Phylogenetic conservation of behavioral variation and behavioral syndromes

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1   **Abstract**

2   Individuals frequently differ consistently from one another in their average behaviors (i.e.

3   "animal personality") and in correlated suites of consistent behavioral responses (i.e.

4   "behavioral syndromes"). However, understanding the evolutionary basis of this

5   (co)variation has lagged behind demonstrations of its presence. This lag partially stems

6   from comparative methods rarely being used in the field. Consequently, much of the

7   research on animal personality has relied on "adaptive stories" focused on single species

8   and populations. Here we used a comparative approach to examine the role of phylogeny in

9   shaping patterns of average behaviors, behavioral variation, and behavioral correlations. In

10   comparing the behaviors and behavioral variation for five species of Gryllid crickets we

11   found that phylogeny shaped average behaviors and behavioral (co)variation. Variation in

12   average exploratory behavior and response to cues of predator presence attributable to

13   phylogeny was greater or comparable to the magnitude of "personality variation".

14   Likewise, magnitudes of variation were concordant with evolutionary relationships and

15   behavioral correlations were consistent across species. These results suggest that

16   phylogenetic constraints play an important role in the expression of animal personalities

17   and behavioral syndromes and emphasize the importance of examining evolutionary

18   explanations within a comparative framework.

19   **Introduction**

20   Behavioral syndromes, correlations between behaviors at the among-individual

21   level (Dingemanse et al. 2012), have been documented across taxa (Brommer and Class

22   2017). Behavioral syndromes can conceptually be thought of as correlations between

23   individual averages and stem from underlying genetic correlations and correlations due to

24   developmental plasticity and other sources of permanent environmental covariance

25   (Dingemanse et al. 2012, Dingemanse and Dochtermann 2014). Among-individual variation

26   in behavior, often referred to as "personality variation", has been found to be similarly

27   ubiquitous (Bell et al. 2009). Similar to behavioral syndromes, this personality variation

28   can be thought of as variation across individuals in their average behaviors and likewise

29   stem from genetic and permanent environmental variation (Dingemanse and Dochtermann

30   2013, Dochtermann et al. 2015). Attempts to infer whether general taxonomic patterns

31   exist for both personality variation and behavioral syndromes have generally been

32   conducted via literature reviews and meta-analyses (Bell et al. 2009, Dochtermann 2011,

33   Garamszegi et al. 2012, 2013, Dochtermann et al. 2015, Brommer and Class 2017). These

34   synthesis efforts have shown that among-individual variation is common (average

35   repeatability ~ 0.37, Bell et al. 2009), that the magnitude of behavioral syndromes is

36   generally weak (average r ~ 0.19, Garamszegi et al 2012, 2013), and that there is general

37   alignment between nested patterns of correlations at the phenotypic, among-individual,

38   within-individual, and genetic levels (Dochtermann 2011, Brommer and Class 2017).

39   Despite the observation that both among-individual variation and behavioral

40   syndromes are common, we have a poor understanding of the evolution of either. This gap

41   in our understanding is partly because comparative approaches have rarely been used in

42   studies of among-individual behavioral variation and behavioral syndromes (White et al.

43   2020), despite having been the backbone of studies of morphological evolution. Such

44   approaches allow for direct comparison across species of behavioral (co)variation and are

45   necessary for a proper understanding of the importance of phylogeny in shaping

46   "personality" and behavioral syndromes (Royauté et al. 2020, White et al. 2020).

47   Direct assessment of evolutionary hypotheses can also be extended to the study of

48   personality and behavioral syndromes: both among-individual variation and behavioral

49   syndromes have clear connections to quantitative genetic parameters; specifically, additive

50   genetic variation, and additive genetic covariances (Dochtermann and Roff 2010,

51   Dingemanse and Dochtermann 2014). The mathematical relationships between among-

52   individual (co)variances and additive genetic (co)variances (Boake 1989, Dingemanse and

53   Dochtermann 2014, Dochtermann et al. 2015) allows the extension of predictions from

54   quantitative genetics to among-individual variation and behavioral syndromes.

55   One such prediction is that differences in the magnitude of variation present for a

56   trait might be attributable to differences in selection between populations or species.

57   Specifically, Mousseau and Roff (1987) argued that traits with low heritability might be

58   indicative of strong selection having eroded genetic variation. Likewise, because among-

59   individual variation represents the sum of additive genetic variation, dominance (and other

60   epistatic) genetic variation, and permanent environmental variation (e.g. irreversible and

61 developmental plasticity), selection is expected to deplete this variation. Note, however,

62 that drift is often also expected to reduce genetic and, therefore, among-individual

63 variation.

64   Selection is likewise expected to shape additive genetic covariances and

65 correlations, both by the loss of variation in single traits and changes to the magnitude and

66 directions of covariances (Roff 1997). For example, correlational selection is expected to

67 produce genetic correlations (Phillips and Arnold 1989, Armbruster and Schwaegerle

68 1996). As in the case of among-individual variances, these effects on genetic correlations

69 are expected to carry over to behavioral syndromes. In other words, behavioral syndromes

70 are expected to reflect the effects of selection on genetic correlations. Therefore, if

71 behavioral syndromes differ across populations, species, or other groupings, then this

72 suggests differences in genetic correlations and correlational selection (i.e. the "adaptive"

73 hypothesis, Bell 2005). In contrast, if behavioral syndromes are conserved across groups

74 then this would suggest that either behavioral syndromes stem from pleiotropic effects (i.e.

75 the "constraints" hypothesis, Bell 2005) or that selection is similar across groups.

76   While these topics have been addressed for other types of traits, particularly

77 morphological and chemical characteristics (Aguirre et al. 2014, Hine et al. 2014,

78 McGlothlin et al. 2018), addressing them for behavior remains important for several

79 reasons. First, considerable behavioral research assumes an adaptive framework for both

80 among-individual variation and behavioral syndromes, thereby minimizing the importance

81 of phylogeny and minimizing the potential role of phylogenetic constraints. Second,

82 behaviors, life-history, and physiological traits exhibit substantially lower heritabilities

83 than do morphological traits (Mousseau and Roff 1987, Stirling et al. 2002, Dochtermann et

84 al. 2019). Consequently, the role of phylogeny and selection in constraining and shaping

85 morphology may not generalize to traits with lower heritabilities and thus greater

86 plasticity.

87   Here, we compared the behavior of five closely related cricket species: *Gryllus*

88 *integer, Gryllus assimilis, Gryllus lineaticeps, Gryllodes sigillatus,* and *Acheta domesticus*. For

89 each species we measured exploratory behavior and response to cues of predator

90 presence. By working with the same behavioral assays in five closely related species we

91   were able to assess the importance of phylogeny for average behaviors and to evaluate

92   predictions about trait (co)variation. Specifically, we addressed the following questions:

93   1. Does the average expression of behavior differ among species?

94   We predicted that species would differ but do so in a manner constrained by

95   phylogeny. Put another way, more closely related species will have more similar

96   average behaviors.

97   2. Do among-individual variances differ among species?

98   We did not have species level predictions but, because selection and drift should

99   both reduce among-individual variance, we predicted that among-individual

100   variation would differ across species independent of phylogeny.

101   3. Do within-individual variances differ among species? Within-individual variation,

102   typically disregarded as residual variation, includes phenotypic plasticity—

103   specifically reversible plasticity or "phenotypic flexibility" not captured by factors

104   and covariates of a statistical model (Piersma and Drent 2003, Whitman and

105   Agrawal 2009, Piersma and Van Gils 2011, Westneat et al. 2015, Berdal and

106   Dochtermann 2019). Differences across groups in the magnitude of within-

107   individual variation therefore are, in part, differences in the magnitude of plasticity.

108   We did not have *a priori* expectations as to species differences or phylogenetic

109   signal for within-individual variances.

110   4. Do behavioral syndromes differ among species?

111   Because behavioral syndrome structure has been conserved at the genetic level

112   across cricket populations of *G. integer* (Royauté et al. 2020), we predicted that

113   syndromes would similarly be phylogenetically conserved and shared across

114   species.

115   **Methods**

116   *Cricket Acquisition, Housing, and Rearing Conditions*

117   Data used in this study were originally collected for various studies investigating the

118   effects of development on behavioral variation and the presence of behavioral constraints

119   and behavioral syndromes (Royauté et al. 2019, Royauté et al. 2020). *A. domesticus* males

120   and females were obtained as nymphs (~ 1 mm in size) from a commercial supplier

121   (Fluker's Cricket Farm, Port Allen, LA, U.S.A.) and were measured once mature. *G. integer*

122   females were captured in Aguila, AZ, *G. lineaticeps* males and females were caught in

123   Dunnigan, CA, and the *G. assimilis* males and females were caught in Maricopa County, AZ.

124   These species were all captured during the summer of 2017. *G. sigillatus* individuals were

125   taken from an outbred population established by S. Sakaluk with crickets collected from

126   California and currently maintained in Fargo, ND. For *G. lineaticeps* and *G. assimilis*, the

127   same individuals that were caught in the field were measured, while lab reared offspring of

128   *G. integer* were measured. All species were reared under a 12:12 light: dark photoperiod at

129   a temperature of 25-28°C. All individuals were housed in 0.71-liter containers with

130   transparent covers that included food, shelter, and water filled glass vials plugged with

131   cotton balls. *A. domesticus* were exposed to a mixture of high and low quality diets

132   described in Royauté et al. (2019), while all other species included in this study were fed ad

133   libitum food (commercially purchased chicken feed).

134   *Behavior Trials*

135   To measure exploratory behavior and anti-predator responses we repeatedly

136   recorded individuals' activity levels in an open field arena, followed by their responses to

137   cues of predator presence created from diluted *Eublepharis macularius* excreta (see details

138   below). *A. domesticus* were measured between March 2015 and October 2016, *G. lineaticeps*

139   were measured from August 2017 to September 2017, *G. assimilis* were measured between

140   September 2017 and October 2017, *G. integer* were measured between May 2018 and June

141   2018, and *G. sigillatus* were measured in May 2019. All trials were conducted in a plastic

142   arena (60 cm x 60 cm and 15 cm high) with a Plexiglas lid. The arena was split into four 30

143   cm × 30 cm arenas separated by a divider, allowing up to four crickets to be tested at one

144   time. Open field trials were always conducted first followed by antipredator response trials

145   either immediately after or on another day to minimized potential carryover effects from

146   exposure to cues of predator presence. After each behavioral assay, arenas were

147   thoroughly cleaned with 70% ethanol wipes to avoid accumulation of any chemical traces

148   of conspecifics. Mass at the time of behavioral trials was recorded to the nearest 1 mg. All

149   individuals were measured in each assay for a maximum of three repetitions, with some

150   individuals measured fewer times due to escape or natural mortality (Table 1). In total, we

151   conducted 2478 behavioral assays across a total of 460 individuals (Table 1).

152

Table 1. Number of individuals, by species, for which behavior was assayed in a first,
second, and third repetition.

Species	Behavioral Assay	Repetition 1	Repetition 2	Repetition 3	Total Trials
Acheta domesticus	Open field	281	263	225	769
	Antipredator	262	235	220	717
Gryllus assimilis	Open field	16	16	16	48
	Antipredator	16	16	15	47
Gryllus integer	Open field	92	91	74	257
	Antipredator	88	88	72	248
Gryllus lineaticeps	Open field	21	17	11	49
	Antipredator	21	13	11	45
Gryllodes sigillatus	Open field	50	50	49	149
	Antipredator	50	50	49	149
Total		896	837	743	2478

153   *Open field behavior*

154   Individual crickets were left to rest for 30 seconds under a 5 cm diameter cup after

155   being introduced into the lower right section of the arena (Figure S1). After these 30

156   seconds we allowed the individuals to move freely through the arena for 220 seconds. We

157   measured each individual's exploratory propensity by calculating the number of *unique*

158   *zones* visited (UZ) by the cricket with Ethovision X (Noldus Information Technology,

159   Wageningen, The Netherlands). This behavioral protocol has previously been used with *A.*

160   *domesticus* and *G. integer* to evaluate genetic and individual differences in activity and

161   exploratory behaviors (Royauté et al. 2015, Royauté and Dochtermann 2017, Royauté et al.

162   2019, Royauté et al. 2020).

163   *Predator cue response*

164   To measure responses to cues of potential predator presence, we collected excreta

165   from three adult leopard geckos, *Eublepharis maculariu*s, that were fed a mixed diet of *A.*

166   *domesticus, G. sigillatus, G. lineaticeps*, *G. integer*, and *G. assimilus*. Leopard geckos were

167   housed according to the standards of the Institutional Animal Care and Use Committee of

168 North Dakota State University (Protocol A14006, A17015, and A19067) and the Animal

169 Behavior Society (2020). Collected excreta was frozen and then finely ground and diluted

170 with deionized water (1 ml H2O: 5 mg of excreta). This solution was then applied to 15 cm

171 diameter filter paper disks with a 5 cm diameter central cutout that allows crickets to be

172 left to rest unexposed to the predator cues (Royauté and Dochtermann 2017, Royauté et al.

173 2019, Royauté et al. 2020). Each predator cue disk was left to dry for a minimum of 2 hours

174 then stored at -23°C until needed for trials. Predator cue disks were allowed to warm to

175 room temperature before use in antipredator trials and discarded after a single use.

176 Between each trial, cue disks were stored at 4°C for a maximum of 14 days.

177 We placed the predator cue disk at the bottom of a 15 cm diameter arena and left

178 the cricket to rest for a minimum of 30 seconds under a 5 cm diameter cup in the

179 nontreated central cutout. We then removed the cup and allowed the cricket to move freely

180 for 220 seconds and estimated the distance travelled in cm (AP distance) using Ethovision

181 X (Figure S1). Previous studies with this protocol show that crickets had heightened

182 activity levels in the presence of this diluted gecko excreta compared to water controls

183 (Royauté and Dochtermann 2017). Consistent with this, *G. sigillatus* crickets have been

184 found to increase their activity after direct exposure to predators (Bucklaew and

185 Dochtermann 2020). Greater activity during these antipredator response assays, i.e.

186 greater *AP distance*, was therefore interpreted as a greater responsiveness to predator

187 cues.

188 *Data Analysis: Univariate Models*

189 To assess differences in behavioral responses between species for means and

190 variances we analyzed behavioral data using separate univariate mixed-effects models for

191 unique zones visited and AP distance (square root transformed). We included species,

192 temperature (Celsius, mean centered), mass (using among- and within-individual centering

193 (Van de Pol and Wright 2009)), and sex as fixed effects. Individual ID was included as a

194 random effect. We compared the fit of four univariate mixed models structured as follows:

195   1) Model 1: V_i = & V_w = A null model where the among- (V_i) and within-individual (V_w)

196   variances were kept constant between species.

2) Model 2: $V_i \neq$ & V_w = A model where the among-individual variance differed between species, but the within-individual variance was kept constant.

3) Model 3: V_i = & $V_w \neq$ The within-individual variance differs between species, but the among-individual variance was kept constant.

4) Model 4: $V_i \neq$ & $V_w \neq$ Both the among and within-individual variances were allowed to vary between species.

These models were specified using the `MCMCglmm` package for Bayesian mixed models (Hadfield 2010) using Markov-chain Monte Carlo (MCMC) with 1.3 million iterations, 300,000 iteration burn-in, a thinning interval of 1000, and an inverse-Wishart prior. AP distance and unique zone models were fit with Gaussian and Poisson error distributions, respectively.

To determine whether species differed in average behavior, Models 1 and 4 were run with and without species as a fixed effect and compared based on deviance information criterion (DIC) values. If species differ in average behavior, models with species included as a fixed effect would be expected to have lower (DIC) values. Average behavioral differences among species reported in the Results section were then qualitatively assessed using posterior-modal estimates for each species (Congdon 2006).

We then compared DIC values among models 1 through 4 to determine whether either among- or within-individual variances differed among species following Royauté et al. (2019) and Royauté and Dochtermann (2020). The model with the lowest DIC value was considered the best model and models with Δ DIC>5 were considered to have a substantively poorer fit (Barnett et al. 2010). Models with ΔDIC<5 were considered as having comparable support relative to the best model (Barnett et al. 2010). All models were specified with the same fixed effect structure as specified above to prevent biased estimates of variance components and repeatability (Spiegelhalter et al. 2003, Nakagawa and Schielzeth 2010b, Westneat et al. 2011).

Data Analysis: Phylogenetic Signal

As our primary questions were about differences in behavioral averages and variances, our results and discussion focus on the above model comparisons. However, we also calculated the variation in behavior directly attributable to phylogeny. To do so, we fit

227   mixed effects models with the same fixed effects, prior structure, and chain parameters as

228   above but omitting Species as a fixed effect. Species was instead incorporated as a random

229   effect, along with individual ID, with the relationship among species modeled according to

230   the current phylogeny (Figure 1, Weissman and Gray (2019)). From these models we then

231   estimated the strength of phylogenetic signal as the proportion of variation attributable to

232   the hierarchical pattern of relatedness among species (i.e. λ, Pagel 1999, Hadfield and

233   Nakagawa 2010, Nakagawa and Santos 2012). From the same models we also estimated

234   the proportion of variation attributable to among-individual differences (i.e. τ, repetability,

235   Dingemanse and Dochtermann 2013). We estimated both phylogenetic signal and

236   repeatability as unadjusted values; that is, we included the variation attributable to fixed

237   effects in the ratio denominator (Nakagawa and Schielzeth 2010a).

238   *Data Analysis: Bivariate Models*

239   Behavioral syndromes were estimated using bivariate mixed-effects models with

240   unique zones traveled and AP distance as response variables, also using the `MCMCglmm`

241   library (Hadfield 2010), and analyzed separately for each individual species. We fit models

242   using temperature (Celsius, mean centered), mass (using among- and within-individual

243   centering on subjects (Van de Pol and Wright 2009)), and sex as fixed effects and individual

244   ID was fit as a random effect. These models were fit with 2.6 million iterations, a 600,000

245   burn-in period, a thinning interval of 2000, and a prior that was flat for correlations.

246   Among-individual correlations were estimated for all species, while within-individual

247   correlations were only assessed when individuals were measured for unique zones

248   traveled and antipredator activity during the same testing period (Dingemanse and

249   Dochtermann 2013). Consequently, we were unable to assess within-individual covariation

250   of *G. lineaticeps* and *G. assimilis* due to the fact that these species were not measured for

251   each behavior in immediate succession. Because model comparisons as used above for

252   single traits could not be conducted for correlations (due to software imposed model

253   limitations), differences in behavioral correlations across species were assessed based on

254   whether 95% HPD intervals overlapped. Overlap of 95% intervals is an over-conservative

255   comparison metric (Royauté and Dochtermann 2020), but this did not affect our species

256   comparison results here. All analyses were conducted in R 3.4.4 (Team 2018).

Results

Differences in average behavior among species

259   Species differed in average behaviors: the inclusion of species as a fixed effect

260 substantially improved model fit for both behaviors (Table 2, Table S1). The monophyletic

261 group of *G. assimilis*, *G. integer*, and *G. lineaticeps* exhibited the lowest number of unique

262 zones visited (Figure 1a) but differences in AP distance were less obviously associated with

263 phylogenetic structure (Figure 1b). Consistent with this, phylogenetic signal was stronger

264 for unique zones visited (λ : 0.27) than for AP distance (λ : 0.16; Table S2).

Table 2. DIC values for statistical models with and without the inclusion of species as a fixed effect. The effect of species was evaluated in a model where variances did not (Model 1) or did (Model 4) differ by species. For both behaviors and both models, the inclusion of species substantially improved model fit, as indicated by the lower DIC values for models with species included as a fixed effect.

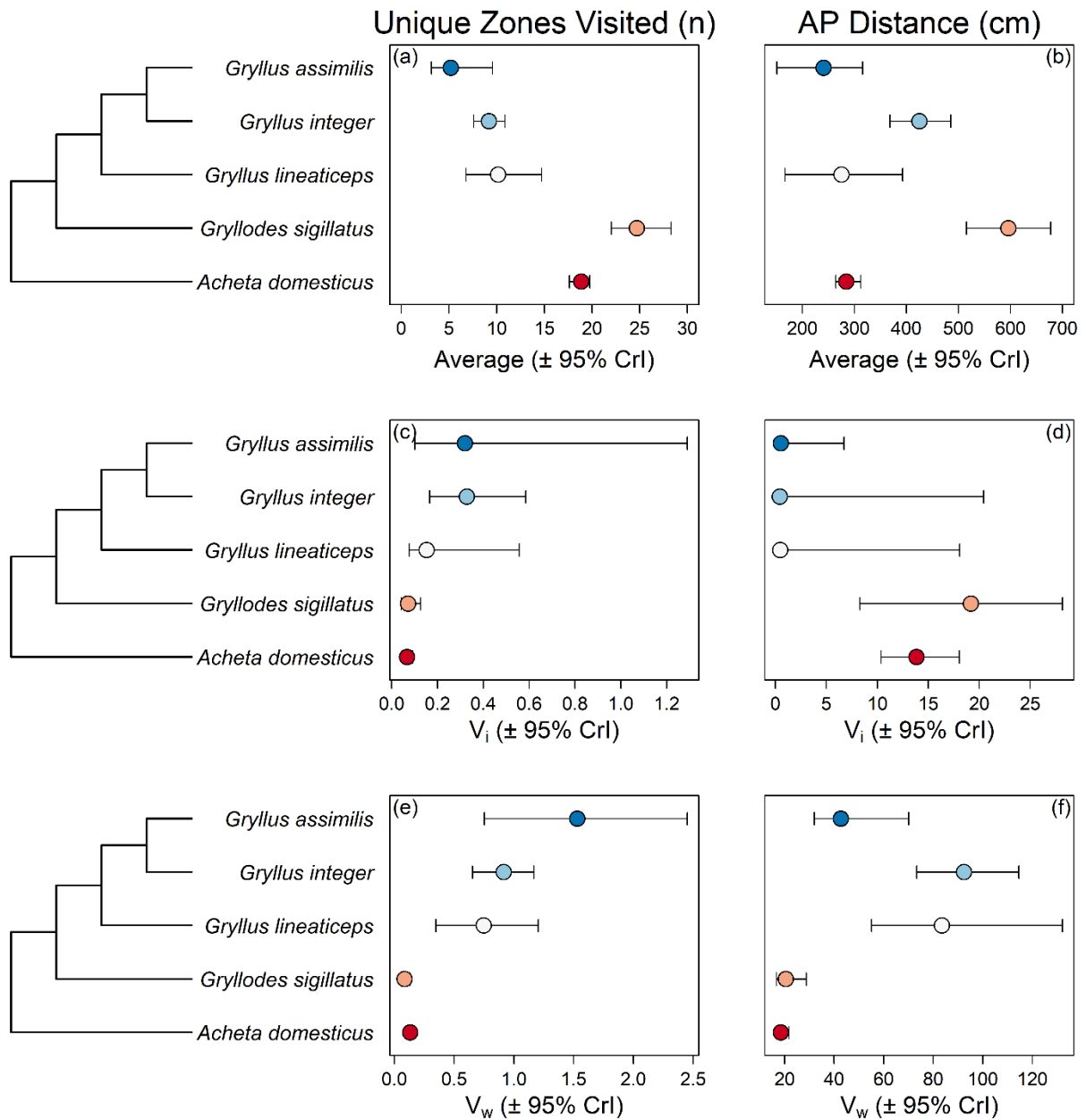|	**Behavior**	**DIC with species**	**DIC without species**	**DIC(without) – DIC(with)**
Model 1 (V_i = & V_w =)	AP Distance	8025.51	8058.71	32.2
	Unique Zones Visited	8982.97	8456.17	526.8
Model 4 (V_i ≠ & V_w ≠)	AP Distance	7763.82	7780.94	17.12
	Unique Zones Visited	8338.21	8344.88	6.67

Figure 1. Species posterior-modal values with 95% HPD credibility intervals. (a) Average unique zones visited. (b) Average AP distance in centimeters. (c) Among-individual variances in unique zones traveled. (d) Among-individual variances in AP distance. (e) Within-individual variances in unique zones traveled. (f) Within-individual variances in AP distance.

Differences in variances among species

The best fit model for unique zones visited was Model 4, which allowed both among and within-individual variances to vary across species. All other models were poorly supported (ΔDIC>8; Table 3). This indicates that both among- and within-individual

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269   variances differed among species in open field trials. For AP distance, Models 3 and 4 fit

270   comparably well (Table 3). Both of these models support differences among species in

271   within-individual variances for AP distance. The difference between the models therefore

272   suggests mixed support for species differences in among-individual variances for AP

273   distance.

274   The monophyletic group of *G. assimilis, G. integer,* and *G. lineaticeps* exhibited higher

275   among-individual variation for unique zones visited and lower among-individual variation

276   for AP distance (Figure 1c & d). This monophyletic group also exhibited higher within-

277   individual variation for both unique zones visited and AP distance than observed for *A.*

278   *domesticus* and *G. sigillatus* (Figure 1e & f).

Table 3. DIC and ΔDIC values of model fit for AP distance and unique zones visited.

Model (variance constraints)	Behavior	DIC	ΔDIC
Model 1 (V_i = & V_w =)	Unique Zones Visited	8982.97	644.76
Model 2 (V_i ≠ & V_w =)	Unique Zones Visited	8420.69	82.48
Model 3 (V_i = & V_w ≠)	Unique Zones Visited	8346.44	8.23
Model 4 (V_i ≠ & V_w ≠)	**Unique Zones Visited**	**8338.21**	**0**
Model 1 (V_i = & V_w =)	AP Distance	8025.51	263.31
Model 2 (V_i ≠ & V_w =)	AP Distance	8010.04	247.84
Model 3 (V_i = & V_w ≠)	**AP Distance**	**7762.20**	**0**
Model 4 (V_i ≠ & V_w ≠)	AP Distance	7763.82	1.62

279   *Differences in behavioral correlations among species*

280   Among-individual behavioral correlations were of similar magnitude for *A.*

281   *domesticus, G. assimilis, G. lineaticeps*, and *G. sigillatus* (0.3 : 0.5, Figure 2a, Table S3) while

282   the correlation for *G. integer* was estimated to be slightly higher (0.66, Figure 2a, Table S3).

283   Importantly, the lower bounds of the HPD intervals for *G. assimilis, G. integer, G. lineaticeps*,

284   and *G. sigillatus* also overlapped with 0 (Figure 2, Table S3). This is perhaps unsurprising

285   given the small sample sizes for *G. assimilis* and *G. lineaticeps*.

286   Behavioral correlations at the within-individual level ranged from 0.1 to 0.35 for *A.*

287   *domesticus*, *G. sigillatus*, and *G. integer*, with *G. integer* having the lower bound of its HPD

288   interval overlapping with 0 (Figure 2b). The overlapping of 0 indicates that behavioral

289   plasticity might not be integrated in this species. Behavioral correlations at either level did

290   not show obvious patterns relative to phylogeny and were not significantly different across
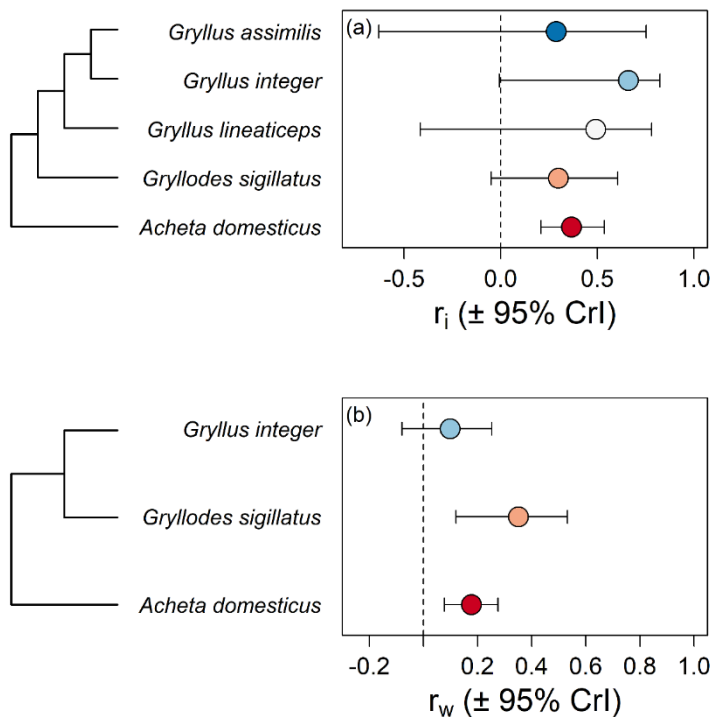
291   species (Figure 2).

Figure 2. Species posterior-modal values with 95% HPD credibility intervals. (a) Among-individual behavioral correlations of unique zones visited and AP distance. (b) Within-individual differences of behavioral correlations of unique zones traveled and AP distance. Within-individual correlations for *G. assimilis* and *G. lineaticeps* were not calculated as behavior trials were not performed in close succession.

292   **Discussion**

293   Our results demonstrate that species differed in their exploratory behavior and

294   response to cues of predator presence at all levels of variation but that behavioral

295   syndromes were conserved across species. These results suggest an important influence of

296   phylogenetic constraints on how behaviors evolve.

297   Species differed from one another in their average behaviors (Table 2), in a manner

298   consistent with phylogenetic relationships. Specifically, the monophyletic group of *G.*

299   *assimilis, G. integer,* and *G. lineaticeps* were generally similar in average unique zones

300   visited (Figure 1a). In contrast, while average AP distance differed by species, it did not do

301   so in a manner clearly concordant with phylogeny (Figure 1b). Indeed, phylogenetic signal,

302   the proportion of variation attributable to the hierarchical pattern of relatedness among

303   species, was higher for unique zones visited than for AP distance (Table S2). Interestingly,

304   and relevant for future research, phylogeny explained considerably more variation in our

305   measure of exploratory behavior—unique zones visited—than did among-individual

306   variation, i.e. "animal personality" ($\tau = 0.15$ versus $\lambda = 0.27$; Table S2).

307   The species we examined also differed in among-individual variation in exploratory

308   (unique zones visited) and predator response (AP distance) behaviors, again in a manner

309   consistent with phylogenetic relationships (Figures 1c, d). Unfortunately, phylogenetic

310   methods have been developed primarily with the goal of understanding differences in trait

311   averages rather than trait (co)variances. Our comparisons of "personality" variation and

312   syndromes among species are therefore based on the model comparison methods

313   identifying the presence of species differences and subsequent qualitative comparisons of

314   species level estimates. Nonetheless, the concordance between patterns of the magnitude

315   of among-individual variation and the currently described phylogeny suggests

316   phylogenetic constraints on the magnitude of "personality" variation. Of the five species,

317   the monophyly of *G. assimilis, G. integer,* and *G. lineaticeps* exhibited the highest among-

318   individual variation in unique zones visited and the lowest among-individual variation in

319   AP distance (Figure 1c and d). While the expression of average behaviors and behavioral

320   syndromes might be expected to exhibit phylogenetic signal, we did not expect this to be

321   the case for among-individual variances. One possible explanation would be bottlenecks at

322   more basal phylogenetic nodes leading to reduced genetic variation present in subsequent

323   groupings. While this could explain the lower among-individual variation in AP distance for

324   *G. assimilis, G. integer,* and *G. lineaticeps*, it does not explain that those same species exhibit

325   higher within-individual variation for unique zones visited.

326   Differences observed in among-individual variation could also be attributable to

327   selection differentially acting upon these species by reducing the additive genetic variation

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328 present in a population or species (Mousseau and Roff 1987). Our results therefore suggest
329 the possibility that exploratory behavior, for which unique zones visited is a proxy, has
330 been under stronger selection for *A. domesticus* and *G. sigillatus* than for the other species.
331 Importantly, because we do not know the strength and direction of selection acting on
332 these phenotypes, the data presented here only suggests this possibility and cannot be used
333 to distinguish between the effects of selection and drift for either behavior.

334   Alternative explanations for the observed differences in among-individual variances
335 stem from differences across source populations and sampling of these populations. For
336 example, individual *A. domesticus* used in this study were from a captive population where
337 inbreeding could have reduced genetic variation over generations. This potentially explains
338 the low among-individual variation the species shows for unique zones visited (Figure 1c)
339 but is contradicted by the high among-individual variation in AP distance (Figure 1d). In
340 contrast, *G. assimilis* and *G. lineaticeps* behavior was measured for field-caught individuals.
341 If individuals of these species experienced different developmental environments from one
342 another, we would predict higher among-individual variation in behavior because
343 permanent environmental variation contributes, on average, 50% of the observed among-
344 individual variation in behavior present in populations (Dochtermann et al. 2015). This
345 explanation is not, however, supported: while *G. assimilis* and *G. lineaticeps* indeed showed
346 high relative among-individual variation in unique zones visited, the same was not the case
347 for AP distance (Figure 1c and d). Moreover, for both behaviors, *G. assimilis* and *G.*
348 *lineaticeps* were very similar to *G. integer*, for which lab reared individuals were measured.
349 To summarize, the conflicting patterns of among-individual variation observed between AP
350 distance and unique zones visited prevents clear interpretation.

351   Estimated within-individual variances include variation from a variety of sources,
352 including plasticity in response to short-term environmental variation and measurement
353 error (Dingemanse et al. 2012, Berdal and Dochtermann 2019). In comparing species, if we
354 assume measurement error is similar among species, differences in within-individual
355 variation will primarily represent differences in plasticity. This short-term plasticity, also
356 referred to as phenotypic flexibility (Piersma and Van Gils 2011), allows individuals to
357 respond flexibly to an environment (Westneat et al. 2015). As was the case for among-
358 individual variation and average unique zones visited, *G. assimilis, G. integer,* and *G.*

16

359   *lineaticeps* were grouped together and exhibited similar magnitudes of within-individual

360   variation (Figure 1e and f). For both behaviors, this group exhibited considerably higher

361   within-individual variation than observed for *A. domesticus* and *G. sigillatus*, differences

362   supported by our model comparison results (Table 3). In other words, the *Gryllus* genus

363   exhibited greater behavioral plasticity.

364   One possible explanation for this pattern is that our sample of *G. assimilis, G. integer,*

365   and *G. lineaticeps* were of individuals either caught from the field or the direct offspring of

366   field inseminated and subsequently captured individuals. In contrast, the population of *G.*

367   *sigillatus* we sampled had been in captivity for around 75 generations and the population of

368   *A. domesticus* was reared for production purposes for some undetermined but large

369   number of generations. Consequently the differences in within-individual variation could

370   be attributable to exposure to a frequently changing environment (Relyea 2001) in the case

371   of *G. assimilis, G. integer,* and *G. lineaticeps* and the loss of plasticity in *A. domesticus* and *G.*

372   *sigillatus*. This possibility could be assessed for crickets via experimental evolution with

373   populations experiencing different levels of environmental heterogeneity.

374   With regard to behavioral correlations, Bell (2005) proposed two hypotheses for the

375   expression of behavioral syndromes within a population relevant to the species level

376   comparisons we performed. The first of these, the constraints hypothesis, chiefly attributes

377   behavioral syndromes to the presence of pleiotropy, with the expression of genes affecting

378   multiple behaviors. This hypothesis can be extended to other mechanistic connections

379   constraining independent trait expression. Second, the adaptive hypothesis states that

380   behavioral syndromes are the adaptive outcome of correlated selection. While pleiotropy

381   and other mechanistic connections can evolve and be adaptive, syndromes attributable to

382   the adaptive hypothesis are expected to respond more quickly to changes in selection (Roff

383   1997). Consequently, phylogenetic similarity in behavioral syndromes provides indirect

384   support for the constraints hypothesis. Due to among-individual correlations not

385   substantively differing among species (Figure 2), our results therefore support the

386   constraints hypothesis, despite species differing in variances and average expressions of

387   behaviors (Figure 1).

388   While a comparative approach has only rarely been used for examining behavioral

389   variation, three particular studies are relevant to the interpretation of our results here.

390   First, Blankers et al. (2017) compared the phenotypic variances and (co)variances of seven

391   calling traits of multiple cricket species (including *G. lineaticeps*, which was included in our

392   study). These authors found that the phenotypic covariance matrices differed among

393   cricket species. One of the major differences among species was in the magnitude of

394   variation present in single traits (Blankers et al. 2017). This is consistent with our findings

395   that variances of behaviors differed across species (Figure 1c-f). Unfortunately, these

396   authors compared phenotypic (co)variances, which conflate among- and within-individual

397   (co)variation (Dingemanse et al. 2012). Second, White et al. (2020) compared the among-

398   individual covariance matrices of seven species of fish. Comparable to our results, these

399   authors detected differences in the magnitude of among-individual behavioral variability

400   and also found overall phylogenetic signal and similarity in how variation was expressed

401   across multiple behaviors (White et al. 2020). Finally, Royauté et al. (2020) compared the

402   expression of additive genetic (co)variance (i.e. **G** matrices) in behavior among four

403   populations of *G. integer*. Similar to White et al. (2020) and our results presented here,

404   Royauté et al. (2020) found differences in single trait variances and covariances but the

405   overall structure of trait covariance was generally conserved across populations—

406   indicating support for the constraints hypothesis.

407   More generally, our findings here suggest that behavioral correlations are

408   phylogenetically conserved. Conserved trait correlations like those observed here

409   constrain the divergence of populations and species (Schluter 1996). While the potential

410   for such constraints has been speculated about for behaviors (Dochtermann and

411   Dingemanse 2013), prior demonstrations of such have primarily focused on morphological

412   traits (McGlothlin et al. 2018, Sztepanacz and Houle 2019) and chemical traits (Blows et al.

413   2004, Aguirre et al. 2014).

414   Jointly, our approach allowed us to determine whether there were differences in

415   average behavior, "personality", behavioral plasticity, and behavioral syndromes among

416   species. Our results demonstrate phylogenetic conservation of behavioral averages,

417   behavioral variation, and behavioral syndromes. This finding is potentially surprising given

418   that behavior is often assumed to be more flexible and labile than other types of traits (but

419   see Zuk and Spencer 2020) and suggests an important role for phylogenetic constraints as

420 an alternative to the dominant adaptive explanations commonly employed when discussing

421 animal personality and behavioral syndromes.

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550

Supplemental Materials

Table S1. Fixed effects coefficients for Model 4 (Table 3). The intercept estimate is for *Acheta domesticus* females (fixed effect coefficients are contrasts versus these values).

AP Distance (square-root transformed)

	posterior mean	95% credibility interval		effective sample size	pMCMC
		lower	upper		
Intercept	16.97	16.25	17.66	1000	<0.001
Gryllus assimilis	-1.44	-4.18	1.22	1000	0.298
Gryllus integer	3.67	2.31	5.18	1000	<0.001
Gryllus lineaticeps	-0.52	-4.00	2.95	1000	0.782
Gryllodes sigillatus	7.43	5.69	9.17	1000	<0.001
Temp2	0.65	0.34	0.96	1000	<0.001
SexM	-0.40	-1.44	0.72	1000	0.454
Mass (w/in individual centered)	0.72	-0.77	2.29	1000	0.344
Mass (b/w individual centered)	0.02	-0.76	0.98	800.8	0.972

Unique zones visited

	posterior mean	95% credibility interval		effective sample size	pMCMC
		lower	upper		
Intercept	2.929	2.869	2.984	1000	<0.001
Gryllus assimilis	-1.227	-1.757	-0.628	1000	<0.001
Gryllus integer	-0.728	-0.891	-0.526	1000	<0.001
Gryllus lineaticeps	-0.593	-0.979	-0.219	1000	0.004
Gryllodes sigillatus	0.282	0.160	0.411	1000	<0.001
Temp2	0.043	0.016	0.070	815.1	0.002
SexM	0.111	0.010	0.201	1000	0.022
Mass (w/in individual centered)	-0.022	-0.175	0.135	1000	0.792
Mass (b/w individual centered)	0.120	0.043	0.212	899.8	0.006

551

Table S2. Variance estimates (posterior modes with 95% credibility intervals) for models including phylogenetic structure as a random effect. Models were fit with temperature (centered), sex, and mass (within and between individual centered) as fixed effects. Phylogeny was modeled according to the trees shown in Figure 1 and with uniform branch lengths. Subject was also included as a random effect. Variance ratios are presented as unadjusted ratios; that is, variance due to fixed effects is included in the denominator. Ratios for unique zones include the distribution specific variance (DSV) in the denominator. λ and τ correspond to unadjusted phylogenetic signal and unadjusted repeatabilities respectively.

	Variance estimate (95% CrI)	Variance ratios* (95% CrI)
AP Distance		
Phylogeny	12.73 (2.10 : 106.61)	λ : 0.16 (0.05 : 0.68)
Subject	13.34 (10.21 : 17.66)	τ : 0.19 (0.07 : 0.27)
Fixed Effects	1.10 (0.32 : 2.60)	0.01 (0 : 0.04)
Residual	38.34 (33.96 : 41.50)	0.50 (0.24 : 0.71)
Unique Zones Visited		
Phylogeny	0.12 (0.03 : 1.07)	λ : 0.27 (0.09 : 0.72)
Subject	0.11 (0.07 : 0.13)	τ : 0.15 (0.05 : 0.23)
Fixed Effects	0.01 (0 : 0.02)	0.01 (0 : 0.03)
Residual	0.26 (0.23 : 0.31)	0.48 (0.16 : 0.58)
DSV**	0.06 (0.03 : 0.11)	*NA*

* while the ratios for any single MCMC estimate will sum to 1, the posterior modes can sum to other values due to uncertainty across the MCMC chain

** estimated as $ln\left(\frac{1}{exp(\beta_0)} + 1\right)$ following Nakagawa & Schielzeth (2010)

552

Table S3. Among- and within-individual correlations by species. Correlation estimates are posterior modes and are presented along with 95% credibility intervals (CrI).

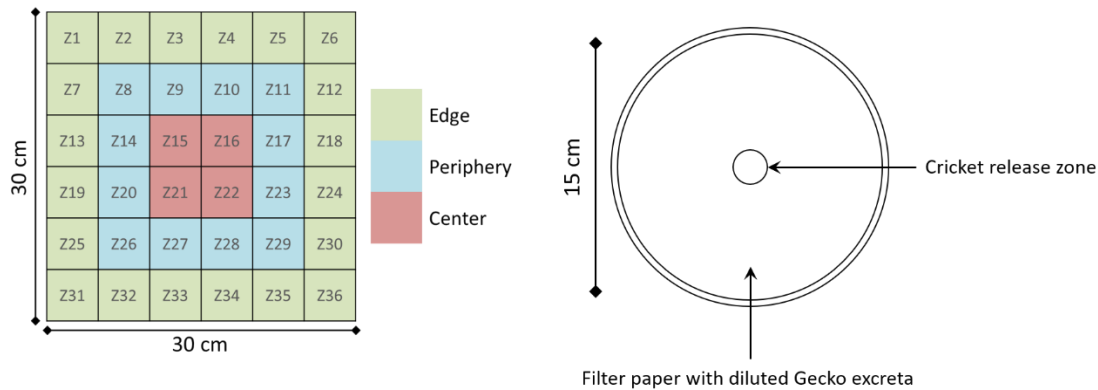| Species	Among-individual correlation (95% CrI)	Within-individual correlation (95% CrI)
Gryllus assimilis	0.37 (0.21 : 0.54)	*NA*
Gryllus integer	0.29 (-0.63 : 0.75)	0.10 (-0.08 : 0.25)
Gryllus lineaticeps	0.66 (-0.01 : 0.82)	*NA*
Gryllodes sigillatus	0.49 (-0.42 : 0.78)	0.35 (0.12 : 0.53)
Acheta domesticus	0.3 (-0.05 : 0.6)	0.18 (0.08 : 0.27)

Figure S1. Schematics of the open field (left) and predator cue arenas (right). The open field arenas were subdivided into 36 unique "zones" during video processing. For the anti-predator response trials the cricket was introduced, under a container, to the center point. This cricket release zone did not have predator cues present.