1   Social relationships among captive male pygmy slow lorises (*Nyctycebus pygmaeus*): Is

2   forming male same-sex pairs a feasible management strategy?

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4   Running head: social relationships among captive male pygmy slow lorises

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Abstract

Little is known about the social behavior of pygmy slow lorises, in particular, the social relationships of same-sex individuals have rarely been investigated. The Slow Loris Conservation Centre was built at the Japan Monkey Centre to enhance the welfare of confiscated slow lorises, promote their conservation, improve public education and perform scientific research on the species. In the course of improving housing conditions, several same-sex pairs of pygmy slow lorises were formed. We monitored their behaviors and fecal glucocorticoid metabolite (FGM) levels to understand whether male same-sex pairings could be a feasible management strategy. The subjects were 10 male and 6 female lorises for comparison, all of whom were over five years old. We successfully formed five pairs of male lorises after eight formation attempts. Male pairs initially showed some aggressive behaviors; however, the rate decreased approximately 10 days after introduction. All of the male pairs eventually exhibited extensive affiliative social behaviors, including allogrooming and social play, during the dark (active) phase, and sleep site sharing during the light (inactive) phase. The rate of sleep site sharing during the light phase was higher than expected, suggesting that the pairs preferred to stay near each other. There was no evidence of increased stress after a long period of male–male social housing. Female same-sex pairs and male-female pairs demonstrated a high level of affiliative behaviors right after introduction. These results highlight the flexibility and high sociability of this species and indicate that such same-sex pairings are a feasible option for their social management.

Keywords

slow loris, sociality, animal welfare, conservation, social management

Introduction

Illegal pet trade is one of the most serious threats to the protecting endangered primates . Slow lorises (*Nycticebus spp.*) are in danger of extinction. The IUCN red list currently classifies them into nine species, among which the pygmy slow loris (*Nycticebus pygmaeus*) is categorised as Endangered (IUCN 2020, 2020). Although their commercial trade has been banned since 2007 by their inclusion in CITES Appendix I, one of the causes of their loss remains their frequent illegal trade (Musing, Suzuki, & Nekaris, 2015; Nekaris, 2007) . Thus, an important conservation goal for this species is to reduce their use in the illegal pet trade through, among other strategies, public education (Norconk et al., 2020). Slow lorises are confiscated in both range and non-range countries, including Japan (Fuller, Eggen, Wirdateti, & Nekaris, 2018; Kitade & Naruse, 2020; Musing et al., 2015). Once confiscated, lorises are typically transferred to sanctuaries, rehabilitation centres or zoos (Fuller et al., 2018). Sanctuaries and rescue centres can often become strained past their capacity due to the unscheduled arrival of the confiscated animals (Moore, Wihermanto, & Nekaris, 2014), which can result in suboptimal captive conditions, mainly due to the lack of available space. Therefore, it is important to develop a practical strategy to house these individuals to ensure their well-being.

Little is known about the social behavior of wild pygmy slow lorises due to the inherent difficulty of nocturnal observation. The species was historically considered solitary, but studies have revealed that they are not asocial. Some slow loris species have been found to have spatial overlaps in home ranges between adult males and females (Wiens & Zitzmann, 2003), and friendly interactions between individuals have been reported (Wiens, 2002). Javan slow loris spend as much as 65% of their time in spatial

67   proximity, including body contact for an average of 18% of their time (Nekaris, 2014).

68   Studies have also demonstrated that male slow lorises are often found with scars, which

69   has led some researchers to conclude that slow loris venom is used for intraspecific

70   competition (Nekaris, Moore, Rode, & Fry, 2013). A recent study investigating the

71   ranging patterns of pygmy slow loris in Cambodia found that the home ranges of some

72   individuals overlapped with others to a small degree. Adult pygmy slow lorises display

73   primarily solitary sleeping patterns, except for adult females with infants, who always

74   sleep with their young (Starr & Nekaris, 2020). These solitary sleeping patterns are

75   consistent with greater slow loris behavior (Wiens & Zitzmann, 2003), but different from

76   Javan slow lorises, who often share sleep sites with conspecifics (Nekaris, 2014). Activity

77   budgets differ across species and studies, with the proportions of social behaviors ranging

78   from 0%–18% (Musing, Suzuki, & Nekaris, 2015; Nekaris, 2007). Overall, the current

79   available evidence suggests that slow lorises are not gregarious, but do form spatial

80   groups (Wiens & Zitzmann, 2003), including dispersed family groups (Javan slow loris

81   and greater slow loris), and polygynous groups (pygmy slow loris) (Poindexter & Nekaris,

82   2020).

83   Slow lorises display social behaviors in captivity, not only between mother and

84   infant (Fitch-Snyder & Ehrlich, 2003), but also between adults (Ehrlich & Alan, 1977). A

85   previous study reported that two polygynous groups of greater slow lorises participated

86   in various social behaviors, including allogrooming and social play (Ehrlich & Alan,

87   1977). Researchers believe that such stimulation from conspecifics can be a form of social

88   enrichment (Fitch-Snyder, Schulze, & Larson, 2001). One survey focusing on the

89   husbandry practices of North American zoos for lorisid primates revealed that

90   approximately 55% of slow lorises are housed alone (Fuller, Kuhar, Dennis, & Lukas,

4

91   2013). Conspecific aggression during animal introductions was listed as one reason for

92   the single housing. Although aggression can be a source of injury and stress in primates

93   (Honess & Marin, 2006; Tennenhouse, Putman, Boisseau, & Brown, 2017; Yamanashi et

94   al., 2016), and can compromise welfare if it continues over a long period or if the level

95   of aggression is severe, moderate aggression is a natural behavior (Bloomsmith, 2001)

96   that often occurs during the introduction phases of gregarious primates, and often

97   decreases once their social relationships are established (Kutsukake et al., 2018). A well-

98   established, cohesive social environment can offer ever-changing stimulation to animals

99   in captivity. Successful social management can help to maximise the positive benefits of

100 social groups and minimise negative effects, such as stress and aggression, but discussion

101 on the practical social management of slow lorises is still limited.

102   Ideally, social structures in captivity should mimic the social structures seen in

103 the wild for a particular species (Price & Stoinski, 2007); however, social structures in

104 wild primates can vary in group size and composition depending on resource availability,

105 predator presence, and other factors. In captivity, such environmental constraints differ

106 (e.g. less resource competition and absent predators), which could allow for increased

107 social group flexibility in captivity (Price & Stoinski, 2007). For example, same-sex

108 groups are sometimes formed to control breeding or to manage surplus animals (Fàbregas

109 & Guillén-Salazar, 2007; Sha, Alagappasamy, Chandran, Cho, & Guha, 2013; Stoinski,

110 Kuhar, Lukas, & Maple, 2004; Stoinski, Lukas, Kuhar, & Maple, 2004; Yamanashi,

111 Nogami, Teramoto, Morimura, & Hirata, 2018), even in species where such social

112 compositions are non-existent in the wild (e.g. chimpanzees). Similarly, wild orangutans

113 are semi-solitary species (van Schaik, 1999), but are sometimes kept in social groups in

114 zoos (Amrein, Heistermann, & Weingrill, 2014; Weingrill, Willems, Zimmermann,

5

115 Steinmetz, & Heistermann, 2011). Such flexible groups enable us to maximise space, and

116 avoid suboptimal housing. However, the degree of social flexibility is species-dependent,

117 and needs to be carefully investigated for each species. In slow lorises, same-sex pairs

118 have also been formed in some captive settings (Fuller et al., 2013; Moore, Cabana, &

119 Nekaris, 2015). In one study conducted at a rehabilitation centre in Indonesia, confiscated

120 slow lorises housed with conspecifics, particularly in same-sex pairs, displayed fewer

121 stereotypic behaviors (Moore et al., 2015). However, the social relationships between

122 same-sex slow lorises have rarely been investigated either in the wild or in captivity, and

123 it is not well understood whether same-sex housing is a viable social management option

124 for *Nycticebus spp.*

125   The purpose of this study was to investigate the social behaviors of male pairs

126 of pygmy slow lorises, in order to consider whether same-sex pairing could be a feasible

127 management strategy for this species. If slow lorises are motivated to interact with

128 conspecifics, and benefit from being together, then we would consider that same-sex

129 pairings are a feasible option. We particularly focused on male–male pairings in this study,

130 because males often exhibit competitive tendencies, (Van Schaik, 1994) which often

131 makes their social management more complicated than female groups (Sha et al., 2013;

132 Stoinski, Lukas, et al., 2004).

133   For the purposes of this study, we explored five variables. First, we investigated

134 the types of social relationships observed between adult males. Second, we determined

135 how often sleep sites were shared, in order to understand whether the lorises preferred to

136 stay together during their light phases (i.e. when the individuals are typically resting or

137 sleeping). To exclude the potential influences of environmental factors, such as locational

138 preference or temperature, we also recorded temperatures, and calculated the chance rate

139 of sleep site sharing. Third, we observed the process that occurred during the formation

140 of the social relationships, and its effects on the individuals' physiological stress levels.

141 Fourth, we compared the social relationship formation process across pairs of different

142 sex combinations. Fifth, we considered the stability of each social relationship by

143 comparing social behaviors observed during two different study periods.

144   If the pygmy slow lorises were motivated to interact with conspecifics, then we

145 hypothesised that male pygmy slow lorises would display affiliative social behaviors, and

146 prefer to share sleep sites during their light phases. In this case, physiological stress levels

147 may decrease after social relationships have been formed, or they may remain the same.

148 If slow lorises were not motivated to interact with conspecifics, then we hypothesised that

149 they would choose to keep distance between each other. In the worst case, they would

150 continue fighting without showing any sign of affiliation. Physiological stress levels in

151 this case would then likely increase over time as a cost of the social environment.

152

153 **Methods**

154 *Subjects*

155   The subjects of this research were 16 pygmy slow lorises living at the Japan

156 Monkey Centre (JMC), in Inuyama, Aichi, Japan (10 males and 6 males). Fifteen of the

157 individuals were confiscated at the airport between 2006 and 2007, and the final male

158 was born at JMC. Specific information on each subject is given in Supplementary Table

159 1. Prior to the establishment of the Slow Loris Conservation Centre (SLCC) at JMC, the

160 subjects were housed individually in small cages due to space limitations (0.1–0.3 m^3),

161 except for one father-son pair. Once the centre was established, the lorises were

162 introduced to larger enclosures, as described below. This study was carried out in

7

163   accordance with the recommendations in the "Guide for Animal Research Ethics" of the

164   Japan Monkey Centre, and the legal requirements of the country, and ASP Principles for

165   Ethical Treatment of Non-Human Primates. The study protocol was approved by the

166   institutional committee of the Japan Monkey Centre (#2015001, #2016001, #2017003).

167

168   *Slow loris conservation centre*

169   The SLCC was established to improve the living conditions of confiscated

170   pygmy slow lorises, and to serve as a place for environmental education, and slow loris

171   research. The facility had previously been used for nocturnal primates, and was renovated

172   by keepers and researchers to become the SLCC. Animal welfare is the first priority at

173   SLCC, thus the renovations were carried out to match the needs of slow lorises. The

174   facility uses a reverse-lighting cycle. Lighting levels were measured on 19 December

175   2019, and were found to be between 0.00 and 0.18 lux during the dark phase, depending

176   on the distance from the light in each room. We used red-filmed light in each room during

177   dark phase observations, in order to minimise the effects of lighting on the slow lorises

178   (Ariana, Marco, & Nekaris, 2020; Fuller et al., 2016; Starr, Nekaris, & Leung, 2012).

179   Enclosure size varied from 2.0 to 16.0 m^3. For the male–male pair experiments, we used

180   rooms that were about 8.0 m^3. Branches were installed to facilitate locomotion, and nest

181   boxes and hiding places were added to each enclosure (Fitch-Snyder, Schulze, & Streicher,

182   2008). The number of nest boxes was equal to, or more than, the number of individuals

183   in the enclosure. The animals also used the branches and the floor for resting and sleeping.

184   Diets mainly comprised of gum, insects and vegetables were provided in multiple

185   locations in each enclosure (Cabana & Nekaris, 2015; Cabana & Plowman, 2014). The

186   mean temperature was 25.8 ± 2.08 °C (measured between January and December 2017).

187   Organised tours with a limited number of zoo visitors were held several times a month.

188   Participants of the organised tour could explore the centre using red light with educational

189   staff, who described the ecology and conservation of slow lorises and the activities of

190   JMC. Social housing that restricted breeding was necessary to accommodate all the slow

191   lorises housed at the facility when it was first established.

192

193   *Social introduction*

194   Each pair was formed in an enclosure that were unfamiliar to both individuals.

195   When necessary, we set up acclimation phases by placing small cages in close proximity

196   for several days or by allowing the individuals to spend time in adjacent cages. In the

197   beginning of this study, we conducted social introduction on a trial-and-error basis before

198   establishing the described acclimation protocol. We judged whether the pairing could

199   continue based on the behaviors exhibited. If the individuals immediately displayed

200   aggressive behavior, and little to no affiliative behaviors were observed, we decided to

201   stop the pairing and change the combination. If some affiliative behaviors were observed

202   with few or no aggressive behaviors, or if no social behaviors were observed at all, then

203   we continued the pairing. With this protocol, we formed five male–male pairs from eight

204   formation attempts with nine individuals (one male formed two pairs with different

205   individuals during different periods of the study, Supplementary Table). A tenth male

206   always behaved aggressively; therefore, he was kept solitary at all times, and was not

207   included further in the study.

208

209   *Data collection*

210   Behavioral data were collected between July 2016 and January 2020. The

9

211   behaviors were recorded using infrared cameras (Zcam-CV, Bohankobo). The cameras

212   were attached to the ceiling and recorded each enclosure. In most cases, it was difficult

213   to identify the individuals from the recorded videos due to the video's resolution,

214   therefore, we recorded aggressive and non-aggressive interactions for the lorises without

215   identifying each individual. An ethogram is provided in Table 1. We recorded

216   aggressive behaviors using all-occurrence sampling methods (Martin & Bateson, 2007).

217   We counted the bouts of aggressive behaviors. We included contact aggression in the

218   category of aggressive interactions, as it is often difficult to differentiate non-contact

219   aggression (i.e. chasing) from non-aggressive behaviors (i.e. following behaviors to

220   inspect conspecifics) on the recorded videos. We recorded affiliative social behaviors that

221   involved direct social contact (allogrooming, social play, co-feeding, nest box sharing)

222   every 5 minutes during the dark phase. We categorised the affiliative behaviors based on

223   (Fitch-Snyder & Ehrlich, 2003). We often observed that the two lorises entered a nest box,

224   and categorised such behaviors as a form of affiliative social behavior. We sometimes

225   observed the animals grooming each other or playing socially inside of the nest box, but

226   could not observe all the behaviors that occurred inside the boxes. In addition to

227   monitoring dark phase behaviors, we determined whether the lorises used the same sleep

228   sites during their light phase. We primarily did so using recorded videos, but also used

229   long-term data from Tsuge–Poplar, which were obtained by keepers (including the second

230   author (KN)) during the light phase.

231

232   Behavioral data recording

233   1. Description of social behavior in five pairs of male slow lorises

234   Behaviors were recorded from the 5 pairs of male slow lorises (9 individuals) for

10

235   a total of 150 hours between November 2016 and August 2017. We recorded their

236   behaviors 5 hours a day from the start of the dark phase for 5 days, 3–4 months after the

237   start of the social housing for each pair. We recorded the behavior for another 5 hours for

238   5 days and 1 year after the start of social housing for the Olive–Kunugi pair because the

239   pair took a longer time to exhibit affiliative behaviors.

240

241   2. Did they choose to stay in the same sleeping site during light phase?

242   Subjects were 5 pairs of male slow lorises (N = 9). A total of 450 days of sleeping

243   site data were obtained between August 2016 and October 2019. Sleeping occurred

244   primarily in their nest boxes, but the ground and branches were sometimes also used.

245   Room temperature was recorded daily by keepers. To check whether sleep sites were

246   shared by chance, we used sleeping site data collected over a month for each pair during

247   the period when they formed affiliative relationships (four pairs, except for the father–

248   son pair, for which data were collected for 16 days only because recordings were only

249   available for this time period). To determine whether a relationship between sleep site

250   sharing and temperature existed, we used data from three of the pairs. We selected the

251   three pairs based on our ability to collect sleep site sharing data and room temperature

252   data simultaneously for these three pairs during a period when they performed affiliative

253   behaviors with almost no aggressive behaviors in their light phase. We collected data from

254   the Tsuge–Poplar pair for 281 days, the Nagi–Poplar for 56 days and the Olive–Kunugi

255   for 66 days.

256

257   3. Process of social group formation and changes in fecal glucocorticoid metabolites in

258   two pairs of male pygmy slow lorises

11

259　　　　For this part of the experiment, we selected two pairs of male slow lorises as

260　subjects (N = 4). We recorded their behaviors 5 hours a day from the day after introduction

261　every day for 20 days, with additional sporadic observations after this period, for a total

262　of 260 hours between August 2016 and August 2017. We started each observation from

263　the start of the dark phase. Since the social relationships were established over different

264　time periods for the different pairs, the timing of the observations varied.

265

266　4. Sex differences in forming social relationships

267　　　　Two pairs of male–male, female–female, and male–female pairs (N = 10). Two

268　males experienced two types of pairings during different periods of the study. Total

269　observation time was 144 hours between August 2016 and January 2020. We observed

270　each female–female and male–female pair for 12 days for 2 hours from the start of the

271　dark phase each day. Observations were made for the first 6 days after social housing

272　began, and then for another 6 days one month after the start of social housing. Any injury

273　incidents that occurred were recorded by keepers between August 2016 and January 2020.

274

275　5. Stability of social relationship

276　　　　Subjects were 3 pairs of male lorises (N = 6). Behaviors were compared between

277　two periods; after 3–4 months, and after 8–12 months from the start of the social housing.

278　We recorded their behaviors 3 hours each day from the start of the dark phase, for six

279　days per observation period. The total observation time was 108 hours between November

280　2016 and August 2017.

281

282　Fecal glucocorticoid metabolite (FGM) analysis

283   The feces from 2 pairs of male lorises were collected by keepers (N = 99). Fecal

284   samples were collected 1–15 days before the start of social housing (11–14 days of sample

285   collections for each pair) and during 0–31 days after the start of social housing (20–32

286   days of sample collections for each pair), between 15 July and 15 September, 2016. We

287   could not identify the individual feces once the pairs were housed together. Collected

288   feces were stored in freezers (−20°C) until use.

289   We extracted FGM based on the methodologies developed by Kinoshita et al.

290   (2011). Feces were placed in a vacuum oven for about 18 hours at 60°C. The dried

291   samples were pulverised using a hammer and contaminants were removed as much as

292   possible using mesh. Approximately 0.02 g of the powdered samples were placed in a

293   conical tube and 3 ml of 80% methanol was added to each tube. The tubes were shaken

294   for 30 mins at ambient temperature, and then centrifuged for 10 mins at 3000 rpm. The

295   supernatant was then placed into new 2.0 ml tubes, and dried in a vacuum oven at 60°C.

296   The dried samples were reconstituted with the assay buffer before an assay. Cortisol levels

297   were measured using enzyme immunoassay kits (Salimetric salivary cortisol kits: #1-

298   3002). Parallelism was confirmed, and the recovery rates were 85.6% on average. Inter-

299   assay variabilities were 3.92% (high) and 12.4% (low), respectively, and intra-assay

300   variabilities were 4.41% on average. Data with CV values that were higher than 10% were

301   removed from the analysis.

302

303   *Statistical analysis*

304   We used R 3.6.1 to perform all the statistical tests described below (R

305   Development Core Team, 2019).

306

307   Did they choose to stay in the same sleeping site during light phase?

308   We performed two analyses, in order to determine whether the pairs chose to

309   share sleep sites during the light phase. First, we compared the rate of sleep site sharing

310   with the rate that could have occurred by chance. The chance level was calculated based

311   on Cohen's kappa calculation (Martin & Bateson, 2007). For example, suppose the two

312   individuals, A and B, used two locations, x and y, for sleeping, and that data was obtained

313   for 30 days. Individual A used sleeping location x a total of 11 times, and y a total of 19

314   times. Individual B used sleeping location x a total of 20 times, and y a total of 10 times.

315   The chance proportion of sharing the location x can be calculated by: $\frac{11}{30} \times \frac{20}{30}$ and that of

316   sharing the location y can be calculated by: $\frac{19}{30} \times \frac{10}{30}$. The chance proportion of sharing

317   the same sleeping site can then be calculated as $\left(\frac{11}{30} \times \frac{20}{30}\right) + \left(\frac{19}{30} \times \frac{10}{30}\right)$. We calculated the

318   chance proportion for each pair. Then, we checked whether the observed proportion of

319   sharing the sleep sites was higher than the chance proportion calculated as above by

320   binomial test. We used the function 'pbinom' in R.

321   We also checked the relationship between room temperature and sleep site

322   sharing using Man–Whitney's U tests for comparing temperatures when sleep sites were

323   shared, and when they did not. We used the function 'wilcox.exact' of the package

324   'exactRankTests' to perform the analyses (Hothorn, Hornik, van de Wiel, & Zeileis, 2006).

325

326   Changes in FGM levels

327   Fecal samples could not be assigned to specific individuals once the pairs were

328   housed together. We calculated the mean values from each individual for each day for the

329   period before social housing to perform statistical analyses on the FGM levels among

14

330   three conditions: 15 days before social housing ('before'), 15 days right after social

331   housing began ('soon after', 0–14 days) and days 15–31 ('later') after social housing

332   began for each pair. Among the four subjects, Nagi lived in a small cage and Poplar, Olive

333   and Kunugi each lived in one of the enclosures (8.0 m^3) during the 'before' period. We

334   used Kruskal–Wallis tests to compare the FGM levels across the three conditions. If we

335   statistically significant differences were determined, then we used Man–Whitney U tests

336   for multiple comparisons with the Bonferroni correction.

337

338   Sex differences in forming social relationships

339   The effect of sex on the formation process of social relationships was compared

340   using a generalised linear model. We used the function 'glm' with Poisson distribution in

341   R. Independent variables included sex (male–male, male–female, female–female),

342   introduction phase (0 months or 1 month), pairs and the interaction of sex and introduction

343   phase. Dependent variables included the number of times we observed aggressive and the

344   number of sampling points we observed affiliative behaviors. We also looked for

345   differences in the rate of affiliative behaviors one month after the start of social housing,

346   by constructing a model that included the independent variables of sex and pair. We

347   compared Akaike Information Criteria (AIC) among models composed of various

348   combinations of the aforementioned factors to identify the models that produced the

349   lowest AIC values. We used the dredge function in the package 'MuMIn' to choose the

350   best models (Barton & Barton, 2019). The AIC tables, including the models containing

351   △AIC < 2 from the final model and the parameter estimates for the best models, are given

352   in the supplementary materials.

353

15

354   Stability of social relationships

355   We used a Mann–Whitney U test to compare the rates of social behaviors

356   between two periods for each pair. We used a proportion test to compare the probability

357   of sharing the sleeping sites.

358

359   **Results**

360   1. Social behaviors observed in the five male–male pairs

361   All five pairs performed some affiliative behaviors. On average, the pairs

362   continued for 595 ± 334 days (mean of four pairs), except for the father-son pair, who had

363   lived together from the birth to the death of the son (Supplementary Table 2).

364   Allogrooming, nest box sharing during dark phase and sleep site sharing during light

365   phase were observed in all pairs (Table2, Figure 1). Social play was observed in three of

366   the pairs (Nagi-Poplar, Shuro-Yanagi, Yashi-Hiiragi: Supplementary video). Unlike other

367   pairs, one pair (Olive–Kunugi) did not show any affiliative behaviors during the

368   observations made three months after the start of the social housing; however, they did

369   show affiliative behaviors similar to the other pairs after one year had passed.

370

371   2. Did they choose to stay in the same sleeping site during light phase?

372   All pairs were observed to remain at the same sleeping site during the light phase.

373   They used multiple locations for sleeping together. The proportion of sleep site sharing

374   was above the calculated chance levels for all the pairs (Table 3).

375   There was no statistically significant relationship between temperature and sleep

376   site sharing for two of the pairs. The proportion of sleep site sharing was high for all the

377   three pairs (Tsuge–Poplar: 96.4%; Nagi–Poplar: 67.9%; Olive–Kunugi: 84.8%). Nagi–

16

378 Poplar had an average temperature of (mean ± SD): 26.2°C ± 0.98°C when they shared a

379 sleeping site, and an average temperature of 26.3°C ± 0.73°C when they slept alone

380 (Man–Whitney's U test: W = 328, P = 0.81). Olive–Kunugi had an average temperature

381 of 25.4°C ± 0.75°C when they shared a sleeping site, and an average temperature of

382 25.7°C ± 0.75°C when they did not (Man–Whitney's U test: W = 218, P = 0.25). In the

383 case of Tsuge–Poplar, the average temperature was relatively high when they shared sleep

384 sites with an average temperature 24.7°C ± 0.73°C, and an average temperature of

385 24.2°C ± 0.67°C when they slept alone (Man–Whitney's U test: W = 817, P = 0.029). It

386 should be noted that they did not share sleep sites for 10 days out of 281, and that the

387 temperatures when they did not share sleep sites were within the range of those when they

388 shared.

389

390 3. Process of social group formation and changes in FGM in two pairs of male slow lorises

391 The male–male pairs showed some forms of aggression in the beginning of the

392 study that ceased after about 10 days. One pair (Nagi–Poplar) gradually increased their

393 affiliative behaviors after 14 days. It took more time for another pair (Olive–Kunugi) to

394 exhibit comparable levels of affiliative social behaviors (Figure 2). Sleep site sharing

395 during the light phase was observed after affiliative behaviors were observed during the

396 dark phase. Aggressive behaviors were observed most often after intense following of

397 another individual, during which the follower grabbed the other male and a short-time

398 jostling occurred.

399 Changes in FGM levels are shown in Figure 3. In the case of Nagi–Poplar, there

400 was a significant increase in FGM levels soon after the start of the experiment (Man–

401 Whitney's U test: 'before' vs 'soon after': W = 22, P < 0.001), but levels decreased to

402   lower than those observed before the social housing began (Man–Whitney's U test: 'soon

403   after' vs 'later': W = 227, P < 0.001; 'before' vs 'later': W = 242, P < 0.001). There was

404   no statistically significant change in FGM levels for Olive–Kunugi (Kruskal–Wallis test:

405    $X^2 = 2.5$, df = 2, $p = 0.28$).

406

407   4. Sex differences in forming social relationships

408   The sex of each animal did make a difference in social behavior during the

409   beginning phase of the social housing (Figure 4). Only male–male pairs showed

410   aggressive behaviors, while the other types of pairs did not show any aggressive behaviors

411   (Figure 4-a). The final model of aggressive behaviors included sex, introduction phase

412   and the interaction of sex and introduction phase (Supplementary tables 3, 4). Parameter

413   estimates of the final model indicated that aggression decreased one month after the start

414   of social housing, compared with the beginning phase. Female–female and male–female

415   pairs showed affiliative behaviors from the beginning, while male–male pairs showed

416   such behaviors later (Figure 4-b). The final model of affiliative behaviors included sex,

417   introduction phase, pair and the interaction of phase and sex (Supplementary table 5, 6).

418   All types of pairs showed similar affiliative behaviors by the end of the experiment. The

419   final model explaining the factors associated with the rate of affiliative behaviors one

420   month after the start of the social housings only included the pair factor, not sex

421   (Supplementary table 7, 8). Some female–female and male–female pairs showed sleep

422   sites share from the beginning, while male–male pairs showed such behaviors later

423   (Figure 4-c).

424   Injuries were rarely observed, with a total of six incidents requiring veterinary

425   interventions between 2016 and 2019. Four individuals out of 16 received some form of

18

426   injury (three males and one female).

427

428   5. Stability of social relationship

429   Social relationship stability differed across the pairs. One pair showed similar

430   levels of social behaviors between the two observation periods, another pair increased the

431   amount of affiliative behaviors and one pair decreased the amount of affiliative behaviors

432   and increased aggressive behaviors (Table 4). During the periods when aggressive

433   behaviors were observed, without any affiliative behaviors, sleep sites were not shared

434   during the light phase.

435

436   **Discussion**

437   All five pairs of male slow lorises formed affiliative relationships by the end of

438   the first observation period. Allogrooming, social play, nest box sharing during the dark

439   phase, and sleep site sharing during the light phase were the main behaviors observed.

440   Some pairs ate together at the same feeding platform in close proximity at times, even if

441   there were other places to forage. The rate of affiliative social behaviors was high in the

442   father-son pair, but some pairs also showed comparable levels of such behaviors. One

443   pair (Olive–Kunugi) did not show any aggressive or affiliative behaviors at times when

444   the other pairs would have; however, even this pair showed affiliative behaviors without

445   any aggressive behaviors by the end of the first observation period. This is surprising

446   considering reports of the solitary nature of wild lorises, but they can express social

447   flexibility (Starr & Nekaris, 2020). In an environment without resource competition or

448   mating pressure, males can coexist, even into adulthood. It is imperative that enclosures

449   have adequate space and options to separate or hide from the group in captivity (Global

19

450   Fereration of Animal Sanctuaries, 2013), especially during the introductory phase.

451   Additionally, it is necessary to closely monitor their relationships considering the fact

452   that the quality of relationship of the Yanagi–Shuro pair deteriorated later.

453   All pairs shared sleep sites during the light phase. Sleep site sharing is often

454   observed in various wild, nocturnal strepsirrhines (Bearder et al., 2003; Nekaris &

455   Bearder, 2007; Rode et al., 2013), as researchers often analyze grouping tendencies by

456   observing daytime sleep sites in the wild (Bearder et al., 2003). Although it is rare for

457   multiple males to share sleep sites, it has been reported in the slender loris and in giant

458   mouse lemurs (Nekaris, 2003; Rode et al., 2013). This result is not consistent with those

459   observed in the solitary sleeping patterns of wild pygmy slow lorises (Starr & Nekaris,

460   2020). Given their small body size, sleeping together may have a thermoregulatory

461   advantage at lower temperatures, but room temperature in this experiment was set within

462   the range recommended by the husbandry manual (Fitch-Snyder et al., 2001), and there

463   was no evidence of increased sharing at lower room temperatures. On the contrary, one

464   pair (Tsuge–Poplar) tended to increase their sharing rate when the temperature increased,

465   which was not expected. Consideration has to be made for the fact that they rarely slept

466   alone, and for the temperature range, which leads us to conclude that the trend was not

467   strong. Furthermore, all the pairs used multiple sleep sites together, and the rate of sleep

468   site sharing was above the chance level for all pairs. These results suggest that the pygmy

469   slow lorises in this study preferred to stay together with other individuals during the light

470   phase. In addition, relationships with both aggressive and affiliative behaviors during the

471   dark phase suggest that sleep site sharing could only be observed during periods when

472   their relationship was more affiliative. Therefore, sleep site sharing is a simple and good

473   indicator of their relationship status.

474   The two pairs of males that showed aggression at the beginning of the experiment,

475   had stopped displaying aggressive behavior around day 10. Most aggressive behaviors

476   did not appear to be serious, and veterinary interventions were rare. We did not record

477   any deaths that were suspected to be related to the wounds incurred by the aggressions.

478   There are previous studies that have reported a few cases of death that may have been

479   related to trauma or infection secondary to bite wounds (Fuller, Lukas, Kuhar, & Dennis,

480   2014). In particular, slow lorises are venomous primates (Nekaris et al., 2013), which

481   results in a need for close monitoring whenever adult pairs are formed to avoid serious

482   injury or death. Results of the changes in FGM levels for Nagi–Poplar indicates that their

483   FGM levels increased during their phase of heightened aggression, and decreased in the

484   later phase. The end FGM levels were even lower than that in the period when they were

485   housed alone in the small cage or an enclosure. This result suggests that forming pairs in

486   a more enriched enclosure is beneficial to reducing the level of physiological stress. The

487   results from Olive–Kunugi were not consistent with that of Nagi–Poplar. There was no

488   significant change in the level of FGM for Olive–Kunugi, which is counter intuitive. This

489   might be related to the fact that Olive and Kunugi already lived in an enriched

490   environment prior to the start of this study. Nevertheless, combined with the behavioral

491   results and FGM levels, there was no evidence of increased stress over a long period of

492   social housing. Instead, forming same-sex pairs increased the opportunity for affiliative

493   social behaviors.

494   There were sex-dependent differences in the formation process of social groups.

495   Aggressive behaviors were observed in the male–male pairs at the start of the experiment.

496   The male–female and female–female pairs showed affiliative behaviors from the

497   beginning. This low level of aggressive behaviors between females is interesting,

498   considering the fact that both adult males and females in the wild do not overlap their

499   home range with same-sex individuals (Starr & Nekaris, 2020). The details in the

500   behaviors seen in female–female pairings will be reported in another paper (Alejandro et

501   al. in prep). Injury risk was relatively high in the male–male pairs, but an injury was

502   observed in a female-female pair once. Nevertheless, all types of pairs showed affiliative

503   behaviors by the end of the experiment, without any serious incidence of aggression.

504   All-male groups have been formed in several captive primate species, including

505   gorillas (Stoinski, Kuhar, et al., 2004), chimpanzees (Morimura, Idani, & Matsuzawa,

506   2010; Yamanashi, Teramoto, Morimura, Nogami, & Hirata, 2018), proboscis monkeys

507   (Sha et al., 2013) and white crowned mangabeys (Fàbregas & Guillén-Salazar, 2007). All

508   of these species are gregarious, and some of these species also have naturally occurring

509   all-male groups in the wild, the type of species that typically form polygynous groups

510   [e.g. gorillas (Gatti, Levréro, Ménard, & Gautier‑Hion, 2004) and proboscis monkeys

511   (Murai, 2004)]. When mating competition is removed from the environment, as in all-

512   male captive groups, coexistence of multiple adult males is more feasible. Fundamentally,

513   male bonding is less easily occurred compared with females because males tend to

514   compete for mating, which cannot be shared easily, in addition to food resources (Van

515   Schaik, 1994). In some species, particularly male philopatric species, social bonding

516   among males might be advantageous for mating success (Van Schaik, 1994). Pygmy slow

517   lorises are not such species, rather they are territorial, and rarely overlap their home

518   ranges with other same-sex adult individuals (Starr & Nekaris, 2020; Wiens & Zitzmann,

519   2003). Additionally, it is normal for other species to exhibit aggression, even after they

520   establish their social relationships (Leeds, Boyer, Ross, & Lukas, 2015), which has led to

521   the recommendation for some species to form social groups at an early age (Sha et al.,

522   2013; Stoinski, Lukas, et al., 2004). Surprisingly, in the case of male pygmy slow lorises,

523   aggressive behaviors were very rare once they formed affiliative relationships, even when

524   they were introduced after sexual maturation. Kinship and familiarity could not explain

525   these relationships, except for the father-son pair. We are still not sure what the proximate

526   and ultimate mechanisms of this flexible trait are, but the results of this study indicate

527   that this species has a high motivation to interact with conspecifics if conditions are

528   appropriate.

529   There were some limitations to our study pertaining to the understanding of

530   social behaviors, and optimal social management regimes. First, we could not record

531   social behaviors without any contact nor vocalisations. Studies have reported that

532   vocalisations often accompany social behaviors (Daschbach, Schein, & Haines, 1981;

533   Zimmermann, 1985). A recent study in Javan slow loris reported that the lorises used

534   ultrasonic vocalisations for communication (Geerah, O'Hagan, Wirdateti, & Nekaris,

535   2019), and reported that lorises emitted vocalisations in the context of following or

536   leading each other, and entering a sleep site. In this study, we sometimes observed that

537   the pygmy slow lorises looked into a nest box where another individual was already inside,

538   or kept in social proximity for a while in front of the nest box before entering the box

539   together. It would be interesting to explore the details of pygmy slow loris communication

540   prior to social behavior initiation in future studies. A second limitation is the fact that the

541   judgement criteria used to test pair compatibility during the first introduction trial was

542   obscure. We introduced two unfamiliar individuals based on published suggestions to

543   minimise aggression during the introduction of a male and female pair (Fitch-Snyder et

544   al., 2001). We judged their compatibility based on behaviors displayed during their first

545   encounters. One male always showed aggressive tendencies to all individuals introduced,

546   thus, we did not form any long-lasting pair with him. We are not sure whether his

547   aggression was a personality trait or the result of some past experience. Since all other

548   pairs managed to form affiliative relationships by the end of the study, it is possible that

549   this individual may have successfully formed an affiliative relationship if given more time

550   or tried with a different individual.

551   In conclusion, male slow lorises appeared to be motivated to interact with

552   conspecifics. All pairs formed affiliative relationship by the end of the study, and they all

553   preferred to sleep together during their light phases. Furthermore, there was no evidence

554   of increasing physiological stress levels over a long period of time. These results suggest

555   that living with conspecifics increases the behavioral options of pygmy slow lorises, and

556   enables facilities to more efficiently use their available space and improve animal well-

557   being. We believe that providing opportunities to interact with conspecifics is one of the

558   most important parts of captive care for this species. Forming same-sex pairs might be a

559   feasible strategy for their social management. Although adult male–male pairings might

560   not be the first choice considering the risk of aggression during the introductory phase,

561   such flexibility in social housing should be considered to reduce the number of individuals

562   housed under suboptimal conditions in sanctuaries, zoos and rehabilitation centres.

563

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24

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747

Table 1. Ethogram used for the study

Category	Behaviors	Definition
Affiliative behaviors	Social grooming	Lick fur and apply toothcomb while grasping other animal with one or both hands.
	Play	Mock fight (mutual biting, hiting, classping but no other signs of threat; low intensity, prolonged, neither animal tries to get away)
	Co-feeding	Eat foods from the same dish in proximity
	Sharing a nest box	Enter into a same nest box during dark phase
Aggressive behaviors	Attack	Bite, hit hard with hands or push with apparent force; lunge and bite at the same time, butt with head with apparent force
	Fight	Mutual hitting and biting with apparent force; wrestle vigorously

753   Table 2. The rate of social behaviors among five pairs of male slow lorises (5 hours for 5

754   days/column)

	Yashi–Hiiragi	Shuro - Yanagi	Nagi–Poplar		Olive–Kunugi	Tsuge–Poplar
Sampling period (months)	Father-son	4	4	3	12	3
Aggression	0	0	0	0	0	0
Affiliation	0.38 ± 0.043	0.14 ± 0.059	0.31 ± 0.14	0	0.27 ± 0.15	0.11 ± 0.084
Sleep site share(days)	5	5	3	0	4	5

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757   Table 3. The proportion of sleep site sharing in the five male pairs

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	Number of sleeping site used	Rate of sleeping site share	Rate of sleeping site share expected by chance	P
Nagi-Poplar	3	0.68	0.38	<0.001
Shuro-Yanagi	2	1.00	0.85	<0.001
Olive-Kunugi	3	0.81	0.68	0.018
Tsuge-Poplar	4	0.90	0.46	<0.001
Yashi-Hiiragi	3	0.94	0.64	<0.001

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761   Table 4. Changes in social behaviors in the three male pairs between two periods

	Shuro - Yanagi			Nagi-Poplar			Olive-Kunugi		
Sampling period (months)	4	8		4	8		3	12	
Aggression	0	1.2 ± 1.3	W = 6, P = 0.061	0	0	W =18, P =1	0.17 ± 0.37	0	W = 21, P =1
Affiliation	0.15 ± 0.048	0	W =36, P = 0.0022	0.28 ± 0.13	0.26 ± 0.064	W = 19, P = 0.98	0	0.29 ± 0.19	W = 0, P = 0.0022
Sleeping site share(days)	6	0	X^2=3.4, P = 0.066	4	5	X^2=0, P = 1	0	4	X^2=8.3, P < 0.001

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764   Figures

765   Figure 1. A photo of two males (Shuro and Yanagi) sharing a nest box

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768

769   Figure 2. Changes in the social behavior from the start of the social housing in two pairs.

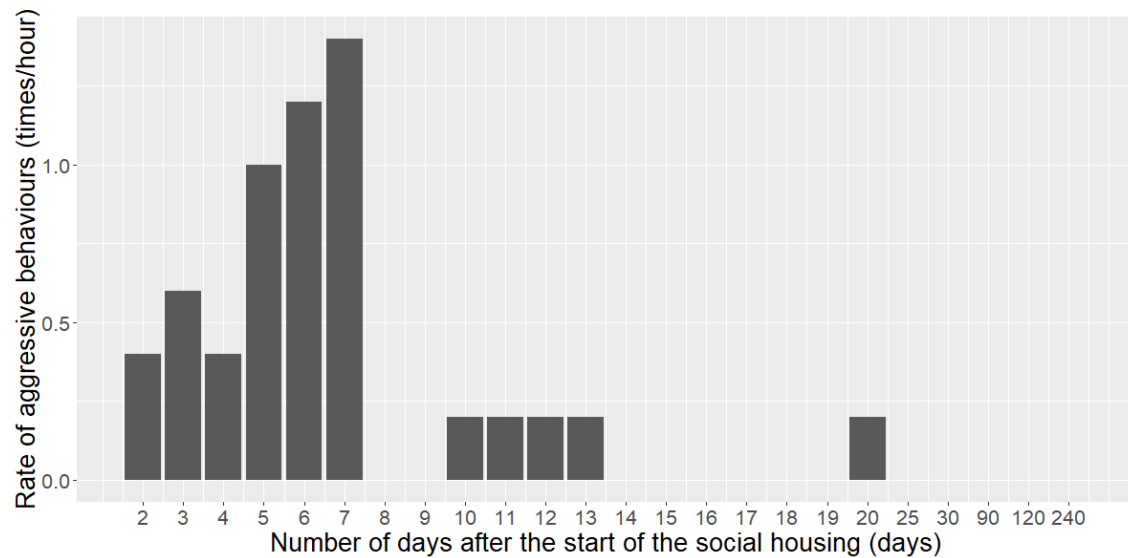770   Figure 2-a. Changes in aggressive behaviors for Nagi–Poplar.

771

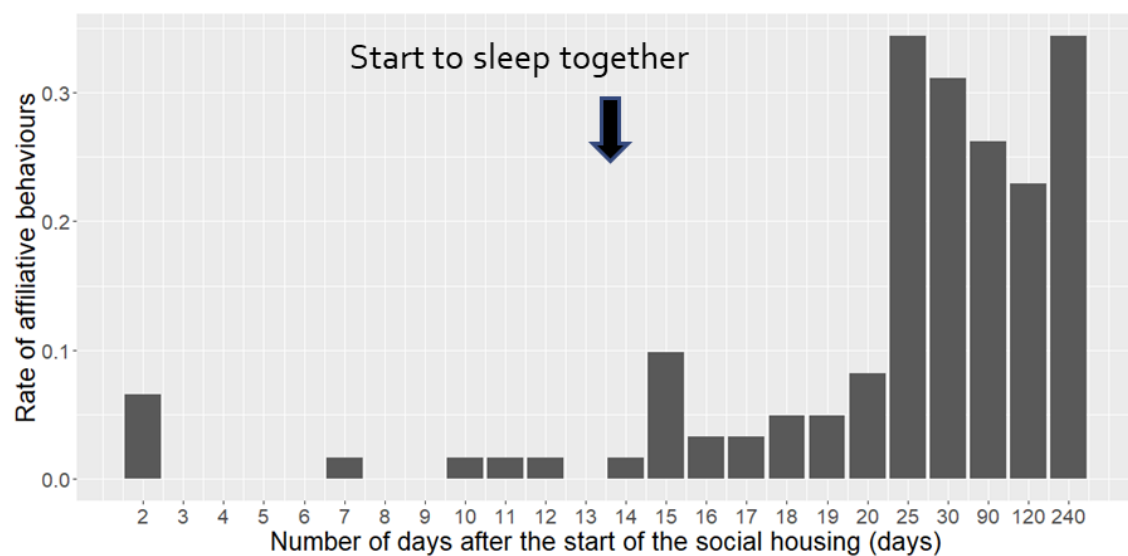Figure 2-a. Changes in aggressive behaviors for Nagi–Poplar.

772   Figure 2-b. Changes in affiliative behaviors for Nagi–Poplar.

773

Figure 2-b. Changes in affiliative behaviors for Nagi–Poplar.

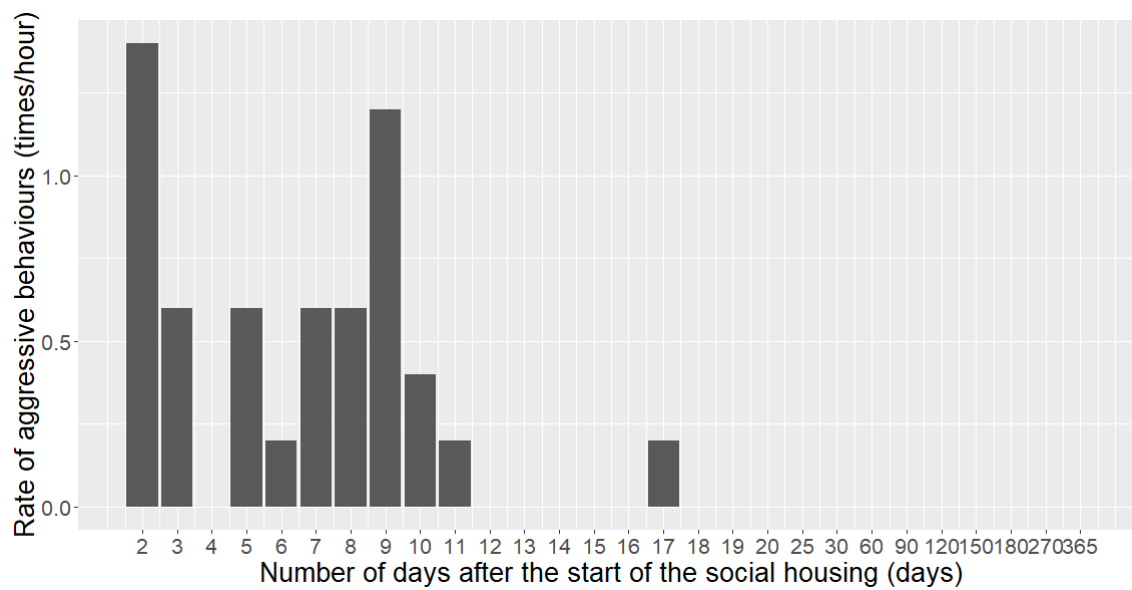774   Figure 2-c. Changes in aggressive behaviors for Olive–Kunugi
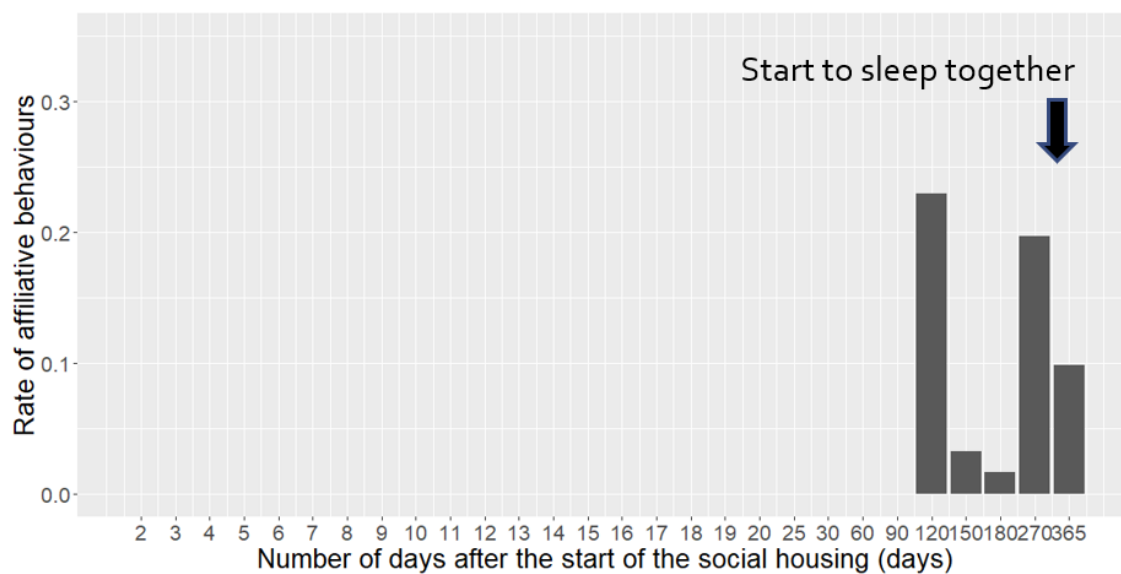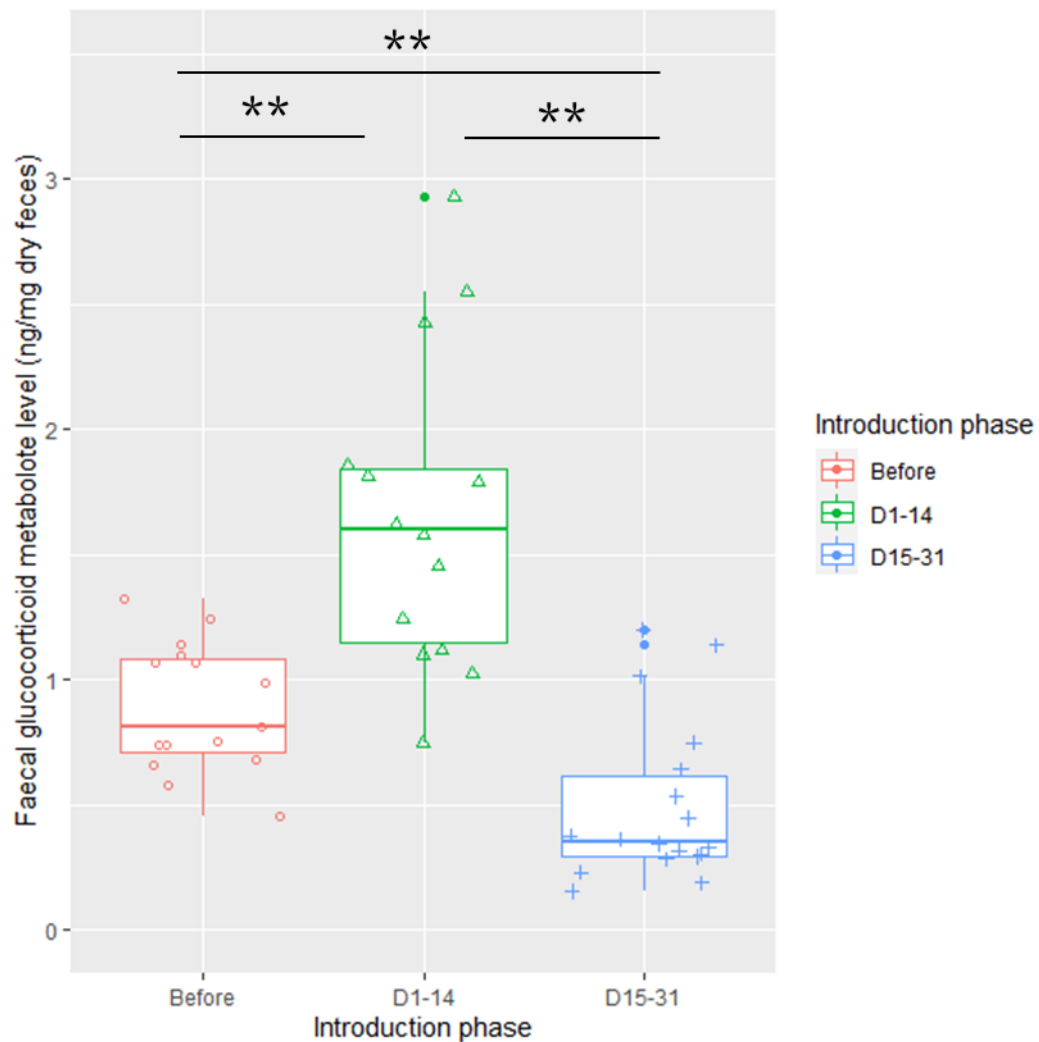
775

Figure 2-d. Changes in affiliative behaviors for Olive–Kunugi
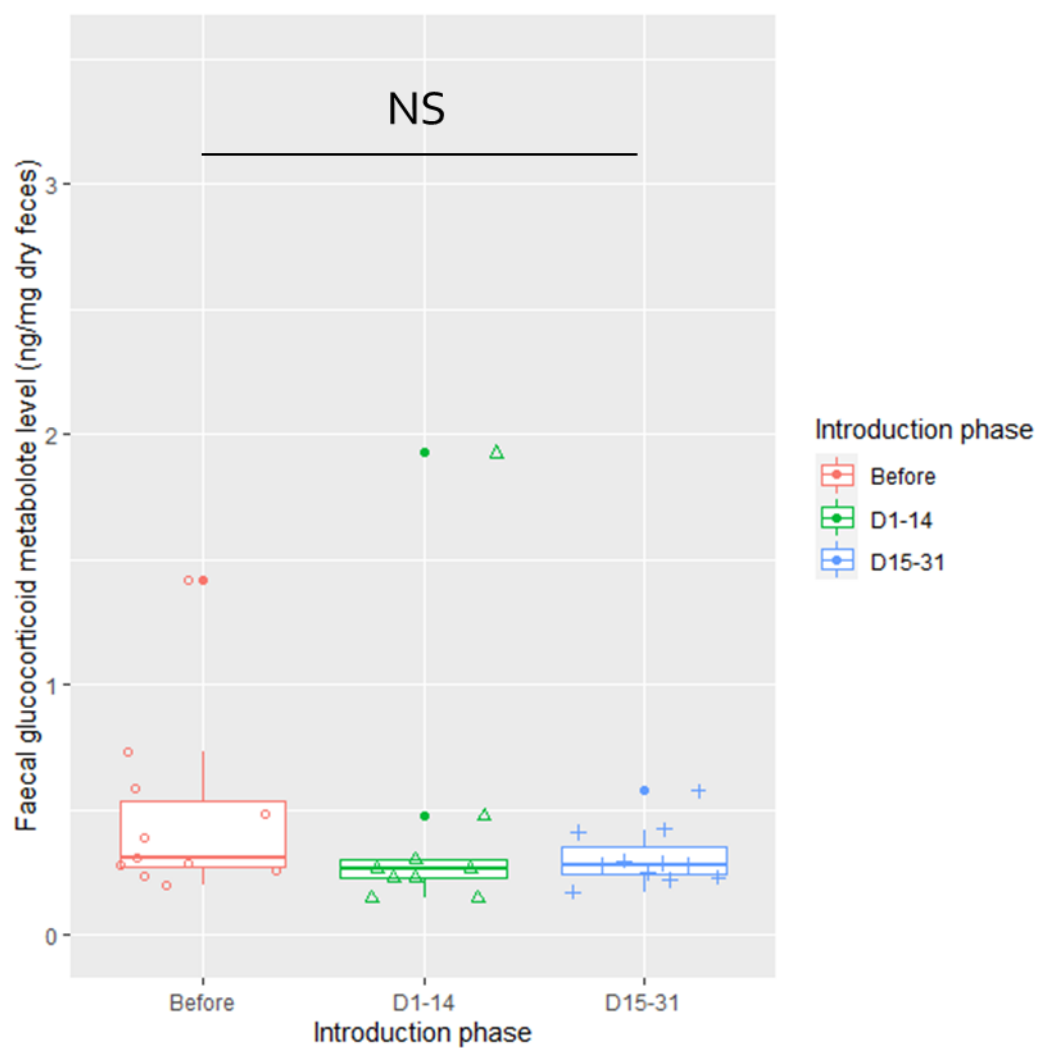

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779   Figure 3. Changes in FGM levels before and after social housing.

780   Figure 3-a. Nagi–Poplar

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782   Figure 3-b. Olive–Kunugi

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786   Figure 4. Sex differences in social behaviors during the beginning phase of social housing.

787   FF represents data from the two female-female pairs, MF represents data from the male–

788   female pairs, and MM represents data from the male–male pairs. The data were obtained

789   on the first 6 days (0M), then again for 6 days one month after the start of the social

790   housing (1M). The data of MM final derived from the study 1.

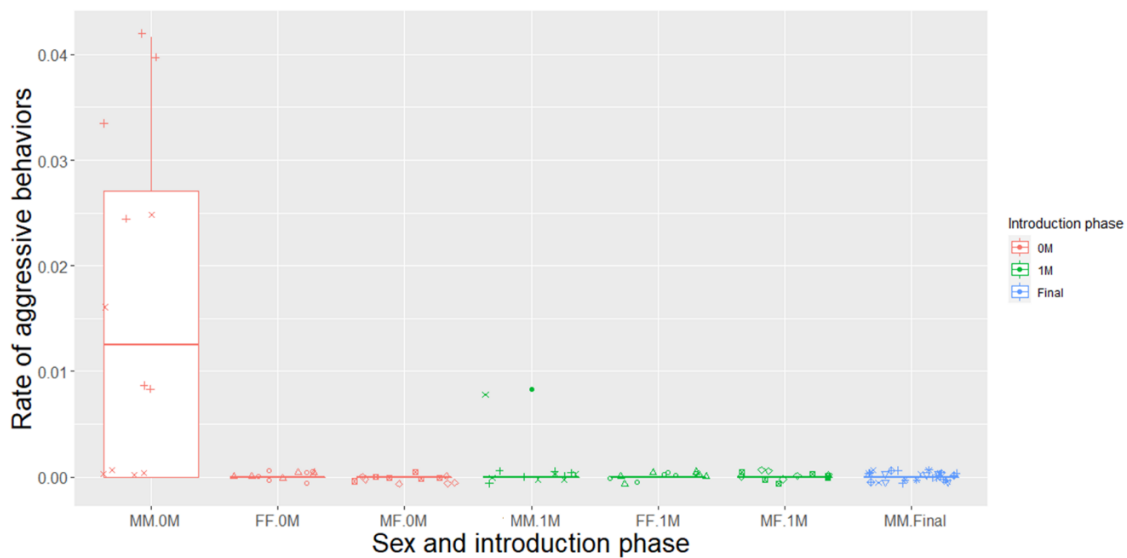791

792   Figure 4-a. Sex differences in aggressive behaviors
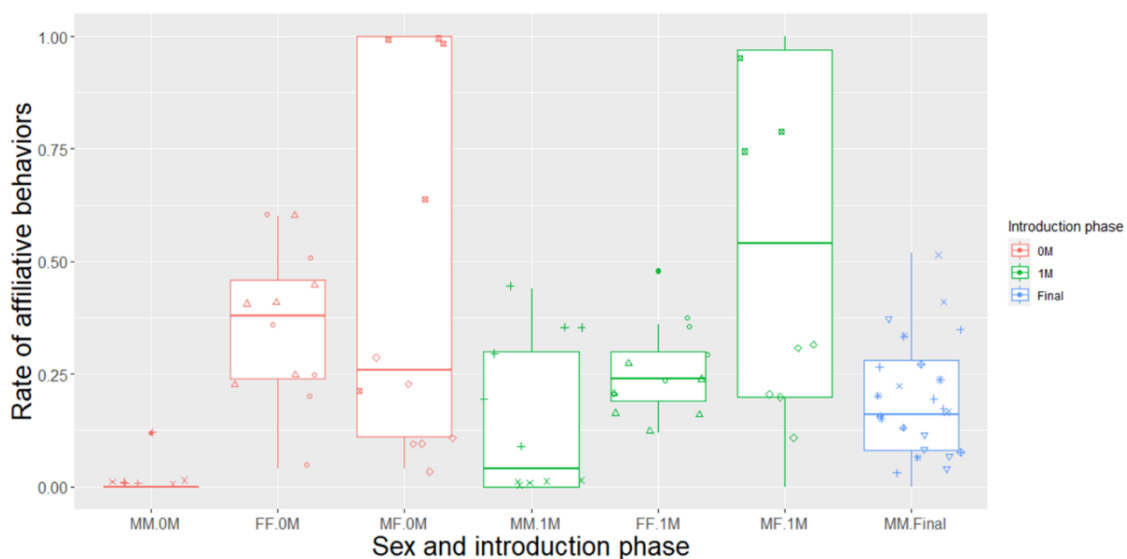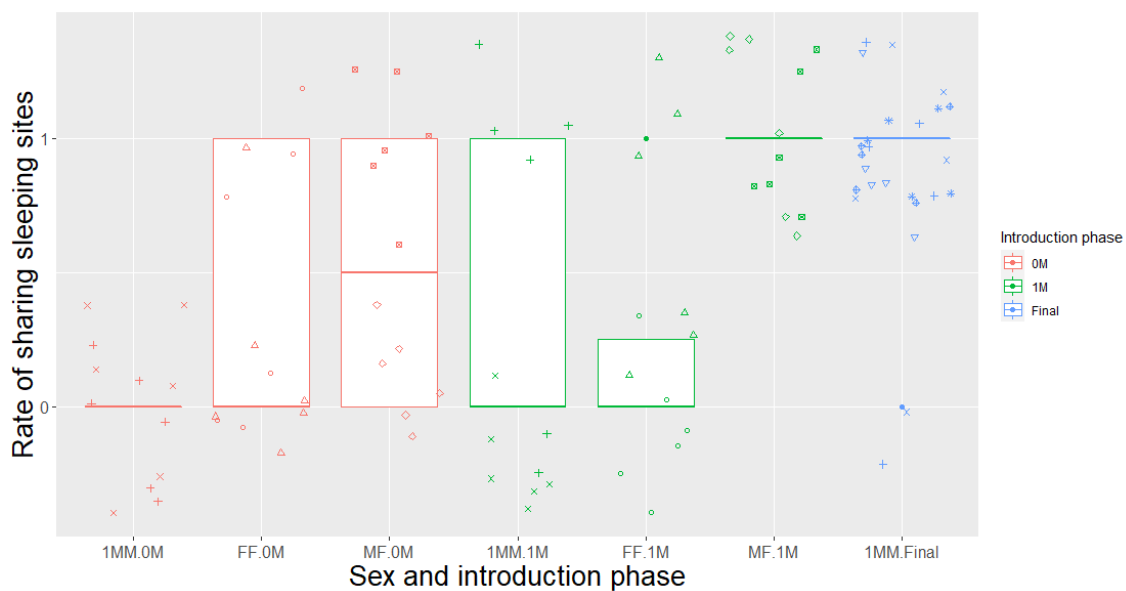
793

794   Figure 4-b. Sex differences in affiliative behaviors

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796   Figure 4-c. Sex differences in sleep site sharing

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800   Supplementary table 1. Subject information

801

Name	Sex	Date of arrival	Origin	Pairing partners
Yanagi	M	2006/12/7	Confiscation	Shuro
Tsubaki	F	2007/8/2	Confiscation	Tsuge
Mizuki	F	2007/8/2	Confiscation	Nanten
Momiji	F	2007/8/2	Confiscation	Poplar
Olive	M	2007/8/28	Confiscation	Kunugi
Nagi	M	2007/8/28	Confiscation	Poplar
Kunugi	M	2007/8/28	Confiscation	Olive
Nanten	F	2007/8/28	Confiscation	Mizuki
Hiiragi	M	2007/8/28	Confiscation	Yashi
Aoki	M	2007/8/28	Confiscation	Unsuccessful
Shuro	M	2007/8/28	Confiscation	Yanagi
Kaede	F	2007/8/28	Confiscation	Mimoza
Poplar	M	2007/8/28	Confiscation	Nagi, Tsuge, Momiji
Tsuge	M	2007/8/28	Confiscation	Poplar, Tsubaki
Mimoza	F	2007/8/28	Confiscation	Kaede
Yashi	M	2010.03.28	Born at the JMC	Hiiragi

802

803   Supplementary table 2. Duration of each pair

Pair	Type of pair	Start	End	Reason of ending the pair
Nagi - Poplar	MM	2016/8/1	2017/3/31	To treat an injury of Poplar
Olive - Kunugi	MM	2016/8/14	2019/1/21	To form a male-female pair
Yashi - Hiiragi	MM	2016/9/16	2018/5/30	Yashi passed away from sickness.
Shuro - Yanagi	MM	2016/11/29	2017/12/12	Shuro passed away from sickness.
Tsuge - Poplar	MM	2017/5/26	2019/10/14	To form a male-female pair
Kaede - Mimoza	FF	2017/1/7	2017/7/2	To treat an injury of Mimoza
Nanten - Mizuki	FF	2017/9/12	2018/11/5	To form a male-female pair
Poplar - Momiji	MF	2019/10/14	Ongoing	
Tsuge - Tsubaki	MF	2019/11/27	Ongoing	

804

805 Supplementary table 3. AIC table for explaining the factors associated with occurrence
806 of aggressive behaviors

AIC	Independent variables
70.0	Sex + Phase + Sex : Phase
70.6	Sex + Phase + Pair + Sex : Phase
71.4	Sex + Phase
72.0	Phase + Pair
72.0	Sex + Phase + Pair

807

808 Supplementary table 4. Parameter estimates for the best-fit model to explain the factors
809 associated with occurrence of aggressive behaviors

	Factor	estimate	SE	z	p
	Intercept	0.51	0.22	2.3	0.022
Sex	Female- female	-21	4428	-0.0050	1.0
	Male - female	-21	4428	-0.0050	1.0
Phase	1 month	-3.0	1.0	-2.9	0.0035
Sex Phase	Female-female : 1month	21	4487	0.0050	1.0
	Male-female : 1month	3.0	6345	0	1.0

810

811 Supplementary table 5. AIC table for explaining the factors associated with the rate of
812 affiliative behaviors

AIC	Independent variables
329	Sex + Phase + Pair + Sex : Phase

813

814 Supplementary table 6. Parameter estimates for the best-fit model to explain the factors
815 associated with the rate of affiliative behaviors

816

	Factor	estimate	SE	z	p
	Intercept	-20	1436	-0.014	0.99
Sex	Female-female	22	1436	0.016	0.99
	Male-female	23	1436	0.016	0.99
Phase	1 month	2.7	0.60	4.4	<0.001
Pair	Mizuki-Nanten	-0.11	0.15	-0.74	0.46
	Nagi-Poplar	19	1436	0.013	0.99
	Olive-Kunugi	NA	NA	NA	NA

	Poplar-Momiji	-1.6	0.15	-11	<0.001
	Tsuge-Tsubaki	NA	NA	NA	NA
Sex : Phase	Female-female : 1month	-3.0	0.62	-4.9	<0.001
	Male-female : 1month	-2.5	0.61	-4.1	<0.001

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818

819 Supplementary table 7. AIC table for explaining the factors associated with the rate of

820 affiliative behaviors one month after the start of the social housings

AIC	Independent variables
156	Pair
156	Sex + Pair

821

822 Supplementary table 8. Parameter estimates for the best-fit model to explain the factors

823 associated with the rate of affiliative behaviors one month after the start of the social

824 housings

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	Factor	estimate	SE	z	p
	Intercept	2.1	0.14	14	<0.001
Pair	Mizuki-Nanten	-0.50	0.2	-2.1	0.032
	Nagi-Poplar	-0.11	0.21	-0.52	0.60
	Olive-Kunugi	-20	2334	-0.0090	0.99
	Poplar-Momiji	-0.50	0.24	-2.1	0.032
	Tsuge-Tsubaki	1.1	0.17	6.3	<0.001

826

827 Supplementary video

828 https://youtu.be/f37WEaY11FQ