developmental consequences and biological
293 significance of mother-infant bonding. *Prog. Neuropsychopharmacol Biol. Psychiatry* 35, 1232-
294 1241.

295 Payne, E., DeAraugo, J., Bennett, P., McGreevy, P., 2016. Exploring the existence and potential
296 underpinnings of dog–human and horse–human attachment bonds. *Behav. Proc.* 125, 114-121.

297 Pettijohn, T.F., Wong, T.W., Ebert, P.D., Scott, J.P., 1977. Alleviation of separation distress in 3
298 breeds of young dogs. *Dev. Psychol.* 10, 373–381.

299 Pierantoni, L., Albertini, M., Pirrone, F., 2011. Prevalence of owner-reported behaviours in dogs
300 separated from the litter at two different ages. *Vet. Rec.* 169, 468.

301 Prato-Previde, E., Valsecchi, P., 2007. Effect of abandonment on attachment behavior of adult pet
302 dogs. *J. Vet. Behav.* 2, 87.

303 Prato-Previde, E., Ghirardelli, G., Marshall-Pescini, S., Valsecchi, P., 2009. Intraspecific
304 attachment in domestic puppies (*Canis familiaris*). *J. Vet. Behav.* 4, 89-90.

305 Tuber, D.S., Hennessy, M.B., Sanders, S., Miller, J.A., 1996. Behavioral and glucocorticoid
306 responses of adult domestic dogs (*Canis familiaris*) to companionship and social separation. *J.*
307 *Comp. Psychol.* 110, 103–108.

308 Trouple-Cremel, O., Zauche-Gaudron, C., 2006. Infant-mother attachment to infant-sibling
309 attachment: evolution of the paradigms of research. *Psychol. Fr.* 51, 205-215.

310 van der Horst, F.C.P., van der Veen, R., van Ijzendoorn, M.H., 2007. John Bowlby and ethology: an
311 annotated interview with Robert Hinde. *Attachment & Human Development*, 9:4, 321-335.

312 Waters, E., Cummings, E.M., 2000. A secure base from which to explore close relationships. *Child*
313 *Dev.* 71, 164–172.

314 Walker, J.K., Waran, N.K., Phillips, C.J.C., 2014. The effect of conspecific removal on the
315 behaviour and physiology of pair-housed shelter dogs. *Appl. Anim. Behav. Sc.* 158, 46-65.

316

317 Tab. 1: procedure of the ASST as adapted for the study of intraspecific attachment bond.

Episodes	Subjects involved	Description
1	Dog1+ Dog2	dogs were free to move and explore the room
2	Dog1+ Dog2 + Stranger	Stranger 1 entered the room and she could greet the dogs if they were seeking attention. Then she sat on the chair and had to ignore the dogs, but she could not move them away if they approached her
3	Dog1+ Stranger	Dog2 is taken in another room 20 m away from the experimental room. Stranger 1 sat on the chair.
4	Dog1+ Dog2	Dog2 is led into the experimental room; in the meanwhile, Stranger left the room
5	Dog1	Dog2 is taken in another room 20 m away from the experimental room. Dog1 is in complete isolation.
6	Dog1+ Stranger	Stranger 1 entered the room and could greet the dog, then she sat
7	Dog1+ Dog2	Dog2 is led into the experimental room; in the meanwhile, Stranger left the room

318 Dog1 = dog tested. Dog2 = dog acting as the presumed attachment figure. Stranger = unfamiliar person.

319 Tab. 2: summary of the remarkable results raising from the comparison between episodes 5
 320 (isolation), 6 (with stranger) and 7 (with the familiar dog) for the dogs tested with their own mother.

321

	Comparison (median values)	Min-max range	Results	Summary
Socially oriented	Ep. 6 vs 7 (17.0 vs 9.5)	6-83 vs 0-45	W=94.50, p=0.032	7>6 ⁺
Following	Ep. 6 vs 7 (2.0 vs 0.0)	0-70 vs 0-9	W=112.00, p=0.099	≈
Seeking attention	Ep. 6 vs 7 (0.0 vs 0.0)	0-58 vs 0-2	W=122.00, p=0.121	≈
Proximity far from door	Ep. 6 vs 7 (37.0 vs 5.1)	0-120 vs 0-119	W=73.50, p=0.005	6>7*
Total proximity	Ep. 6 vs 7 (37.0 vs 52.0)	0-120 vs 0-119	W=157.00, p=0.874	≈
Close to door	Ep. 5 vs 6 (77.0 vs 10.5)	9-120 vs 0-100	W=233.50, p=0.002	5>6*
	Ep. 5 vs 7 (77.0 vs 52.0)	9-120 vs 0-116	W=113.50, p=0.125	≈
	Ep. 6 vs 7 (10.5 vs 52.0)	0-100 vs 0-116	W=106.00, p=0.076	≈
Oriented to door	Ep. 5 vs 6 (90.5 vs 75.0)	38-117 vs 32-110	W=90.00, p=0.023	5>6 ⁺
	Ep. 5 vs 7 (90.5 vs 77.0)	38-117 vs 37-111	W=92.00, p=0.027	5>7 ⁺
	Ep. 6 vs 7 (75.0 vs 77.0)	32-110 vs 37-111	W=145.50, p=0.601	≈
Behaviours against door	Ep. 5 vs 6 (7.0 vs 0.0)	0-47 vs 0-101	W=57.00, p=0.001	5>6*
	Ep. 5 vs 7 (7.0 vs 0.0)	0-47 vs 0-26	W=67.00, p=0.001	5>7*
	Ep. 6 vs 7 (0.0 vs 0.0)	0-101 vs 0-26	W=150.50, p=0.645	≈
Whining	Ep. 5 vs 6 (13.0 vs 8.5)	0-62 vs 0-47	W=128.50, p=0.287	≈
	Ep. 5 vs 7 (13.0 vs 5.4)	0-62 vs 0-34	W=105.00, p=0.068	≈
	Ep. 6 vs 7 (8.5 vs 5.4)	0-47 vs 0-34	W=143.00, p=0.542	≈
Barking	Ep. 5 vs 6 (0.0 vs 0.0)	0-19 vs 0-4	W=108.00, p=0.016	5>6*
	Ep. 5 vs 7 (0.0 vs 0.0)	0-19 vs 0-1	W=105.00, p=0.013	5>7*
	Ep. 6 vs 7 (0.0 vs 0.0)	0-4 vs 0-1	W=161.50, p=0.968	≈

322 ≈=no statistically significant differences; *= statistically significant, p<0.0167; ⁺= 0.0167<p<0.005

323

324 Tab. 3: summary of the remarkable results raising from the comparison between episodes 5
 325 (isolation), 6 (with stranger) and 7 (with the familiar dog) for the dogs tested with an older female
 326 dog living in the same household.

327

	Comparison (median values)	Min-max range	Results	Summary
Socially oriented	Ep. 6 vs 7 (15.5 vs 12.5)	0-75 vs 2-86	W=1031.50, p=0.909	≈
Following	Ep. 6 vs 7 (1.1 vs 0.0)	0-6 vs 0-9	W=879.50, p=0.014	6>7*
Seeking attention	Ep. 6 vs 7 (0.5 vs 0.0)	0-105 vs 0-16	W=888.00, p=0.019	6>7 ⁺
Proximity far from door	Ep. 6 vs 7 (23.0 vs 11.5)	0-120 vs 0-119	W=963.00, p=0.299	≈
Total proximity	Ep. 6 vs 7 (23.0 vs 48.0)	0-120 vs 0-120	W=888.00, p=0.041	7>6 ⁺
Close to door	Ep. 5 vs 6 (93.5 vs 10.0)	26-120 vs 0-120	W=638.00, p<0.001	5>6*
	Ep. 5 vs 7 (93.5 vs 46.0)	26-120 vs 0-120	W=471.00, p=0.004	5>7*
	Ep. 6 vs 7 (10.0 vs 46.0)	0-120 vs 0-120	W=813.00, p=0.002	7>6*
Oriented to door	Ep. 5 vs 6 (82.0 vs 64.5)	9-120 vs 0-106	W=856.00, p=0.013	5>6*
	Ep. 5 vs 7 (82.0 vs 60.2)	9-120 vs 11-111	W=858.50, p=0.015	5>7*
	Ep. 6 vs 7 (64.5 vs 60.2)	0-106 vs 11-111	W=1032.00, p=0.914	≈
Behaviours against door	Ep. 5 vs 6 (5.2 vs 0.0)	0-56 vs 0-82	W=753.00, p<0.01	5>6*
	Ep. 5 vs 7 (5.2 vs 0.0)	0-56 vs 0-26	W=847.50, p=0.007	5>7*
	Ep. 6 vs 7 (0.0 vs 0.0)	0-82 vs 0-26	W=904.00, p=0.024	7>6 ⁺
Whining	Ep. 5 vs 6 (19.0 vs 9.0)	0-66 vs 0-63	W=854.50, p=0.012	5>6*
	Ep. 5 vs 7 (19.0 vs 2.2)	0-66 vs 0-70	W=798.00, p=0.001	5>7*
	Ep. 6 vs 7 (9.0 vs 2.2)	0-63 vs 0-70	W=992.50, p=0.518	≈
Barking	Ep. 5 vs 6 (0.0 vs 0.0)	0-50 vs 0-42	W=943.50, p=0.109	≈
	Ep. 5 vs 7 (0.0 vs 0.0)	0-50 vs 0-74	W=904.50, p=0.014	5>7*
	Ep. 6 vs 7 (0.0 vs 0.0)	0-42 vs 0-74	W=082.50, p=0.222	≈

328 ≈=no statistically significant differences; *= statistically significant, p<0.0167; ⁺= 0.0167<p<0.005

329

330

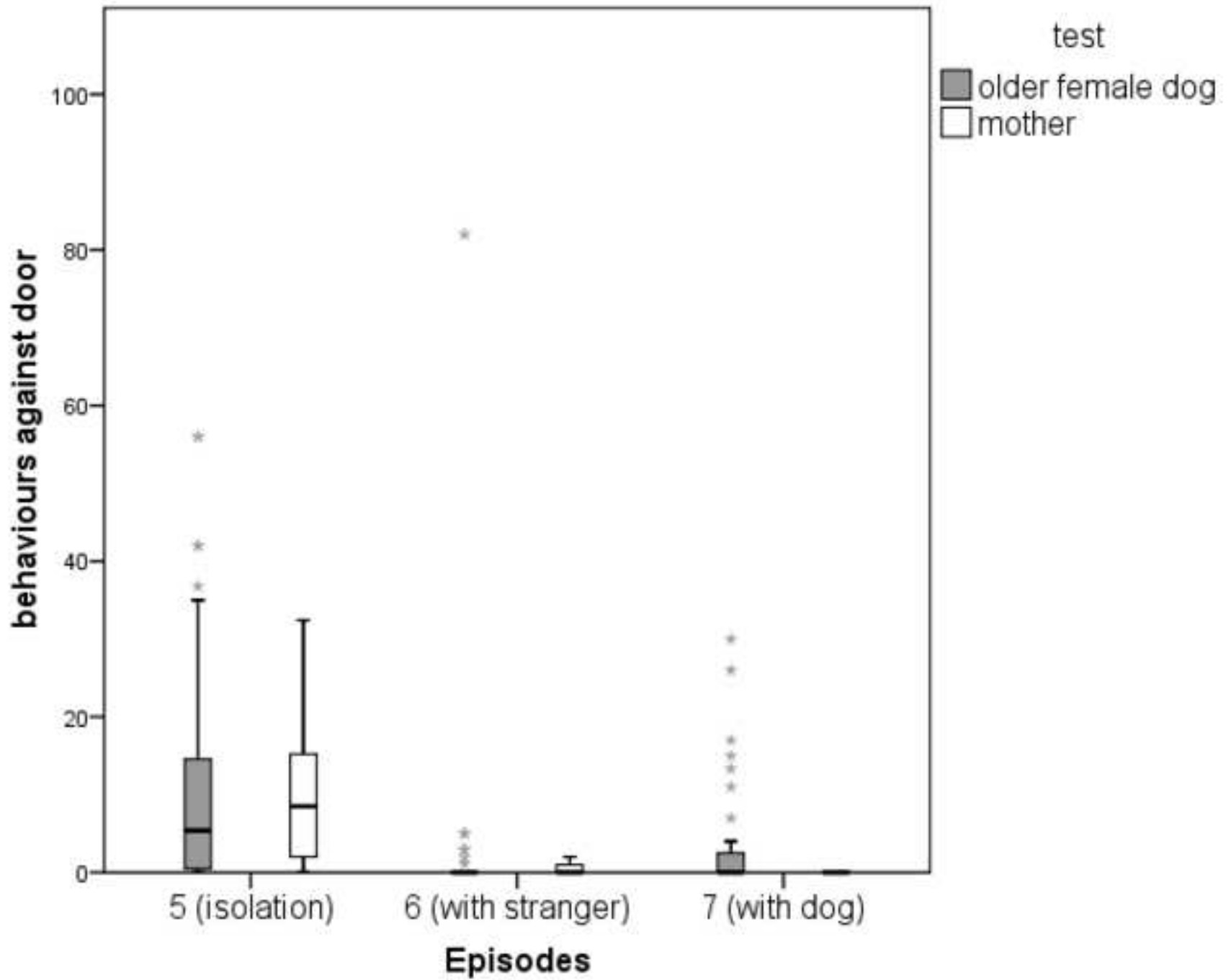
331

332

333 Fig. 1: results concerning the duration of behaviours against the door in episode 5 (isolation), 6
 334 (with stranger) and 7 (with the familiar dog) in both the MO (dogs tested with mother) and the FO
 335 (dogs tested with an older female dog living in the same household) groups. For each box, the
 336 bottom and top horizontal lines represent the lowest and highest values, the lowest and top edge of
 337 the grey/white box represent the lower and upper quartile, the horizontal line within the grey/white
 338 box represents the median, and the stars represent the outliers.

339

Figure
[Click here to download high resolution image](#)



AUTHOR DECLARATION TEMPLATE

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from **chiara.mariti@unipi.it**

Signed by all authors as follows:

CHIARA MARITI 12/08/2016



BEATRICE CARLONE 12/08/2016



EMILIA VOTTA 12/08/2016



EVA RICCI 12/08/2016



CLAUDIO SIGHIERI 12/08/2016



ANGELO GAZZANO 12/08/2016

