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Agathe Gaffard, Clotilde Loiseau, Vincent Bretagnolle, Olivier Pays, Jérôme Moreau. Sublethal effects of pesticide residues differ between strains of captive Grey partridge: consequences in terms of rearing conditions.. *Applied Animal Behaviour Science*, 2022, 257, pp.105791. 10.1016/j.applanim.2022.105791 . hal-03859896

HAL Id: hal-03859896

<https://u-bourgogne.hal.science/hal-03859896v1>

Submitted on 24 Jan 2023

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1 **Sublethal effects of pesticide residues differ between strains of captive Grey partridge:**
2 **consequences in terms of rearing conditions**

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23 **ABSTRACT**

24 Over the last 50 years, farmland bird populations have declined steeply in Europe and North
25 America. Reintroduction or reinforcement for populations unable to self-maintain are popular

26 management tools to overcome extinction risk through captive rearing and release. However,
27 released birds tend to have lower survival rates than their wild conspecifics. Here, we aimed
28 to mimic the diet shift that Grey partridge (*Perdix perdix*) encounters once released (poultry
29 food to natural grain) and compared (i) differences in responses to this transition depending
30 on strain (wild or farm) and (ii) the consequences of ingesting organic or conventional grains
31 on individual body mass, haematocrit and behavioural traits (anti-predator responses, activity,
32 exploration) that are likely critical for post-release survival. Our experimental procedure
33 involved 40 farm-strain and 40 wild-strain partridges fed with commercial poultry food
34 during early life then with wheat and corn for 3 months, from either organic or conventional
35 (clopyralid residues) agriculture. The results suggest that the haematocrit and body mass
36 index of farm-strain partridges were lower at the end of the experiment, probably due to diet
37 shift. Birds fed conventional grains (with clopyralid residues) exhibited lower flight duration
38 and flight initiation distance, and were more likely to translocate by running rather than
39 flying. The findings suggest that, when rearing partridges of farm-strain origin, birds fed
40 organic grains experience better post-release survival than birds fed conventional grains
41 containing pesticide residues.

42

43 **KEYWORDS:** captivity, conservation, strain, *Perdix perdix*, pesticide residues

44

45 **1. INTRODUCTION**

46 Over the last 50 years, farmland bird populations have declined by 20% in Europe (Burns et
47 al. 2021) and 74% in North America (Stanton et al. 2018). The main causes of decline are
48 related to agricultural intensification, including habitat loss, seasonal shifts in cultivation
49 practices, and increased use of agrochemicals (Robinson and Sutherland 2002; Newton 2004).
50 Reintroductions after strong decline (e.g. the process of rearing animals in captivity and

51 releasing them into the wild) or reinforcement for populations unable to self-maintain are two
52 management tools that have become increasingly popular in the past 30 years to overcome
53 extinction risk, even on a local scale (Seddon et al. 2007). Reinforcing wild populations has
54 also been commonly used in game birds for hunting purposes, leading to game farm
55 implementation, which bred originally wild-strain birds in captivity for decades (Buner et al.
56 2011). However, released farm-strain individuals such as common pheasant (Musil and
57 Connelly 2009), chukar partridge (Yolcu et al. 2016) and grey partridge (Parish and Sotherton
58 2007) tend to have lower survival rates than their wild conspecifics (Sokos et al. 2009).
59 Understanding why farm-strain birds are less fit to persist in wild environments may help us
60 to improve reintroduction projects through changes in rearing conditions.

61 Captive environments are known to impair genetic diversity, lead to genetic drift, and
62 enhance adaptation to captivity, all of which can be detrimental when such individuals are
63 released into the wild (Frankham 2008; Keller et al. 2012). Indeed, many studies have
64 reported behavioural deficiencies in farm-strain birds that impair survival (Rymešová et al.
65 2012, 2013; Homberger et al. 2014; Yolcu et al. 2016), especially in regard to excessive
66 boldness (Madden and Whiteside 2014), inhibited escape responses (Pérez et al. 2010), lower
67 group vigilance (Rantanen et al. 2010b) and poor anti-predator defences (Håkansson and
68 Jensen 2008; Rantanen et al. 2010a). Upon release, animals have to cope with risk-taking,
69 foraging and novel environment discovery (Réale et al. 2007). Fleeing quickly in life-
70 threatening situations, and showing a high level of exploration and activity to find food,
71 sexual partners and territory, should collectively increase fitness (Sih et al. 2004; Smith and
72 Blumstein 2008; Homberger et al. 2013). Moreover, farm-strain birds display higher body
73 mass, lower glycogen reserves and lower oxidative capacity than wild ones as a result of
74 unlimited access to high-nutrient food in captivity (Putala and Hissa 1995, 1998). In addition
75 to making them less fit, captivity accustoms their metabolism to conditions that sharply

76 diverge from those that await them in nature (i.e. food unpredictability and quality, variable
77 diet, vulnerability to predators and/or diseases). Food quality during captivity may thus also
78 play a role in post-release survival and the success of the reintroduction process (Madden et
79 al. 2020). Traditionally, farm-strain birds are fed with highly-digestible commercial poultry
80 food (Liukkonen-Anttila et al. 1999, 2000) including genetically modified soya, wheat and
81 corn that differs from their natural diet (in terms of energy supply, digestibility and quality).
82 However, whether in processed or unprocessed form, grains used in farm-strain bird diets may
83 contain pesticide residues used in conventional agriculture (Kumar et al. 2019). Even small
84 quantities of pesticides in food may cause sublethal effects when eaten over an extended time
85 (Parween et al. 2016; Moreau et al. 2021). In game birds, exposure to pesticide treatments are
86 known to deteriorate the immune system (Lopez-Antia et al. 2015b) and haematocrit (Lopez-
87 Antia et al. 2015a), but also induce dysfunctions of metabolic pathways associated with the
88 ability to store fat (Lopez-Antia et al. 2013; Moreau et al. 2021), and interfere with the
89 endocrine system (Lopez-Antia et al. 2015c; Khalil et al. 2017). The latter results in
90 behavioural changes in general activity (Moreau et al. 2021), risk-taking behaviour (Kobiela
91 et al. 2015) and vigilance (de Faria et al. 2018), which may have positive or negative effects
92 on survival (i.e. foraging, reproduction, predation) depending on environmental context
93 (Smith and Blumstein 2008).

94 Therefore, strain (wild or farm) and food quality may affect physiological or
95 behavioural traits in released individuals, which could alter the fitness and long-term
96 demographics of population though simultaneous effects that have received limited attention
97 (but see Homberger et al. 2013, 2015 and 2021). In the present study, we used Grey partridge
98 (*Perdix perdix*), an iconic game bird of European farmlands that is currently declining, as a
99 test case (Sotherton et al. 2010; Aebischer and Ewald 2012; Bro 2016). Several million birds
100 are annually reared and released in Europe, including ~2 million in France (Bro 2016;

101 Madden 2021). Mortality rates of released farm-strain partridges are very high at 60–80%
102 (Parish and Sotherton 2007; Buner and Schaub 2008; Rymešová et al. 2013; Bro 2016;
103 Homberger et al. 2021). Although many studies have investigated morphological, behavioural
104 and physiological deficiencies, as well as post-release survival of farm-strain grey partridges
105 (Putaala and Hissa 1995, 1998; Rantanen et al. 2010a, 2010b; Keller et al. 2012; Homberger
106 et al. 2013, 2015, 2021; Rymešová et al. 2013), none have explored the potential interactions
107 between strain (wild or farm) and sublethal effects of pesticides. We thus aimed to mimic the
108 diet shift that partridges encounter once released (poultry food *vs.* natural grain) to investigate
109 (i) differences in responses to this transition depending on strain, and (ii) the consequences of
110 ingesting organic or conventional grains on physiology and behaviour traits that are likely
111 critical for post-release survival. Our experimental procedure involved 8-month-old grey
112 partridges, either bred in captivity for 60 generations (farm strain) or 2 generations (wild
113 strain). Partridges were fed from birth with poultry food. At the beginning of the experiment,
114 they were fed with natural grain grown by either organic or conventional agriculture.
115 Individual conditions were monitored throughout captivity and several behavioural traits were
116 also quantified for each bird (see Methods for details). We predicted that birds that had been
117 in captivity the longest (farm strain) would lose weight and display a lower haematocrit in
118 comparison to wild-strain birds due to diet shift. Furthermore, we predicted that birds fed
119 conventional grains would be even more affected in terms of physiology and behaviour due to
120 potential pesticide residue ingestion.

121

122 **2. MATERIALS AND METHODS**

123 **2.1 Experimental design**

124 The study took place in a commercial game farm in Southwest France, and lasted 3 months,
125 from December 2019 to March 2020. Tests were performed on 80 8-month-old captive-born

126 grey partridges; 40 were from a game farm strain (60 to 80 generations in captivity, ‘farm-
127 strain’ hereafter) and 40 were from F3 generations of wild trapped birds (two generations in
128 captivity, ‘wild-strain’ hereafter). From birth to the beginning of the experiment
129 (corresponding to the diet shift from poultry food to natural grain), all birds had the same
130 experience, living in two large pens depending on their strain (100 m × 10 m × 4 m) at the
131 game farm. They were fed ground feed mixture for game birds (STARGIB entretien; see ESM
132 1 for composition) mainly made from imported conventional soya bean, maize, wheat and
133 sunflower, likely containing pesticide residues (Klüche M. et al. 2020). Before
134 experimentation commenced, each bird was sexed (according to the presence of secondary
135 sexual characters) and fitted with an alphanumeric metal ring.

136 In December 2019, four experimental groups of 20 partridges were established
137 randomly, including 10 males and 10 females, each placed in a grassy holding pen (100 m ×
138 10 m × 4 m) including a game bird feeder, several drinkers equally spaced in the pen, and two
139 sheets of metal providing shelter. The first group comprised 20 wild-strain partridges fed *ad*
140 *libitum* with grains obtained from harvests of certified organic crops (i.e. purchased from
141 organic producers), composed of half wheat and half corn. The second group of 20 wild-strain
142 partridges was fed *ad libitum* with grains purchased from producers in conventional
143 agriculture (i.e. for which different pesticides were used during the cropping season) also
144 composed of half wheat and half corn. Similarly, the third and the fourth groups were
145 composed of 20 farm-strain partridges each, either fed *ad libitum* with organic or
146 conventional wheat and corn.

147 A previous study showed that conventional grains contain pesticide residuals whereas
148 organic grains do not, and that the energy content for both types is equivalent (Moreau et al.
149 2021). In the present study, only conventional grown wheat had detectable and quantifiable
150 pesticide residues (analysed by GIRPA, a referenced laboratory based at Beaucouze, Loire

151 Atlantique, France). We detected 0.014 mg/kg clopyralid, above the limit of quantification set
152 at 0.010 mg/kg. Clopyralid is an organochlorine herbicide that is considered relatively non-
153 toxic to terrestrial and aquatic animals, although available data concerning toxicity to birds
154 are scarce (EFSA 2018). No pesticide residues were detected in organic grains. Birds were
155 under constant surveillance, with water and food provided each day by the farmer from the
156 18th of December (week 0) to the 10th of March (week 12). All groups were kept under a
157 natural light cycle, and 11 partridges died during experimentation (4 in the wild-strain organic
158 group, 3 in the wild-strain conventional group, 1 in the farm-strain organic group, and 2 in the
159 farm-strain conventional group; Fisher's exact test; $p = 0.57$).

160 **2.2 Monitored parameters**

161 During the experiment, birds in each group were monitored to assess (i) their physiological
162 condition through haematocrit, which reflects nutritional state and physiological stress (Ots et
163 al. 1998), and body mass index, (which reflects energy capital accumulated by feeding (Peig
164 and Green 2009), and (ii) their behaviour in relation to anti-predator responses, activity, and
165 exploration (Réale et al. 2007; de Faria et al. 2018).

166 2.2.1 Physiological conditions; haematocrit and body mass index

167 Morphological measurements and blood samples were taken the day before release, while in
168 their pens, in December (week 0) and after 3 months of food type (week 12). To assess
169 haematocrit, a blood sample was taken from the brachial vein using a sterile needle (\emptyset 0.06
170 mm) and heparinised micro-capillary tubes (10 μ L). Capillary tubes were then centrifuged at
171 5000 rpm for 5 min immediately afterwards to determine haematocrit levels; the proportion of
172 the tube filled with red blood cells was divided by the total sample volume in the capillary
173 tube. (Biard et al. 2015). Length was measured with an electronic calliper to the nearest 0.1
174 mm. In order to compare the body mass index of grey partridges, right and left tarsus lengths
175 were measured twice with a calliper (accuracy 0.1 mm) at the beginning of the experiment

176 (week 0), since in adulthood tarsus lengths remain stable. Body mass was recorded at week 0
177 and week 12 to the nearest 1 g using a spring scale (Pesola 500 g). From body mass and mean
178 tarsus length (right and left), we evaluated the body mass index with the scaled mass index
179 (Peig and Green 2009; Moreau et al. 2021).

180 2.2.2 Behavioural tests

181 A set of behavioural tests were performed in week 9 during three consecutive days with
182 identical weather (from the 18th to the 20th of February); 21 birds were tested on day 1, 27 on
183 day 2, and 27 on day 3. Each evening, partridges of different groups were randomly caught,
184 placed in cardboard boxes (five birds per box, 66 cm × 38 cm × 17.5 cm), and left inside for
185 the night under controlled conditions (7–11°C). Catching birds the night before the
186 experiment enabled us to decrease the capture stress that can affect behavioural tests. The
187 following day, each bird was processed using the same sequence, which consisted in (i) the
188 cage test and (ii) the open-field test. The cage test consisted of measuring flight initiation
189 distance (FID) and escape attempt strategy (as birds could not escape) in a cage outside. The
190 open-field test consisted of measuring activity, exploration, escape strategy and duration of
191 escape in an open-field test. The behaviour of each bird was always recorded by the same
192 observers. After the full series of measurements, birds were released into their pens.

193 2.2.3 Cage test

194 Partridges were placed by a single operator in a square, wire cage (46 cm × 32 cm × 36 cm) in
195 a ploughed field. Another operator was hidden behind a hedge, 100 m from the cage, and they
196 watched the bird's behaviour through binoculars. After 5 min of acclimatisation, the hidden
197 operator approached the bird. The FID, the distance at which the individual begins to fly when
198 a predator, or human, approaches (Frid & Dill, 2002), was estimated as the distance at which
199 the bird reacted (tried to run or fly inside the cage) using the straight-line approach of the
200 operator. The escape attempt strategy (running or flying) of the bird was also recorded.

201 Approaches towards birds were performed by the same operator to standardise approach
202 speed and operator dress, thus limiting detectability bias. After the test, birds were caught by
203 the first operator, placed in a cardboard box (66 cm × 38 cm × 17.5 cm) and brought directly
204 to the open-field test in a nearby building (5 m × 5.5 m × 1.50 m, 7–11°C, 30.5 lux).

205 *2.2.4 Open-field test*

206 A grid was set on the floor of the building that included 110 numbered squares, each 50 × 50
207 cm. Partridges were brought in a cardboard box (66 cm × 38 cm × 17.5 cm) and given 3 min
208 for acclimatisation to the box. The operator, hidden in a corner behind a sheet, then opened
209 the cardboard box to let the bird out. The exploration behaviour of the partridge was assessed
210 by counting the number of different squares visited during 7 min. Activity, considered any
211 behaviour except resting, as measured as the number of times each square was visited. At the
212 end of exploration and activity assessment, a stuffed fox (40 cm × 80 cm) on castors, hidden
213 with the operator, was launched towards birds at a speed of ~1 m/s. The responses to this
214 stimulus were then assessed as (i) their escape strategy (running or flying) and (ii) their
215 duration of flight (recorded with a chronometer). After the test, partridges were caught with a
216 landing net and returned to their cage (see Fig. 1).

217 **2.3 Statistical analysis**

218 We analysed the effects of strains and treatments on the physiological conditions of birds at
219 three different times. First, at the beginning (week 0) and at the end (week 12) of the
220 experiment, we tested whether strain (wild vs. farm), food type (organic vs. conventional),
221 and their combination affected haematocrit and body mass index, accounting for sex
222 differences using analysis of variance (ANOVA). Although no effect of food type was
223 involved at week 0 since all birds were fed with the very same food until that date, food effect
224 from the start of the experiment was included in the model to test for possible initial sampling
225 bias. A third model investigated whether trends existed for haematocrits or body mass index

226 between week 0 and week 12, including the effects of strain, sex, and food nested within
227 strain. Pairwise post-hoc comparison tests between the four groups were tested using
228 Bonferroni correction in the case where interactions between strain and food type were
229 significant. We chose to nest the effects of food and strain because at the beginning of the
230 experiment we already had a strain effect (see the results below). Similarly, we analysed the
231 effects of strain and food type on behavioural traits, including their interactions and sex
232 differences. Activity and exploration were square root transformed before analysis. Both
233 escape strategy during cage and open-field tests could be in the form of flight or running,
234 hence we used a binomial modelling framework in the logistic regression model and escape
235 strategy (0 for running, 1 for flying) as a response variable. Finally, for birds that flew away
236 during the open-field test, duration of flight was also square root transformed before analysis.
237 The normality of residuals and the distribution of residuals against fitted values were checked
238 for each model. Effect of blood sample hours on haematocrit variation was also checked at
239 week 0 using simple linear model (LM). For all tests, *p*-values were calculated at the 5%
240 significance level. All statistical tests were performed using R software (v. 3.5.0, R Core
241 Team 2018) and the *smatr* package (Warton et al. 2012) for scale mass index computation
242 (Peig and Green 2009). Results are presented as means \pm standard error (SE).

243 **2.4 Ethical approval**

244 All experiments complied with French laws on animal experimentation, and all experimental
245 protocols were approved by the Committee of Animal Experimentations of the Deux-Sèvres
246 French Department (APA FIS#9465-201703101551625).

247

248 **3. RESULTS**

249 Numbers of monitored partridges differed depending on the test undertaken (this is specified
250 on each figure). Concerning physiological condition comparison, 65 partridges were

251 monitored (11 deaths at the end of the experiment and four without all data). Concerning
252 behavioural tests, 73 partridges were monitored because at the time of the test, seven
253 partridges were already dead.

254 **3.1 Partridge physiological conditions**

255 Time of blood samples did not affect haematocrit variation (LM $\chi^2 = 1.77$, $df = 1$, $P = 0.19$).
256 Farm-strain partridges had slightly but significantly lower haematocrits than wild birds at the
257 onset of the experiment, with haematocrits of birds in the conventional food type group
258 significantly lower than in the other groups (i.e. highlighting a sampling bias, Fig. 2.a, Table 1).
259 At week 12, farm-strain partridges still had lower haematocrits (Fig. 2.a). Haematocrits of all four
260 groups increased with both food types (significant intercepts when comparing week 0 and 12)
261 with a significant effect of sex (Fig. 2.a, Table 1). Indeed, haematocrits of males (mean \pm SE at
262 week 0; $42.96 \pm 3.17\%$ at week 12; $46.91 \pm 3.53\%$) increased more than those of females ($43.33 \pm$
263 3.37% and $44.85 \pm 3.27\%$; Fig. 2.a, Table 1). Thus, there was no statistically significant effect of
264 food type on haematocrit.

265 Conversely, the body mass index of farm-strain partridges was significantly higher than
266 that of wild birds at the onset of the experiment (Fig. 2.b, Table 1), and female body mass index
267 was higher than that of males (week 0, 388.8 ± 31.8 g for females, 357.5 ± 28.7 g for males). At week
268 12 a significant sex effect was evident, with female body mass index remaining higher than that of
269 males (week 12, 365.1 ± 37.1 g for females, 331.2 ± 22.8 g for males). In addition, body mass
270 index decreased during the food type experiment only for farm-strain partridges regardless of food
271 type, whereas this index stayed stable for wild birds (Fig. 2.b, Table 1).

272 **3.2 Partridge behaviour**

273 Strain and food type affected behavioural traits in different ways (Table 2). During the
274 cage test, farm-strain partridges fed conventional grains showed lower FID (46.1 ± 22.2 m) than
275 other birds (farm-strain birds fed organic grains = 63.1 ± 13.2 m; wild-strain birds fed organic

276 grains = 56.7 ± 16.9 m; wild-strain birds fed conventional grains = 65.2 ± 18.9 m; Fig. 3.a).
277 Moreover, strain and food type interacted significantly to impact escape strategy; farm-strain
278 partridges fed conventional grains were less likely to escape by flying in comparison to other
279 groups (Table 2, Table 3). Concerning the open-field test, farm-strain partridges were less active
280 (Table 2, Fig. 3.b) and less likely to try to escape by flying in response to the stuffed fox in
281 comparison with wild-strain birds (Table 2, Table 3). Finally, for birds that escaped by flying,
282 duration of flight was higher for wild-strain partridges fed organic grains, and farm-strain birds
283 that consumed conventional grains displayed the shortest flight duration among the four groups
284 (Table 2, Fig. 3.c).

285

286 **4. DISCUSSION**

287 In this study, we compared the effects of captive-reared bird strain (farm vs. wild) and
288 interactions with potential sublethal effects of low doses of pesticide ingestion through food
289 (organic vs. conventional grains), though only clopyralid was detected. Regardless of food
290 type, the body mass index of farm-strain birds was higher at the onset of the experiment, and
291 thus decreased as the experiment progressed, while that of wild-strain birds remained stable.
292 Conversely, haematocrit of farm-strain partridges was lower at the onset of the experiment but
293 increased over time for all groups. We also found that farm-strain partridges had lower
294 activity and tended to flee by running rather than flying during the open-field test. Finally, we
295 found an interaction between strain and food type in behavioural traits involved in anti-
296 predator responses, with farm-strain birds fed conventional grains (containing clopyralid
297 residues) showing lower FID and shorter flight duration, and they were less likely to try to
298 escape by flying during cage tests than other groups. Such an interaction between strain and
299 food type resulted in the worst performance for farm-strain birds fed conventional grains.
300 These results suggest that these birds could be disadvantaged in the wild.

301 Captive-bred grey partridges are traditionally fed *ad libitum* using feeders containing
302 commercial poultry food; however, when released, these birds have to cope with an
303 unpredictable and strikingly different type of food, based on winter grains (Perkins et al.
304 2007). Our experimental design somewhat mimicked this transition as diet of birds shifted
305 from poultry food to natural wheat and corn grains at the onset of the experiment. As
306 predicted, farm-strain partridges showed higher body mass than wild-strain birds at the
307 beginning of the experiment, highlighting morphological differences between the two strains.
308 Indeed, captive-reared birds are often larger than their wild counterparts as a result of
309 unlimited access to high-nutrient food over several generations of captivity (Putala and Hissa
310 1995, 1998). Regardless of treatment type, we found that the body mass index of farm-strain
311 birds decreased, which could suggest that those birds may be less inclined to properly
312 assimilate new/unfamiliar food. Indeed, captivity adaptation could have led to shortened
313 intestines, caeca and gizzards of our farm-strain partridges, in comparison with wild birds
314 (Putala and Hissa 1995; Liukkonen- Anttila et al. 2002), although we did not examine the
315 digestive systems of our birds in order to keep them alive. These organs play a role in food
316 digestion and assimilation, and the larger they are, the more effective they are at processing
317 fibrous food such as natural grains. As expected, wild-strain partridges seemed to cope better
318 with this dietary change, based on their relatively stable body mass index, consistent with
319 previous studies (Putala and Hissa 1995). An alternative hypothesis may explain the decrease
320 in body mass of farm-strain partridges (i.e. the shift to a smaller group and/or a different
321 group composition). Indeed, the stress responses and behaviour of individuals depends on the
322 group to which they belong, as reported previously for body condition (Creel et al. 2013;
323 Homberger et al. 2015). Testing this hypothesis would have required performing enough
324 replicates for each group, which was not feasible here due to space constraints in the game
325 farm.

326 Haematocrit is often positively correlated with body condition as it reflects nutritional
327 state and physiological stress, although extrapolating from an individual's state should be
328 done with caution (Ots et al. 1998; Fair et al. 2007). However, in the current study, we found
329 the opposite trend between fat storage and red blood cells. Indeed, at the beginning of the
330 experiment, farm-strain partridges (i.e. those with the higher body condition) had lower
331 haematocrit than wild birds. Although we detected significant sampling bias at the onset of
332 the experiment, haematocrit increased over the course of the study for all four groups. This
333 overall increase in haematocrit may be linked to the mating period, which begins in February
334 for partridges, since haematocrit can change according to hormones (Fair et al. 2007). This
335 hypothesis was corroborated by the haematocrits of males, which were higher those of
336 females at the end of the experiment, highlighting differences between sex, even though
337 haematocrit increased for all birds (Fair et al. 2007).

338 We also found that farm-strain partridges were less active and preferred to flee by
339 running rather than by flying during open-field tests. Physiological and morphological
340 adaptations due to long-term captivity (e.g., higher body mass for farm-strain partridges at
341 week 0) may have induced behavioural changes in our farm-strain partridges. For example,
342 farm-strain partridges may have weaker endurance due to smaller breast muscles, lower
343 glycogen content, and consequently lower release and/or production of glucocorticoids in
344 pectoral muscles (Putala and Hissa 1995). Moreover, they may to have lower excretion of
345 glucocorticoids as a response to stress, which can affect anti-predator responses (Homberger
346 et al., 2013, 2014, 2015). As animals selected over many generations for high reproductive
347 rates, with food provided *ad libitum*, one may expect a decrease in energy-demanding
348 behaviours for farm-strain birds, such as escape by flying (Beilharz et al. 1993; Schutz and
349 Jensen 2001). In addition, being isolated from predators may result in lacking appropriate
350 anti-predator responses (Pérez et al. 2010; Rantanen et al. 2010b). Upon release, animals have

351 to cope with risk-taking, foraging and novel environment discovery (Réale et al. 2007).
352 Fleeing quickly in life-threatening situations (e.g., by flying rather than running), and
353 displaying a high level of activity to find food, sexual partners and territories are expected to
354 increase fitness (Sih et al. 2004; Smith and Blumstein 2008; Homberger et al. 2013). Based on
355 our results, our farm-strain partridges could therefore be expected to suffer higher mortality
356 rates than their wild counterparts after release (Rantanen et al. 2010a; Rymešová et al. 2012,
357 2013; Homberger et al. 2014). Interestingly, Homberger et al. (2021) found that bolder birds
358 (i.e., those with higher activity and lower tonic immobility responses) survived longer after
359 release despite negative associations postulated and repeatedly reported between proactivity,
360 bold behaviour, survival and longevity (Dammhahn et al., 2018; Smith & Blumstein 2007).
361 Under fluctuating environmental conditions or changing needs at different stages of the life
362 cycle, the advantages and disadvantages of a particular behaviour type may change or even be
363 reversed (Sih et al., 2004). Individuals can acquire their own behavioural strategies and adjust
364 behaviour according to environmental context, making it difficult to predict their probability
365 of survival based solely on captive experiments.

366 We also detected an interaction between strain and food type, since farm-strain birds
367 fed conventional grains showed lower capacity to detect (i.e., lower FID) and escape a
368 predator in the field (i.e., running instead of flying). Many studies have described sublethal
369 adverse effects of pesticides on behaviour, caused by neurotoxicity (Mitra et al. 2011; Moreau
370 et al. 2022, submitted). For example, organomercury fungicides act mainly through the central
371 nervous system, resulting in disruption to risk-taking behaviour (Kobiela et al. 2015);
372 abamectin insecticides may inhibit GABA receptors and cause environment perception
373 disorder (de Faria et al. 2018); neonicotinoids impact acetylcholine esterase activity, leading
374 to a decrease in foraging activity and ultimately to starvation and body weight loss (Eng et al.
375 2017, 2019). Although clopyralid is considered relatively non-toxic to birds (EFSA 2018),

376 some long-term studies have reported weight loss and damage to stomach, liver and kidney at
377 15 mg/kg per day for mammals, specifically rats and rabbits (Hayes et al. 1984; EFSA 2018).
378 Our current results suggest that chronic ingestion of clopyralid residues may induce
379 neurotoxicity and alter anti-predator responses of farm-strain partridges, whereas wild-strain
380 partridges seemed to be unaffected by conventional grain ingestion.

381

382 **5. CONCLUSION**

383 Currently, most commercial game farms breed partridges from farm-strain birds and feed
384 them commercial poultry food (Sokos et al. 2009; Homberger et al. 2014). Our results suggest
385 that game farms produce birds that have lower fitness (i.e., less likely to survive and/or
386 reproduce) when released into the wild compared with wild strains, and this is aggravated by
387 food type, (i.e. when fed conventional grains containing pesticide residues). Our results
388 therefore suggest that feeding captive-bred individuals from game farms with organic grains
389 may, to some extent, compensate for this disadvantage, and may therefore contribute to
390 improving post-release survival rates. This strategy may be preferred by partridge farmers
391 who may be more inclined to change the food they provide their partridges rather than
392 completely changing their breeding strategy.

393

394 **6. ACKNOWLEDGMENTS**

395 We thank Julien Blandin for letting us work on his farm, and for taking care of partridges
396 during the study. We also thank the Fédération des Chasseurs (79), its president Guy Guédon,
397 and Frédéric Audurier and his team for their support. We are grateful to Landry Boussac for
398 his assistance during part of the monitoring, and to Karine Monceau for her help during data

399 analysis. Finally, we thank Oxford Science Editing service for carefully improving the
400 English of the manuscript.

401

402 **7. DECLARATIONS**

403 **Funding:** This work was supported by the University of La Rochelle, the French National
404 Centre of Scientific Research (CNRS), and the French National Research Institute for
405 Agriculture, Food and the Environment (INRAE). This study was partly funded by the
406 projects ANR JCJC PestiStress (#19-CE34-0003-01), the BioBird project funded by the
407 regional government of Nouvelle-Aquitaine, and the French National program EC2CO
408 (Ecosphère Continentale et Côtière).

409

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600 **Table 1 Effects of strain (wild vs. farm), sex and food type (organic vs. conventional) on the physiological condition of grey partridges**
601 **(haematocrit and body mass index) at the beginning and end of the food experiment (week 0 before food type and week 12), and**
602 **differences at 12 weeks based on the combined effect of food and strain.** Wild birds fed organic grains served as a reference in models. $\beta \pm$
603 SE values were extracted from minimal models including only significant factors. Farm, farm-strain; Conv., conventional. Significant effects are
604 in bold.

Response variables	Beginning of the experiment (week 0)					End of the experiment (week 12)					Difference between week 12 and week 0			
	Fixed factors	F	df	p	$\beta \pm$ SE	F	df	p	$\beta \pm$ SE	F	df	p	$\beta \pm$ SE	
Haematocrit					Intercept = 44.42 \pm 0.66				Intercept = 46.08 \pm 0.91				Intercept = 2.30 \pm 0.84, p <0.01	
	Strain	6.99	1	<0.01	Farm = -2.06 \pm 0.78	6.34	1	<0.01	Farm = -1.63 \pm 1.15	0.01	1	0.98		
	Sex	0.25	1	0.62		6.21	1	<0.01	Males = 2.05 \pm 0.82	10.23	1	<0.01	Males = 2.52 \pm 0.76	
	Food	1.54	1	0.22		0.91	1	0.35						

	Strain	8.10	1	<0.01	Farm Conv.	0.27	2	0.61		2.45	2	0.09	
	x Food				= -4.19 ± 1.47								
Body mass					Intercept =				Intercept =			Intercept = -6.03 ± 6.46	
index					375.70 ± 5.81				376.26 ± 8.73				
	Strain	14.44	1	<0.001	Farm = 25.52	0.24	1	0.63		26.30	1	<0.001	Farm = -31.61 ± 8.15
					± 6.80								
	Sex	20.83	1	<0.001	Male = -31.19	19.44	1	<0.001	Male = -34.05	0.53	1	0.53	
					± 6.83				± 7.88				
	Food	1.37	1	0.25		0.42	1	0.52					
	Strain	2.36	1	0.13		2.50	2	0.12		0.19	2	0.83	
	x Food												

605 **Table 2 Effects of strain (wild vs. farm), sex and food type (organic vs. conventional) on**
606 **partridge behaviour during cage and open-field tests.** Linear models were conducted for
607 FID and square root transformed activity, exploration, and duration of flight. Logistic
608 regression was conducted for escape strategy. Wild birds fed organic grains served as a
609 reference in models. $\beta \pm SE$ values were extracted from minimal models including only
610 significant factors. Significant effects are in bold.

Tests	Response	Fixed factors	F	df	p	$\beta \pm SE$
						Intercept = 57.73 ± 4.71
Cage test	FID (n = 73)	Strain	1.09	1	0.30	
		Food	1.98	1	0.16	
		Sex	0.24	1	0.62	
		Strain x Food	8.97	2	<0.01	Farm-strain conventional =
						Intercept = -0.65 ± 0.54
Open-field test	Escape strategy (n = 73)	Strain	0.71	1	0.39	
		Food	1.11		0.29	
		Sex	0.68	1	0.40	
		Strain x Food	4.17	2	<0.05	Farm-strain conventional =
						Intercept = 4.68 ± 0.41
Open-field test	Exploration (square root, n = 73)	Strain	0.08	1	0.78	
		Food	2.16	1	0.15	
		Sex	0.89	1	0.34	
		Strain x Food	0.11	2	0.11	
						Intercept = 9.14 ± 0.87
Open-field test	Activity (square root, n = 73)	Strain	14.26	1	<0.001	Farm-strain = - 3.35 \pm 0.88
		Food	3.52	1	0.06	
		Sex	0.22	1	0.90	

	Strain x Food	0.30	2	0.58	
					Intercept = 3.04 ± 0.81
Escape strategy (n = 73)	Strain	8.19	1	<0.01	Farm-strain = - 1.80 ± 0.70
	Food	1.15		0.28	
	Sex	0.70	1	0.40	
	Strain × Food	1.84	2	0.17	
					Intercept = 3.17 ± 0.20
Duration of flight (for birds that flew, n = 57)	Strain	12.85	1	<0.001	Farm-strain = -0.94 ± 0.26
	Food	7.30	1	<0.01	Conventional = -0.67 ± 0.25
	Sex	2.07	1	0.16	
	Strain × Food	5.07	2	0.03	Farm-strain conventional =

611

612

613 **Table 3 Summary of escape strategy (flying versus running) during cage and open-field**
614 **tests with the percentage of partridges who tended to escape by flying rather than**
615 **running depending on strain (wild vs. farm) and food type (organic vs. conventional**
616 **grains).**

	Wild strain		Farm strain	
	Organic	Conventional	Organic	Conventional
Partridges flying during cage test	39%	50%	72%	37%
Partridges flying during open-field test	89%	94%	78%	53%

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621 **Figures captions**

622 **Fig. 1** Schematic representation of the experimental design, involving consecutives (A)
623 outside FID test (B) inside open-field behavioural tests on game farm or wild grey partridges
624 fed organic or conventional grains.

625

626 **Fig. 2** Temporal variation of (a) haematocrit and (b) body mass index over time, from the
627 beginning (week 0) to the end (week 12) of the food type experiment, with different strains (wild
628 vs. farm) and food types (organic vs. conventional grains). The horizontal line corresponds to the
629 median, the top and bottom of boxes are the first and third quartiles, and whiskers show the lower
630 and higher values included in the 95% confidence interval. Black points correspond to the mean,
631 open dots correspond to outliers. Stars correspond to significant effects of post-hoc tests and
632 Bonferroni correction for multiple comparisons when the interaction between food type and strain
633 was significant (i.e., $p < 0.05$).

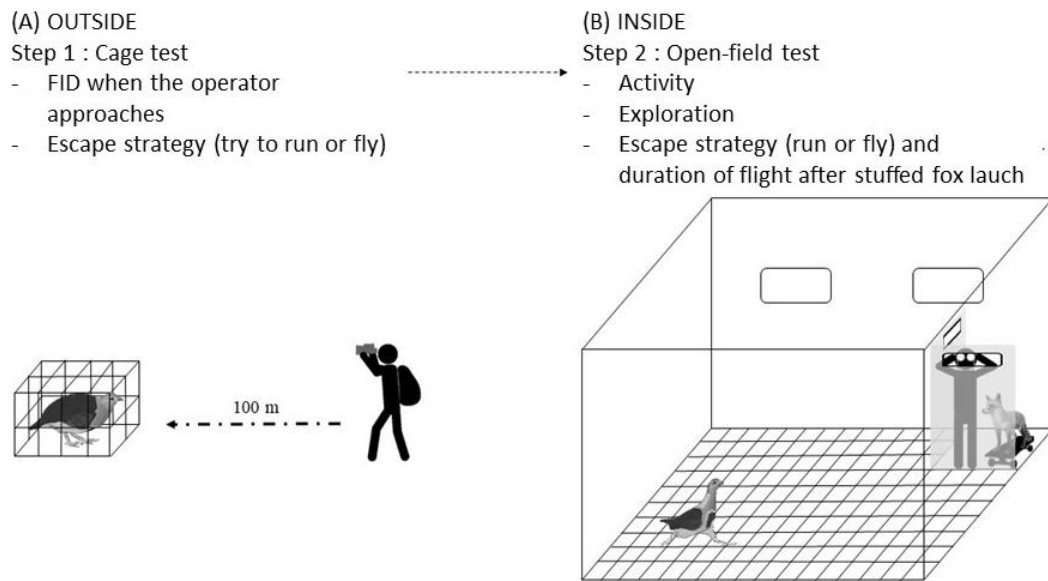
634

635 **Fig. 3** Variation in (a) flight initiation distance, (b) activity and (c) duration of flight according to
636 strain (wild vs. farm) and food type (organic vs. conventional grains). The horizontal line
637 corresponds to the median, the top and bottom of boxes are the first and third quartiles, and
638 whiskers show the lower and higher values included in the 95% confidence interval. Black points
639 correspond to the mean, open dots correspond to outliers.

640

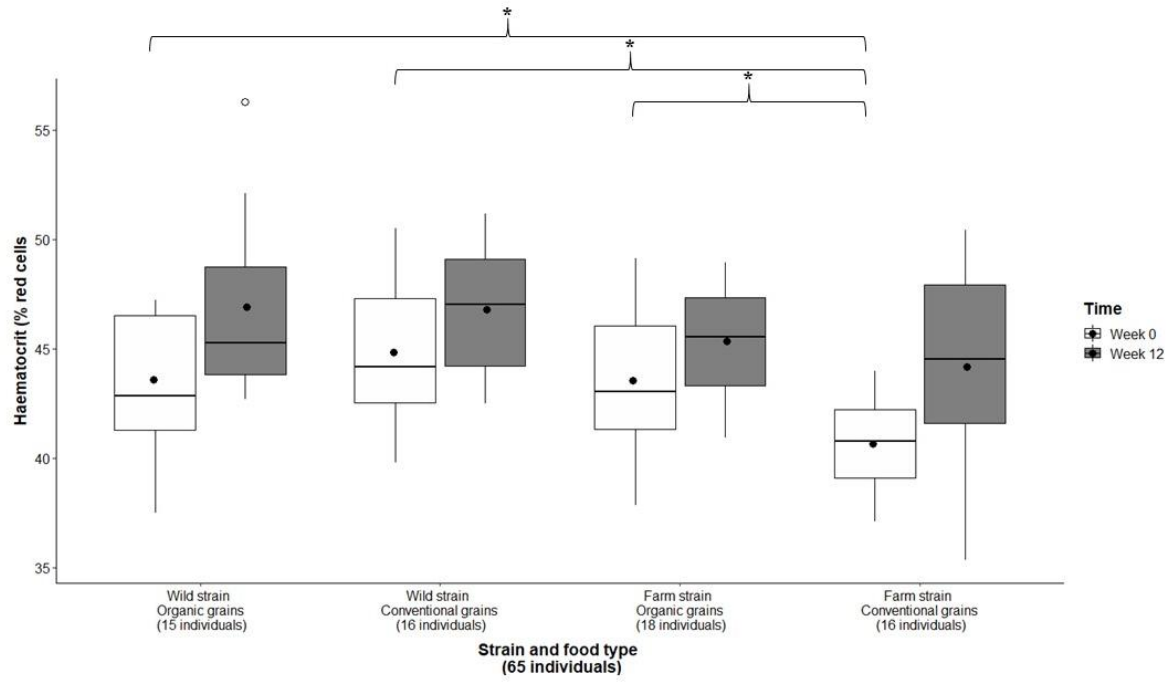
641

642 Fig 1



643

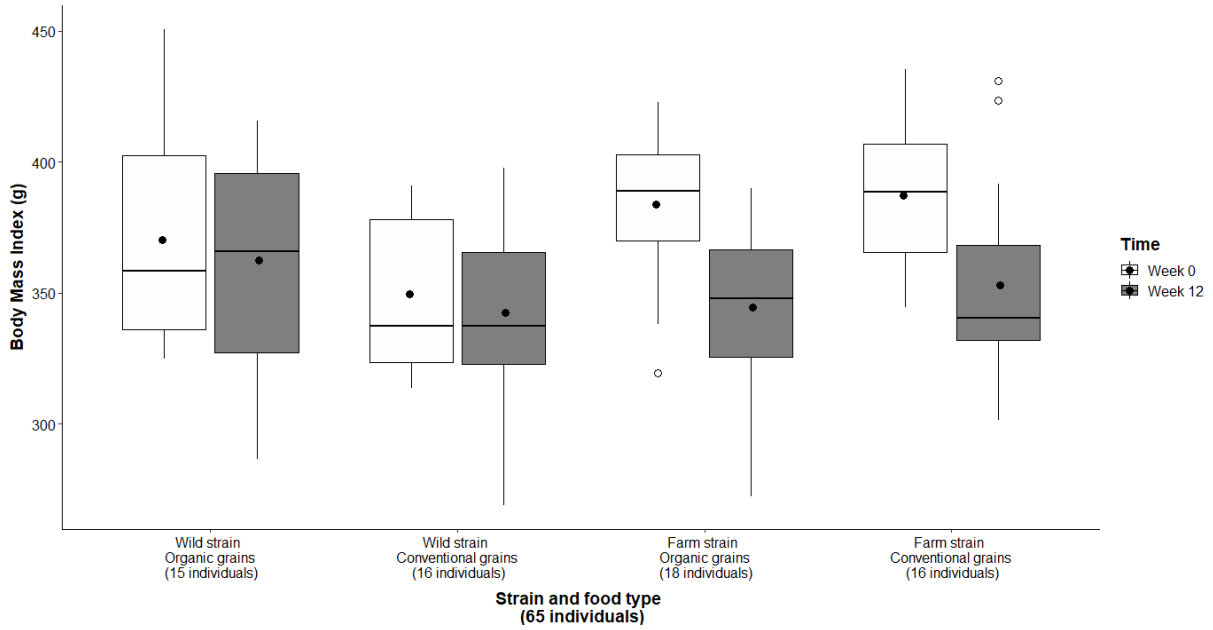
644 Fig 2.a



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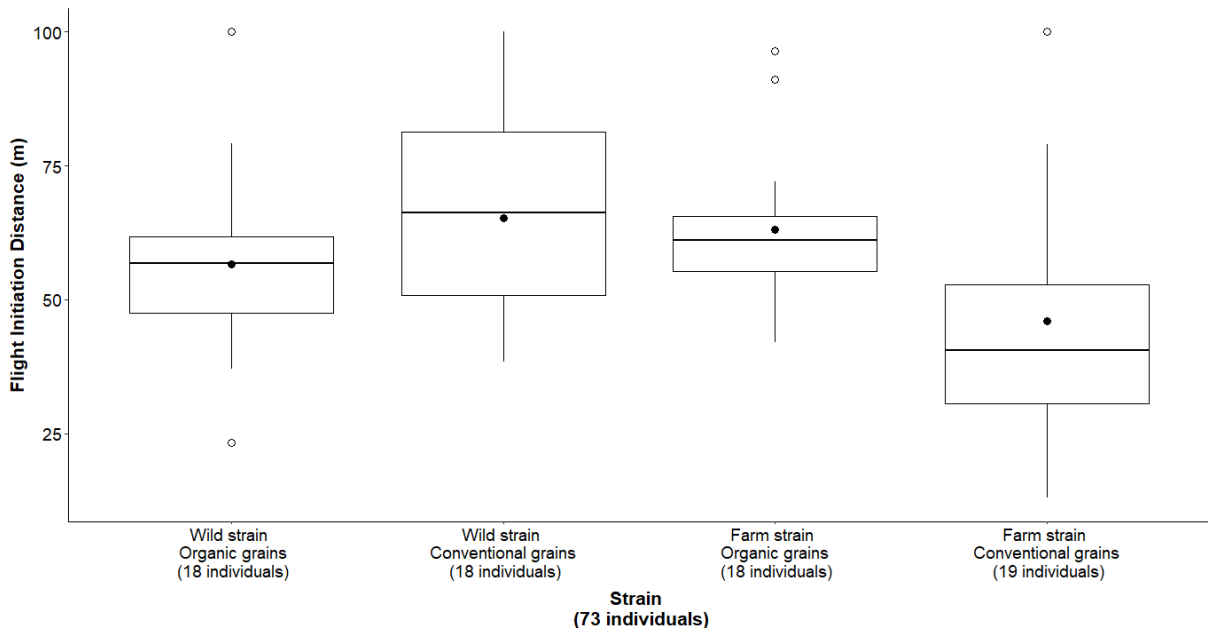
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647 Fig 2.b



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649 Fig 3.a

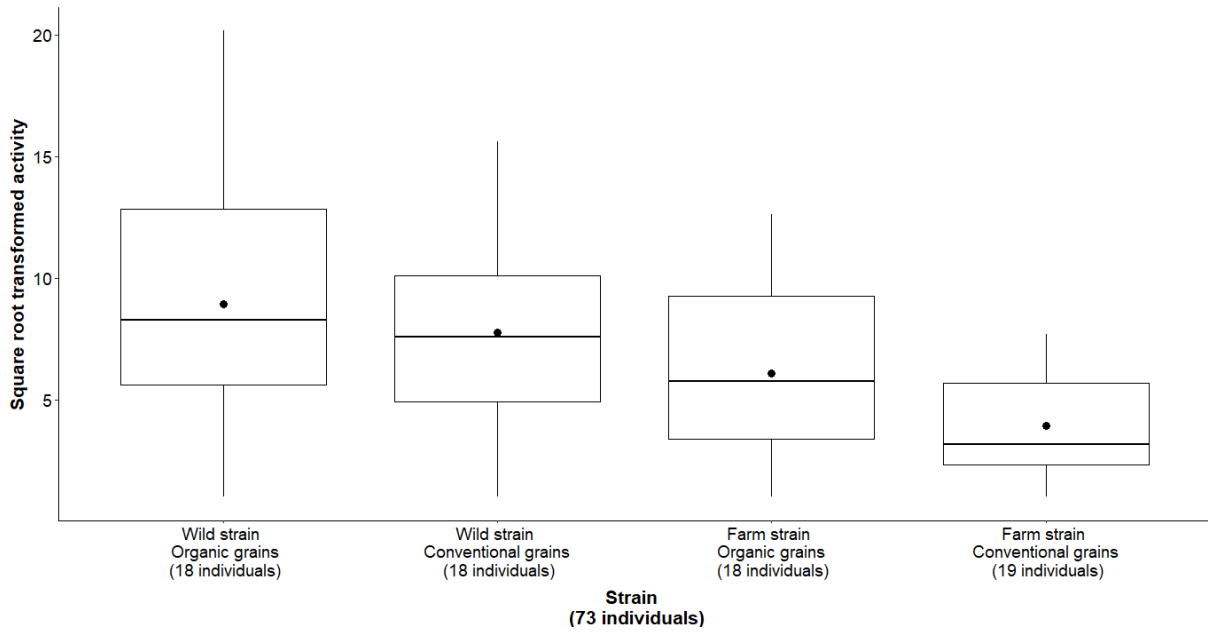


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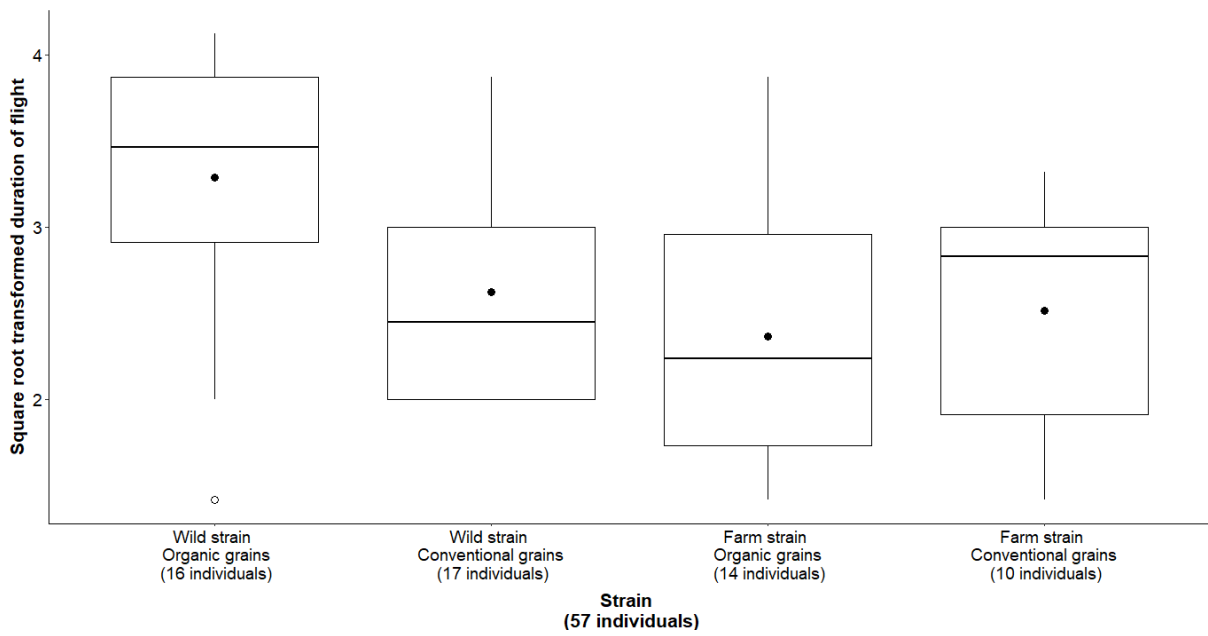
653 Fig 3.b



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656 Fig 3.c



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