1   Consistent variations in personality traits and their potential for genetic improvement in the

2   biocontrol agent *Trichogramma evanescens*

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Abstract

Improvements in the biological control of agricultural pests require improvements in the phenotyping methods used by practitioners to select efficient biological control agent (BCA) populations in industrial rearing or field conditions. Consistent inter-individual variations in behaviour (i.e. animal personality) probably affect BCA efficiency, but have never been taken into account in the development of phenotyping methods, despite having characteristics useful for phenotyping: repeatable (by definition), often heritable, etc. We developed a video-tracking method targeting animal personality traits and evaluated the feasibility of its use for genetic improvement in the BCA *Trichogramma evanescens*, by phenotyping 1,049 individuals from 24 isogenic lines. We found consistent individual variations in boldness, activity and exploration. Personality differences between the 24 isogenic lines suggested a genetic origin of the variations in activity and exploration (broad-sense heritability estimates of 0.06 to 0.11) and revealed a trade-off between exploration and fecundity.

Key words: biocontrol, animal personality, *Trichogramma*, genetic improvement, intraspecific variation, behavioural ecology, behavioural syndrome, genetic correlations, pace-of-life

Introduction

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40   The demand for more sustainable agriculture is increasing worldwide (Godfray et al.,

41   2010; Willer & Lernoud, 2019). Various elements can be used in the development of sustainable

42   strategies, and biological control (BC) is one such element that is currently attracting

43   considerable attention (van Lenteren 2012). Most BC methods are based on the choice, rearing

44   and introduction of biological control agent (BCA) populations able to control the target pests

45   (Eilenberg, Hajek, & Lomer, 2001). Choosing the right BCA is key to the success of pest

46   regulation programmes and is based on (i) the ability of the BCA to control pest populations in

47   the field, (ii) its potential to adapt to the release environment, (iii) its expected impact on local

48   biodiversity, and (iv) the feasibility of mass-rearing and storing the BCA in industrial conditions

49   (Briese, 2000; Kruitwagen, Beukeboom, & Wertheim, 2018; Sforza, 2010). The identification

50   of BCA species or populations with as many of the desired features as possible is time-

51   consuming and complex, particularly given that the choice of non-indigenous species before

52   use as BCAs is constrained by increasingly strict regulations for the protection of biodiversity

53   (Lommen, Jong, & Pannebakker, 2017).

54   Phenotyping is key for (i) the efficient characterisation of traits related to the desirable

55   features of BCAs listed above, (ii) smart choices of BC taxa when screening the available

56   natural enemy diversity and (iii) the management of phenotypic evolution in industrial contexts

57   involving rearing procedures and quality control (Kruitwagen et al., 2018; Lommen et al.,

58   2017). However, the phenotyping methods currently used in the choice of BCAs or for quality

59   control are mostly low-throughput and based on single proxies of fitness, such as predation or

60   parasitism rate, size, sex ratio, longevity, or developmental rate (Hopper, Roush, & Powell,

61   1993; Prezotti, Parra, Vencovsky, Coelho, & Cruz, 2004; Roitberg, Boivin, & Vet, 2001; Smith,

62   1996). These proxies are intuitively correlated with fitness under laboratory conditions, but

63   their actual relevance for biocontrol, in industrial mass-rearing or field conditions, remains a

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64 matter of debate (Lommen et al., 2017; Roitberg et al., 2001). This situation calls for drastic

65 improvements in the phenotyping capacities of the community involved in BC research and

66 innovation.

67   Behavioural traits are among the most promising of the traits to which more attention

68 could be paid in BCA phenotyping procedures. Most behavioural traits are likely to affect the

69 performance of BCA both during industrial mass rearing and in the field (Roitberg, 2007;

70 Wajnberg, 2009; Wajnberg, Roitberg, & Boivin, 2016). Indeed, studies of BCA behavioural

71 traits have suggested that these traits could (i) facilitate the selection of BCAs that are specific

72 to the targeted pest, (ii) improve release strategies (through studies of the BCA response to pre-

73 release handling or BCA mating behaviour, for example), and (iii) predict the efficiency of

74 target pest suppression by the BCA (Mills & Kean, 2010). However, there have been few

75 studies of BCA behavioural traits for the development of phenotyping methods, and behaviour

76 has been largely neglected by those using BC (Mills & Kean, 2010; Wajnberg, Bernstein, &

77 Alphen, 2008).

78   As a consequence, the current state-of-the-art for insect behavioural studies displays

79 several key limitations. The first limitation is the lack of diversity of possible target traits for

80 phenotyping. Indeed, although many studies have focused on traits relating to foraging

81 behaviour (Lirakis & Magalhães, 2019; Mills & Wajnberg, 2008), tools for measuring other

82 aspects of behaviour remain scarce. A second limitation is the insufficient focus on the

83 intraspecific variation of traits. Such variation has been comprehensively investigated for only

84 a limited number of BCA species and a limited number of traits (Kruitwagen et al. 2018; Lirakis

85 and Magalhães 2019). This situation is detrimental because the investigation of only a fraction

86 of the available intraspecific variability makes it difficult to identify the populations displaying

87 the highest performance for biocontrol, and prevents the development of efficient genetic

88 improvement programmes based on selective breeding and controlled evolution (Wajnberg

89   2004; Bolnick et al. 2011; Lommen et al. 2017; Kruitwagen et al. 2018, Lirakis and Magalhães

90   2019). A third limitation is the reliance of most choices in BC exclusively on comparisons

91   between average trait values for species or populations (Lommen et al., 2017). Published studies

92   have suggested that individual variation can affect the characteristics of the population thought

93   to be important for BC (Biro & Stamps, 2008; Michalko, Pekár, & Entling, 2019; Réale, Reader,

94   Sol, McDougall, & Dingemanse, 2007; Wolf & Weissing, 2012).

95   One way to overcome each of these three limitations would be to apply approaches used

96   in the field of animal personality to BC. Indeed, these approaches provide a framework offering

97   (i) sets of behavioural traits rarely studied in BC and displaying features (repeatability,

98   heritability) that make them good candidates for use in genetic improvement for BC, and (ii)

99   phenotyping methods suitable for analyses of intraspecific variation, including inter-individual

100   variation. Animal personality research focuses on inter-individual differences in behaviour that

101   are consistent over time and context (Dingemanse, Kazem, Reale, & Wright, 2009; Réale et al.,

102   2007). Interest in animal personality has increased over the last few decades, and studies have

103   been performed on diverse taxa, including insects (Amat, Desouhant, Gomes, Moreau, &

104   Monceau, 2018; Bell, Hankison, & Laskowski, 2009; Dingemanse et al., 2009; Gosling, 2001;

105   Kralj-fiser & Schuett, 2014; Mazué, Dechaume-Moncharmont, & Godin, 2015; Monceau et al.,

106   2017; Réale et al., 2007; Sih, Bell, & Johnson, 2004; van Ooers & Sinn, 2011) and, more

107   specifically, insects used as BC agents (Gomes, Desouhant, & Amat, 2019; Michalko et al.,

108   2019). Réale et al. (2007) described five main categories of personality traits: boldness,

109   exploration, activity, aggressiveness and sociability. Personality traits have been shown to be

110   correlated with traits relevant for pest control, such as foraging capacity, fecundity, growth,

111   survival (Biro & Stamps, 2008), dispersal ability (Cote, Fogarty, Weinersmith, Brodin, & Sih,

112   2010) and insecticide resistance (Royauté, Buddle, & Vincent, 2014). These traits are probably,

113   therefore, of interest in the context of BC. Moreover, personality traits are repeatable, by

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114 definition, and can be heritable (Dochtermann, Schwab, & Sih, 2014; Réale et al., 2007;

115 Stirling, Reale, & Roff, 2002), making them suitable tools for genetic improvement. From a

116 methodological point of view, animal personality provides valuable information for the design

117 of phenotyping and genetic improvement strategies in BC. Indeed, animal personality studies

118 are based on standardised methods designed to measure inter-individual variation and to

119 investigate correlations between traits (e.g. by looking for behavioural syndromes) (Réale et

120 al., 2007; Sih et al., 2004). This is particularly relevant to the objective of selecting several

121 combined BC traits rather than a single trait, as recently recommended by Lommen et al. (2017)

122 and Kruitwagen et al. (2018). The investigation of correlations between traits is also important,

123 to detect trade-offs that may constrain genetic improvement programmes or affect BC traits if

124 mass-rearing causes uncontrolled trait selection (Mackauer, 1976).

125 　　　　In this study, we assessed the potential for BCA phenotyping based on concepts and

126 methods used in the field of animal personality. We used the egg parasitoid *Trichogramma*

127 *evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) as a test species.

128 *Trichogramma* micro-wasps are used worldwide in augmentative BC against lepidopteran pests

129 (Hassan, 1993; van Lenteren, 2012). Their economic importance (Robin & Marchand, 2020;

130 Thibierge, 2015) justifies investments in research and development aiming to improve their

131 genetic potential. Our aims were (i) to determine whether behavioural traits meeting the criteria

132 of personality traits could be measured in these micro-wasps of approximately 0.5 mm in

133 length; (ii) to investigate the relationships between personality traits and traits classically

134 measured on BCAs in the laboratory, and (iii) to determine whether personality traits could be

135 used in genetic improvement strategies for BCAs. We developed a method based on the video-

136 tracking and measuring, at individual level, of multidimensional behavioural traits relating to

137 boldness, activity and exploration. We investigated the relationships between these traits, and

138 between these traits and individual fitness traits relevant for mass rearing (offspring number,

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139 longevity, tibia length). We then compared the traits between 24 near-isogenic strains, to obtain

140 a first insight into the broad-sense heritability of these traits. We looked for genetic correlations

141 potentially constraining the use of these traits for genetic improvement.

Methods

Laboratory rearing of T. evanescens

144 We used 24 near-isogenic lines (hereafter referred to as "lines") of *Trichogramma*

145 *evanescens*. Lines were created from inbred crosses in populations established from individuals

146 sampled in different parts of France (geographic origins detailed in Table 7 in the appendix),

147 from 2010 to 2016, and reared in the laboratory at 18 ± 1 °C, $70 \pm 10\%$ RH and 16:8 h L:D

148 (details of the protocol followed to create the lines are provided in the appendix). Genetic

149 diversity within lines was below 1.1 alleles per locus at 19 microsatellite loci (unpublished

150 data), and individuals within lines were considered genetically identical. We created two

151 sublines for each line (Lynch & Walsh, 1998), to disentangle the confounding effects of rearing

152 tubes and lines (which may be caused by maternal effects). We considered variation between

153 lines to be of genetic origin, and variation within lines to be of environmental origin. We reared

154 *Trichogramma evanescens* individuals on sterilised *Ephestia kuehniella* Zeller 1879

155 (Lepidoptera: Pyralidae) eggs, renewed every 10 days, at 25.5 ± 1 °C, $70 \pm 10\%$ RH and 16:8

156 h L:D (Schöller & Hassan, 2001). We kept populations in glass tubes (height: 73 mm, diameter:

157 11 mm), and fed adults with honey *ad libitum*.

Measurement of variables

General experimental design

160 The following experimental design was used to measure phenotypic traits in

161 *Trichogramma* females (Figure 1). We used *T. evanescens* females that had mated within the

162 last 24 hours, randomly chosen from each line. We checked the physical integrity of these

163 females, which were isolated in glass tubes before the beginning of the experiment (height: 73

164 mm, diameter: 11 mm) and fed with honey, *ad libitum*. On the first two days, we assessed the

165 behavioural traits of the females. We estimated the number of offspring on days 3 to 5, and

166 longevity from day 6. The experiment lasted from May to July 2019, and was split into 17

167 experimental sessions, in each of which, we used three females per line. The physiological,

168 developmental and behavioural traits of *Trichogramma* wasps, and of *T. evanescens* in

169 particular, are dependent on temperature (Ayvaz, Karasu, Karabörklü, Tunçbilek, 2008;

170 Schöller & Hassan, 2001). Moreover, as Suverkropp et al. (2001) showed that *T. brassicae*

171 individuals have similar levels of activity throughout the day at temperatures of about 25 °C or

172 higher, we assumed that our *T. evanescens* individuals had similar responses to temperature

173 throughout the day. Therefore, we performed the behavioural experiments at 25.5 ± 1 °C, 70 ±

174 10% RH. We then measured female longevity and offspring number at 18 ± 1 °C, 70 ± 10%

175 RH, to ensure that the females would live long enough for the final stages of the experiment

176 (Cônsoli & Parra, 1995; Schöller & Hassan, 2001).

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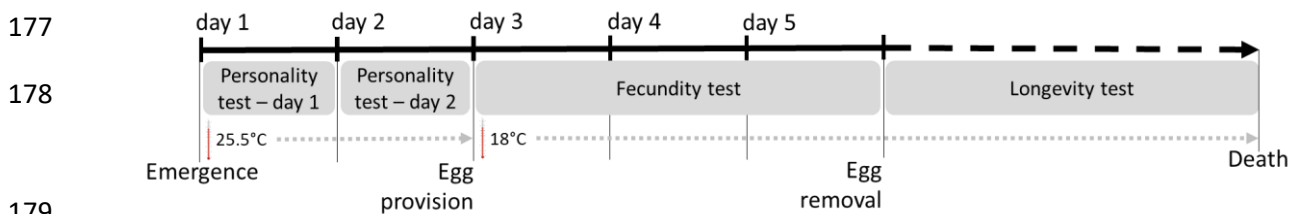
180 **Figure 1.** Overview of the experimental design, for one session.

181 *Behavioural trait measurement*

182 We observed individuals in an arena composed of two sheets of glass (24 cm x 18 cm),

183 one for the floor and one for the ceiling. The 2 mm space between them was maintained by

184 walls made of a black rubber sheet. We placed this arena on an LCD screen (Samsung© 28"

185 LED, 3840*2160 pixels, 60 Hz) displaying a white circle with a diameter of 5.5 cm on a dark

186 background (Figure 2.a). The LCD screen was turned on one hour before the beginning of the

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187   experiment, to ensure that a stable temperature of 25.5 ± 1 °C was achieved in the area. The

188   conditions in the growth chamber in which the experimental design was set up were as follows:

189   22.5 ± 1 °C and 70 ± 10% RH. We used a fine paintbrush to introduce a randomly chosen

190   female into the centre of the arena while the screen was showing a white background. The glass

191   ceiling was replaced, and we then switched to a background with a white circle on a dark

192   background, with the female positioned in the middle of the white circle. We observed the

193   behaviour of the female for 90 seconds, with video recording at 25 frames per second (with a

194   resolution of 1080 p), with a Nikon© D750 camera (Figure 2.a).

195   We then analysed the videos files, determining the 2D spatial position (x-y coordinates)

196   and body orientation (in radians) of the female on each frame, with C-trax software (Branson,

197   Robie, Bender, Perona, & Dickinson, 2009). We independently determined the exact position

198   of the border between the white circle and the black background with ImageJ software

199   (Abràmoff, Magalhães, & Ram, 2004). We thus defined regions of interest of 0.5 cm on either

200   side of the border, for investigation of the behaviour of the insect near the border (Figure 2.b).

201   We imported the C-trax and ImageJ output files into R software v.3.6.1 (R Core Team 2019)

202   and cleaned our data to remove tracking artefacts. We used the "trajr" package (Mclean &

203   Volponi, 2018) to calculate speed and sinuosity, in each region of interest. We calculated seven

204   variables linked to three personality traits — boldness, exploration and activity — as defined

205   by Réale et al. (2007).

206   Boldness is the reaction of the individual to a risky situation (Réale et al., 2007). We

207   estimated boldness by measuring three variables. The first was the time until the female first

208   entered the dark area (area 3 in Figure 2.b). Higher values indicated that the female took longer

209   to cross the border, which we interpreted as meaning that the female was less bold. The second

210   and third variables were the absolute difference in speed between areas 2 and 1 (Figure 2.b) and

211   the absolute difference in sinuosity between areas 2 and 1 (Figure 2.b). We considered high

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212 values for these two variables to indicate a larger change in behaviour at the border, which we

213 interpreted as meaning that the female was more affected by the border and was, therefore, less

214 bold. Exploration represents the individual's reaction to a new environment (Réale et al., 2007).

215 Exploration was estimated in area 1 as (1) the total area explored per unit time, and (2)

216 the mean sinuosity of the pathway (Figure 2.b). For this variable, we hypothesised that the

217 females with the most winding pathways remained closer to their release point, indicating a

218 lower level of exploration.

219 Finally, we measured activity in area 4, so exploration and activity were measured in

220 different areas of the experimental arena. Activity was estimated as (i) the proportion of time

221 the female was considered to be active (with a speed of more than 0.01 centimetres per second),

222 referred to hereafter as "activity rate", and (ii) mean speed (Figure 2.b), considering faster

223 movement to be indicative of a higher level of activity.

224 We estimated the repeatability of measurements, by conducting two observations per

225 female, with 24 hours between the two measurements, a time interval corresponding to 20% of

226 the mean lifespan of this species. Females were tested in a random order on day 1, and then in

227 the same order on day 2, to ensure that all individuals had exactly the same time interval

228 between two measurements. Between behavioural experiments, each female was placed back

229 in its glass tube and fed with honey, *ad libitum*, in a growth chamber at 25.5 ± 1 °C, 70 ± 10%

230 RH and 16:8 h L:D. Behavioural trait measurements were obtained for 776 to 996 females from

231 the 24 lines.

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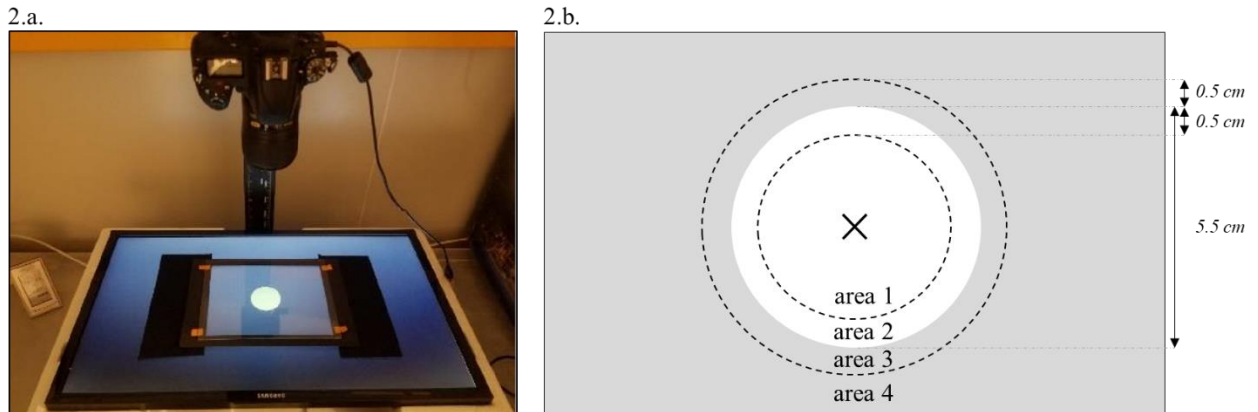
Figure 2. Experimental set-up of the behavioural experiment. Fig. 2.a. shows a photograph of the experimental setup: the LCD screen displaying the white circle on a dark background, the arena and the Nikon© D750 camera above. Fig. 2.b. represents the defined areas of the arena. The grey shading corresponds to the dark background, the white part indicates the white circle, and the dark cross is the site at which the female was placed at the start of the experiment. The dotted lines represent the virtual borders defined between areas 1 and 2, and between areas 3 and 4.

Offspring number, longevity and tibia length measurement

After the second day of behavioural observation, females were kept in their glass tubes at 18 ± 1 °C, $70 \pm 10\%$ RH and 16:8 h L:D and fed with honey, *ad libidum*. We provided each female with a piece of paper 4.50 cm x 0.85 cm in size, covered with *E. kuehniella* eggs, *ad libidum*. *E. kuehniella* eggs were removed 72 hours later and placed in conditions of 25.5 ± 1 °C, $70 \pm 10\%$ RH and 16:8 h L:D. Three days later, we counted the number of parasitised eggs (considered as black eggs), to estimate the size of the progeny of each female over a period of 72 hours, providing a proxy for female fitness. From day 6, we measured female longevity (the females were still kept in the same individual tubes with honey, but with no *E. kuehniella* eggs, at 18 ± 1 °C, $70 \pm 10\%$ RH and 16:8 h L:D). Tubes were checked every day at 5 p.m., until the death of the female. Dead females were conserved in ethanol, for subsequent measurement of tibia length on a micrograph (obtained with an Axioplan II microscope), with ImageJ software (Abràmoff et al., 2004). Images were acquired at the Microscopy Platform of Sophia Agrobiotech Institute, INRA, UNS, CNRS, UMR 1355-7254, INRA PACA, Sophia Antipolis.

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254   Not all individuals lived long enough for all the phenotypic measurements to be made. We

255   therefore collected progeny measurements for 929 females, longevity measurements for 655

256   females and tibia size measurements for 959 females, from all 24 lines.

257   ***Data analysis***

258   We used the R software v.3.6.1 for all statistical analyses. For each variable, we first

259   fitted a linear mixed model with the lme4 package (Bates, Maechler, Bolker, & Walker, 2015),

260   with individual, line, subline and session as random effects. For each variable, data

261   transformations were chosen after graphical inspection of the distribution of model residuals,

262   estimated with the "simulateResiduals" function of the DHARMa package (Hartig, 2019). We

263   performed logarithmic transformations for all behavioural variables except for the area explored

264   within area 1. We addressed several questions regarding the data, and the data analysis for each

265   of these questions is presented below.

266   *Are the measured behavioural traits repeatable?*

267   We first estimated the repeatability of the behavioural traits measured with generalised

268   linear mixed models, using the rptR package (Stoffel, Nakagawa, & Schielzeth, 2017). The

269   "rptGaussian" function of the rptR package was used to provide repeatability estimates. As

270   repeatability can be defined as the proportion of variation explained by between-individual

271   variation (Nakagawa & Schielzeth, 2010), we included only two random effects in these

272   models: individual (assuming that the effects of line and subline on variation were included in

273   the individual effect) and session, with individual as a grouping factor. In subsequent analyses,

274   we considered only traits that were significantly repeatable.

275   *Do the measured traits identify individual behavioural strategies?*

276   Based on methods generally used in animal personality studies, we first investigated

277   correlations between behavioural traits and then summarized the data by principal component

278   analysis (PCA). We first obtained a single value for each trait for each individual, by extracting,

279   from the linear mixed model described above, linear predictors for each individual, with the

280   "ranef" function of the lme4 package. We used these values to measure the phenotypic

281   correlation between traits, by calculating Spearman's rank correlation coefficients, to determine

282   whether individuals adopted different strategies, or whether it was possible to describe

283   behavioural syndromes. We estimated bootstrapped 95% confidence intervals from 1000

284   bootstraps, to assess the significance of the Spearman's rank correlation coefficients obtained

285   (Nakagawa & Cuthill, 2007), using the "spearman.ci" function of the RVAideMemoire package

286   (Hervé, 2020). *P*-values were adjusted by the false discovery rate method (Benjamini &

287   Hochberg, 1995). We then performed PCA with the "PCA" function of the FactoMineR

288   package (Le, Josse, & Husson, 2008), using both values obtained for each individual (days 1

289   and 2, when available). We estimated two synthetic personality scores based on the first two

290   axes of the PCA. We used the "fviz_pca_biplot" function of the factoextra package

291   (Kassambara & Mundt, 2019) to obtain a graphical representation of the correlation between

292   repeatable behavioural traits and the distribution of individual values along the two first axes

293   of the PCA.

294   *Are the measured traits correlated with fitness-related traits?*

295   We studied the correlation between behavioural and fitness-related traits, using the same

296   linear mixed model as described in the introduction to this section. We extracted linear

297   predictors (using the "ranef" function of the lme4 package (Bates et al., 2015)) for each

298   individual and each personality score from this model. We assessed the correlation between the

299   linear predictors of these personality traits and scores, and offspring number, body size and

300   longevity, by calculating Spearman's rank correlation coefficients. We estimated bootstrapped

301   95% confidence intervals to assess significance of the Spearman's rank correlation coefficients

302   obtained, with the same R function and method as described above. *P*-values were adjusted by

303   the false discovery rate method.

Are the measured traits heritable?

305   We sought to establish a first estimate of broad-sense heritability for each trait. To this

306   end, we followed the simple design proposed by Lynch and Walsh (1998) for clonal

307   populations, and approximated the proportion of the variance explained by genetic factors with

308   an estimate of the proportion of variance explained by the line effect in our generalised linear

309   mixed models. This estimate was obtained with the "rptGaussian" function of the rptR package

310   (Stoffel et al., 2017), with models including line, subline, individual and session as random

311   effects, and line as a grouping factor.

Do personality traits differentiate the isogenic lines?

313   We compared the personality scores of the 24 lines, taking into account variation due to

314   individual, subline and session effects. With the values of each personality score extracted from

315   the PCA (see above), we first fitted a linear mixed-effects model with the "lmer" function of

316   the lme4 package (Bates et al., 2015), with line as a fixed effect and individual, subline and

317   session effects as random effects. We performed a Tukey all-pairs comparison on lines with the

318   "glht" function of the multcomp package (Hothorn, Bretz, & Westfall, 2008). We graphically

319   represented the distribution of each line along the two personality scores, for the same PCA as

320   described above, estimated from individual values. We then used the "plot.PCA" function of

321   the FactoMineR package to represent only mean point values for each line on the graph.

Are personality traits genetically correlated with fitness-related traits?

323   We investigated the genetic correlation between genetic traits, using the same linear

324   mixed model as described in the introduction to this section. We first extracted linear predictors

325   for each line and trait, with the "ranef" function of the lme4 package. We then used these values

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326 to calculate Spearman's rank correlation coefficients. We estimated bootstrapped 95%

327 confidence intervals, to assess significance of the Spearman's rank correlation coefficients, and

328 adjusted the *p*-values as described above.

329 **Results**

330 *Are the measured behavioural traits repeatable?*

331 Repeatability estimates for the seven behavioural traits ranged from 0.04 to 0.35 (Table

332 1). The repeatability estimates had confidence intervals excluding zero for all traits except for

333 "time to first crossing of the border between the white and black areas" (Table 1). Only

334 repeatable traits were considered in the subsequent analysis.

335 **Table 1.** Estimated repeatability (R) and 95% confidence intervals (between square brackets) for behavioural traits.

336 Repeatable traits (R-value in bold type) were used to estimate personality scores.

Personality trait category	Variable assessed	R [95% CI]
Activity	Mean speed in area 4	**0.35** [0.29; 0.40]
	Activity rate in area 4	**0.08** [0.01; 0.14]
Boldness	Change of speed in the border area (area 2)	**0.10** [0.04; 0.17]
	Change of sinuosity in the border area (area 2)	**0.12** [0.04; 0.19]
	Time to first crossing of the white/black border	0.04 [0.00; 0.11]
Exploration	Sinuosity in area 1	**0.24** [0.17; 0.30]
	Area explored in area 1	**0.18** [0.12; 0.24]

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338 *Do the measured traits identify individual behavioural syndromes?*

339 All repeatable variables were correlated with at least one other variable (Table 2),

340 indicating the existence of a behavioural syndrome. We combined these six variables into two

341 personality scores based on the first two axes of a PCA, which accounted for 56.8% of the

342 variance (Table 3). The first axis (personality score 1, PC1) was positively correlated with the

343 "area explored in area 1" and inversely correlated with "sinuosity in area 1" and with the

344 "change of sinuosity in the border area 2" (Table 3). Highly positive values of PC1

345 corresponded to a high exploration score (Figure 3). The second axis (personality score 2, PC2)

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346 and correlated mostly with "mean speed in area 4", "activity rate in area 4" and the "change of

347 speed in border area 2" (Table 3). High positive values of PC2 correspond to high activity scores

348 (Figure 3).

349 **Table 2.** Phenotypic correlation between behavioural variables, with Spearman's rank correlation coefficient Rho

350 and 95 percent confidence intervals (between square brackets), based on a number of individual values from N =

351 977 to N = 1009. Correlation coefficients with confidence intervals excluding zero are shown in bold, and

352 correlation coefficients remaining significantly different from zero after Benjamini and Hochberg correction are

353 indicated with an asterisk. The personality trait category to which each variable belongs is indicated in brackets:

354 activity (A), boldness (B) and exploration (E).

	(B) Change of speed in border area 2	(A) Mean speed in area 4	(A) Activity rate	(B) Change of sinuosity in border area 2	(E) Sinuosity in area 1
(A) Mean speed in area 4	**0.31** **[0.25; 0.37] ***				
(A) Activity rate in area 4	**0.10** **[0.04; 0.16] ***	**0.38 *** **[0.32; 0.43] ***			
(B) Change of sinuosity in border area 2	**0.11** **[0.05; 0.17] ***	**0.07** **[0.01; 0.14] ***	**-0.12** **[-0.18; -0.06] ***		
(E) Sinuosity in area 1	**-0.07** **[-0.14; -0.01] ***	**0.13** **[0.07; 0.19] ***	**-0.16** **[-0.22; -0.10] ***	**0.38** **[0.32; 0.44] ***	
(E) Area explored in area 1	**0.11** **[0.04; 0.16] ***	0.01 [-0.04; 0.08]	**0.29** **[0.23; 0.34] ***	**-0.28** **[-0.34; -0.22] ***	**-0.56** **[-0.61; -0.52] ***

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356 **Table 3.** Parameters from the first two principal components (PC1 and PC2) of the PCA for the behavioural

357 variables measured. Component loadings represent the relationship between the principal components and the

358 variables from which they are constructed. The personality trait category to which each variable belongs is

359 indicated in brackets: activity (A), boldness (B) and exploration (E).

Parameter	PC1	PC2
Eigenvalue	1.87	1.54
Percentage of variance explained	31.23	25.58
Component loading		
(A) Mean speed in area 4	0.16	0.84
(A) Activity rate in area 4	0.43	0.60
(B) Change of speed in area 2	0.19	0.51
(B) Change of sinuosity in area 2	-0.56	0.34
(E) Area explored in area 1	0.81	-0.09
(E) Sinuosity in area 1	-0.81	0.27

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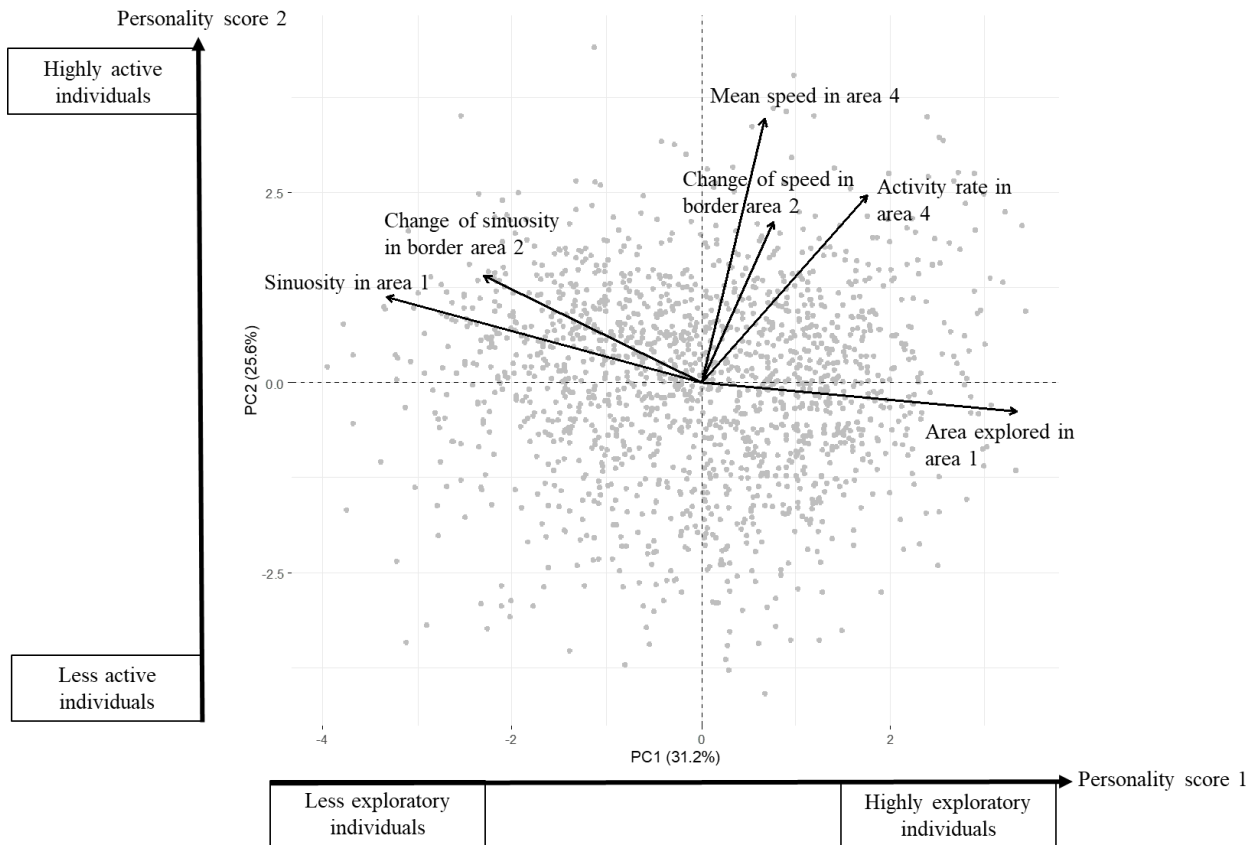
Figure 3. Graphical representation of the first two axes of the PCA on individual values (grey points) for repeatable behavioural traits (in black type).

Are the measured traits correlated with fitness-related traits?

Active females (i.e. those with higher PC2 values) had significantly larger numbers of offspring and significantly longer tibias (Table 4). Higher rates of exploration (i.e. higher PC1 values) were not significantly correlated with any of the fitness-related traits measured. None of the behavioural variables or personality scores was significantly correlated with longevity (Table 4).

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373 **Table 4.** Phenotypic correlation between behavioural traits (behavioural variables and personality scores) and

374 other life history traits (with Spearman's rank correlation coefficient Rho and 95% confidence intervals (between

375 square brackets) calculated from 959 individual values). Correlation coefficients with confidence intervals

376 excluding zero are shown in bold, and correlation coefficients that remained significantly different from zero after

377 Benjamini and Hochberg correction are indicated with an asterisk. The personality trait category to which each

378 variable belongs is indicated in brackets: activity (A), boldness (B) and exploration (E).

	Offspring number	Longevity	Tibia length
Behavioural variables			
(A) Mean speed in area 4	**0.20 [0.14; 0.26] ***	-0.05 [-0.12; 0.03]	**0.19 [0.12; 0.25] ***
(A) Activity rate in area 4	-0.01 [-0.08; 0.06]	-0.06 [-0.13; 0.02]	-0.07 [-0.13; 0.00]
(B) Change of speed in border area 2	**0.13 [0.06; 0.19] ***	-0.08 [-0.15; 0.00]	**0.16 [0.10; 0.21] ***
(B) Change of sinuosity in border area 2	**0.11 [0.04; 0.17] ***	0.002 [-0.07; 0.08]	0.05 [-0.01; 0.11]
(E) Area explored in area 1	-0.05 [-0.11; 0.01]	-0.05 [-0.13; 0.02]	-0.02 [-0.09; 0.04]
(E) Sinuosity in area 1	0.01 [-0.05; 0.07]	0.05 [-0.02; 0.13]	0.05 [-0.01; 0.11]
Personality scores			
Exploration score 1 (PC1)	-0.01 [-0.07; 0.06]	-0.05 [-0.13; 0.03]	-0.03 [-0.09; 0.03]
Activity score 2 (PC2)	**0.17 [0.10; 0.23] ***	-0.01 [-0.10; 0.07]	**0.15 [0.09; 0.21] ***

379

380 *Are the measured traits heritable?*

381 Broad-sense heritability estimates for behavioural traits and personality scores ranged

382 from 0.01 to 0.11. Confidence intervals excluded zero for all traits linked to activity and

383 exploration, whereas they included zero for the two traits linked to boldness (Table 5). Fitness-

384 related traits (offspring number, tibia length and longevity) displayed broad-sense heritability

385 ranging from 0.04 to 0.28, with all confidence intervals excluding zero (Table 5).

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391 **Table 5.** Broad-sense heritability (H²) of traits measured with 95% confidence intervals (between square brackets).

392 Heritability estimates are shown in bold if their 95% confidence interval did not include zero. The personality trait

393 category to which each behavioural variable belongs is indicated in brackets: activity (A), boldness (B) and

394 exploration (E).

	H² [95% CI]
Behavioural variables	
(A) Mean speed in area 4	**0.11** [0.05; 0.18]
(A) Activity rate in area 4	0.02 [0.00; 0.04]
(B) Change of speed in border area 2	0.01 [0.00; 0.03]
(B) Change of sinuosity in border area 2	0.01 [0.00; 0.03]
(E) Area explored in area 1	**0.06** [0.02; 0.10]
(E) Sinuosity in area 1	**0.06** [0.02; 0.11]
Personality scores	
Exploration score 1 (PC1)	**0.08** [0.03; 0.13]
Activity score 2 (PC2)	**0.05** [0.02; 0.10]
Fitness-related traits	
Offspring number	**0.12** [0.05; 0.19]
Tibia length	**0.05** [0.01; 0.09]
Longevity	**0.28** [0.14; 0.39]

395

396 *Do personality traits differentiate between lines?*

397   We found significant differences in personality scores between lines (Figure 4.a and

398 4.b), and the 24 lines were distributed along the first two axes of the PCA (Figure 5). We were

399 therefore able to distinguish between lines that were very active and exploratory (e.g., lines 3

400 and 12), and lines that were less active and exploratory (e.g., lines 14 and 21); we were also

401 able to distinguish between lines that were very exploratory but not very active (e.g., lines 9

402 and 10) and lines that were active but not very exploratory (for example line 4).
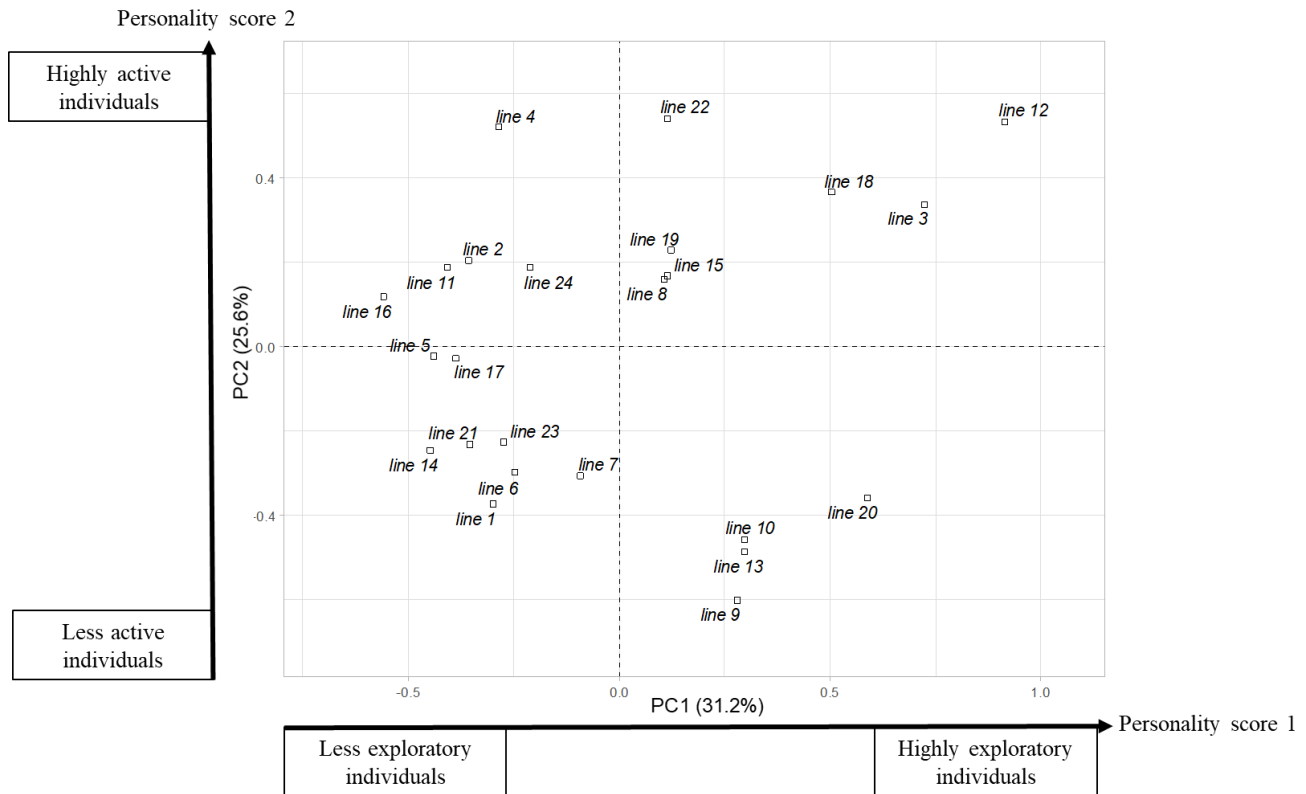
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4.a.

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4.b.

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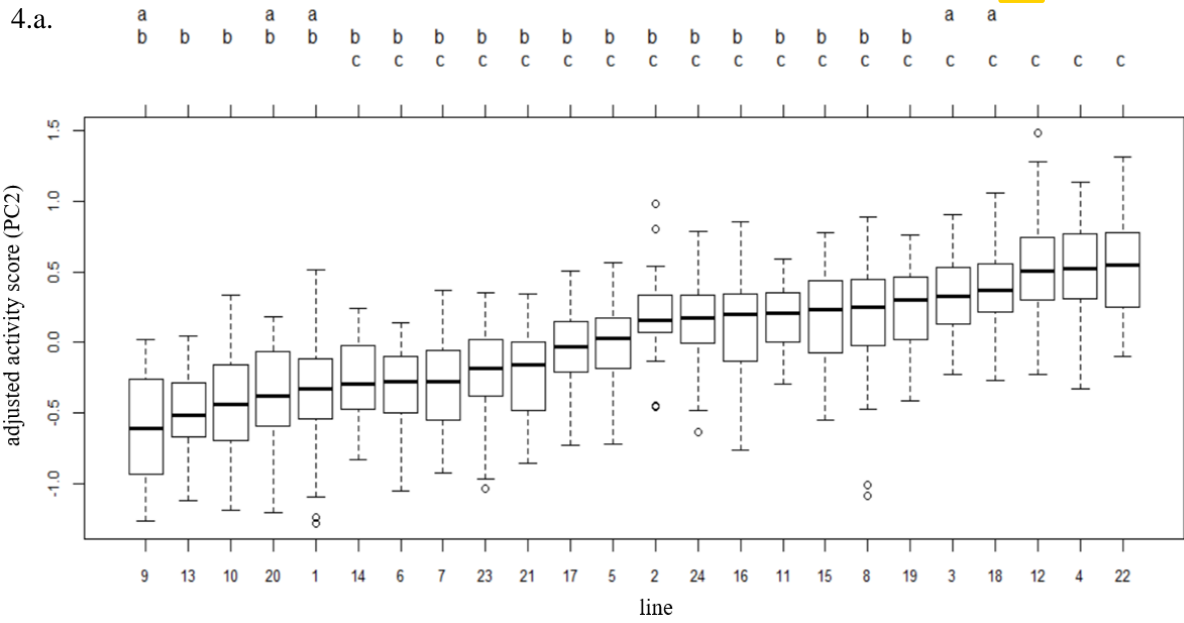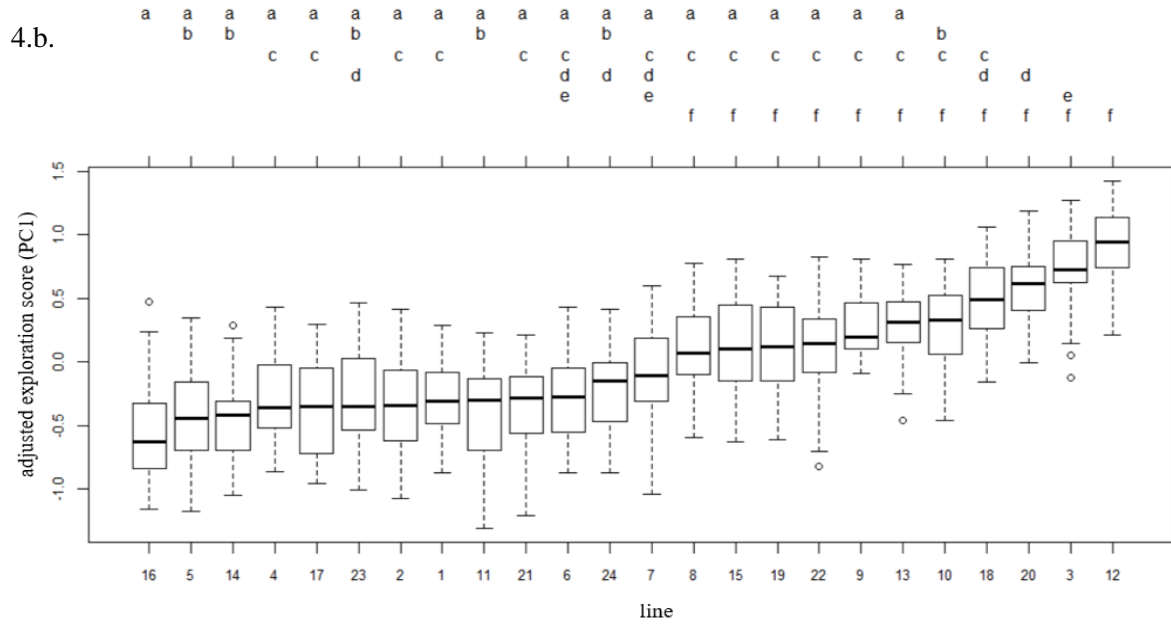
Figure 4. Boxplot of the adjusted values of personality score 1 (Fig. 4.a) and personality score 2 (Fig. 4.b) after the elimination of variation due to individual, subline and session effects, and compact letter display after Tukey all-pair comparisons. Two lines with no letters in common are considered to be significantly different (with a p-value <0.05).

409

Figure 5. Distribution of the mean points for the 24 lines (centroids) along the first two axes of the PCA.

411 *Are personality traits genetically correlated with fitness-related traits?*

412   The only genetic correlation between personality scores and fitness-related traits that

413 remained significant after FDR correction was the positive correlation between exploration

414 score (PC1) and offspring number (Table 6).

415 **Table 6.** Genetic correlation between personality and other life history traits (Spearman's rank correlation

416 coefficient, based on the trait estimates of 24 near-isogenic lines, with associated p-values in brackets). Correlation

417 coefficients with confidence intervals excluding zero are shown in bold, and correlation coefficients that remained

418 significantly different from zero after Benjamini and Hochberg correction are indicated with an asterisk. The

419 personality trait category to which each behavioural variable belongs is indicated in brackets: activity (A), boldness

420 (B) and exploration (E).

	Offspring number	Longevity	Tibia length
Behavioural variables			
(A) Mean speed in area 4	-0.16 [-0.54; 0.33]	-0.32 [-0.62; 0.06]	**0.51 [0.21; 0.70]**

(A) Activity rate in area 4	**-0.53 [-0.78; -0.14]**	0.15 [-0.28; 0.54]	-0.21 [-0.62; 0.28]
(B) Change of speed in border area 2	-0.03 [-0.44; 0.37]	**-0.45 [-0.72; -0.10]**	0.35 [-0.05; 0.63]
(B) Change of sinuosity in border area 2	0.29 [-0.11; 0.63]	0.15 [-0.31; 0.56]	0.19 [-0.20; 0.54]
(E) Area explored in area 1	**-0.60 [-0.79; -0.26] ***	0.01 [-0.40; 0.43]	-0.28 [-0.65; 0.16]
(E) Sinuosity in area 1	**0.63 [0.33; 0.82] ***	0.25 [-0.20; 0.62]	-0.03 [-0.43; 0.39]
Personality scores			
Exploration score 1 (PC1)	**-0.64 [-0.83; -0.29] ***	-0.09 [-0.52; 0.32]	-0.19 [-0.58; 0.24]
Activity score 2 (PC2)	-0.10 [-0.52; 0.39]	-0.22 [-0.66; 0.19]	**0.41 [0.01; 0.67]**

Discussion

We investigated whether animal personality could be used to develop or improve phenotyping methods for the BCA *Trichogramma evanescens*. We first developed an automated phenotyping method based on automated pathway analysis, providing a set of behavioural trait measures that proved repeatable over time and heritable (i.e. personality traits). We then identified differences in life history strategies between individuals as behavioural traits were correlated together and combined them into personality scores, which were correlated with other life history traits. Finally, we observed differences in personality scores between the 24 genotypes of *T. evanescens* and found a negative genetic correlation between exploration and fecundity.

Evidence of personality traits in Trichogramma evanescens

Personality has never before been assessed in a species as small as *Trichogramma evanescens*. Based on other video-tracking studies in other species (Branson et al., 2009; Charalabidis, Dechaume-Moncharmont, Petit, & Bohan, 2017), we designed and developed a video-tracking approach measuring a large number of variables relating to the movements of *T. evanescens* individuals during their presence in the different areas (white, black and border areas) within an experimental arena. Here, we chose to work on seven variables that (i) could be calculated with methods commonly used in trajectory and movement studies (speeds, trajectory length and sinuosity estimates) (Mclean & Volponi, 2018) and (ii) we considered to

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441 be associated with some of the commonly defined personality traits defined by Réale et al.

442 (2007): boldness, exploration and activity.

443 For each of the seven behavioural variables, we assessed repeatability, broad-sense

444 heritability and phenotypic and genetic correlations between personality traits and between

445 these traits and other life history traits, according to methods generally used in animal

446 personality studies (Réale et al., 2007). For six of the seven behavioural variables, we observed

447 significant repeatability (R) (values ranging from 0.10 to 0.35, Table 1). The R values obtained

448 were within the range of R values commonly observed for behavioural traits, although most

449 were lower than the mean R value obtained for animal behaviour (0.35) (Bell et al., 2009).

450 However, personality has rarely been studied in parasitoid insects, and a recent study on the

451 parasitoid wasp *Venturia canescens* reported a similar R value for activity and a lower R value

452 for exploration (about 0.10, whereas we obtained R values for exploration-related variables of

453 0.18 and 0.24 (Gomes et al., 2019)).

454 The broad-sense heritability of the variables (ranging from 0.06 to 0.11, Table 5) was

455 lower than the mean value for animal behaviour (0.24) in the meta-analysis by Dochtermann et

456 al. (2019). Stirling et al. (2002) found no significant differences in heritability between

457 behavioural and life-history traits in their meta-analysis, whereas we found that heritability

458 values for personality traits were lower than heritability values of two classical fitness-related

459 traits (offspring number and longevity) in *T. evanescens* (Table 5).

460 Behavioural traits could be grouped together into two continuums or behavioural

461 syndromes (Réale et al., 2007; Sih et al., 2004; Sih, Cote, Evans, Fogarty, & Pruitt, 2012): a

462 continuum extending from individuals with low levels of exploratory behaviour to highly

463 exploratory individuals, and a continuum extending from individuals with low levels of activity

464 to highly active individuals (Figure 3). Bold (or shy) behaviour and active behaviour have been

465 shown to be correlated with fecundity traits in several species (Biro & Stamps, 2008), but rarely

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466 in insects (Monceau et al., 2017). In this study, we found a weak but significant phenotypic

467 correlation between behavioural traits, fecundity and body length, as shy or active females

468 produced more offspring, and had longer tibias (Table 4). Finally, an analysis of genetic

469 correlations showed that the lines with the most exploratory individuals had the smallest

470 numbers of offspring (Table 6). These correlations seem to be compatible with the pace-of-life

471 syndrome (POLS) hypothesis, a currently debated hypothesis (Royauté, Berdal, Garrison, &

472 Dochtermann, 2018), according to which, behavioural traits are related to morphological,

473 physiological and other life-history traits (Réale et al. 2010).

474 ***Potential of personality traits for use in genetic improvement of the biocontrol agent***

475 **Trichogramma evanescens**

476 In this study, our aim was to evaluate the possibility of using personality traits as traits

477 of interest in biological control, and of integrating these traits into genetic improvement

478 programmes for the BCA *T. evanescens*. The six repeatable behavioural traits we measured

479 were correlated with each other, and could be combined into two continuums. For each

480 individual and continuum, we estimated a personality score corresponding to the position of the

481 individual along the continuum, a common method in animal personality studies (Mazué et al.,

482 2015; Monceau et al., 2017). We found that it was possible to capture a large proportion of the

483 behavioural trait variance with two scores (36.2% of the total variance explained by personality

484 score 1, and 26.4% explained by personality score 2). This finding highlights the utility of

485 calculating a few synthetic indices (or scores), rather than measuring large numbers of

486 variables, to obtain relevant information for BC. We therefore systematically present our results

487 considering all the traits individually and summarized as two personality scores.

488 The relevance of the behavioural traits or synthetic scores to the context of BC was

489 demonstrated by the phenotypic correlations between these traits and scores and the traits

490 classically measured in BC (fecundity, longevity and body length) (Hopper et al., 1993; Prezotti

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491 et al., 2004; Roitberg et al., 2001; Smith, 1996). In this study, active females (i.e. with high

492 values for "mean speed in area 4" and "personality score 2") produced more offspring and had

493 longer tibias (Table 4). By contrast, we found that bold females (i.e. with low values for "change

494 of speed in border area 2" and "change of sinuosity in border area 2") produced a small number

495 of offspring (Table 4). In several species, activity and boldness behaviours have been shown to

496 be correlated with traits of ecological importance, such as dispersal (Sih et al., 2004), which is

497 also a trait linked to field efficiency in BC (Fournier & Boivin, 2000). Our results indicate that

498 active females produce more offspring, which is predictive of a high degree of efficiency in

499 rearing conditions and in the field. Note, however, that we did not assess survival or body

500 condition in the offspring. The same females also displayed shyer behaviour, which would

501 presumably have a negative impact on field efficiency. Further studies are, therefore, required

502 to assess the full ecological relevance of these lines in BC. The relevance of the variables

503 measured will be confirmed only if they are shown to be correlated with BC performance in

504 industrial and field conditions.

505 Most of our data analyses aimed to evaluate the added value of the measured behavioural

506 traits for genetic improvement strategies, breeding programs. We found that personality scores

507 differ among isogenic lines (Figure 4.a. and Figure 4.b.) and that these differences highlight

508 contrasted behaviours, as evidenced by their distribution along the two personality scores in

509 Figure 5. This may make it possible to differentiate between these behaviours and to select for

510 them, should they prove relevant in terms of BC efficiency. We also observed a negative genetic

511 correlation between the personality score relating to exploration and offspring production. It

512 will probably be important to take this trade-off into account in BC, as it may oppose

513 performance in rearing and performance in the field. Indeed, as for activity and boldness,

514 exploration behaviours are also correlated with traits linked to field efficiency in BC, such as

515 dispersal (Fournier & Boivin, 2000; Sih et al., 2004).

516   Given these results, and the ease with which all the traits can be assessed and personality

517   scores obtained through short (90 seconds) automated video-tracking measurements, the new

518   method described here may provide useful criteria for the selection of candidate BCA taxa

519   (populations, strains, sibling species, etc.) or for quality control purposes. However, the high

520   level of intra-isogenic line variability observed (Figure 4.a and Figure 4.b), accounting for the

521   relatively low broad-sense heritability of the traits and scores (between 0.01 and 0.11; Table 5),

522   constrains the use of this method, as it may be necessary to phenotype large numbers of

523   individuals for reliable comparisons between taxa or reared populations. The low heritability

524   also constitutes an obstacle to the implementation of ambitious experimental evolution

525   programmes. Oriented experimental evolution may be fastidious for traits displaying such a

526   high degree of environmentally induced variability. As a comparison, breeding programmes for

527   livestock animals generally make use of traits with higher heritability. Heritability values for

528   morphological, physiological, behavioural or other traits linked to fitness and considered in

529   these breeding programmes generally range from 0.17 to 0.70 in sheep, pigs, cows and fish

530   (Juengel et al., 2019; Kavlak & Uimari, 2019; Moretti, de Rezende, Biffani, & Bozzi, 2018;

531   Vargas Jurado, Leymaster, Kuehn, & Lewis, 2016).

532   **Conclusion**

533   In conclusion, the use of methods and concepts of animal personality to develop

534   phenotyping methods and associated data analyses for BC led to the rapid phenotyping of traits

535   rarely used in BC that were repeatable, heritable and correlated with fitness-related traits.

536   However, it will be possible to consider the actual potential of these traits and of the

537   phenotyping method satisfactory only after investigating the relationships between the

538   laboratory-measured traits and BC performance indices in real BC situations, in industrial

539   production settings or in field releases. This first study has driven the launch of large-scale field

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540 experiments, which are currently underway and aim to generate field-release performance

541 indices.

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Conflict of interest disclosure

558 The authors of this article declare that they have no financial conflict of interest with the content

559 of this article.

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