1   **Effects of domestication on neophobia: A comparison between the domesticated Bengalese**

2   **finch (*Lonchura striata* var. *domestica*) and its wild ancestor, the white-rumped munia**

3   **(*Lonchura striata*)**

4

5   Kenta Suzuki[a,c,d,e] *, Maki Ikebuchi[c,d,e], Hiroko Kagawa[b,c,d,e], Taku Koike[c], Kazuo Okanoya[b,c,d,e]

6   [a] *Faculty of Health Sciences, Nihon Institute of Medical Science, Moroyama-machi 350-0435,*

7   *JAPAN*

8   [b] *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo,*

9   *Meguro-ku, Tokyo 153-8902, JAPAN*

10   [c] *Laboratory for Biolinguistics, RIKEN Brain Science Institute (BSI), Wako, Saitama 351-0198,*

11   *JAPAN*

12   [d] *Okanoya Emotional Information Project, Exploratory Research for Advanced Technology*

13   *(ERATO), Japan Science and Technology Agency (JST), Wako, Saitama 351-0198, JAPAN*

14   [e] *Cognition and Behavior Joint Research Laboratory, RIKEN Center for Brain Science (CBS), Wako,*

15   *Saitama 351-0198, JAPAN*

16   *Corresponding author: Faculty of Health Sciences, Nihon Institute of Medical Science, 1276

17   Shimogawara, Moroyama-machi, Iruma-gun, Saitama 350-0435, JAPAN. Tel.: +81-49-294-9000,

18   Fax: +81-49-294-9009.

19   E-mail address: kentszk@gmail.com (K. Suzuki).

20   **ABSTRACT**

21   Bengalese finches (*Lonchura striata* var. *domestica*) have more complex song traits than their wild

22   ancestors, white-rumped munias (*Lonchura striata*). Domesticated finches are likely able to allocate

23   more resources to song development rather than allocating resources to mechanisms aimed at coping

24   with predation, which are no longer needed under domesticated conditions. Here, we aimed to

25   examine the effects of changes in selection pressure due to domestication on the behaviour of

26   Bengalese finches and to contemplate the possible evolutionary mechanisms underlying these

27   changes. To do so, we compared neophobic responses to novel-object conditions as an assessment of

28   reactions to potential predators. We studied groups of Bengalese finches and white-rumped munias

29   and found that Bengalese finches were more likely to eat the food provided to them under

30   novel-object conditions. Bengalese finches had a shorter latency time to eat, and this latency time

31   was less affected by the novel object in the case of Bengalese finches compared to white-rumped

32   munias. Therefore, Bengalese finches have reduced neophobic responses due to domestication. The

33   behavioural strategies of white-rumped munias appear to be more suitable for natural environments,

34   which include unpredictable risks, whereas Bengalese finches have likely adapted their behaviour to

35   the conditions of artificial selection.

36

37   **Keywords:** Bengalese finch (*Lonchura striata* var. *domestica*); Domestication; Neophobia;

38   Neophobic response; White-rumped munia (*Lonchura striata*)

39 **Abbreviations**

40 BF, Bengalese finch; WRM, white-rumped munia.

41

42 **1. Introduction**

43   The Bengalese finch (BF, *Lonchura striata* var. *domestica*) is a domesticated variety of the

44 wild white-rumped munia (WRM, *Lonchura striata*), which was imported from China to Japan

45 approximately 250 years ago (Washio, 1996; Okanoya, 2004a; Svanberg, 2008). BFs sing

46 phonologically and syntactically complex songs, as opposed to the stereotypical simple songs sung

47 by WRMs (Honda and Okanoya, 1999; Okanoya, 2004a, b). The complex songs of BF have not

48 been selected artificially (Washio, 1996). Complex song as a high-quality sexual trait is

49 believed to have evolved through the domestication process in BFs (Okanoya, 2004a, b). WRMs are

50 thought to experience strong natural selection pressures in wild environments. Conversely,

51 domesticated BFs experience safe and resource-rich conditions under human-controlled rearing

52 conditions (i.e., no environmental perturbations, no predation, abundant food, and low risk of

53 parasitism and injury). Thus, we hypothesised that the relaxation of natural selection pressures and

54 the presence of artificial selection may have led BFs to allocate fewer resources to behaviours

55 associated with efforts for survival and more to efforts associated with reproduction.

56   Domestication results in species being removed from many natural selection pressures;

57   however, domesticated species are exposed to artificial selection pressures exerted by their captive

58   environments and by humans (Fox, 1968; Boice, 1973; Ratner and Boice, 1975; Price, 1984). This

59   change in selection pressure results in changes in physiological and behavioural traits (Hale, 1969;

60   Price, 1984, Künzl and Sachser, 1999; Lepage et al., 2000). Domesticated species have reduced

61   behavioural expressions related to ontogenetic survival, such as fear responses (Desforges and

62   Wood-Gush, 1975; Schütz et al., 2001; Campler et al., 2009), and have enhanced behavioural traits

63   that are not directly related to survival, such as sexual and reproductive behaviours (Künzl and

64   Sachser, 1999). Therefore, these behavioural changes have been a major target of domestication

65   effects.

66   In natural environments, birds must be vigilant of unpredictable risks such as predation

67   when foraging or eating; this is particularly important while approaching novel conditions such as

68   novel places, objects, and food items. Neophobia is the aversion that an animal displays while

69   approaching novel conditions (Greenberg, 2003). In birds, the most well-established behavioural

70   responses to novel conditions (neophobic responses) are the reactions to potential predators

71   (Greenberg, 1990). In the wild, the presence of unpredictable risks is considered to enhance induced

72   neophobia (Brown et al., 2020). Domesticated BFs are likely able to allocate more resources to

73   reproduction (e.g., song development) as a trade-off for a reduction in the behaviours and strategies

74   aimed at coping with predation (e.g., neophobic responses). In the present study, to examine the

75 effects of changes in selection pressure due to domestication on the behaviour of BFs, and to

76 contemplate the evolutionary mechanisms underlying these effects, we compared neophobic

77 responses to novel conditions in BFs and their wild ancestors, WRMs. To the best of our knowledge,

78 this is the first study that evaluates neophobia in Bengalese finches and white-rumped munias, which

79 also explores the evolutionary mechanisms of behavioural changes in domesticated animals,

80 including the evolution of complex songs through human self-domestication.

81

82 **2. Materials and methods**

83 *2.1 Birds used in this study*

84 Seventy BFs and 60 WRMs were used in the experiment. All the birds were sexually

85 mature. BFs (n = 10) were bought from local suppliers and others (n = 60) were bred in our

86 laboratory. WRMs were captured in the wild in Taiwan. These captured birds were reared for

87 more than a year in our laboratory before being used in this study. Birds were housed in a

88 group of 8 (one group of WRM) or 10 per cage resulting in us using seven groups of BFs and six

89 groups of WRMs for our experiments. Sex ratios of the birds that made up the groups were different

90 for each cage (BF-1 and WRM-1: all females, BF-2, 3 and WRM-2: all males, BF-4, 5 and WRM-3,

91 4: 50% of each sex, BF-6, 7 and WRM-5, 6: 6 males and 4 females). Birds were housed in stainless

92 steel cages (370 × 415 × 440 mm) within an animal-rearing room at RIKEN Brain Science Institute

93   (BSI) and given finch seed mixture, shell grit, and vitamin-enhanced water *ad libitum*. The light

94   cycle was kept constant at 14 h light and 10 h dark. The room was maintained at an ambient

95   temperature of approximately 25 °C, with a humidity of 50 %. The birds were acclimatized for at

96   least four months to the animal-rearing room before performing the experiments. All experimental

97   procedures and the housing conditions of the birds were approved by the Animal Experiments

98   Committee at RIKEN (#H20-2-231, #H22-2-217), and all the birds were cared for in accordance

99   with the Institutional Guidelines of RIKEN for experiments using animals.

100

101   *2.2 Novel-object experiment*

102   Before the experiment, each group of birds was transferred from their home cage in the

103   animal-rearing room to a testing cage (with the same dimensions and characteristics as the home

104   cage) in a sound-proof box. To avoid the effects of isolation stress, experiments were conducted

105   using the same groups of birds that were housed together during keeping. The test cage was

106   equipped with two wooden perches and with food and water. Experiments were conducted under two

107   conditions: the control (non-object) condition and the novel-object condition. Experiments were

108   carried out under the different conditions on different days. New food and water were provided at the

109   beginning of the experiments. Under the novel-object condition, a novel object (a small toy dog,

110   approximately 15 mm × 15 mm × 20 mm) was placed on the food. The behaviour of birds was

111 recorded for 60 min using a video camera. We quantified the number of groups of birds that ate food

112 during the observation period (at least one bird in the group) and the latency time (the time that

113 passed before first birds started eating the food) under both conditions. If the birds did not approach

114 or eat the food during the test period, the latency time of the birds was set at the maximum time

115 (3600 s). All groups were used only once under both conditions. All tests were conducted between

116 13:00 and 15:00 to avoid other factors that could affect our results, such as diurnal changes in

117 activity, hunger, and hormone levels.

118

119 *2.3 Statistical analyses*

120   The numbers of groups of BFs and WRMs that ate the food during the observation period

121 were compared with Fisher's extract test. The latency times to start eating the food are represented as

122 the mean ± standard error of the mean (SEM). The latency times in BFs and WRMs were analysed

123 using a repeated measures two-way analysis of variance (ANOVA) (birds × conditions) with

124 post-hoc Bonferroni/Dunn tests. The difference in the effect of the novel object on the latency times

125 (calculated as the latency times under the novel-object conditions - the latency times under control

126 conditions) between BFs and WRMs were analysed by an unpaired *t*-test with a Welch's correction.

127 Values of $p < 0.05$ were considered significant. We used three different statistical software packages

128 for analyses. Statistical analyses were performed using R statistical software (version 4.03, R

129   Foundation for Statistical Computing, Vienna, Austria) for Fisher's extract test, Stat View software

130   (version 5, SAS Institute Inc., Cary, NC, USA) for repeated measures 2-way ANOVA with post-hoc

131   Bonferroni/Dunn tests, and GraphPad Prism software (version 4, GraphPad Software Inc., San

132   Diego, CA, USA) for *t*-tests with a Welch's correction.

133

134   **3. Results**

135   *3.1 Responses of BFs and WRMs to novel objects*

136   We examined the effects of domestication on behavioural responses to novel objects in

137   BFs and WRMs. Under control conditions, all groups of birds (both BFs and WRMs) ate the food at

138   some point during the observation time (BFs: 7/7, 100 %; WRMs: 6/6, 100 %). Thus, there were no

139   differences between BFs and WRMs ($p = 1.00$). However, under the novel-object conditions, six of

140   the seven groups of BFs (6/7, 85.7 %) ate the food at some point during the observation time,

141   whereas none of the WRMs groups ate the food (0/6, 0 %). Therefore, there were significant

142   differences between BFs and WRMs ($p < 0.01$). In BFs, there were no significant differences in the

143   number of groups that ate the food between the control and novel-object conditions ($p > 0.90$).

144   However, WRMs were significantly less likely to eat the food under the novel-object conditions ($p <$

145   0.01).

146

147   *3.2 Latency times in BFs and WRMs to start eating food under control and novel-object conditions*

148   The latency times to start eating the food were compared between BFs and WRMs (Fig.

149   1). Under the control conditions, the latency times were 25.6 ± 9.0 s for BFs and 159.0 ± 46.7 s for

150   WRMs. Under the novel-object conditions, the latency times were 1204.9 ± 524.9 s for BFs, but

151   none of the WRMs approached the food within the observation time (3600.0 ± 0.0 s). The latency

152   times was significantly affected by the birds (BF or WRM, $F(1, 11) = 16.9$, $p < 0.01$), conditions

153   (novel object or control, $F(1, 11) = 69.4$, $p < 0.0001$), and interactions (birds $\times$ conditions, $F(1, 11)$

154     $= 12.7$, $p < 0.01$). Latency times to eat were significantly shorter in BFs than in WRMs regardless of

155   the presence or absence of novel objects (control conditions: $p < 0.05$, novel-object conditions: $p <$

156   0.01). In both species, the latency times were extended by the presence of the novel object (BFs: $p <$

157   0.05, WRMs: $p < 0.0001$), and the extended times were significantly longer in WRMs than those in

158   BFs (BFs: 1379.3 ± 530.7 s, WRM: 3441.0 ± 46.7 s; $t = 3.87$, $p < 0.01$).

159

160   **4. Discussion**

161   We compared responses to novel conditions in BFs and their wild ancestor, WRMs, and

162   examined the effects of changes in selection pressure due to domestication. Our findings indicate

163   that these two birds responded differently to novel-object conditions. BFs had significantly lower

164   neophobia levels (more groups of BFs ate the food, and BFs had shorter latency times to eat under

165   novel-object conditions) than those of their wild ancestors, the WRMs. The novel objects extended

166   the latency times to eat in both birds, but latency times were significantly longer in WRMs than

167   those in BFs. Therefore, it is likely that BFs have reduced neophobic responses due to domestication.

168   Under control conditions, the proportion of the groups that ate the food was the same in BFs and

169   WRMs. However, BFs ate the food sooner than WRMs. It is probable that the WRMs were more

170   cautious about the experimental situation (the soundproof box is different from the WRMs' normal

171   environment). In a previous report, male WRMs had higher faecal corticosterone concentrations than

172   those in male BFs (Suzuki et al., 2012; 2014a, b). Therefore, WRMs are considered to have a higher

173   level of baseline vigilance than that in BFs. This strain difference with respect to vigilance seems to

174   have been further increased under novel-object conditions.

175   Our results are similar to those of a previous study that found that domesticated ducks

176   (*Anas platyrhynchos* var. *domesticus*) had lower levels of neophobia than those of wild mallards

177   (*Anas platyrhynchos*) (Desforges and Wood-Gush, 1975). In addition, laboratory mice (*Mus*

178   *musculus*) had lower levels of neophobia than those in wild mice (Meddock and Osborn, 1968). In a

179   comparison of dogs (*Canis lupus familiaris*) and wolves (*Canis lupus*), dogs had lower levels of

180   neophobia than those in wolves, but dogs had an overall lower interest in novel objects (Moretti et

181   al., 2015). In the present study, BFs showed a fear response to novel objects and were considered to

182   have no diminished interest in novel objects.

183   This study was conducted using groups of birds, and, as such, there may have been a social

184   effect. Because it was difficult to identify individuals in this study, the analysis was conducted as a

185   group-by-group comparison, and it was therefore not possible to take into account the differences in

186   the responses of each individual. Wolves and dogs spend more time approaching a novel object in

187   groups than as individuals, so risk sharing may increase vigilance (Moretti et al., 2015). Since this

188   experiment was conducted using groups, there may have been an effect of risk sharing. Responses to

189   novel objects may also be influenced by the identities of the group members. In the Gouldian finches

190   (*Chloebia gouldiae*), shy birds took more risks when they were paired with bold partners, and bold

191   birds took fewer risks when they were paired with shy partners (King et al., 2015). Therefore, in

192   experiments conducted using groups, individual differences may be reduced, and more of the

193   characteristics of the groups may be expressed. Conversely, in experiments conducted using

194   individuals, there is a high possibility that individual differences will be noticeable. Future studies on

195   BFs and WRMs should take into consideration the reactions of each individual bird to novel-object

196   conditions. In a previous study, we compared the tonic immobility response, which can be used as a

197   measure of fear responses, between individual BFs and WRMs and found that BFs had lower fear

198   responses (Suzuki et al., 2013). Therefore, we believe that the fear response is lower in BFs than that

199   in WRMs, even on an individual basis.

200   In this study, the effects of sex ratio and environmental or rearing conditions (bought from

201   local supplier, wild-caught, or bred in the laboratory) were ruled out because the groups in the cages

202   were neither separated by sex nor by environmental or rearing conditions. Moreover, previous

203   studies have shown that sex and environmental or rearing conditions of the white-rumped munia

204   (captive-born, bought from a supplier, or captured) did not affect the fear response (tonic immobility

205   reactions) (Suzuki et al., 2013). Additionally, the conditions under which Bengalese finches and

206   white-rumped munias were bred or reared did not affect the corticosterone levels, which is known to

207   affect fear responses (Suzuki et al., 2012). All the munias used in this experiment were wild-caught;

208   however, differences in rearing conditions have not been an issue in previous experiments.

209   Therefore, the magnitude of the impact of these factors is small, but it must be considered in future

210   research.

211   In summary, this is the first study that evaluates neophobia in Bengalese finches and

212   white-rumped munias. Our results suggest that the domestication process led to differences in the

213   responses of BFs and WRMs to novel conditions. The behavioural strategies of WRMs seem to be

214   suitable for the natural environment, which includes unpredictable risks, whereas BFs might have

215   adapted their behaviours to the conditions of captivity and artificial selection. BFs are likely able to

216   allocate the resources that would be needed in the wild to cope with predators to song development,

217   due to domestication. Our results can provide insights into the evolutionary mechanisms of

218   domestication phenotypes (domestication syndrome), including human self-domestication processes

219   of other domesticated species.

220

Acknowledgements

225

Declarations of interest

227   None

228

Author contributions

230   **Kenta Suzuki:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology,

231   Visualization, Writing - original draft; **Maki Ikebuchi:** Conceptualization; **Hiroko Kagawa:**

232   Conceptualization, Writing - review & editing; **Taku Koike:** Data curation, Investigation; **Kazuo**

233   **Okanoya:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

234

Funding

238

239   **References**

240

241   Boice, R., 1973. Domestication. Psychol. Bull. 80, 215–230. https://doi.org/10.1037/h00348.

242

243   Brown, G.E., Demers, E.E., Goldman, J.A., Singh, A., Chivers, D.P., Ferrari, M.C., 2020.

244   Unpredictable risk enhances induced neophobia in northern red-bellied dace. Anim. Behav. 168,

245   121–127. https://doi.org/10.1016/j.anbehav.2020.08.012.

246

247   Campler, M., Jöngren, M., Jensen, P., 2009. Fearfulness in red junglefowl and domesticated White

248   Leghorn chickens. Behav. Processes 81, 39–43. https://doi.org/10.1016/j.beproc.2008.12.018.

249

250   Desforges, M.F., Wood-Gush, D.G.M., 1975. A behavioural comparison of domestic and mallard

251   ducks. Habituation and flight reactions. Anim. Behav. 23, 692–697.

252   https://doi.org/10.1016/0003-3472(75)90145-1.

253

254   Fox, M.W., 1968. The influence of domestication upon behavior of animals. in: Fox, M.W. (Ed),

255   Abnormal behaviour in animals. Saunders, Philadelphia, London, pp. 64–76.

256

257   Greenberg, R.S., 1990. Ecological plasticity, neophobia, and resource use in birds. Stud. Avian Biol.

258   13, 431–437.

259

260   Greenberg, R.S., 2003. The role of neophobia and neophilia in the development of innovative

261   behaviour of birds. in: Reader, S.N. and Laland, K.N. (Eds), Animal innovation. Oxford university

262   press, NY, pp. 175–196.

263

264   Hale, E.B., 1969. Domestication and the evolution of behaviour. in: Hafez, E.S.E. (Ed) The

265   Behaviour of Domestic Animals, 2nd Edition. Baillière, Tindall and Cassell, London, pp. 22–42.

266

267   Honda, E., Okanoya, K., 1999. Acoustical and syntactical comparisons between songs of the

268   white-backed munia (*Lonchura striata*) and its domesticated strain, the Bengalese finch (*Lonchura*

269   *striata* var. *domestica*). Zool. Sci. 16, 319–326. https://doi.org/10.2108/zsj.16.319.

270

271   King, A.J., Williams, L.J., Mettke-Hofmann, C., 2015. The effects of social conformity on Gouldian

272   finch personality. Anim. Behav. *99*, 25–31. https://doi.org/10.1016/j.anbehav.2014.10.016.

273

274   Künzl, C., Sachser, N., 1999. The behavioral endocrinology of domestication: A comparison

275   between the domestic guinea pig (*Cavia aperea*f.*porcellus*) and its wild ancestor, the cavy (*Cavia*

276   *aperea*). Horm. Behav. 35, 28–37. https://doi.org/10.1006/hbeh.1998.1493.

277

278   Lepage, O., Øverli, Ø., Petersson, E., Järvi, T., Winberg, S., 2000. Differential stress coping in wild

279   and domesticated sea trout. Brain Behav. Evol. 56, 259–268. https://doi.org/10.1159/000047209.

280

281   Meddock, T.D., Osborn, D.R., 1968. Neophobia in wild and laboratory mice. Psychonom. Sci. 12(5),

282   223–223. https://doi.org/10.3758/BF03331280.

283

284   Moretti, L., Hentrup, M., Kotrschal, K., Range, F., 2015. The influence of relationships on

285   neophobia   and   exploration   in   wolves   and   dogs.   Anim.   Behav.   107,   159–173.

286   https://doi.org/10.1016/j.anbehav.2015.06.008.

287

288   Okanoya, K., 2004a. Song syntax in Bengalese finches: proximate and ultimate analyses. Adv. Stud.

289   Behav. 34, 297–346. https://doi.org/10.1016/S0065-3454(04)34008-8.

290

291   Okanoya, K., 2004b. The Bengalese finch: a window on the behavioral neurobiology of birdsong

292   syntax. Ann. N. Y. Acad. Sci. 1016, 724–735. https://doi.org/10.1196/annals.1298.026.

293

294   Price, E.O., 1984. Behavioral aspects of animal domestication. Q. Rev. Biol. 59, 1–32.

295   https://doi.org/10.1086/413673.

296

297   Ratner, S.C., Boice, R., 1975. Effects of domestication on behaviour. in: Hafez, E.S.E. (ed), The

298   behaviour of domestic animals, third ed., Williams & Wilkins, Baltimore, pp. 3–19.

299

300   Schütz, K.E., Forkman, B., Jensen, P., 2001. Domestication effects on foraging strategy, social

301   behaviour and different fear responses: a comparison between the red junglefowl (Gallus gallus) and

302   a   modern   layer   strain.   Appl.   Anim.   Behav.   Sci.   74(1),   1–14.

303   https://doi.org/10.1016/S0168-1591(01)00156-3.

304

305   Suzuki, K., Ikebuchi, M., Bischof, H. J., Okanoya, K., 2014a. Behavioral and neural trade-offs

306   between song vcomplexity and stress reaction in a wild and a domesticated finch strain. Neurosci.

307   Biobehav. Rev. 46, 547–556. https://doi.org/10.1016/j.neubiorev.2014.07.011.

308

309   Suzuki, K., Ikebuchi, M., Okanoya, K., 2013. The impact of domestication on fearfulness: a

310   comparison of tonic immobility reactions in wild and domesticated finches. Behav. Processes. 100,

311   58–63. https://doi.org/10.1016/j.beproc.2013.08.004.

312

313   Suzuki, K., Matsunaga, E., Yamada, H., Kobayashi, T., Okanoya, K., 2014b. Complex song

314   development and stress hormone levels in the Bengalese finch. Avian Biol. Res. 7(1), 10–17.

315   https://doi.org/10.3184/175815514X13903270812502.

316

317   Suzuki, K., Yamada, H., Kobayashi, T., Okanoya, K., 2012. Decreased fecal corticosterone levels

318   due to domestication: a comparison between the white-backed munia (*Lonchura striata*) and its

319   domesticated strain, the Bengalese finch (*Lonchura striata* var. *domestica*) with a suggestion for

320   complex song evolution. J. Exp. Zool. A 317, 561–570. https://doi.org/10.1002/jez.1748.

321

322   Svanberg, I., 2008. Towards a cultural history of the Bengalese Finch (*Lonchura domestica*). Der

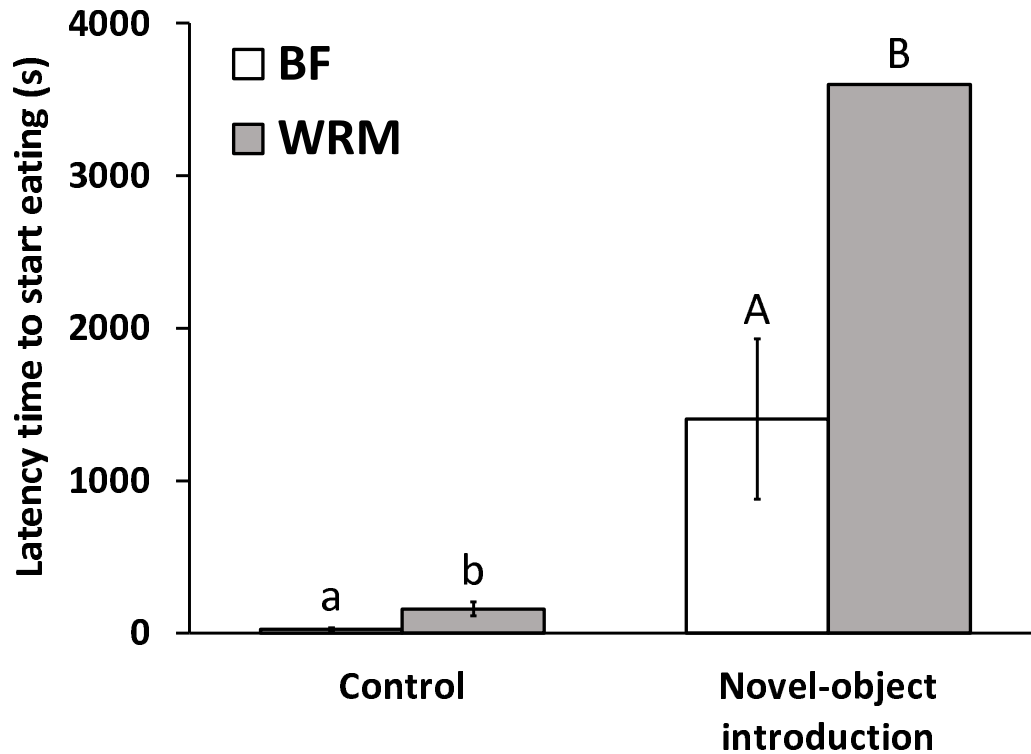323   Zoologische Garten 77(5–6), 334–344. https://doi.org/10.1016/j.zoolgart.2008.05.003.

324

325   Washio, K., 1996. Chasing the mystery of the Bengalese finch. Modern Literature Inc., Tokyo. (in

326   Japanese)

327

328

Fig. 1. Latency times (in seconds) to start eating food in Bengalese finches (BFs, *Lonchura striata*

var. *domestica*) and white-rumped munias (WRMs, *Lonchura. striata*). Bars indicate means, and

vertical lines indicate standard errors of mean (SEM). BFs had significantly shorter latency times to

start eating the provided food than those WRMs under both conditions (control and exposure to a

novel object, i.e., a small dog toy). Latency times were extended under novel-object conditions in

both BFs and WRMs. Different letters indicate significant differences between groups (a vs b: $p <$

0.05, A vs B: $p < 0.01$, a vs A: $p < 0.05$, b vs B: $p < 0.0001$).