



HAL
open science

Captive Blue-and-yellow macaws (*Ara ararauna*) show facial indicators of positive affect when reunited with their caregiver

Aline Bertin, Baptiste Mulot, Raymond Nowak, Marie-Claire Blache, Scott A. Love, Mathilde Arnold, Annabelle Pinateau, Cécile Arnould, Léa Lansade

► To cite this version:

Aline Bertin, Baptiste Mulot, Raymond Nowak, Marie-Claire Blache, Scott A. Love, et al.. Captive Blue-and-yellow macaws (*Ara ararauna*) show facial indicators of positive affect when reunited with their caregiver. *Behavioural Processes*, 2023, 206, pp.104833. 10.1016/j.beproc.2023.104833 . hal-04168459v2

HAL Id: hal-04168459

<https://hal.inrae.fr/hal-04168459v2>

Submitted on 17 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Captive Blue-and-yellow macaws (*Ara Ararauna*) show facial indicators of positive affect**
2 **when reunited with their caregiver.**

3 Aline Bertin^a, Baptiste Mulot^b, Raymond Nowak^a, Marie-Claire Blache^a, Scott Love^a,
4 Mathilde Arnold^b, Annabelle Pinateau^b, Cécile Arnould^{a*}, Léa Lansade^{a*}

5 ^a CNRS, IFCE, INRAE, Université de Tours, PRC, 37380, Nouzilly, France; ^bZooParc de
6 Beauval & Beauval Nature, Saint-Aignan, France

7

8 *co-last authors

9 *corresponding author: aline.bertin@inrae.fr, ^aPRC, CNRS, IFCE, INRAE, Université de
10 Tours, 37380, Nouzilly, France.

11 Baptiste Mulot : baptiste.mulot@zoob Beauval.com; Raymond Nowak :
12 raymond.nowak@inrae.fr; Marie-Claire Blache : marie-claire.blache@inrae.fr; Scott Love :
13 scott.love@inrae.fr; Mathilde Arnold : mathilde.arnold@zoob Beauval.com; Annabelle
14 Pinateau : annabelle.pinateau@gmail.com; Léa Lansade : lea.lansade@inrae.fr; Cécile
15 Arnould : cecile.arnould@inrae.fr.

16 Declarations of interest: none

17

18

19 *Keywords:* Animal sentience; Human-animal bond; Facial expression; Positive emotions;
20 Emotional expression

21

22 **Abstract**

23

24 In mammals, human-animal bonding is recognized as a source of positive affect for
25 companion or farm animals. Because this remains unexplored in birds, we investigated
26 captive parrots' perspective of the human-animal relationship. We used a classical separation-
27 reunion paradigm and predicted that variations in parrots' facial displays and behaviours
28 would indicate their appraisal of the relationship. The test was divided into three phases of
29 two minutes each: the bird was placed in an unfamiliar environment with a familiar caregiver
30 (union), then the bird was left alone (separation) and finally, the caregiver returned (reunion).
31 The test was repeated 10 times for each bird and video recorded in order to analyze their
32 behaviour. The data show significantly higher crown and nape feather heights, higher redness
33 of the skin and higher frequency of contact-seeking behaviours during the union and reunion
34 phases than during the separation phase during which they expressed long distance contact
35 calls. We observed the expression of eye pinning during the union and reunion phases in one
36 out of five macaws. We argue that variation in facial displays provides indicators of parrot's
37 positive appraisal of the caretaker presence. Our results broaden the scope for further studies
38 on parrots' expression of their subjective feelings.

39

40

41

42

43

44

45

46

47 **1. Introduction**

48 Interacting and bonding with companion animals such as dogs, cats, horses or birds is
49 known to provide multiple benefits for human well-being (e.g. Beck 2014; Beck and Katcher
50 1996; Katcher and Beck, 1987). Comparatively, reciprocal studies dealing with the benefit for
51 animals of bonding with humans are rarer and focused on mammals. To investigate this bond,
52 the separation-reunion paradigm - derived from human psychology to investigate attachment-
53 related behaviours (Ainsworth and Bell, 1970) - has been used on farm animals and more
54 recently on companion dogs, cats or horses (Topál et al., 2005; Nagasawa et al., 2009; Payne
55 et al., 2015; Rehn et al., 2014; Vitale et al., 2019; Lundberg et al., 2020). In farm animals, the
56 existence of a positive affiliative bond towards humans was recognized as beneficial for
57 animal welfare (Rault et al., 2020). For example, lambs express distress vocalizations when
58 isolated but when they are reunited with a familiar caregiver they search physical contact and
59 display appeasement. This indicates that the caregiver acts as a social support for the lambs
60 (Nowak et al., 2015; Price and Thos, 1980; Boivin et al., 2001; Coulon et al., 2013; Rault et
61 al., 2011). In dogs, the mere return of the owner after a brief separation is known to induce
62 contact-seeking behaviour and to have a positive effect on oxytocin levels – a neuropeptide
63 thought to be involved in the expression of prosocial behaviours and positive emotions -
64 (Rhen, 2014).

65 Emotions are characterized by their valence (positive or negative) and arousal level
66 (high or low; Mendl et al., 2010). Two types of positive emotions or states are commonly
67 distinguished. Those characterized by high arousal levels such as joy, excitement,
68 consummatory or appetite motivational states and, those characterized by low arousal levels
69 such as calmness, relaxation, safeness, social bonding or post-consummatory behaviours
70 (Mendl et al., 2010; Richardson et al., 2016; Carver, 2001). In previous studies, we showed
71 that specific facial displays such as crown and nape feather ruffling were associated with

72 activities having a positive valence and low arousal levels, like quiet positive social
73 interactions, maintenance or resting in Blue-and yellow macaws or Sulphur-crested cockatoo
74 (*Cacatua galerita*) (Bertin et al., 2018a; 2020). Macaws were also found to blush (i.e. increase
75 in blood flow in vascularized tissues) and ruffle their crown feathers when engaged in a
76 mutual interaction with their familiar caregiver (Bertin et al., 2018a), a context recognized as
77 rewarding for captive parrots (Pepperberg and McLaughlin 1996). Head feather ruffling was
78 also observed in Japanese quail performing dust-bathing, a behaviour considered as rewarding
79 with high arousal level (Bertin et al., 2018b).

80 Psittaciformes are highly social with primate-like cognitive capacities (Olkowicz et al.
81 2016; Gutiérrez-Ibáñez et al., 2018). Despite being wild non-domesticated animals, they form
82 strong affiliative bonds with people when captive (Baker, 2012; Anderson, 2014; Bond and
83 Diamond, 2019). We propose that, similarly to mammalian domesticated animals, a human-
84 animal social bond is a source of positive affect for these birds. To reveal attachment-related
85 behaviours, it is necessary to place the individual in a slightly stressful situation (Bowlby,
86 1982). Here we used a separation-reunion test in an unfamiliar environment as a slightly
87 stressful situation. The test was an adaptation of the Ainsworth Strange Situation test in which
88 human infants are separated and reunited from a parent or a stranger in a novel environment
89 (Ainsworth 1979). The original test compared the child's proximity seeking, during distress,
90 to the attachment figure or the stranger (Ainsworth 1979). Because our parrots were not used
91 to being approached by strangers and they show fear or aggressive behaviours in general
92 towards them, it was not possible to test parrots' behaviours when reunited with an unfamiliar
93 human. As a consequence, our study did not aim to determine whether parrots show a
94 preference for a specific attachment figure which, is one of the criteria that define a social
95 bond as attachment *per se* (a special affectional and emotional relationship between two
96 specific individuals).

97 We hypothesized that if the parrots were socially bonded toward their most familiar
98 caregiver then, this person should act as a social support to the birds (i.e: the ability of social
99 partners to enhance one's ability to cope with a challenging situation). Evidence of this social
100 support could consist of signs of positive-like emotional responses (e.g. calmness, relaxation)
101 during the test despite being placed in an unfamiliar environment and separated from
102 conspecifics. We predicted that this bond would be expressed by variations in behaviour
103 during the different phases of the test: union, separation, reunion. Specifically, based on our
104 previous research, we expected that macaws would express feather ruffling and blushing
105 when in presence of the familiar caregiver and a potentially enhanced response (rebound
106 effect) of these behaviours during the reunion phase.

107 **2. Animals and methods**

108 *2.1. Birds and housing conditions*

109 We observed five hand-reared Blue-and-yellow macaws (two males and three females
110 between 7 and 12 years old), not exposed to public visitors, at the Zooparc de Beauval Saint
111 Aignan (41110, France). All birds are part of a free-flying show. They had been trained daily
112 (i.e. handled daily) since weaning and were thus in close contact with humans, especially their
113 caregivers. During free-flight training the birds were taught to land on the caregivers' arm
114 when called after unrestricted outdoor flight. The five birds were housed in two adjacent
115 aviaries of similar sizes with an indoor area (250 cm x 520 cm x 260 cm) freely connected to
116 an outdoor area (250 cm x 850 cm x 260 cm) and were mixed with a pair of Sulphur-crested
117 cockatoo. The aviaries were equipped with several tree branches, perches and ropes.
118 Enrichments were provided daily (cardboard and newspaper). Parrots were fed daily with
119 fresh fruits and vegetables, germinated seeds (wheat, corn, sunflower, rice, and oat), millet
120 seeds, oyster shells, and a commercial mix for exotic birds.

121 *2.2 Test apparatus and procedure*

122 All the tests took place inside a (3m l x 3m L x 2m h) barnum installed indoors,
123 unfamiliar to the birds and approximately 60 meters away from their aviaries. In order to
124 obtain profile images of the heads of the birds, the barnum was equipped with a perch (1.15 m
125 high, 80 cm long) placed perpendicularly to a small window allowing the passage of the
126 camera recorder mounted on a tripod. All the walls and the roof were opaque and composed
127 of white fabric. A lamp (Somikon photo light SLH3), placed on the floor, was used to have
128 even lighting conditions. All the tests were filmed by a Sony FS5 4K camera.

129 All birds were tested individually 10 times on 10 different days. All the tests were
130 conducted between 10am and 12pm for two successive weeks. Each bird was tested only once
131 per day and rank order of the birds was counterbalanced between days. All birds were tested
132 with the same animal caretaker. We worked with the animal caretaker who was the most
133 familiar to all of them (more than 10 years of caring for them). The caretaker chosen was also
134 the only one spending quality time with them daily to work on bonding (like playing with
135 them, providing tactile contacts or talking to them). As we observed pet-like behaviours
136 (supplementary data, Figure S1) during the daily routine in presence of the caretaker -
137 characteristic of an affiliative social bond with humans in pet macaws (Jordan, 2003) - we
138 assumed that, more than being familiar, a positive social bond had been established between
139 the birds and the caretaker. For each test session, a bird was called by its name, placed in its
140 usual transport cage (the same used for transport before free-flight shows) and taken to the
141 barnum. Then the bird was put on the perch by hand. The test was divided into three phases of
142 two minutes each:

- 143 1. Union: the familiar caregiver stayed with the bird
- 144 2. Separation: the caregiver left the barnum and thus the bird alone
- 145 3. Reunion: the caregiver returned (reunion).

146 On the first day of testing, the birds were not willing to stay on the perch. They flew back
147 to their transport cage or perched on the caregiver's arms. Therefore, from day 2 to day 10,
148 the transport cage was left outside of the barnum and the birds were brought by hand inside
149 the barnum. The caregiver remained motionless in front of the bird at approximately 50 cm,
150 was asked to keep the arms behind her back. The caregiver was allowed to whisper the same
151 words to all parrots ("oui, c'est bien") to encourage them to stay on the perch. At the end,
152 each parrot received a nut and was placed back in its transport cage.

153 *2.3. Facial display characterization*

154 We used a protocol similar to Bertin et al. (2018b) on video-recordings obtained from
155 day 2 to day 9. We used the VLC software to search images of clear profiles and extract them
156 (Fig. 1). To homogenize the time when the profiles were extracted between birds, the profiles
157 were searched within twelve 30-s sequences. We extracted images every second with the
158 software and kept the first clear profile found within the 30-s sequence for analysis. We
159 extracted one clear profile per 30-s sequence for all birds (12 images per bird per test). We
160 used the software ImageJ, an image analysis program, to measure crown feather height, nape
161 feather height and area of the pupil. To correct for variation in the distance of the bird from
162 the camera, we used an invariant distance on each bird. For each bird, we measured manually
163 before the experiment, the real distance between the top of the beak and its tip with a digital
164 calliper (± 0.01 mm) (Fig. 1; a). Then, for each image, we used the function "straight" to draw
165 this distance on the image and then the function "set scale" to convert the distance in pixels to
166 distance in real centimetres. To measure crown feather height, we drew an angle with a
167 vertical plane going from the top of the beak (boundary between the nostril and the beak) and
168 the middle of the pupil (Fig. 1; b) and a 90° angle from the middle of the pupil to the top of
169 the green feathers (Fig. 1; c). The length of the line was determined with the function
170 "analyse" and "measure". To measure nape feather height, we estimated the length of a line

171 adjacent to the white skin of the jaw and going from the top of the blue feathers on the head to
172 the tip of the black feathers of the throat (Fig. 1; d). The pupil area was assessed with the
173 function “oval”, with which we circled the black pupil and then used the function “measure”
174 to obtain the area of the circle.

175 To assess the presence of blushing (redness of the skin), we drew a 10 x 10 pixels
176 square on the bare skin at the external corner of the eye, between the blue feathers of the head
177 and the black feathers of the jaw (Fig. 1; e). With the function “measure” of image J, we
178 obtained the mean red (R), mean green (G), and mean blue (B) values of the square. Similarly
179 to some carotenoid-based studies (Pérez-Rodríguez and Viñuela 2008; Passarotto et al.,
180 2021), we calculated the redness of the skin as $R / (R+G+B)$. In order to control for the
181 balance of the white within the images, a 10 x 10 pixels square was also drawn on the white
182 wall of the barnum for each image. Because the values of redness obtained in the white wall
183 (mean Redness: 0.33 ± 0.001) did not differ significantly from a theoretical redness obtained
184 in a white or grey standard reference (0.33) (t-test, $P > 0.05$), no colour correction of the
185 images was deemed necessary. The same well-trained experimenter, blind to the phase, made
186 all the measures (540 images in total).

187

188 *2.3 Analysis of behaviour*

189 We assessed the number of times each bird expressed “contact seeking behaviours”
190 towards the animal caregiver: the bird stretched his body horizontally towards the caregiver,
191 and tried, or not, to grasp the caregiver's clothes with its beak, or the bird “begged” to be
192 taken on hand by lifting one leg in front of its breast. These behaviours were scored during the
193 three phases to make sure that they were specifically displayed in the presence of the
194 caregiver. We also recorded the number of times the birds expressed “long distance contact
195 calls” during the three phases. Macaws commonly emit these loud calls when they are

196 separated from their flock (Luescher 2008) (Video 1, supplementary data). Furthermore, one
197 out of the five birds expressed eye pinning (i.e. a rapid constriction of the pupil) (Gregory and
198 Hopkins 1974). The occurrence of this behaviour (i.e. the number of times the pupil
199 contracted to half its size, Figure S2, supplementary data) was recorded during the three
200 phases for this bird by two independent observers.

201

202 *2.4 Statistical analyses*

203 For all the parameters recorded, data was averaged across sessions per phase and per
204 individual. To test the effect of the phase we used the function `aovperm` of the `Permuco`
205 package in R 3.4.2 to run permutation tests with the phase as a fixed factor and the individual
206 as a random factor nested within phase with 10^6 permutations (Kherad-Pajouh and Renaud,
207 2015). All tests were two-tailed with significance considered at $P < 0.05$. The same function
208 was used for post-hoc comparisons. Because the usefulness of using corrections for multiple
209 comparisons in cases of low sample size is highly debated and results in a loss of power
210 (Garcia 2004; Nakagawa 2004; Garamszegi 2006), we present the original P -values. The data
211 are represented as boxplots with medians and interquartile distribution ranges. Only
212 descriptive data were given for the occurrence of eye pinning as it was displayed only by one
213 out of the five birds. The concordance between observers on the frequency of eye pinning was
214 evaluated with a spearman test and was highly significant ($Rho=0.97$; $P < 0.01$).

215

216 *2.5 Ethical note*

217 The Zooparc de Beauval (41110, Saint Aignan) kindly provided access to their birds.
218 Only video-recorded observations were conducted. Behavioural observations are not
219 considered as animal experimentations and are beyond the scope for ethical consideration
220 regarding French and European animal experimentation regulations. The Val de Loire Ethics

221 Committee for Animal Experimentation (N° CE19 – 2022-1503 – 5) reviewed the protocol
222 and attributed a positive recommendation.

223

224 **3. Results**

225 We found a significant effect of the phase on crown feather height ($F = 0.001$; $P =$
226 0.001) and nape feather height ($F < 0.001$; $P = 0.0008$) with in both cases the heights were
227 more important during the union and reunion phases compared to the separation phase
228 whereas heights did not differ significantly between the union and reunion phases (Fig. 2A
229 and 2B). We found a significant effect of the phase on redness of the skin ($F = 0.01$; $P =$
230 0.023) with post-hoc trends towards a higher redness during the union and reunion phases
231 than during the separation phase ($P = 0.06$ for all comparisons) whereas it did not differ
232 significantly between the union and reunion phases (Fig. 2C). The pupil surface did not differ
233 significantly between the three phases ($F = 0.14$; $P = 0.15$; Fig. 2D). The occurrence of
234 contact seeking behaviours differed significantly between the phases ($F = 0.09$; $P = 0.04$)
235 with higher occurrences during the union and reunion phases compared to the separation
236 phase whereas the occurrence did not differ significantly between the union and reunion
237 phases (Fig. 3A). The number of long distance contact calls differed significantly between the
238 phases ($F = 0.01$; $P = 0.001$) with a significant higher number of calls during the separation
239 phase than during the union and reunion phases (Fig. 3B). Eye pinning was observed in one
240 bird and only during the union and reunion phases (union: 3 [1-3]; separation: 0 [0-0];
241 reunion; 3 [1-3]).

242

243 **4. Discussion**

244 Our study shows variations in facial displays and behaviours during the different
245 phases of the tests. The presence of long distance calls during the separation phase and of

246 specific facial displays associated with contact seeking behaviours during the union and
247 reunion phases show that the familiar caregiver acted as a social support for the parrots placed
248 in the unfamiliar environment.

249 As expected, we found variation in crown and nape feather heights and redness of the
250 bare skin depending on the phase. We argue that lower heights of the crown and nape
251 feathers, lower redness of the skin and the occurrence of long distance calls during the
252 separation phase indicate that parrots have perceived this phase more negatively compared to
253 the other two phases. Macaws are highly social birds inhabiting dense forest. They are known
254 to produce loud calls also called “screams” especially at daybreak and dusk. These calls
255 frequently emitted in the wild, are essential in keeping track of companions (Luescher 2008).
256 For some authors, the sleeking of feathers might be associated with stressful situations and the
257 activation of the sympathetic nervous system but this remains understudied (Moris 1956). In
258 addition, feathers sleeking could also be observed in neutral situations such as locomotion
259 (Bertin et al., 2018a). A control neutral situation would have been necessary to determine
260 whether the feathers were sleeked or in a neutral position (neither sleeked or ruffled) during
261 the separation phase. No fear-related behaviours were observed except on day 1 suggesting
262 that the birds habituated to the experimental situation and that the phase of separation may
263 only have had a mild negative valence for the birds.

264 Bare skin blushing and ruffling of crown and nape feathers were observed during the
265 union and reunion phases. We previously observed ruffling of both crown and nape feathers
266 in macaws or cockatoos engaged in intraspecific positive social interactions or comfort
267 behaviours such as resting (Bertin et al., 2018a; Bertin et al., 2020); activities which are
268 commonly considered as reflecting calm and relaxed states and low level of threat in birds and
269 vertebrates (Mattiello et al., 2019; Ritters et al., 2019; Mendl et al., 2010; Richardson et al.,
270 2016; Luescher, 2006 (e.g. Mattiello et al., 2019; Ritters et al., 2019; Richardson et al., 2016;

271 Luescher, 2006). The similar facial displays observed in the present study during the union
272 and reunion phases suggest that these phases had a more positive emotional valence for the
273 parrots than the separation phase during which head feathers were sleeked. In our previous
274 study on macaws, crown ruffling and bare skin blushing were also more frequent when the
275 caretaker was actively engaging with the parrot than during a control phase with no mutual
276 interaction whereas nape feathers remained ruffled during both phases (Bertin et al., 2018a).
277 In humans, consistent with Darwin's observation (Darwin 1872), recent research has
278 demonstrated that faces do change color with emotion (Benitez-Quiroz et al., 2018;
279 Thorstenson et al., 2018). For example, emotions such as anger and happiness elicit dilated
280 arteries, facilitating blood flow to the skin surface and increasing facial redness. These
281 changes in color allow the emitter to successfully transmit and observers to visually interpret
282 emotion even in the absence of facial muscle movement (Benitez-Quiroz et al., 2018).
283 Trichromatic color vision in humans is thought to have been naturally selected, in part, for
284 detecting color changes on conspecific faces to discriminate emotions, or other socially
285 relevant physiological states (Changizi, Zhang, & Shimojo, 2006). This capacity to produce
286 and perceive facial color changes is also thought to facilitate adaptive social functioning by
287 regulating approach-avoidance behaviours (Thorstenson and Pazda, 2021). In Blue-and-
288 yellow macaws, evolution has favoured the development of a particularly complex face with
289 mobile coloured feathers (black, green, blue, yellow) and bare skin. Given their
290 tetrachromatic vision, it is conceivable that they have evolved a comparable mechanism
291 conveying information about an individual's internal physiological and psychological state.

292 So far, studies on emotional facial expression in birds are scarce. Some authors also
293 described subtle changes in crown and nape feather displays as indicators of the bird's social
294 status during social interactions (Bond and Diamond 2019) or in warning threat in pet parrots
295 (Simone-Freilicher 2015). In crested Psittaciformes like cockatoos, facial/head feather

296 movement including the crest were reported in contexts of alertness, agonistic interactions or
297 play readiness (Kaplan, 2015). All in all these results call attention to the overlooked function
298 of subtle facial visual displays which, similarly to mammals, could convey close-range public
299 information regarding individuals' intention to engage in specific activities or emotions
300 (Waller and Micheletta 2013).

301 When reunited with the caregiver, macaws ceased calling and expressed contact
302 seeking behaviours towards the caregiver as during the union phase. Most parrot species show
303 complex social organization with flock members maintaining non-random affiliative
304 relationships characterized by allopreening, allofeeding and maintenance of close proximity
305 (Seibert 2006). Proximity searching with the caregiver indicated that she acted as a social
306 support and a source of appeasement for the macaws which ceased calling for their mates.
307 Separation distress, proximity seeking and appeasement are two out the three main features
308 characterizing attachment namely: proximity seeking, appeasement (relief from stress due to
309 the social support of the attachment figure); secure base (i.e. increase of exploration of the
310 novel environment) and separation distress (Cassidy 1999). As we were not able to test
311 macaws' responses towards a stranger, we cannot resolve whether our results fulfil all criteria
312 for an attachment-bond. However, to our knowledge, our study constitutes the first attempt to
313 investigate the human-animal bond in a bird species.

314 In humans, non-human primates and dogs, reunion following separation results in a
315 positive emotional state, and an increase in affiliative behaviours (e.g. Kalin et al., 1995;
316 Rhen, 2014). A higher frequency of contact seeking behaviours during the reunion phase than
317 during the union phase could thus have been expected. Our sample size was relatively small
318 and the inter-individual variability was high, which could have masked a potential rebound
319 effect on affiliative behaviours. This high inter-individual variability was also observed for
320 eye surface and the expression of eye pinning. Contrarily to mammals, birds have the control

321 of the dilation and constriction of their pupils (Walls 1963). Unexplored by the scientific
322 community, eye pinning describes the rapid dilation and contraction of the pupils of a bird's
323 eye. In Psittacidae, eye pinning is reported when birds anticipate positive events like stroking,
324 or during pleasant activities like eating a favourite food or courtship but also during negative
325 events like defense of territory (Moustaki 2011). Eye pinning was also described, for the first
326 time in a scientific journal, in a female pet Yellow-fronted amazon (*Amazona ochrocephala*
327 *panamensis*) when she engaged in mutual vocal interactions with the owners (Gregory and
328 Hopkins 1974). In our study, the production of eye pinning was observed only in an 8-year-
329 old female that, according to the caregiver, was the most bonded to her out of the five
330 macaws. It would be interesting to determine in future studies if the production of this
331 behaviour varies according to the quality of the bond established between the bird and the
332 caregiver and if, in a social group, this behaviour may constitute a close-range visual means to
333 express affiliative behaviours towards preferred conspecifics.

334 From a general point of view, our results and interpretation warrant caution due to the
335 lack of control situations. For example, we cannot rule out the hypothesis that the mere
336 familiarity of the caregiver (and not a particular affiliative link) was enough to buffer the
337 effects of social separation and novelty. In mammals, the effectiveness of social support is
338 known to depend on both the familiarity and the nature of the relationship with the social
339 partner (Rault, 2012 for a review). While macaws are known to form strong exclusive bonds
340 with humans (Blanchard, 1997), it would be of interest to conduct further studies with
341 caretakers more or less familiar or affiliated to the tested birds. Observations of facial displays
342 in larger groups of macaws will also be needed to further investigate the function of facial
343 displays and blushing in social interactions varying in emotional valence and intensity.

344

345 **5. Conclusion**

346 Our data reinforce the idea that parrots have the capacity to produce diverse rapid
347 changes in their facial displays according to situations differing in their emotional valence
348 and/or arousal level. Psittaciformes are highly social and very popular as companion animals
349 but the way they perceived humans, is yet relatively unknown. Captive parrots are
350 particularly sensitive to feather plucking or stereotypic behaviours, which are signs of
351 negative welfare (van Zeeland et al. 2009). Care should be taken at the fact that they are very
352 social birds while being almost exclusively raised as a single individual by pet owners.
353 Identifying signs of positive emotions will provide a better understanding of parrots'
354 affiliative behaviours and emotional expression, which is crucial in order to satisfy their social
355 needs. Species-specific repertoires of facial expressions could provide useful tools to better
356 assess their well-being and provide for their needs. Although additional works are needed, our
357 work suggests that facial displays may be used to identify whether captive parrots perceived
358 positively the human-parrot relationships in which they are engaged.

359

360 **Acknowledgements**

361 We are grateful to the Association Beauval Nature pour la Recherche et la Conservation and
362 the Zooparc de Beauval who provided access to the birds and financial support. This funding
363 source had no role in the study or the preparation of the article. We thank all the caretakers of
364 the Zooparc de Beauval.

365

366

367

368

369

370

371 **References**

- 372 Ainsworth, M.D.S., Bell, S.M., 1970. Attachment, exploration, and separation: illustrated by the
 373 behaviour of one-year-olds in a strange situation. *Child Dev.* 41, 49–67.
 374 <https://doi.org/10.7312/stei93738-006>.
- 375 Ainsworth, M. D. S., 1979. Attachment as related to mother-infant interaction. *Adv. Study Behav.* 9,
 376 1-51. [https://doi.org/10.1016/S0065-3454\(08\)60032-7](https://doi.org/10.1016/S0065-3454(08)60032-7).
- 377 Anderson, P. K., 2014. Social dimensions of the human–avian bond: parrots and their persons.
 378 *Anthrozoös* 27, 371-387. <https://doi.org/10.2752/175303714X13903827488006>.
- 379 Baker, P., 2012. Parrots will be parrots—understanding parrots’ behavioural needs. *Vet. Nurs. J.* 27,
 380 457-459. <https://doi.org/10.1111/j.2045-0648.2012.00234.x>.
- 381 Beck, A. M., 2014. The biology of the human–animal bond. *Anim. Front.* 4, 32-36.
 382 <https://doi.org/10.2527/af.2014-0019>
- 383 Beck, A. M., Katcher, A. H., 1996. *Between pets and people: The importance of animal*
 384 *companionship*. Purdue University Press.
- 385 Benitez-Quiroz, C. F., Srinivasan, R., Martinez, A. M., 2018. Facial color is an efficient mechanism to
 386 visually transmit emotion. *Proc. Natl. Acad. Sci. USA.*, 115, 3581-3586.
 387 <https://doi.org/10.1073/pnas.1716084115>.
- 388 Bergman, L., & Reinisch, U. S., 2006. Comfort behaviour and sleep. *Manual of parrot behaviour*, 59.
- 389 Bertin, A., Beraud, A., Lansade, L., Blache, M.-C., Diot, A., Mulot, B., Arnould, C., 2018a. Facial display
 390 and blushing: Means of visual communication in blue-and-yellow macaws (*Ara Ararauna*)?
 391 *PLoS ONE*, 13: e0201762. <https://doi.org/10.1371/journal.pone.0201762>.
- 392 Bertin, A., Cornilleau, F., Lemarchand, J., Boissy, A., Leterrier, C., Nowak, R., Calandreau, L., Blache,
 393 M.C., Boivin, X., Arnould, C., Lansade, L., 2018b. Are there facial indicators of positive
 394 emotions in birds? A first exploration in Japanese quail. *Behav. processes* 157, 470-473.
 395 <https://doi.org/10.1016/j.beproc.2018.06.015>.
- 396 Bertin, A., Beraud, A., Lansade, L., Mulot, B., Arnould, C., 2020. Bill covering and nape feather ruffling
 397 as indicators of calm states in the Sulphur-crested cockatoo (*Cacatua galerita*). *Behav.*
 398 *processes*, 178, 104188. <https://doi.org/10.1016/j.beproc.2020.104188>
- 399 Blanchard, S., 1997. The Parrot to Human Bond. *AFA Watchbird*, 24(1), 32-37.
- 400 Boivin, X., Nowak, R., Garcia, A.T., 2001. The presence of the dam affects the efficiency of gentling
 401 and feeding on the early establishment of the stockperson–lamb relationship. *Appl. Anim.*
 402 *Behav. Sci.* 72, 89–103. [https://doi.org/10.1016/S0168-1591\(00\)00201-X](https://doi.org/10.1016/S0168-1591(00)00201-X)
- 403 Bowlby, J., 1958. The nature of the child’s tie to his mother. *Int. J. Psychol. Psychoanal.* 39, 350–373.
- 404 Bowlby, J., 1982. Attachment and loss: retrospect and prospect. *Am. J. Orthopsychiatry.* 52, 664–678
- 405 Bond, A., Diamond, J., 2019. *Thinking like a parrot: Perspectives from the wild*. University of Chicago
 406 Press.
- 407 Carver, C.S., 2001. Affect and the functional bases of behaviour: On the dimensional structure of
 408 affective experience. *Pers. Soc. Psychol. Rev.*, 5, 345-356.
 409 https://doi.org/10.1207/S15327957PSPR0504_4.
- 410 Cassidy, J., 1999. The nature of child’s ties. In: Cassidy, J., Shaver, P.R. (Eds.), *Handbook of*
 411 *Attachment: Theory, Research and Clinical Applications*. Guilford Press, New York, pp. 3–20.
- 412 Changizi, M. A., Zhang, Q., Shimojo, S., 2006. Bare skin, blood and the evolution of primate colour
 413 vision. *Biol. Lett.* 2, 217-221. <https://doi.org/10.1098/rsbl.2006.0440>.
- 414 Coulon, M., Nowak, R., Andanson, S., Ravel, C., Marnet, P.G., Boissy, A., Boivin, X., 2013. Human-lamb
 415 bonding: oxytocin, cortisol and behavioural responses of lambs to human contacts and social
 416 separation. *Psychoneuroendocrinology.* 38, 499–508.
 417 <https://doi.org/10.1016/j.psyneuen.2012.07.008>.
- 418 Darwin, C., 1872. *The expression of the emotions in man and animals*. New York, NY: D Appleton and
 419 Company. (Reprinted by University of Chicago Press, Chicago, 1965).
- 420 Emery, N., 2016. *Bird Brain: An exploration of avian intelligence*. Princeton University Press.

421 Garamszegi, L. Z., 2006. Comparing effect sizes across variables: generalization without the need for
422 Bonferroni correction. *Behav. Ecol.* 17, 682–687. <https://doi.org/10.1093/beheco/ark005>
423 Garcia, L. V. 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105: 657–663.
424 <https://doi.org/10.1111/j.0030-1299.2004.13046.x>.
425 Gregory, R., Hopkins, P., 1974. Pupils of a talking parrot. *Nature* 252: 637-638.
426 Gutiérrez-Ibáñez, C., Iwaniuk, A.N., Wylie, D.R., 2018. Parrots have evolved a primate-like
427 telencephalic-midbrain-cerebellar circuit. *Sci. Rep.* 8, 9960. [https://doi.org/10.1038/s41598-](https://doi.org/10.1038/s41598-018-28301-4)
428 [018-28301-4](https://doi.org/10.1038/s41598-018-28301-4).
429 Jordan, R., 2003. A guide to macaws as pet & aviary birds, ABK Publications, NSW, Australia.
430 Kalin, N. H., Shelton, S. E., Lynn, D. E., 1995. Opiate systems in mother and infant primates coordinate
431 intimate contact during reunion. *Psychoneuroendocrinology* 20, 735-742.
432 [https://doi.org/10.1016/0306-4530\(95\)00023-2](https://doi.org/10.1016/0306-4530(95)00023-2).
433 Kaplan, G., 2015. *Bird minds: cognition and behaviour of Australian native birds*. CSIRO PUBLISHING.
434 Katcher, A. H., Beck, A. M., 1987. Health and caring for living things. *Anthrozoös* 1, 175-183.
435 <https://doi.org/10.2752/089279388787058461>.
436 Kherad-Pajouh, S., Renaud, O. A., 2015. General permutation approach for analyzing repeated
437 measures ANOVA and mixed-model designs. *Stat. Papers* 56, 947–967.
438 <https://doi.org/10.1007/s00362-014-0617-3>.
439 Lundberg, P., Hartmann, E., Roth, L. S., 2020. Does training style affect the human-horse
440 relationship? Asking the horse in a separation–reunion experiment with the owner and a
441 stranger. *Appl. Anim. Behav. Sci.* 233, 105144.
442 <https://doi.org/10.1016/j.applanim.2020.105144>.
443 Luescher, A. (Ed.), 2008. *Manual of parrot behaviour*. John Wiley & Sons.
444 Mattiello, S., Battini, M., De Rosa, G., Napolitano, F., Dwyer, C., 2019. How can we assess
445 positive welfare in ruminants? *Animals* 9 (10), 758. <https://doi.org/10.3390/ani9100758>
446 Mendl, M., Burman, O.H. and Paul, E.S., 2010. An integrative and functional framework for the study
447 of animal emotion and mood. *Proc. R. Soc. B: Biol. Sci.* 277, 2895-2904.
448 <https://doi.org/10.1098/rspb.2010.0303>.
449 Morris, D., 1956. The Feather Postures of Birds and the Problem of the Origin of Social Signals.
450 *Behaviour* 9, 75-113.
451 Moustaki, N., 2011. *Parrots for Dummies*. John Wiley & Sons.
452 Nagasawa, M., Mogi, K., Kikusui, T., 2009. Attachment between humans and dogs. *Jpn. Psychol. Res.*
453 51, 209-221. <https://doi.org/10.1111/j.1468-5884.2009.00402.x>.
454 Nowak, R., Boivin, X., 2015. Filial attachment in sheep: Similarities and differences between ewe-
455 lamb and human-lamb relationships. *Appl. Anim. Behav. Sci.* 164, 12-28.
456 <https://doi.org/10.1016/j.applanim.2014.09.013>.
457 Olkowicz, S., Kocourek, M., Lucan, R.K., Portes, M., Fitch, W.T., Herculano-Houzel, S., Nemeč, P.,
458 2016. Birds have primate-like numbers of neurons in the forebrain. *Proc. Natl. Acad. Sci.*
459 U.S.A. 113, 7255-60. <https://doi.org/10.1073/pnas.151713111>.
460 Passarotto, A., Rodríguez-Caballero, E., Cruz-Miralles, Á., Avilés, J. M., 2021. Ecogeographical patterns
461 in owl plumage colouration: Climate and vegetation cover predict global colour variation.
462 *Glob. Ecol. Biogeogr.* 31, 515-530. <https://doi.org/10.1111/geb.13444>.
463 Payne, E., DeAraugo, J., Bennett, P., McGreevy, P., 2016. Exploring the existence and potential
464 underpinnings of dog–human and horse–human attachment bonds. *Behav. processes* 125,
465 114-121. <https://doi.org/10.1016/j.beproc.2015.10.004>.
466 Pepperberg, I. M., McLaughlin, M. A., 1996. Effect of avian–human joint attention in allospecific vocal
467 learning by grey parrots (*Psittacus erithacus*). *J. Comp. Psychol.* 110, 286.
468 <https://doi.org/10.1037/0735-7036.110.3.286>.
469 Pérez-Rodríguez, L., Viñuela, J., 2008. Carotenoid-based bill and eye ring coloration as honest signals
470 of condition: an experimental test in the red-legged partridge (*Alectoris rufa*).
471 *Naturwissenschaften* 95, 821-830. <https://doi.org/10.1007/s00114-008-0389-5>.

472 Price, E.O., Thos, J., 1980. Behavioural responses to short-term social isolation in sheep and goats.
473 Appl. Anim. Ethol. 6, 331–339. [https://doi.org/10.1016/0304-3762\(80\)90133-9](https://doi.org/10.1016/0304-3762(80)90133-9).
474 Rault, J. L., Waiblinger, S., Boivin, X., Hemsforth, P., 2020. The power of a positive human–animal
475 relationship for animal welfare. Front. Vet. Sci. 7, 857.
476 <https://doi.org/10.3389/fvets.2020.590867>.
477 Rault, J. L., 2012. Friends with benefits: social support and its relevance for farm animal welfare.
478 Appl. Anim. Behav. Sci. 136, 1-14. <https://doi.org/10.1016/j.applanim.2011.10.002>
479 Rault, J. L., Boissy, A. and Boivin, X., 2011. Separation distress in artificially-reared lambs depends on
480 human presence and the number of conspecifics. Appl. Anim. Behav. Sci. 132, 42-50.
481 <https://doi.org/10.1016/j.applanim.2011.02.011>.
482 Rehn, T., Handlin, L., Uvnäs-Moberg, K. and Keeling, L. J., 2014. Dogs' endocrine and behavioural
483 responses at reunion are affected by how the human initiates contact. Physiol. Behav. 124,
484 45-53. <https://doi.org/10.1016/j.physbeh.2013.10.009>.
485 Richardson, M., McEwan, K., Maratos, F. and Sheffield, D., 2016. Joy and Calm: How an Evolutionary
486 Functional Model of Affect Regulation Informs Positive Emotions in Nature. Evol. Psychol. 2,
487 308-320. <https://doi.org/10.1007/s40806-016-0065-5>.
488 Ritters, L.V., Kelm-Nelson, C.A., Spool, J.A., 2019. Why do birds flock? A role for opioids
489 in the reinforcement of gregarious social interactions. Front. Physiol. 10, 421.
490 <https://doi.org/10.3389/fphys.2019.00421>
491 Seibert, L. M., 2006. Social behaviour of psittacine birds. Manual of parrot behaviour, 1.
492 Simone-Freilicher, E., Rupley, A. E., 2015. Juvenile psittacine environmental enrichment. Vet. Clin.
493 Exot. Anim. 18, 213-231. <https://doi.org/10.1016/j.cvex.2015.01.003>
494 Thorstenson, C. A. and Pazda, A. D., 2021. Facial coloration influences social approach-avoidance
495 through social perception. Cogn. Emot. 35, 970-985.
496 <https://doi.org/10.1080/02699931.2021.1914554>
497 Thorstenson, C. A., Elliot, A. J., Pazda, A. D., Perrett, D. I., Xiao, D., 2018. Emotion-color associations in
498 the context of the face. Emotion 18, 1032–1042. <https://doi.org/10.1037/emo0000358>.
499 Topál, J., Gácsi, M., Miklósi, Á., Virányi, Z., Kubinyi, E., Csányi, V., 2005. Attachment to humans: a
500 comparative study on hand-reared wolves and differently socialized dog puppies. Anim.
501 Behav. 70, 1367-1375. <https://doi.org/10.1016/j.anbehav.2005.03.025>.
502 van Zeeland, Y.R.A., Spruit, B.M., Rodenburg, T.B., Riedstra, B., van Hierden, Y.M., Buitenhuis, B.,
503 Korte, S.M., Lumeij, J.T., 2009. Feather damaging behaviour in parrots: A review with
504 consideration of comparative aspects. Appl. Anim. Behav. Sci. 121, 75-95.
505 <https://doi.org/10.1016/j.applanim.2009.09.006>.
506 Vitale, K. R., Behnke, A. C., Udell, M. A., 2019. Attachment bonds between domestic cats and
507 humans. Curr. Biol., 29(18), R864-R865. <https://doi.org/10.1016/j.cub.2019.08.036>.
508 Waller, B.M., Micheletta, J., 2013. Facial expression in nonhuman animals. Emot. Rev. 5, 54-59.
509 <https://doi.org/10.1177/1754073912451503>.
510 Walls, G. L., The Vertebrate Eye and its Adaptive Radiation, 645-647 (Hafner, New York and London,
511 1963).

512
513

514

515

516

517

518

519 **Figure captions**

520

521 **Fig 1. Characterization of facial displays:** Photographs with schematic representation of the
522 measures of beak height (a); crown feather height (b and c); nape feather height (d) and the
523 position of the 10 x 10 pixels scare for the assessment of redness of the skin (e). A) Head and
524 nape feathers were sleeked. B) Head and nape feathers were ruffled.

525

526 **Fig 2: Median and interquartile distribution ranges of** A) crown feather height; B) nape
527 feather height; C) redness of the skin; D) pupil surface. Different letters indicate significant
528 post-hoc differences. A letter in italic indicates a trend ($0.05 < P < 0.1$).

529

530 **Fig 3: Median and interquartile distribution ranges of** A) frequency of contact seeking
531 behaviours; B) frequency of long distance contact calls. Different letters indicate significant
532 post-hoc differences.

533

534

535

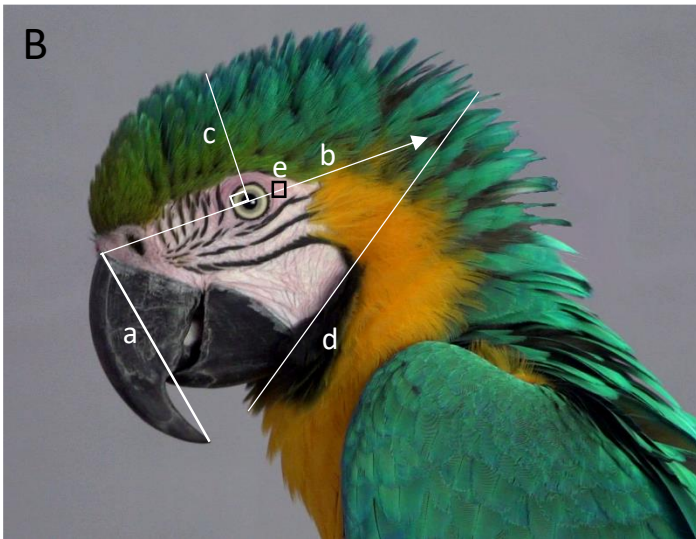
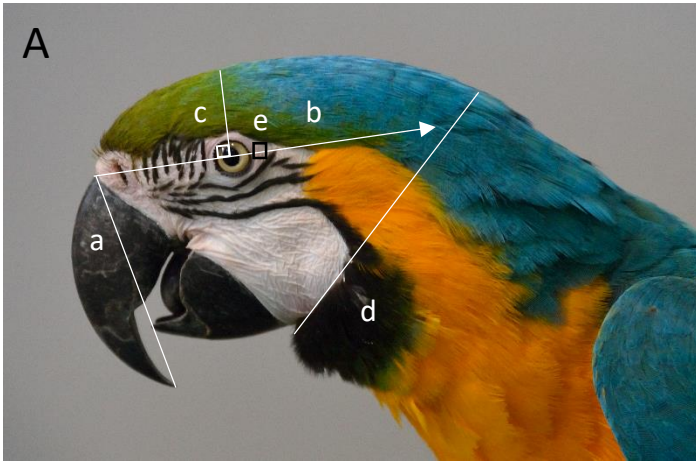
536

537

538

539

540 Figure 1



541

542

543

544

545

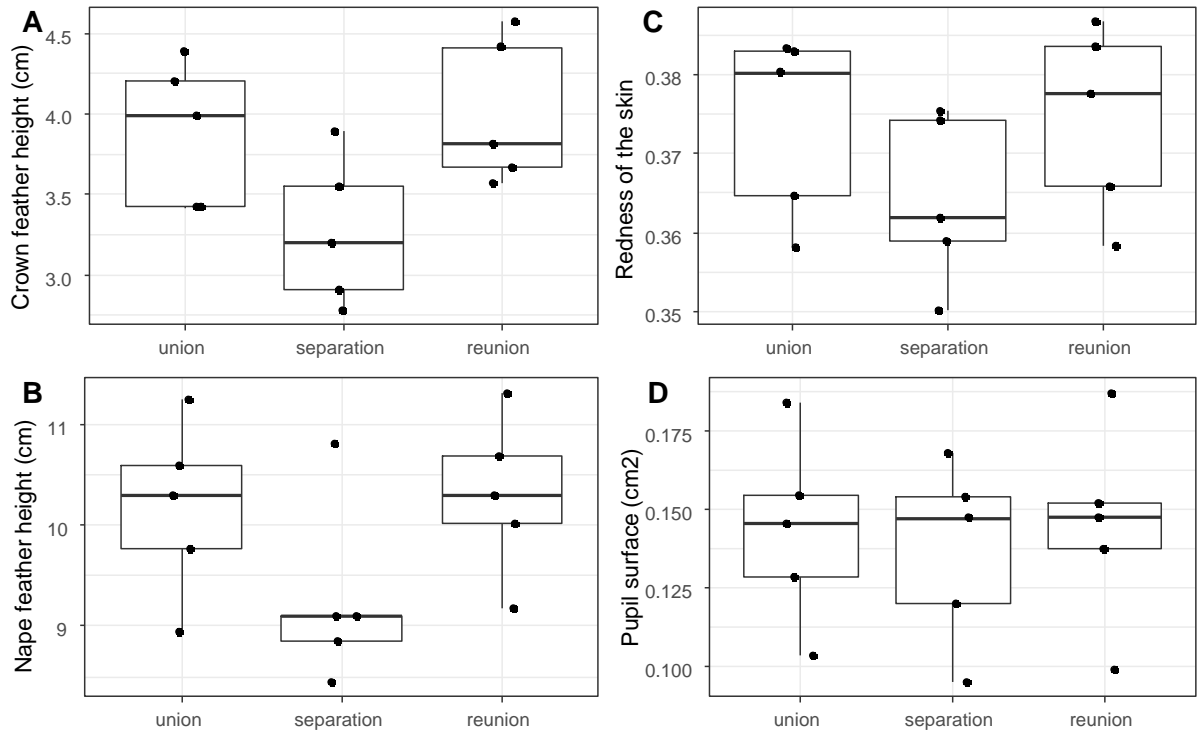
546

547

548

549 Figure 2

550



551

552

553

554

555

556

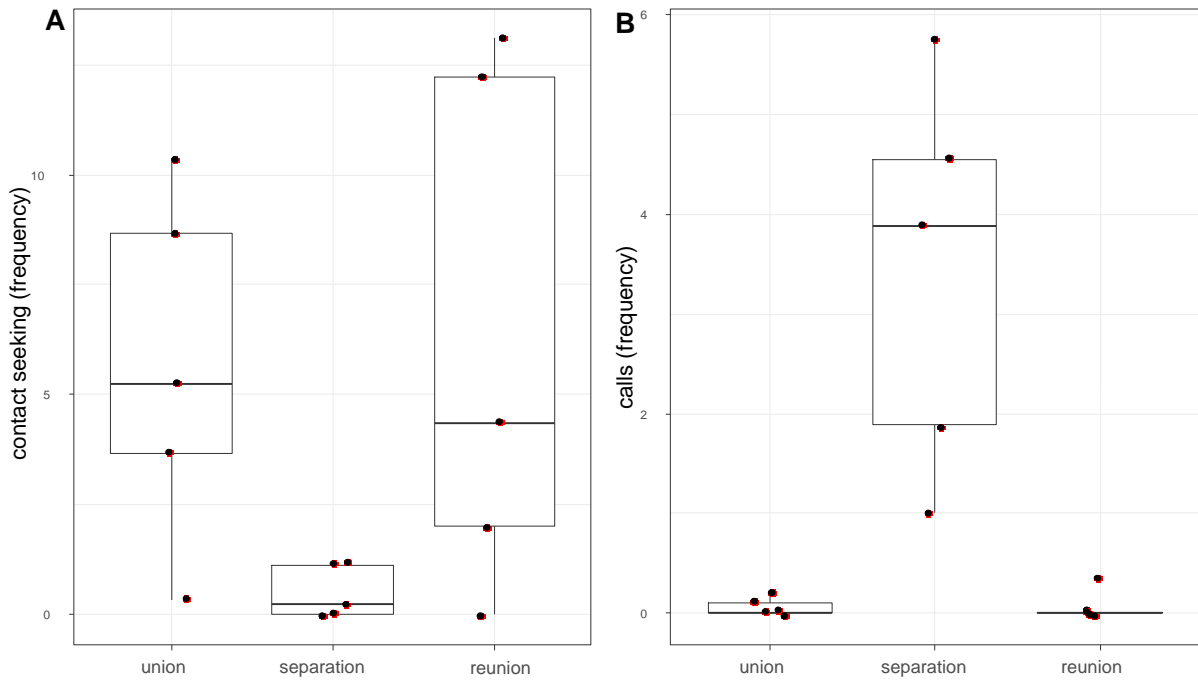
557

558

559

560

561 Figure 3



562

563

564

565

566

567

568

569

570

571

572

573 Supplementary data

574 Figure S1: Examples of pet-like behaviours expressed by the birds in presence of their animal
575 caretaker during their routine interactions. A) cuddling behaviour; B) rolling on the back to have the
576 belly rubbed.

577 A)



578

579 B)



580

581

582

583

584 Figure S2: photography showing the reduction of the pupillary size during the expression of eye-
585 pinning.

586

587

588

589

590

591

592

593

594

595

596



597

598

599

600

601 Movie 1: expression of long distance contact calls during the separation phase.

602

603

604