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

3

4

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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  $\mu$ 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 18<sup>th</sup> to the 20<sup>th</sup> 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,  $388.8 \pm 31.8$  g for females,  $357.5 \pm 28.7$  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,  $365.1 \pm 37.1$  g for females,  $331.2 \pm 22.8$  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 ( $46.1 \pm 22.2$  m) than  
275 other birds (farm-strain birds fed organic grains =  $63.1 \pm 13.2$  m; wild-strain birds fed organic

276 grains =  $56.7 \pm 16.9$  m; wild-strain birds fed conventional grains =  $65.2 \pm 18.9$  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

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401

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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	Fixed factors	Beginning of the experiment (week 0)				End of the experiment (week 12)				Difference between week 12 and week 0			
		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				<b>Intercept = 2.30 <math>\pm</math> 0.84, <i>p</i> &lt;0.01</b>
	Strain	<b>6.99</b>	1	<b>&lt;0.01</b>	<b>Farm = -2.06 <math>\pm</math> 0.78</b>	<b>6.34</b>	1	<b>&lt;0.01</b>	<b>Farm = -1.63 <math>\pm</math> 1.15</b>	0.01	1	0.98	
	Sex	0.25	1	0.62		<b>6.21</b>	1	<b>&lt;0.01</b>	<b>Males = 2.05 <math>\pm</math> 0.82</b>	<b>10.23</b>	1	<b>&lt;0.01</b>	<b>Males = 2.52 <math>\pm</math> 0.76</b>
	Food	1.54	1	0.22		0.91	1	0.35					

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

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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 = $57.73 \pm 4.71$
Cage test	FID (n = 73)	Strain	1.09	1	0.30	
		Food	1.98	1	0.16	
		Sex	0.24	1	0.62	
		<b>Strain x Food</b>	<b>8.97</b>	<b>2</b>	<b>&lt;0.01</b>	<b>Farm-strain conventional =</b>
						Intercept = $-0.65 \pm 0.54$
Open-field test	Escape strategy (n = 73)	Strain	0.71	1	0.39	
		Food	1.11		0.29	
		Sex	0.68	1	0.40	
		<b>Strain x Food</b>	<b>4.17</b>	<b>2</b>	<b>&lt;0.05</b>	<b>Farm-strain conventional =</b>
						Intercept = $4.68 \pm 0.41$
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 = $9.14 \pm 0.87$
Open-field test	Activity (square root, n = 73)	<b>Strain</b>	<b>14.26</b>	<b>1</b>	<b>&lt;0.001</b>	<b>Farm-strain = - 3.35 <math>\pm</math> 0.88</b>
		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)	<b>Strain</b>	<b>8.19</b>	<b>1</b>	<b>&lt;0.01</b>	<b>Farm-strain = - 1.80 ± 0.70</b>
	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)	<b>Strain</b>	<b>12.85</b>	<b>1</b>	<b>&lt;0.001</b>	<b>Farm-strain = -0.94 ± 0.26</b>
	Food	7.30	1	<0.01	<b>Conventional = -0.67 ± 0.25</b>
	Sex	2.07	1	0.16	
	Strain × Food	5.07	2	0.03	<b>Farm-strain conventional =</b>

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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.,  $p < 0.05$ ).

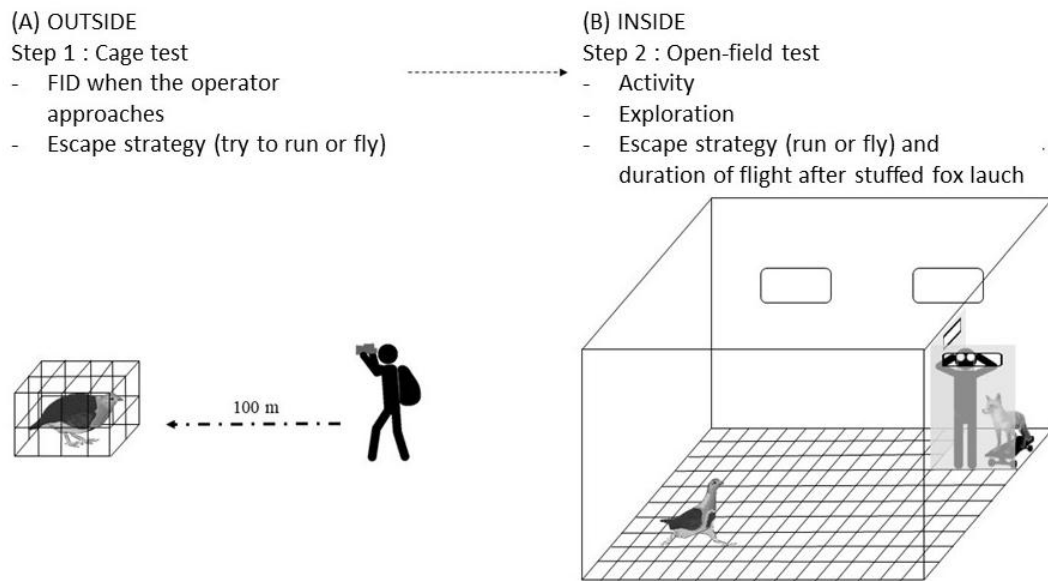
634

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.

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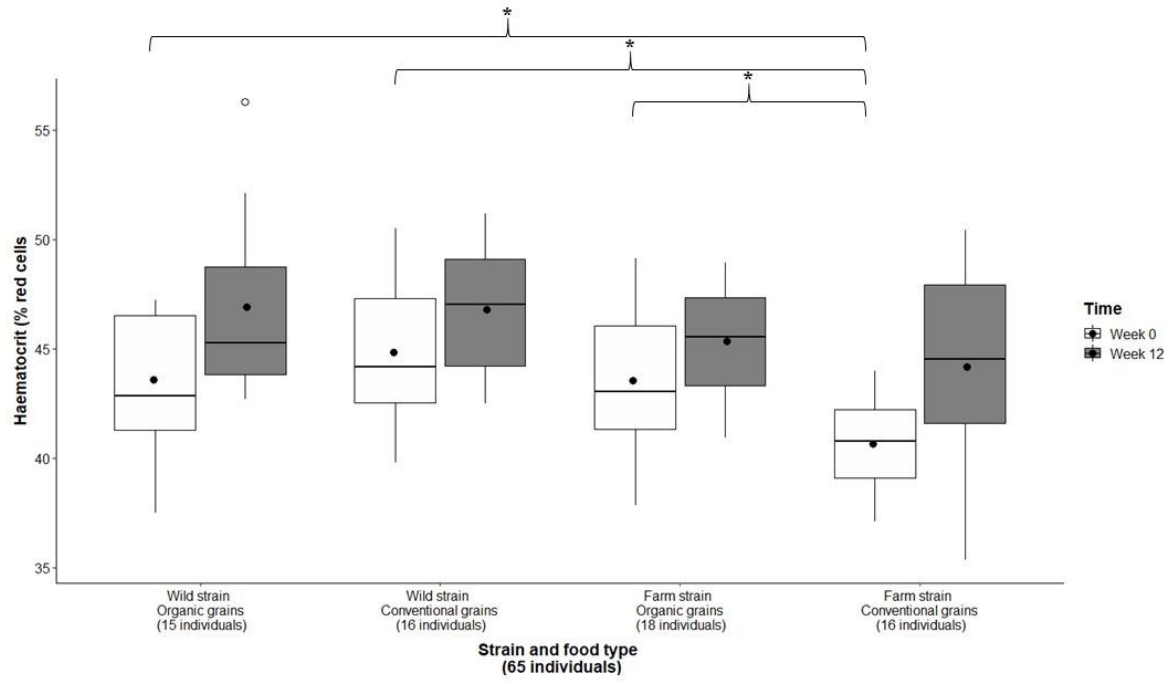
642 Fig 1



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644 Fig 2.a

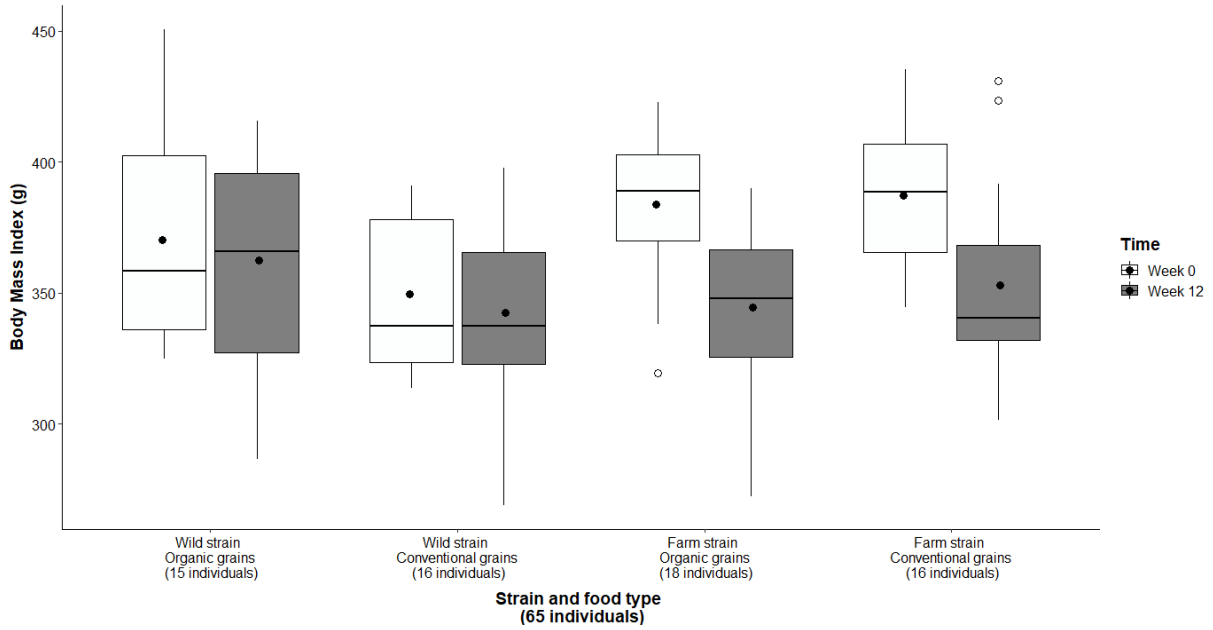




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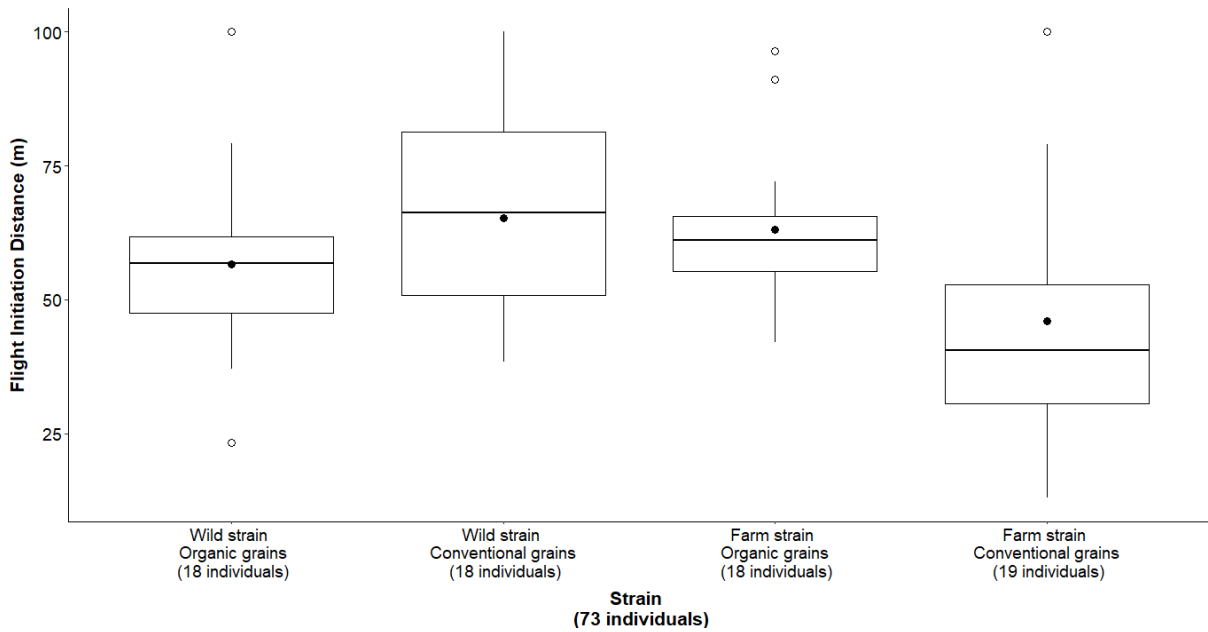
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647 Fig 2.b



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649 Fig 3.a

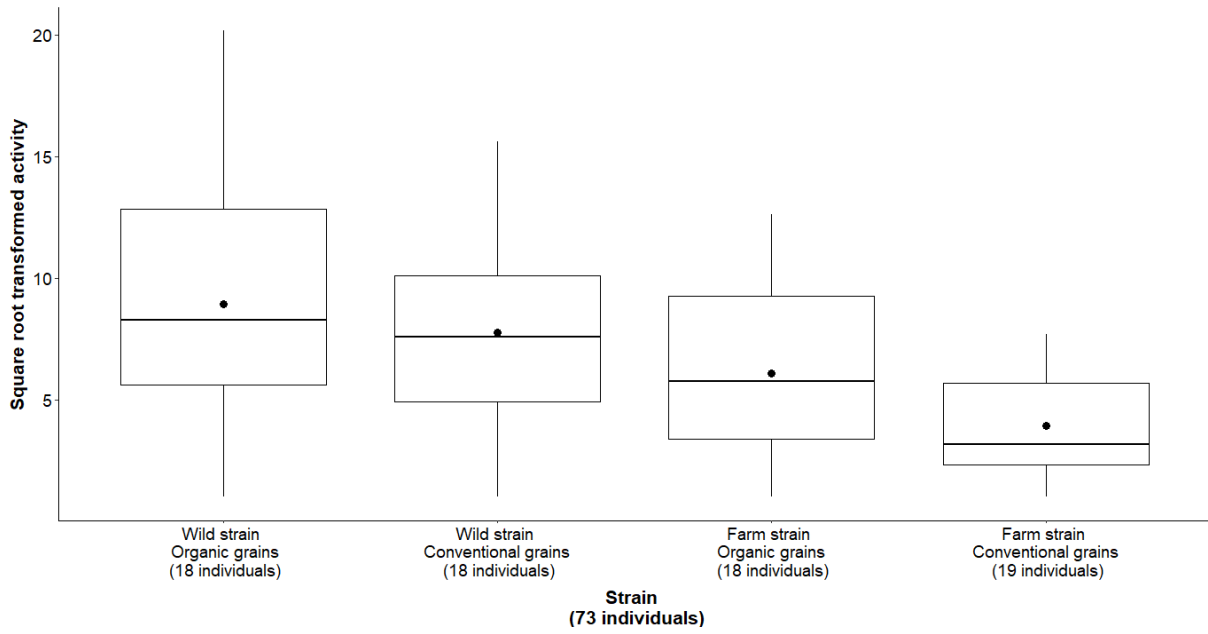


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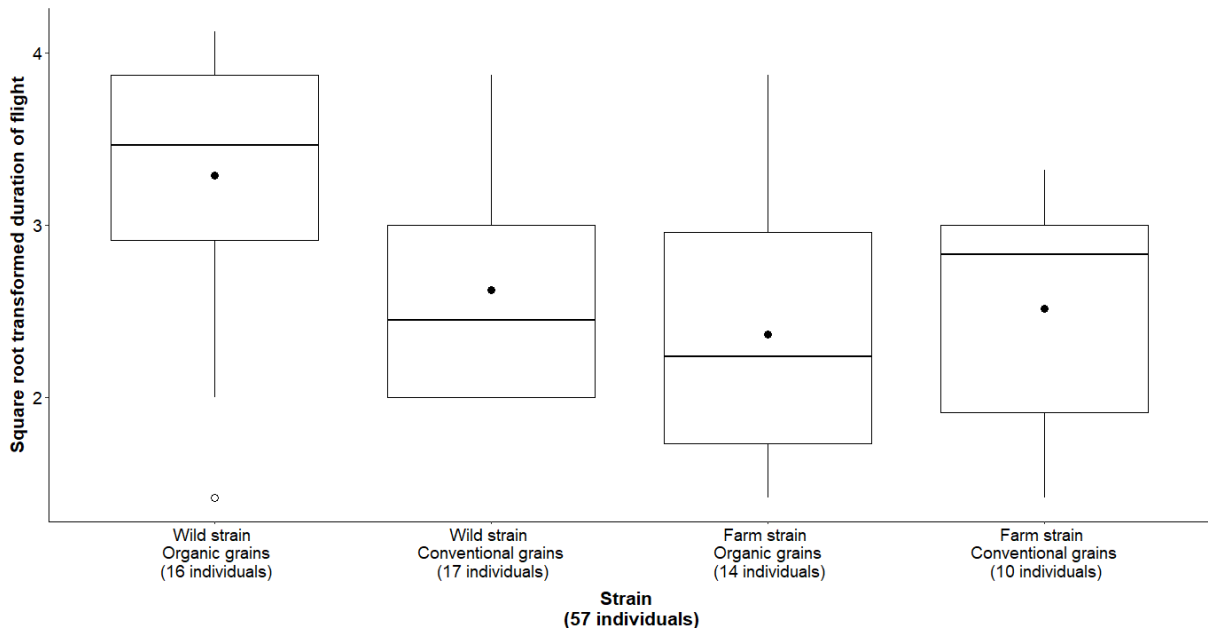
653 Fig 3.b



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656 Fig 3.c



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