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Social experience during adolescence influences how male zebra finches (*Taeniopygia guttata*) group with conspecifics

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1 **Adolescent Social Environment Shapes Sexual and**
2 **Aggressive Behaviour of Adult Male Zebra Finches**
3 **(*Taeniopygia guttata*)**

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13 **ABSTRACT**

14 Adolescence is the pivotal transitional phase during which animals become sexually and
15 socially mature and acquire the skills to cope with a variety of environmental challenges on
16 their own. We investigated in a bird species, the zebra finch, how the social environment
17 experienced during this period influences their behaviour in a sexual context. Zebra finches
18 were kept in pairs (male-female or male-male) or larger mixed-sex groups (3 males and 3
19 females) during adolescence and the long-term consequences were studied on courtship
20 behaviour, aggressiveness and attractiveness in 42 males. To investigate the stability of the
21 observed effects over time, all behavioural tests were repeated approximately four months
22 after the initial recordings. Males that grew up with a single female showed the most intense
23 courtship and highest aggressiveness and were most attractive to females, while group-reared
24 males had lowest courtship and aggressiveness and were the least attractive. The observed
25 differences in courtship and aggressiveness were stable, while the differences in attractiveness
26 disappeared over time. These findings are very similar to earlier studies on guinea pigs,
27 indicating that the observed effects represent a general phenomenon, not restricted to
28 mammals with a similar function and presumably also similar underlying mechanisms.

KEYWORDS

adolescence, social experience, phenotypic plasticity, aggressiveness, courtship, attractiveness, zebra finch

INTRODUCTION

The social environment experienced early in life can strongly affect how an individual deals with social challenges in adulthood, such as aggression towards conspecifics (Benus and Henkelmann 1998; Delville et al. 2003; Arnold and Taborsky 2010), courtship behaviour (Siegeler et al. 2011) mate-choice behaviour (Adkins-Regan and Krakauer 2000; Field and Waite 2004), territoriality (Sundström et al. 2003) and grouping behaviour (Chapman et al. 2008). Most research about social influences on behavioural development has concentrated on the early postnatal phase when the first social experiences have important long-term effects on social and sexual behaviour (Lorenz 1935; Immelmann and Suomi 1981). Only very recently research has started to emphasize and investigate the importance of the social conditions during adolescence (Sachser and Lick 1991; Ferris et al. 2005; Kaiser et al. 2007; Sachser et al. 2011). During this transitional period, when juveniles mature to independent adults, they typically leave the familiar environment of their early development and encounter a range of novel environments, situations and conspecifics. Maturation of morphology and behaviour during adolescence is accompanied and regulated by changes in endocrinological and neuronal systems (Bischof and Rollenhagen 1999; Bischof et al. 2002; Bischof 2003; Sisk and Zehr 2005; Blakemore 2008; Blakemore 2010; Lürzel et al. 2010; Buwalda et al. 2011; Lürzel et al. 2011). Yet, we know very little about the consequences of the social environment during this phase for adult behaviour.

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53 Evidence of phenotypic modifications caused by social influences during adolescence
54 originates mainly from research on mammals (Sachser and Kaiser 2010), including humans
55 (Dishion et al. 1997; Carlo et al. 2007), although social experience during the transition to
56 adulthood can have long-lasting influences on adult behavioural traits in birds as well
57 (Bischof and Clayton 1991, Groothuis and Mulekom 1991; Bischof 1994). Given that such
58 phenotypic changes are potentially adaptive processes enhancing survival and reproductive
59 success, the consequences of the social environment experienced during the transition to
60 adulthood merit study on a broader scale than the previous narrow focus in birds which has
61 mainly concentrated on the influence of the social environment on imprinting or song learning
62 (Eales 1985; Bischof 1994; Bischof 1997). Hence, the aim of this study is to investigate the
63 potential importance of variation in social experience during adolescence on behavioural
64 interactions with conspecifics during adulthood in an avian species, the zebra finch
65 (*Taeniopygia guttata*).

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66 Zebra finches are highly social animals that roost and breed together in colonies. During
67 adolescence (day 40 to 100), individuals experience fundamental changes in the social
68 environment since they are no longer primarily surrounded by the familiar parents and
69 siblings, but start to interact with a range of unfamiliar conspecifics in their colony that may
70 become partners or competitors (Zann 1996). As in other species, sexual dimorphism in
71 plumage and behaviour appears (Sossinka 1980) during this phase, and sexual preferences and
72 adult song performance stabilizes (Bischof 1997). These morphological and behavioural
73 changes are accompanied by hormonal and neuronal reorganisation (Pröve 1983; Adkins-
74 Regan et al. 1990) which can be influenced by the social context experienced during this
75 phase (Pröve 1981; Rollenhagen and Bischof 1994; Bischof et al. 2002; Bischof 2003).

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76 We studied how the number and sex of conspecifics encountered during adolescence
77 influences the behaviour of adult male zebra finches (*Taeniopygia guttata*) in a sexual
78 context, by keeping them in pairs (male-male or male-female) or in groups (3 males and 3

79 females) during this period. When they were adult, we examined courtship and aggressive
80 behaviour in the males. Since we found behavioural differences between males from different
81 social backgrounds, we also tested the males' relative attractiveness to females. To investigate
82 the stability of the observed effects over time, all behavioural tests were repeated about 4
83 months later.

86 METHODS

87 *Subjects*

88 The 42 male zebra finches (*Taeniopygia guttata*) used for this study were bred in five aviaries
89 (6m*2m*3m), each containing 10 to 15 domesticated adult breeding pairs at the University of
90 Bielefeld (for further housing details see below). We tried to avoid the use of siblings
91 throughout the study. The actual subjects used in the study were sired by 24 different breeding
92 pairs in 26 broods and in no case siblings were assigned to the same treatment group. During
93 their first 40 ± 2 days of life, they were reared in the breeding aviaries with their parents,
94 siblings and other zebra finches of different ages and both sexes. Throughout the whole
95 experiment, a commercial zebra finch diet (Elles, Mischfutter für Exoten, L. Stroetmann Saat,
96 48163 Münster, Germany) and water were available ad libitum. Twice a week this diet was
97 supplemented with egg food (Cédé N.V., 9940 Evergem, Belgium) and germinated seeds.

99 *Treatment*

100 At day 40 ± 2 after hatching, birds (males and females) were assigned to one of the three
101 treatment groups: one male and one female (1m/1f), 2 males without a female (2m/0f), or 3
102 males and 3 females (3m/3f). These birds were reared together until adulthood (day 110 ± 2).

103 Siblings were never assigned to the same group, and birds within one group did not differ in
104 age by more than 4 days. During the treatment phase, the different treatment groups had
105 auditory but no visual contact. All groups had access to at least 2 big feeding stations
106 (automatic circular feeders with a central food depot (diameter: 16 cm, height: 20cm)) during
107 this phase, to ensure that the easy access to food was warranted for each bird and a potential
108 difference in food competition between the groups of different sizes was minimized.

109 After 70 ± 2 days in the treatment groups, the males were transferred into individual cages
110 (0.3m*0.4m*0.4m) located in two rooms in our main building where they experienced a
111 constant temperature of 25°C and a light-dark cycle of 14:10 h. Again, birds had auditory but
112 no visual contact with each other. Three weeks later, we began testing the birds' courtship
113 behaviour, aggression and attractiveness (Table 1). The stimulus females used in these tests
114 were colony reared and thus had experience with both male and female conspecifics of
115 different ages.

116 The study was performed in two batches. Males from batch 1 (N=8 for each treatment) were
117 hatched and kept in outdoor aviaries (6m*2m*3m) during their treatment phase (June -
118 December 2010), thus temperature and light conditions were determined by the local weather
119 and the local natural photoperiod. However, all aviaries were roofed and featured with
120 infrared heat lamps to ensure that birds were not directly exposed to rain and cold. Males
121 from batch 2 (N=6 for each treatment) were hatched and kept indoors (October 2010 -
122 February 2011) in standard double cages (0.3m*0.8m*0.4m) (1m/1f and 2m/0f) or in small
123 indoor aviaries (1m*2m*2m) (3m/3f) at a constant temperature of 25°C and a light-dark cycle
124 of 14:10h.

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128 *Male Behaviour and Attractiveness*

129 *Courtship Song*

130 Directed song, which is defined as song that a male produces facing a female in his proximity
131 (Sossinka and Böhner 1980) was quantified as an important component of male courtship
132 behaviour. Each male was individually exposed to an unfamiliar female sitting in a cage
133 attached to the front of the male's cage. Three different females were used; each served
134 equally often as a stimulus for the males of the different treatment groups. The experiment
135 took place in the room where males were individually housed between different tests. Thus,
136 background noise produced by conspecifics was always present. The latency to present the
137 first courtship song and the number of directed motifs sung within the first 10 minutes were
138 recorded. Males that did not sing at all received a latency value of 600 seconds. Recordings
139 took place between 9:00 am and 11:00 am.

141 *Aggression Towards Other Males*

142 One male from each treatment group and an unfamiliar female were housed together in a
143 standard double cage (0.3m*0.8m*0.4m; four perches: two in the upper half and two in the
144 lower half of the cage). Males were identified by using differently coloured rings (violet, light
145 blue and yellow) with care taken that each colour was assigned with equal frequency to each
146 treatment group throughout all replicates. We recorded the number of times one male chased
147 another as a measure of aggressiveness towards other males. Chasing was defined as a flight
148 at another male followed by his immediate displacement. A chase was finished when the
149 chased bird landed. Tests always lasted 3 hours and started between 8:00 and 9:00 am by
150 introducing the female into the cage. Every five minutes the behaviour of a focal male from a
151 different treatment was recorded, resulting in 12 observation periods (a total sample time of 1
152 hour) for each male.

153 To prevent physical harm to the experimental males, they were continuously observed during
154 the tests and these would have been interrupted if the males showed sustained high intensity
155 aggression or actual fighting which could result in injuries. However, this was not necessary
156 since the males showed only low-intensity aggressive behaviour such as threats and chases,
157 which is typical for zebra finches, who are highly social and generally tolerant of
158 conspecifics.

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160 *Male Attractiveness*

161 One male from each treatment group was kept individually in a cage (0.4x0.3x0.2m) with
162 metal bars allowing visual contact from all sides (Figure 1 inset). The three cages containing
163 the experimental males and a fourth, empty cage, representing a neutral location were affixed
164 in the four upper corners of a cubic aviary made of wire mesh (2x2x2m) (Figure 1). The
165 empty cage was included in the setup because a pilot experiment showed that this neutral
166 position prevents females from developing a place preference. A female, unfamiliar to all
167 males was brought into the aviary where she could perch in front of each of the four cages. A
168 light-barrier on the perch automatically recorded the time the female spent in front of each
169 cage. Recordings took place over a period of eight hours from 8:00 am to 5:00 pm. After each
170 hour, the position of males within the setup was changed by moving each cage one position
171 clockwise to ensure that the measured time reflected a true male-preference rather than a
172 place preference. For acclimatisation, the female was placed into the aviary and the males into
173 their cages 15 hours before the test started. The female and each male had mutual visual
174 contact, but the males could not see one another.

175 The same three males remained together in the aggressive behaviour test and the
176 attractiveness test and thus got to know each other as the study progressed.

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178 *Temporal Stability of male courtship rate, aggressiveness and*
179 *attractiveness*

180 Each of the behavioural tests was repeated 4 months after the first measurements. Between the
181 first recordings and the repetitions, all males were kept in visual but not acoustic isolation. In
182 the repetitions, males were tested in new combinations and thus were not familiar with each
183 other beforehand. However, as in the first experiments, the three males that built a triad in the
184 aggressiveness test were later tested together in the attractiveness test. As stimulus birds, new
185 unfamiliar females were used. One 3m/3f male died, resulting in a sample size of $N = 41$ for
186 the directed song measurement. The aggression and attractiveness tests were not repeated for
187 this male nor for the males from the 1m/1f and the 2m/0f treatment partnered with him during
188 the first testing phase, thus reducing the sample size to $N=39$ for these tests.

190 *Data Analysis*

191 *Data Transformations*

192 Some data was transformed because variances were significantly different between treatments
193 (Levene's Test) or residuals were not normally distributed (Kolmogorov-Smirnov-Test). The
194 numbers of motifs sung in the directed song test were log transformed (a value of 1 had to be
195 added to each measure because several birds did not sing at all).

196 For the analysis of aggressiveness, the number of chasings was summed up for each male
197 over all 12 observations and a rank of aggressiveness was assigned to each bird, giving the
198 highest rank to the male with the highest total number of chasings.

199 We calculated an attractiveness score as the relative duration of stay in front of each male for
200 each female tested to correct for the variation between females in overall perching time. Time
201 spent at the empty cage was not included in this calculation. Since fractions can only vary

202 between 0 and 1, we used the logit of the attractiveness score to ensure normality and variance
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2 203 homogeneity. All other measured data did not have to be transformed and were analysed
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5 204 using the raw values for the ANOVA.
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9 206 *Statistics*

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12 207 Data from both batches were analysed in a single statistical model, using an ANOVA with
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15 208 “treatment” and “batch” as fixed factors. Unless specifically mentioned in the results, the
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17 209 effect of the interaction between batch and treatment and the effect of batch on their own were
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20 210 negligible ($p > 0.2$) and were excluded from the final model. When the ANOVA showed a
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22 211 significant treatment effect, pair-wise post-hoc comparisons were done using two-sample t-
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25 212 tests with Sidak adjustment to correct for multiple testing.

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27 213 To test for a relationship between the males’ relative attractiveness and all other behavioural
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30 214 measures, we performed correlation analyses. In case the respective data sets did not differ
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32 215 from a normal distribution (Kolmogorov-Smirnov-Test) we calculated a Pearson-Correlation;
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34 216 otherwise we used a non-parametric Spearman-Rank-Correlation.

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37 217 The change in male attractiveness over time was calculated by subtracting the value from the
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39 218 first recording from his value from the second recording (four months later) for each male.
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42 219 These values were tested against a test-value of zero using a one-sample t-test.

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44 220 Results are always presented as means \pm standard errors. All analyses were performed in
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46 221 SPSSv19 and the significance level α was set at $p < 0.05$.

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RESULTS

Male Behaviour and Attractiveness

Courtship Song

Male zebra finches that grew up in different social environments during adolescence differed significantly in their latency to court an unfamiliar female (ANOVA: $F_{2,39} = 9.27$, $P = 0.001$, Figure 2A) and in their motif rate (ANOVA: $F_{2,39} = 9.98$, $P < 0.001$, Figure 3A). Post-hoc comparisons revealed that males reared in a mixed-sex group (3m/3f) started to court significantly later than males raised with a single female (1m/1f) or a single male (2m/0f) (two-sample t-test with Sidak adjustment: 3m/3f vs. 1m/1f: $t_{26} = 3.92$, $P = 0.001$, 3m/3f vs. 2m/0f: $t_{26} = 3.51$, $P = 0.003$) The latter two groups did not differ in their latency to court an unfamiliar female (two sample t-test with Sidak adjustment: $t_{26} = 0.41$, $P = 0.97$). A similar pattern was observed for the motif rate: 3m/3f males had a significantly lower rate of singing than 1m/1f and 2m/0f males (two-sample t-test with Sidak adjustment: 3m/3f vs. 1m/1f: $t_{26} = 4.72$, $P < 0.001$, 3m/3f vs. 2m/0f: $t_{26} = 3.12$, $P = 0.008$), while the latter ones did not differ in their motif rate (two-sample t-test with Sidak adjustment: $t_{26} = 1.60$, $P = 0.62$).

Aggression Towards Other Males

The social environment experienced during adolescence significantly influenced male zebra finches' aggressiveness towards other males when competing for a female (ANOVA: $F_{2,39} = 3.82$, $P = 0.03$, Figure 4A). Post-hoc comparisons showed that 3m/3f males were significantly less aggressive compared to 1m/1f males (two-sample t-test with Sidak adjustment: $t_{26} = 2.69$, $P = 0.03$). Compared to 2m/0f males, both 3m/3f males (two-sample t-test with Sidak adjustment: $t_{26} = 0.82$, $P = 0.80$) and 1m/1f males (two-sample t-test with Sidak adjustment: $t_{26} = 1.88$, $P = 0.19$) did not differ in aggressiveness. Batch was excluded from the final model

250 since the effect of the interaction between treatment and batch (ANOVA: $F_{2, 36} = 2.45$, $P =$
251 0.10) and of batch (ANOVA: $F_{2, 38} = 3.47$, $P = 0.07$) were not statistically significant and their
252 inclusion did not change the significance of the treatment effect.

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254 *Male Attractiveness*

255 The time female zebra finches spent with a male depended significantly upon the male's
256 social rearing conditions (ANOVA: $F_{2,39} = 7.08$, $P = 0.002$, Figure 5A). Females spent
257 significantly more time near 1m/1f males compared to 3m/3f males (two-sample t-test with
258 Sidak adjustment: $t_{26} = 3.71$, $P = 0.002$). Furthermore, there was a strong tendency for the
259 females to sit longer near 1m/1f males compared to 2m/0f males (two-sample t-test with
260 Sidak adjustment: $t_{26} = 2.41$, $P = 0.06$). The time females spent in front of 2m/0f and 3m/3f
261 males did not differ significantly (two-sample t-test with Sidak adjustment: $t_{26} = 1.29$, $P =$
262 0.50). The males' relative attractiveness was positively correlated with male courtship motif
263 rate (Pearson: $N = 42$, $r_p = 0.354$, $P = 0.02$) and the absolute number of chasings (Spearman:
264 $N = 42$, $r_s = 0.466$, $P = 0.002$). Furthermore, a negative relationship between the males'
265 relative attractiveness and their latency to court a female was observed by trend (Spearman: N
266 $= 42$, $r_s = -0.254$, $P = 0.11$).

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269 *Temporal Stability of male courtship rate, aggressiveness and* 270 *attractiveness*

271 Four months after the first recordings, treatment effects on latencies to court an unfamiliar
272 female (ANOVA: $F_{2,38} = 3.97$, $P = 0.03$, Figure 2B), on motif rate (ANOVA: $F_{2,38} = 4.63$, $P =$
273 0.02, Figure 3B), and on the level of aggressiveness towards other males (ANOVA: $F_{2,35} =$
274 4.8, $P = 0.01$, Figure 4B) remained stable. Group-reared males still started to court an

275 unfamiliar female significantly later than males reared with a single female (two-sample t-test
276 with Sidak adjustment: $t_{24} = 2.52$, $P = 0.048$), and they still started to court later than males
277 reared with a single male, although the significance of this difference was borderline (two-
278 sample t-test with Sidak adjustment: $t_{24} = 2.39$, $P = 0.07$). As during the initial recording,
279 2m/0f and 1m/1f males did not differ in their latency to court (two-sample t-test with Sidak
280 adjustment: $t_{24} = 0.13$, $P = 0.99$). The motif rate of 3m/3f was still significantly lower than the
281 motif rate of 2m/0f males (two-sample t-test with Sidak adjustment: $t_{24} = 2.83$, $P = 0.022$),
282 and tended to be lower than that of 1m/1f males (two-sample t-test with Sidak adjustment: t_{24}
283 $= 2.25$, $P = 0.09$). Males reared with a single male or female still did not differ in their motif
284 rate (two-sample t-test with Sidak adjustment: $t_{24} = 0.59$, $P = 0.91$). Also, as in the first
285 measurement, group-reared males were still significantly less aggressive compared with those
286 reared with a single female (two-sample t-test with Sidak adjustment: $t_{24} = 2.89$, $P = 0.02$),
287 and still did not differ in aggressiveness from those reared with a single male (two-sample t-
288 test with Sidak adjustment: $t_{24} = 0.47$, $P = 0.95$). Males reared with a single female continued
289 to show on average the highest level of aggression, which this time was almost significantly
290 higher than that of males reared with a single male (two-sample t-test with Sidak adjustment:
291 $t_{24} = 2.41$, $P = 0.06$).

292 In contrast to the stable treatment differences in courtship and aggression, females no longer
293 spent different amounts of time in front of the males when the attractiveness test was repeated
294 (ANOVA: $F_{2,36} = 0.84$, $P = 0.44$, Figure 5B). This was due to a significant increase in the
295 relative time females spent in the proximity of the 3m/3f males (one-sample t-test: $t_{12} = 2.19$,
296 $P = 0.049$), and in a significant reduction in the relative time spent in front of the 1m/1f males
297 (one-sample t-test: $t_{12} = -3.60$, $P = 0.004$). The relative time females spent in front of the
298 2m/0f males did not change (one-sample t-test: $t_{12} = 0.21$, $P = 0.84$). Males' relative
299 attractiveness was no longer correlated with courtship motif rate (Pearson, $N = 39$, $r_p = 0.016$,

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300 P = 0.92), latency to court a female (Spearman: N = 39, $r_s = -0.09$, P = 0.59) and the males'
301 absolute number of chasings (Spearman: N = 39, $r_s = -0.011$, P = 0.946).

303 **DISCUSSION**

304 Our experiments clearly showed that the behavioural responses of zebra finch males to social
305 stimuli (potential mate/competitor) strongly depended on the social environment which they
306 experienced during adolescence. Males that grew up with a single female during adolescence
307 showed the most intense courtship and highest aggressiveness towards mates or competitors
308 and were the most attractive to female conspecifics, while group-reared males had lowest
309 courtship and aggressiveness rates and were also the least attractive.

310 When we repeated the tests four months later, the significant differences in courtship (albeit
311 slightly diminished) and aggressiveness persisted. In contrast, the attractiveness of the males,
312 as rated by female zebra finches, no longer differed significantly between treatments. The
313 strength of the social environment's impact during adolescence on the development of these
314 behavioural traits is further corroborated by the fact that males were affected equally in
315 almost all cases in both batches, although these differed in temperature, photoperiod, housing
316 and background noise (see Methods). The only difference between the batches that one might
317 see by trend is that males that were reared in cages/small aviaries (batch 2) tend to be more
318 aggressive than males reared in big aviaries (batch 1) (Figure 4). It could be speculated that
319 this results from the difference in space the birds had during their treatment phases. While the
320 males in big aviaries had some space to escape from aggression, birds reared in small aviaries
321 might not have been easily able to avoid aggressive interactions and thus became more
322 aggressive on their own. However, since the differences between batches are so small, even

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2 323 the small aviaries might have been big enough to allow the avoidance of aggressive
3 324 encounters.

4 325 The outcome of our experiment strongly resembles very similar studies in guinea pigs reared
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6 326 pair-wise or in colonies during adolescence: when two pair-wise reared males were housed
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8 327 with a single unfamiliar female, they started to court earlier and showed higher rates of
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10 328 aggression than two colony-reared males in the same situation (Sachser and Lick 1993, Kaiser
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12 329 et al. 2007). The consistency of these effects across an avian and a mammalian species
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14 330 indicates that the observed influence of social experience during adolescence may represent a
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16 331 general phenomenon.
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23 24 333 *Function of the Behavioural Modifications*

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27 334 A possible function of the observed behavioural modifications may be that the social
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29 335 environment during adolescence prepares individuals for the demands of the social conditions
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31 336 during adulthood. Living in a group requires that individuals get along with other members of
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33 337 their group to minimize costs originating from competition and to benefit from the advantages
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35 338 of living in a social group. The low levels of aggression in our group-reared male zebra
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37 339 finches may directly decrease costs of physical conflicts and their more cautious courtship
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39 340 behaviour may reduce costs indirectly by decreasing the likelihood of courting a female that is
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41 341 already paired, which in zebra finches results in a physical conflict with the female's mate and
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43 342 the female itself (Silcox and Evans 1982; Zann 1996). Indeed, studies on guinea pigs indicate
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45 343 that individuals benefit from such behavioural modifications: guinea pig males reared in a
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47 344 colony during adolescence easily integrated into a new social group without showing signs of
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49 345 stress, whereas males that spent their adolescence in isolation or together with a single female,
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51 346 were involved in a high amount of socio-negative interactions, lost a substantial amount of
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53 347 weight and showed increased cortisol levels when exposed to a group of unfamiliar
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1 348 conspecifics during adulthood (Sachser 1993; Sachser and Renninger 1993). Whether social
2 349 experience in a group during adolescence is indeed beneficial for male zebra finches when
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4 350 they have to interact with other individuals later in life is under investigation at present.

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7 351 The behavioural profile shown by zebra finch males reared with a single female could be
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9 352 interpreted as pathological in terms of not being well adapted to a group-living life-style.
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11 353 Alternatively, their behaviour may be adapted to a social environment where it is not
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13 354 necessary to adjust the behaviour to the continuous presence of other individuals, as suggested
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17 355 by Sachser et al. (2011). In nature, such a situation may occur under low population densities,
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19 356 when the chance to meet a potential mate may be quite small so that the best strategy to
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21 357 maximize reproductive success may be to court each female immediately and with high
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23 358 intensity and to attack every potential competitor. In the ancestor of the wild guinea pig, the
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26 359 wild cavy, a comparable situation exists in the natural habitat at low population densities
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29 360 (Asher et al. 2004). In contrast, under high densities male cavies are less aggressive towards
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31 361 same-sex conspecifics and pursue a queuing strategy to increase reproductive success (Asher
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34 362 et al. 2008). According to the guinea pig /cavy studies the distinct behavioural modifications
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36 363 observed in male zebra finches grown up with a single female or in a group of conspecifics
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38 364 during adolescence can therefore also be interpreted as adaptations to different social
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41 365 environments, for example to local or seasonal differences in population densities.

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46 367 *Temporal Stability of male courtship rate, aggressiveness and*
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49 368 *attractiveness*

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52 369 The experimental treatment affected courtship and aggressive behaviour 3 weeks after
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54 370 animals had been removed from the different social environments and kept under identical
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56 371 conditions. Moreover, the repetitions of the directed song and the aggression measurements
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59 372 four months after the first test clearly demonstrated that the differences between males grown
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373 up under distinct social conditions during adolescence are not temporary, but persist at least
374 for several months, even though the males from all treatments made the same novel
375 experiences between the test repetitions. They were kept either isolated between the tests or
376 spent time with unfamiliar females and males from the other treatments during the tests, but
377 these experiences were not sufficient to override the influence of the social conditions during
378 adolescence, indicating a reduced behavioural plasticity for courtship and aggression in
379 adulthood.

380 The high courtship rate of pair-wise reared males may have directly increased their
381 attractiveness in the first preference tests as has been shown in many species (Gottlander
382 1987; Radesäter et al. 1987; Collins et al. 1994; but see also: Forstmeier and Birkhead 2004).

383 This assumption gets even directly supported by the positive correlation between motif rate
384 and the males' relative attractiveness. Such enhancement of courtship rate may originate
385 from a lack of experience with comparing receptivity, rejection and solicitation behaviour of
386 different females leading to an indiscriminate courting of all presented females, as has been
387 shown for females who are less choosy when they have little experience with male courtship
388 song (Riebel 2000, Lauay et al. 2004). Also, the attractiveness of group-reared males may be
389 reduced because the presence of juvenile males may have inhibited a faithful representation of
390 their father's song, (Tchernichovski and Nottebohm 1998) possibly because they copied song
391 syllables from each other (Jones et al. 1996). Furthermore, courtship activity of a male can be
392 reduced by the presence of another male (Ikebuchi et al. 2009). Since males already knew
393 each other from the aggressiveness test and were able to see each other in the attractiveness
394 tests, the most aggressive male may have suppressed courtship activity of the other males and
395 thereby influenced the choice of the female indirectly. The strong positive correlation between
396 male's relative attractiveness and their level of aggressiveness may underline this idea.
397 Although there is already some evidence that a female zebra finch's preference in a choice
398 test reflects a true sexual preference (Clayton 1990), it seems important to note that an

1 399 observed preference in a choice test like the one performed in this study (which did not allow
2 400 the different birds to interact physically with each other) does not necessarily mean that the
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4 401 females would actually pair with the male they preferred in this test when they can interact
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7 402 physically with him (Rutstein et al. 2007).

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9 403 The attractiveness of the males for females was not stable over time. Four months after the
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11 404 initial assessment, 3m/3f males had become more attractive while the attractiveness of the
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14 405 1m/1f males had decreased, so that male attractiveness no longer depended on their social
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16 406 rearing conditions. This indicates that some aspects of male behaviour or appearance that we
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19 407 did not measure must have changed from the first to the second measurement. Alternatively,
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21 408 the slight reduction in the differences in courtship rate may have been sufficient to prevent
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24 409 females from differentiating between the different phenotypes.

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26 410 In summary, further approaches are needed to shed more light on the impact of social
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29 411 experience during adolescence on the development of zebra finch males' attractiveness.

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35 413 *Adolescence - A Sensitive Period for the Modification of Socially*
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38 414 *Relevant Behaviour?*

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41 415 Our study clearly shows that the development of male zebra finches' courtship and aggressive
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43 416 behaviour is influenced by the social environment they experience during adolescence. It is
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46 417 not possible to decide from our experiments whether this long-lasting influence of the social
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48 418 environment is restricted to the time of adolescence (a sensitive period) or can be observed
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51 419 over the entire lifespan of an animal. Sensitive periods at the time of adolescence have been
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53 420 described for two aspects of social behaviour in birds: for sexual imprinting where the final
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55 421 sexual preference of a zebra finch male is established during adolescence (Bischof 1994;
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58 422 Bischof 2007), and for song learning, where the permanent song of a young male becomes
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61 423 stabilized in its adult appearance (Bischof 1997). In agreement with these studies the

424 behavioural changes (aggressiveness and courtship rate) observed in our study are stable and
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2 425 modifications occur at the same developmental period, namely adolescence. It could therefore
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4 426 be speculated that the development of social behaviour shown here may just be another aspect
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7 427 of social learning during adolescence and that the endocrinological and neural mechanisms
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9 428 regulating imprinting and song learning may also underlie the behavioural changes observed
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12 429 here.

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42 43 441 *Ethical standards*

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46 442 All experimental procedures applied in this study are not regulated by the German animal
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49 443 protection law and do not need a special approval.

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51 444 Animal facilities were approved (dated 18 April 2002) for keeping and breeding zebra finches
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54 445 for research purposes by the local government authority responsible for health, veterinary and
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56 446 food monitoring (Gesundheits-, Veterinär- und Lebensmittelüberwachungsamt). Details of
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59 447 breeding and housing conditions are described in the respective method sections.

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Figure Captions

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Figure 1 **Experimental setup of the attractiveness test.**

Males from different treatments were placed in small cages in the corners of the experimental aviary. A female was brought into the aviary and could perch in front of the male cages. Male attractiveness was assessed by recording the time females spent in front of each male using light-barrier bordered perches attached to the front of each cage (see inset). Arrows indicate positions of the light-barrier bordered perches within the setup, F/W = food & water dispensers.

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Figure 2 **Courtship song: latency.**

Latency until first directed motif sung [s]

(a) Results of the first measurement

(b) Results of the repetition around 4 month later

Data are shown separately for batch 1 (triangles, N = 8 for each group) and batch 2 (circles, N = 6 for 1m/1f and 2m/0f; N = 5 for 3m/3f). Shown are means \pm SEM; ** p < 0.01, * p < 0.05, (*) p = 0.07.

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Figure 3 **Courtship song: motif rate.**

Average number of directed motifs sung within each minute.

(a) Results of the first measurement

(b) Results of the repetition around 4 month later

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594 Data are shown separately for batch 1 (triangles, N = 8 for each group) and
595 batch 2 (circles, N = 6 for 1m/1f and 2m/0f; N = 5 for 3m/3f). Shown are
596 means \pm SEM; *** p < 0.001, ** p < 0.01, * p < 0.05, (*) p = 0.09.

597
598 **Figure 4 Aggression towards other males.**

599 Ranks of chasing, for calculation of ranks see “data transformation”.

600 (a) Results of the first measurement

601 (b) Results of the repetition around 4 month later

602 Data are shown separately for batch 1 (triangles, N = 8 for each group) and
603 batch 2 (circles, N = 5 for each group). Shown are means \pm SEM; * p < 0.05,
604 (*) p = 0.06.

605
606 **Figure 5 Male attractiveness.**

607 Females’ relative duration of stay at the different treatment birds [%]

608 (a) Results of the first measurement

609 (b) Results of the repetition around 4 month later

610 Data are shown separately for batch 1 (triangles, N = 8 for each group) and
611 batch 2 (circles, N = 5 for each group). Shown are means \pm SEM; ** p < 0.01,
612 (*) p = 0.06.

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615 **Table Captions**

616 **Table 1 Time table of experimental procedures.**

617 For more details see text.

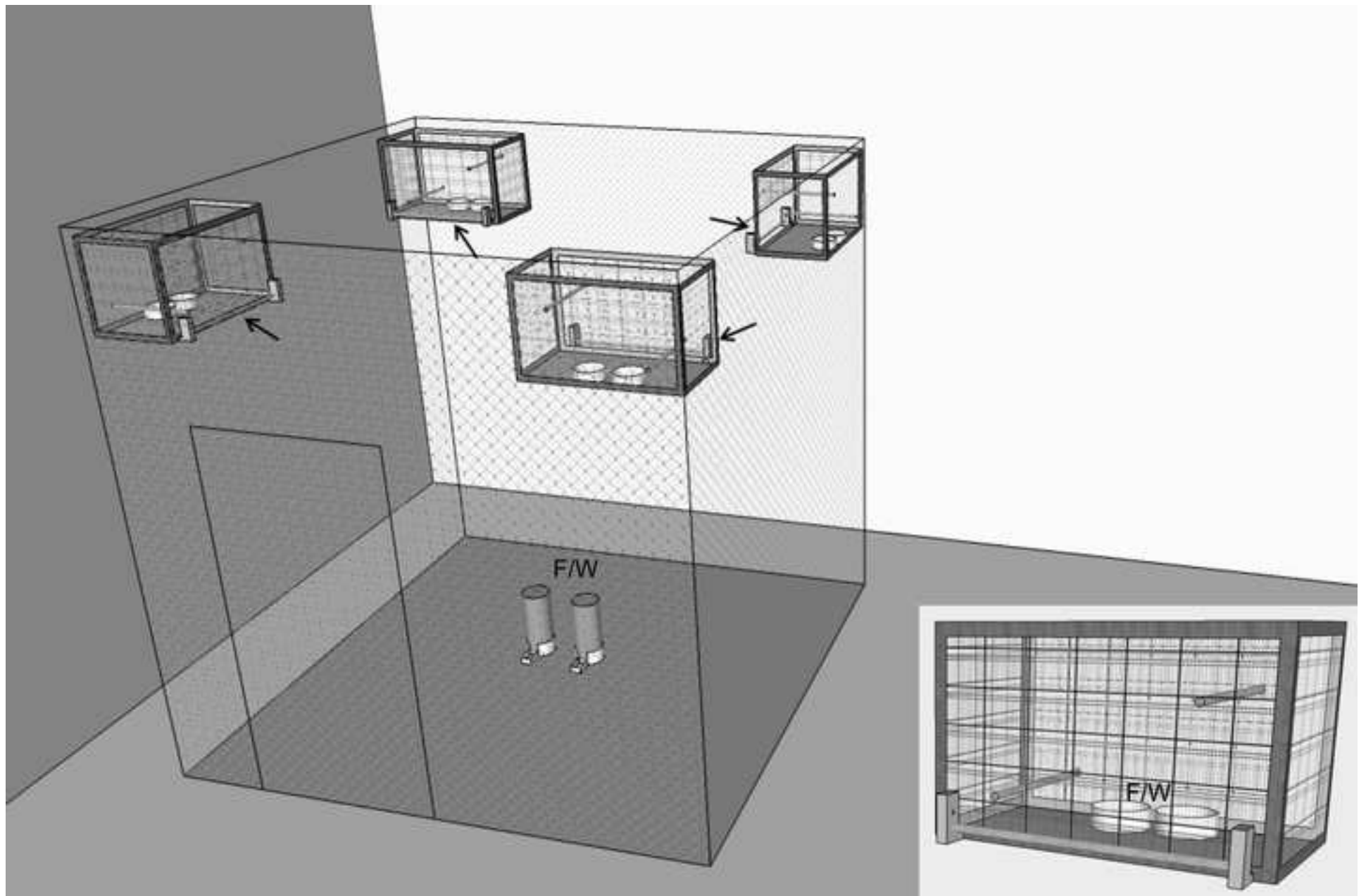


Figure 2

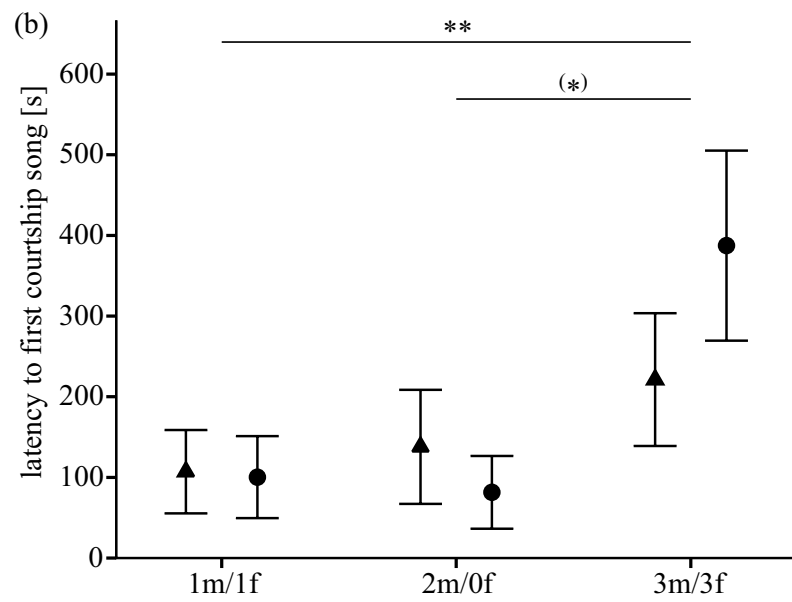
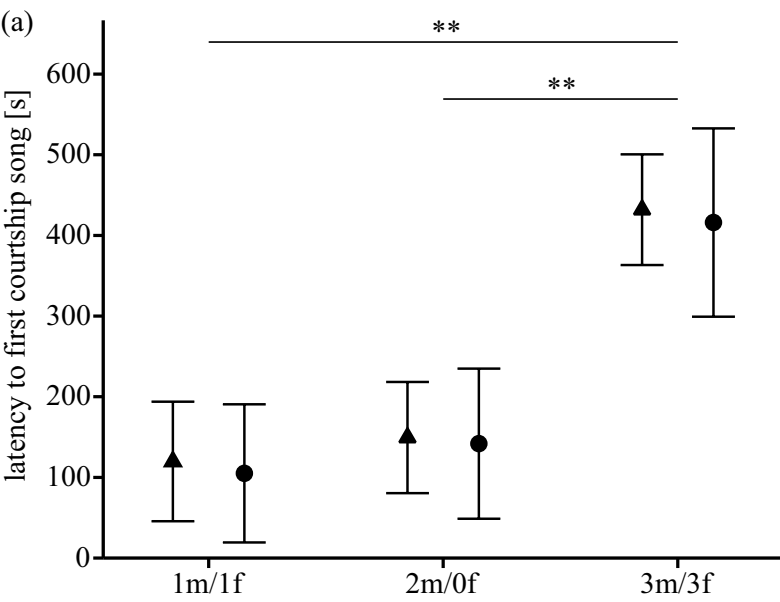


Figure 3

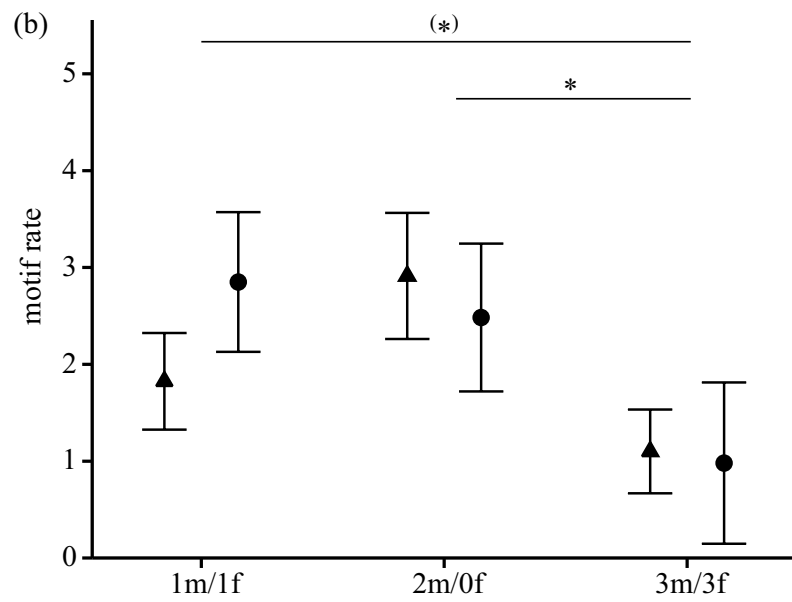
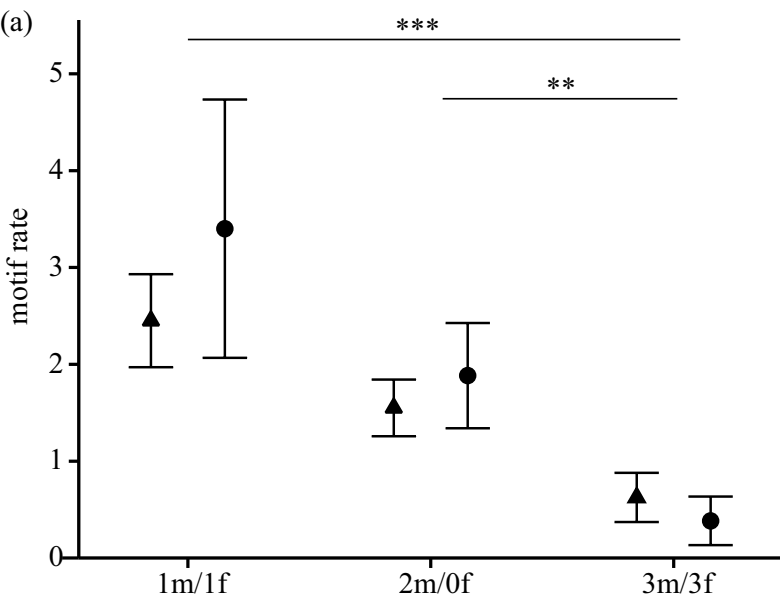


Figure 4

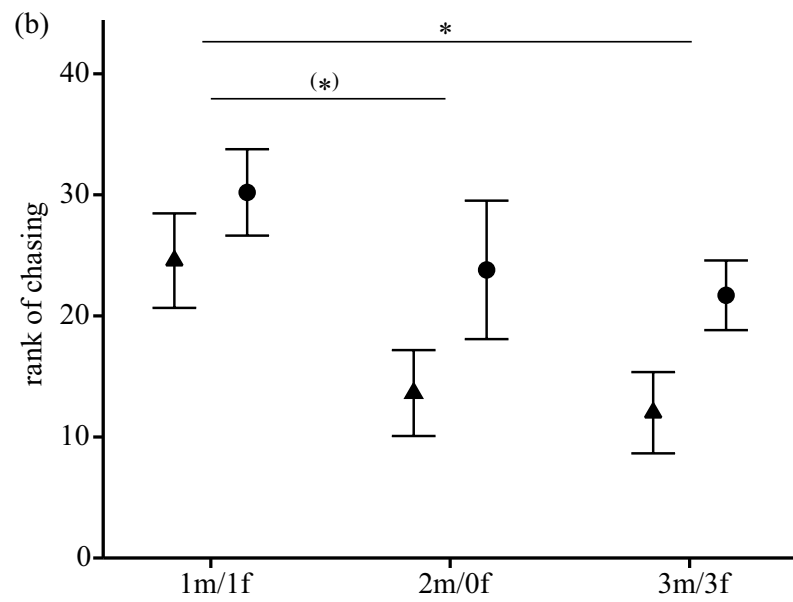
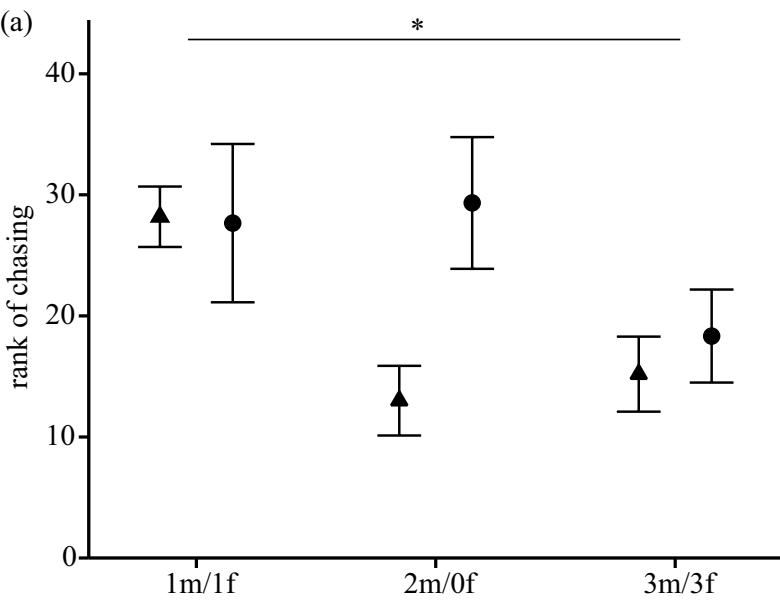
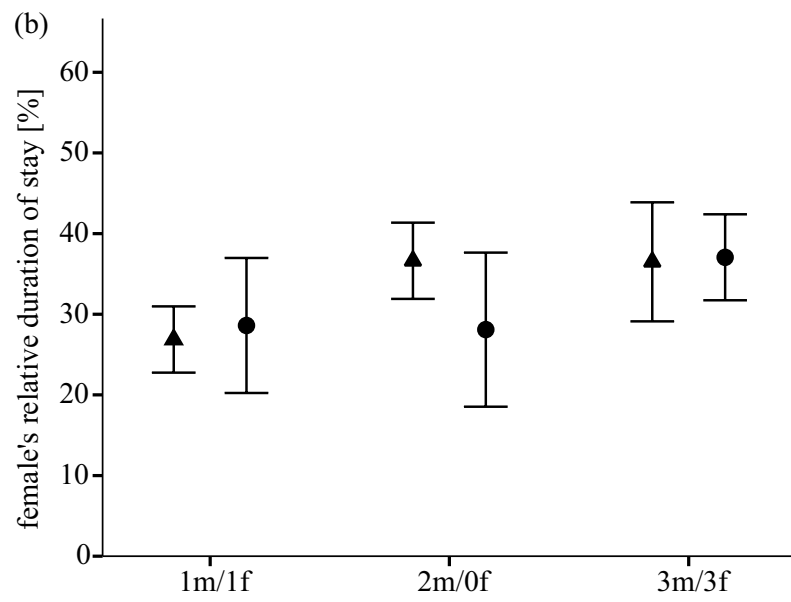
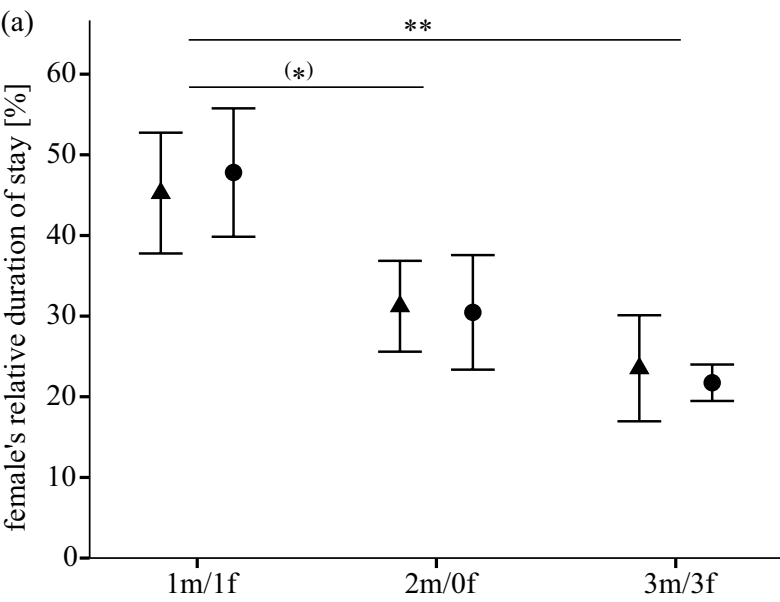


Figure 5



Subjects' Age	Action/ behavioural test type
0	hatching of subjects in breeding aviaries
40 ± 2	subjects transferred to treatment groups
110 ± 2	subjects isolated from treatment groups
131 ± 1	courtship song test
135 ± 5	aggressiveness test
145 ± 6	attractiveness test
253 ± 1	repetition courtship song test
258 ± 4	repetition aggressiveness test
270 ± 6	repetition attractiveness test
