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1 **Can extreme climatic events induce shifts in adaptive potential? A conceptual**  
2 **framework and empirical test with *Anolis* lizards**

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20

21 **Abstract**

22 Multivariate adaptation to climatic shifts may be limited by trait integration that causes

23 genetic variation to be low in the direction of selection. However, strong episodes of selection

24 induced by extreme climatic pressures may facilitate future population-wide responses if

25 selection reduces trait integration and increases adaptive potential (i.e., evolvability). We

26 explain this counter-intuitive framework for extreme climatic events in which directional  
27 selection leads to increased evolvability and exemplify its use in a case study. We tested this  
28 hypothesis in two populations of the lizard *Anolis scriptus* that experienced hurricane-  
29 induced selection on limb traits. We surveyed populations immediately before and after the  
30 hurricane as well as the offspring of post-hurricane survivors, allowing us to estimate both  
31 selection and response to selection on key functional traits: forelimb length, hindlimb length,  
32 and toepad area. Direct selection was parallel in both islands and strong in several limb traits.  
33 Even though overall limb integration did not change after the hurricane, both populations  
34 showed a non-significant tendency toward increased evolvability after the hurricane despite  
35 the direction of selection not being aligned with the axis of most variance (i.e., body size).  
36 The population with comparably lower between-limb integration showed a less constrained  
37 response to selection. Hurricane-induced selection, not aligned with the pattern of high trait  
38 correlations, likely conflicts with selection occurring during normal ecological conditions that  
39 favors functional coordination between limb traits, and would likely need to be very strong  
40 and more persistent to elicit a greater change in trait integration and evolvability. Future tests  
41 of this hypothesis should use G-matrices in a variety of wild organisms experiencing  
42 selection due to extreme climatic events.

43

#### 44 **Introduction**

45 Despite the vast literature focusing on adaptation to climate, predictions for how species will  
46 respond to climate change generally ignore the degree of adaptive potential of populations in  
47 the face of these changes, especially when multiple traits are under selection (Nadeau and  
48 Urban 2019; but see Etterson and Shaw 2001, Etterson 2004, Peschel et al. 2021). Adaptive  
49 responses to climatic pressures can alter ecological responses of populations, lowering their  
50 vulnerability to future changes, or even rescue populations from extinction (Quintero and

51 Wiens 2013, Diniz-Filho et al. 2019). Of course, these adaptive responses depend on standing  
52 genetic variation in the traits under selection, the frequency and severity of climatic  
53 perturbations that induce selection and even on interactions with other species (Grant et al.  
54 2017). For instance, we may expect adaptation to be especially challenging in response to  
55 extreme climatic events that induce very strong episodes of selection, leading to high  
56 mortality and small population sizes that may never rebound. Given that extreme climatic  
57 events are likely to become more frequent and intense with future climate change, such as  
58 accelerating warming rate (Fischer et al. 2021), the potential of populations facing extreme  
59 events to adapt and persist may be limited. However, the scenario may not be so daunting if  
60 populations can increase their adaptive potential in response to predicted extreme climatic  
61 events.

62         In this paper, we lay out a conceptual framework for the intriguing possibility that  
63 directional selection may increase the adaptive potential of populations by reorganizing  
64 genetic variance such that it aligns more with the direction of selection (Pavlicev et al. 2011),  
65 such as when selection is caused by extreme climatic pressures. Then we show how to apply  
66 this framework with a case study of hurricane-induced selection on functional traits in *Anolis*  
67 lizards. Although our data set has some important limitations which we discuss, such as only  
68 phenotypic (and no genetic) data and limited sample sizes, our goal is to stimulate future  
69 research on evolvability and extreme climatic events, a theme that is currently  
70 underappreciated in the literature.

71

## 72 *Genetic integration, constraint and evolvability*

73         Given that selection induced by climate is likely on several traits at once, an important  
74 determinant of population-level responses is phenotypic and genetic integration among traits  
75 (Etterson and Shaw 2001). Theoretical models posit that if selection induced by changes in

76 the climate acts on several traits (i.e., multivariate selection; Lande 1979, Lande and Arnold  
77 1983) that are tightly integrated—showing high genetic correlations—the response to  
78 selection may deviate from the direction of selection (Hellmann and Pineda-Krch 2007,  
79 Chevin 2012). Genetic trait integration is measured by the additive genetic variance-  
80 covariance matrix, the G-matrix ( $G$ ), which has trait variances along its diagonal and trait  
81 covariances in the off-diagonal and characterizes the pattern of trait co-inheritance (Lande  
82 1979, Arnold et al. 2008). The response to selection is then determined by an interaction  
83 between directional selection and the G-matrix:  $\Delta z = G\beta$ ; in which  $\Delta z$  is the evolutionary  
84 response vector and  $\beta$  is the selection gradient, which measures only the direct selection on  
85 traits by accounting for phenotypic trait correlations (Lande and Arnold 1983, Cheverud  
86 1984).

87         High genetic integration among traits results in a very stretched  $G$  that accumulates  
88 additive variance in only one or a few trait combinations (a ‘cigar-shaped’ G-matrix, in  
89 contrast with a ‘ball-shaped’ G-matrix; see Figure 1), while most trait combinations will have  
90 little additive genetic variance (Arnold et al. 2001, Walsh and Blows 2009). Therefore, the  
91 shape of  $G$  (i.e., how genetic variance is distributed along trait combinations) is crucial for  
92 determining how much additive variation will be available in the direction of  $\beta$ , that is, how  
93 high is the evolvability or the ability of a population to adapt (Houle 1992, Hansen and Houle  
94 2008). In highly integrated systems, if the direction of  $\beta$  is not aligned with the combinations  
95 of traits accumulating genetic variance,  $G$  will have low evolvability in the direction of  $\beta$  and  
96  $\Delta z$  will deviate from the direction of selection (Arnold et al. 2001; scenario B in Figure 1).  
97 Thus, whether populations will respond adaptively to climate-induced selection depends on  
98 evolvability: the higher the evolvability, the more likely the response to selection will be  
99 effectively adaptive. However, populations may be able to escape low evolvability if  
100 selection induced by climate events can change the shape of  $G$  to become more aligned with

101 the direction of selection (i.e., more additive genetic variance in the direction of selection;  
102 *e.g.*, Assis et al. 2016).  
103  
104 *Rapid shifts in evolvability and strong selection induced by climatic extremes*  
105 Pavlicev et al. (2011) presented a theoretical model in which directional selection changes the  
106 pattern of trait integration (i.e., changing the shape of  $G$ ), by acting on genes that regulate the  
107 degree of association between traits (the so-called ‘rQTLs’, relationship Quantitative Trait  
108 Loci; Cheverud et al. 2004, Pavlicev et al. 2007). The fundamental idea is that rQTLs change  
109 the effects of loci that control more than one trait (pleiotropic loci), which are one of the  
110 genetic bases for trait correlations together with linkage disequilibrium. Therefore, this model  
111 depends on genetic variation in pleiotropy, which was shown to be produced by interactions  
112 of pleiotropic loci with different rQTLs (i.e., differential epistasis; Pavlicev et al. 2008).  
113 Importantly, selection on rQTLs can change genetic variation without an appreciable effect  
114 on trait means (Cheverud et al. 2004, Pavlicev et al. 2007). Thus, different genotypes at  
115 rQTLs result in different  $G$ -matrices, and if they also show differences in mean fitness,  
116 selection will favor a specific  $G$  and may culminate in changes in the population covariance  
117 structure after several generations.

118         The most interesting result of this model is that  $G$  stretches in the direction of  
119 selection, that is, the combination of traits with the highest additive variance gets aligned with  
120 the direction of selection, increasing evolvability (Pavlicev et al. 2011). This happens because  
121 the rQTL genotype with higher variance along the direction of selection has higher fitness.  
122 The change in  $G$  is gradual across generations until the most advantageous genotype is fixed  
123 by selection (Figure 2A). Hence, this model posits that directional selection can theoretically  
124 change genetic trait integration to increase heritable variation in the direction of selection.  
125 This is different from what is predicted with a more traditional view of selection acting

126 strictly on additive variation without epistasis, which would result in no change in genetic  
127 variance, and therefore no change in evolvability after selection when considering  
128 polygenetic traits and mutation-selection equilibrium (i.e., the genetic variation that is  
129 removed by selection is replenished by mutation; Hill 1982, Burger 1993). However, both  
130 the model of Pavlicev et al. (2011) and evolutionary simulations of the mutational matrix (M-  
131 matrix; Jones et al. 2014, Melo and Marroig 2015) indicate that gene interactions (epistasis)  
132 are a crucial factor to allow the alignment between the M-matrix, the G-matrix and  
133 directional selection. More importantly, an increase in evolvability can happen quite fast,  
134 only after 100 years of directional selection on chipmunks in natural conditions (Assis et al.  
135 2016) and after 50 generations of artificial selection on mice (Penna et al. 2017).

136         If climate-induced selection elicits a change in  $G$  such that more additive variation  
137 accumulates in the direction of selection, populations may be more prone to evolve  
138 adaptively to the same climatic factor in the future, even if traits are strongly integrated  
139 before the action of selection. This scenario may be even more relevant for population  
140 survival in face of rapid climate change if selection induced by climatic events is strong.  
141 Extreme climatic events, which are historically rare but increasing in frequency, may induce  
142 strong episodes of directional selection (Grant et al. 2017). Such selection has the potential to  
143 change not only trait means but also change the pattern of trait integration, culminating in a  
144 shift in evolvability. It is important to note that the Pavlicev et al. (2011) simulations were  
145 done with very weak selection (selection coefficients of 0.001 or 0.05), and still the change in  
146  $G$  could happen in less than 50 generations depending on the level of genetic linkage (Figure  
147 2A, B). Yet, with very strong selection induced by extreme climatic pressures, a small change  
148 in  $G$  could happen very fast, perhaps in just a single generation, but being enough to increase  
149 evolvability in a biologically meaningful amount (Figure 2C).

150 We test this new hypothesis using lizard limb traits subjected to selection induced by  
151 hurricanes to demonstrate how this framework can be applied in future studies. Different  
152 from previous studies showing increased evolvability after selection (Pavlicev et al. 2008,  
153 Assis et al. 2016, Penna et al. 2017), we test this hypothesis on a system in which selection is  
154 not on the main axis of variation, which is body size here and in many other studies. It is  
155 unclear whether evolvability can increase with directional selection that is not aligned with  
156 the axis of greatest variation. In this case study, we use P-matrices as surrogates of G-  
157 matrices, and our results suggest that extreme climatic events could lead to increased  
158 evolvability in some populations. However, we caution that the ultimate test needs to be  
159 performed with G-matrices and larger sample sizes. An analytical framework developed by  
160 Grabowski and Porto (2017) can be used to estimate the minimum sample sizes needed to  
161 find significant changes in evolvability. Nevertheless, we emphasize that having ideal  
162 samples to study unpredictable extreme events in nature is quite difficult, and thus, we must  
163 work with the available data to reach an understanding of potential impacts of these  
164 increasingly common phenomena on biodiversity.

165

166 *Case study: Hurricane-induced selection on lizard limb traits*

167 We focused on two populations of the lizard *Anolis scriptus* that were exposed to  
168 Hurricanes Irma and Maria in 2017, showing strong selection on limb traits induced by this  
169 extreme climatic event (Donihue et al. 2018). In both populations, toepad area became larger,  
170 relative humerus length became longer, whereas relative femur length was shorter in  
171 survivors of the hurricane. These morphological changes were shown to be associated with  
172 higher clinging capacity and reduced aerodynamic drag, performances that are likely relevant  
173 to increase survival in the face of hurricanes (Donihue et al. 2018, Debaere et al. 2021).  
174 These populations were re-surveyed in 2019 and data on offspring of the survivors were



175 collected, allowing for the assessment of the degree to which the evolutionary responses to  
176 selection in the offspring ( $\Delta z$ ) followed the direction of selection ( $\beta$ ). Toepad area is likely  
177 heritable because the offspring of survivors from the hurricane also had large toepads  
178 (Donihue et al. 2020). Although this change could be due to phenotypic plasticity, limb traits  
179 are moderately to highly heritable. Narrow-sense heritability of hindlimb has been measured  
180 in *Anolis sagrei*, exhibiting quite high values ( $0.78 \pm 0.13$ , see Calsbeek and Bonneaud  
181 2008). Significant genetic variance on limb length was also measured in five species of  
182 *Anolis*, where heritabilities varied from 0.45 - 0.54 for femur and tibia, and around 0.15 for  
183 humerus and ulna (McGlothlin et al. 2018). Therefore, the limb structures of *Anolis* that we  
184 are dealing with comprise heritable traits that show additive variance that can be molded by  
185 selection.

186       Because we know that hurricane-induced selection is multivariate and limb traits are  
187 expected to be highly integrated in lizards, we need to analyze more than just a single trait for  
188 a robust understanding of the response to selection and whether evolvability changes after  
189 this episode of strong selection. Morphological integration of limb traits has been  
190 characterized in different *Anolis* species, showing that phenotypic correlations are generally  
191 high among limb elements (Kolbe et al. 2011). Recent studies in *Anolis* species revealed that  
192 genetic correlations are also high between limb traits (McGlothlin et al. 2018, 2021). High  
193 positive between-limb genetic correlations mean that it is easier to change all limb traits in  
194 the same direction (increasing or reducing all limb traits; directions that bear high genetic  
195 variation) than changing just fore- or hindlimb traits or changing fore- and hindlimb in  
196 different directions. We expect a similar pattern of high limb integration for *A. scriptus*  
197 populations (i.e., high positive correlations between fore- and hindlimbs), and that toepad  
198 area will be mostly correlated with the length of the corresponding longest finger or toe on  
199 which it was measured. Moreover, given that hurricane-induced selection resulted in an

200 increase in the lengths of forelimb traits, but a reduction in the lengths of hindlimb traits  
201 (Donihue et al. 2018), we expect the evolutionary response to selection in the offspring to  
202 have not followed the direction of selection because fore- and hindlimbs are genetically  
203 correlated in anoles (McGlothlin et al. 2018, 2021). Yet, we also expect to see an increase in  
204 evolvability in the offspring if hurricane-induced selection reduced correlations between the  
205 limbs (i.e., if selection reduced the overall integration of limb traits due to the opposing  
206 direction of selection on fore- and hindlimbs).

207

## 208 **Methods**

### 209 *Sampling and morphological measurements*

210 *Anolis scriptus* populations were sampled on two small islands in the Turks and Caicos: Pine  
211 Cay and Water Cay (350 and 250 ha, respectively). The islands are presently connected by a  
212 sandbar that was deposited by hurricane storm surge in the mid-1990s. However, the two  
213 populations are genetically distinct (unpublished data), and therefore can be treated as  
214 replicates of the response to extreme climatic events. Both cays are similarly covered by low  
215 vegetation (1 to 3 m in height). We sampled the anole populations along a 2-km transect on  
216 each island. The initial survey began on 28 August 2017 and lasted until 4 September 2017.  
217 We did not permanently mark individuals on the islands, and consequently, direct measures  
218 of individual survival are impossible. Hurricane Irma hit the Turks and Caicos on 8  
219 September 2017 and Hurricane Maria passed through the region on 22 September 2017.  
220 Populations were revisited after the hurricanes between 16 October and 20 October 2017 and  
221 again approximately 18 months after the storms between 1 April 2019 and 8 April 2019. We  
222 caught 71 adult lizards in the initial survey, 93 during the 2017 revisit, and 117 lizards in  
223 2019. This difference in sample size before and after the hurricane reflects an increased  
224 sampling effort after the hurricane. However, we do not expect these differences in sampling

225 to strongly affect selection differentials and response to selection because these metrics are  
226 calculated using sample means of surveyed populations, and means are generally well  
227 estimated with just a few individuals (although standard errors are generally higher with  
228 smaller sample sizes).

229         To identify the most likely offspring of hurricane survivors in 2019, we calculated the  
230 maximum body size of a lizard that had hatched on 1 April 2018, one year before our revisit  
231 (following Donihue et al. 2020). To do this, we used a logistic-by-length model and estimated  
232 growth rates in ecologically similar species to characterize the growth curve of lizards (see  
233 Donihue et al. 2020 for details). By excluding lizards larger than this size threshold, we  
234 conservatively estimated the subset of the sampled population less than a year old and thus,  
235 offspring of hurricane survivors. We retained for further analyses only the sexually mature  
236 adults of each sampling period (i.e., pre-hurricane / post-hurricane / offspring) by using an  
237 adult body size threshold for each sex. See Supplementary Material and Methods for a full  
238 description of the body size thresholds used. Our final sample sizes for each population were  
239 36, 53 and 57 for Pine Cay, and 39, 52 and 57 for Water Cay, for pre-hurricane, post-  
240 hurricane, and offspring surveys, respectively.

241         All morphological measurements were taken by the same researcher on all three  
242 visits. Measurements included the snout-vent length (SVL), eight limb length segments, and  
243 toepad area on the fore- and hindlimbs (as described in Herrel et al. 2008; see also Lowie et  
244 al., 2019). For further details on the morphological measurements, see Supplementary  
245 Material.

246

#### 247 *Selection analysis and response to selection*

248         To measure total hurricane-induced selection (indirect plus direct selection), we  
249 estimated a proxy of selection differentials ( $S$ ), the mean trait difference between post- and

250 pre-hurricane sampling periods ( $S = [\text{mean traits of post}] - [\text{mean traits of pre}]$ ), which  
251 represent the survivors after selection and the population before selection, respectively. The  
252 selection differential should be a difference between the mean of parents of the next  
253 generation, after the hurricane, and the mean of pre-hurricane populations, thus we are  
254 assuming that most survivors reproduced. We calculated separate selection differentials for  
255 each island, given that the populations are genetically distinct, and they differed in mean trait  
256 values in the pre-hurricane survey (Table S1). To measure only the direct selection on traits,  
257 we estimated the corresponding selection gradients ( $\beta$ ) for each island. We used two different  
258 equations:  $\beta = P^{-1}S$  and  $\beta = P^{-1}\Delta z$  (Lande and Arnold 1983), where  $P^{-1}$  is the inverse of  
259 the phenotypic covariance matrix of limb traits in pre-hurricane populations and  $\Delta z$  is the  
260 response to selection. The second equation estimates  $\beta$  as the realized selection because it is  
261 based on the realized response to selection. We calculated the response to selection ( $\Delta z$ ) for  
262 each island as the mean difference in traits between offspring and pre-hurricane populations.  
263 Inverting the P-matrix discounts the effects of trait correlations from the selection vector,  
264 retaining just direct selection on traits (Lande and Arnold 1983). However, given that P-  
265 matrices are estimated with sampling error, their inversion makes more error accumulate in  
266 the smallest eigenvalues (which dominate the inverted P-matrix), biasing estimates of  
267 selection gradients. To correct for this bias, we performed a sampling noise control approach  
268 following Marroig et al. (2012), in which we replace the smallest unreliable eigenvalues of P-  
269 matrices by the last reliable eigenvalue (which is the sixth one for the *A. scriptus* P-matrices,  
270 determined using the second derivative of the distribution of eigenvalues). This procedure has  
271 been shown to improve estimation of selection gradients (for more details, see Marroig et al.  
272 2012). We explain below how we estimated P-matrices. Finally, we measured the vector  
273 correlation between both estimates of  $\beta$  to check if they are similar, and thus ensure that our  
274 assumption of post-hurricane population comprising mostly parents of the next generation

275 holds true. To determine the significance of vector correlations, we randomly sampled 1,000  
276 vectors of 11 elements (number of traits) from a normal distribution of mean zero and unit  
277 SD and calculated their vector correlations. The critical values considering  $\alpha = 0.05$  for this  
278 random distribution is -0.57 and 0.57. Therefore, any vector correlation below or above these  
279 values were considered significant.

280         To estimate uncertainty due to sampling error in our selection analysis, we performed  
281 bootstrapping of individuals caught on each survey in each island. We resampled with  
282 replacement 1,000 times for each population and then we calculated the selection metrics on  
283 resampled populations, resulting in 1,000 values of  $S$  and  $\beta$ . We extracted 95% confidence  
284 intervals (CI) for the selection metrics and if they did not encompass zero, the values were  
285 considered statistically significant. We also calculated 95% CI for mean-standardized  
286 selection to determine if hurricane-induced selection is strong compared to the literature  
287 (Hereford et al. 2004), and for  $\Delta z$  values to determine if the offspring showed significant  
288 changes.

289

### 290 *P-matrices, overall integration and modularity of limb traits*

291 We estimated P-matrices for each sampling period on each island to use as surrogates for G-  
292 matrices. Environmental effects leading to phenotypic plasticity may cause the P-matrix to  
293 differ from the G-matrix (Willis et al. 1991), but the potential for plasticity to impact traits in  
294 this study is likely low because growth after sexual maturity is limited. Moreover,  
295 Cheverud's conjecture has shown that well estimated G-matrices tend to resemble P-matrices  
296 (Cheverud 1988), especially for morphological traits with generally high heritabilities (i.e.,  
297 high contribution of genetic factors to overall variance), which is the case for lizard limb  
298 traits. Moreover, empirical examples of similarity between  $G$  and  $P$  are mounting in the  
299 literature (e.g., Roff 1996, Phillips and Arnold 1999, Marroig and Cheverud 2001, Sodini et

300 al. 2019, Styga et al. 2019). Finally, we compared *A. scriptus* P-matrices with a G-matrix of  
301 the closest relative, *A. cristatellus*, extracted from McGlothlin et al. (2018), and matrices are  
302 substantially similar (see Supplementary Methods and Tables S2, S3). We also confirmed  
303 that P-matrices are reasonably well-estimated using a rarefaction analysis and we compared  
304 all P-matrices across populations of both islands (see Supplementary Methods and Figure  
305 S1). Given that  $P = G + E$  (Falconer and Mackay, 1996), if P-matrices are similar, we may  
306 assume that P and G-matrices are also similar, otherwise the environmental matrix ( $E$ ) in all  
307 populations would have to compensate for differences in G-matrices, which is a very unlikely  
308 scenario (Marroig and Cheverud 2001). Even though it is preferable to use estimates of G-  
309 matrices to test the hypothesis of increased evolvability with strong selection induced by  
310 climatic extremes, because the potential to adapt ultimately depends on heritable additive  
311 variation, estimating G-matrices in wild populations with appropriate sample sizes may be  
312 difficult. Despite this, the growing use of genomic data allowing the estimation of more  
313 precise G-matrices in a wide variety of organisms (Gienapp et al. 2017) will enable  
314 researchers to test this hypothesis more rigorously in the future.

315         To estimate mean overall integration of traits in both Pine and Water Cays, we used  
316 correlation P-matrices and calculated an integration index using the variance of eigenvalues  
317 (egv), which is less prone to bias due to differences in sample size compared to other  
318 integration metrics if negligible eigenvalues are excluded (O’Keefe et al. 2022). This index is  
319 a ratio between the variance of eigenvalues and its theoretical maximum, which simply  
320 corresponds to the number of traits minus one (Pavlicev et al. 2009). To test if egv values  
321 differed between surveys, we used the 1,000 bootstrapped populations to calculate 1,000 P-  
322 matrices for each survey and extract a 95% CI of overall integration for each survey. If the  
323 95% CI did not overlap between-surveys, we considered the differences in egv significant.  
324 We performed this analysis for all traits (including SVL), and separately for forelimbs and

325 hindlimbs (excluding SVL) to check if one of the limbs showed a stronger response to  
326 hurricane-induced selection than the other.

327         We also performed a modularity analysis to explore the extent of integration of fore-  
328 and hindlimb components in relation to between-limb integration, and whether this pattern  
329 changed after the hurricanes. If the selection induced by the hurricane reduced the magnitude  
330 of between-limb correlations in the P-matrix, we expect to see an increase in limb modularity.  
331 We constructed three hypothetical modules: forelimb, hindlimb and toepad, the latter being  
332 composed of toepad area of fore- and hindlimbs as well as the longest finger and longest toe.  
333 Even though toepads are epidermal modifications that likely develop from an embryonic  
334 origin semi-independent from the skeletal limb elements (Weatherbee and Niswander, 2008;  
335 Liu et al., 2015; Gamble 2019), toepads are functionally linked to toes and fingers in  
336 locomotion (Ruibal and Ernst 1965; Elstrott and Irschick 2004), and therefore may constitute  
337 a functional module together with toes and fingers. We then tested whether average  
338 correlations within hypothetical modules (AVG+) were higher than between-modules (AVG-  
339 ) using the empirical correlation P-matrices and a Mantel test to control for non-independence  
340 between correlations (Cheverud 1995, Porto et al. 2013; using function ‘TestModularity’ in  
341 *evolq* R package, Melo et al. 2015). Although the Mantel test is effective in detecting  
342 biologically meaningful modules when the modularity hypotheses are grounded in well-  
343 established developmental and functional studies as is our case here, we also tested  
344 modularity using a more recent test, the Covariance Ratio coefficient (CR), and permutations  
345 to calculate significance of effect sizes using the R package *geomorph* (Adams and Otárola-  
346 Castillo 2013, Adams and Collyer 2019). The CR is based on a ratio of within- and between-  
347 module covariances and gives standardized effect sizes (using Z-score transformations that  
348 take the scale-dependence of traits into account) that characterize the strength of the modular  
349 signal (Adams and Collyer 2019).

350

351 *Evolvability analysis*

352 Evolvability measures the amount of genetic variation in  $G$  in the direction of selection

353 ( $e(\beta) = (\beta^T G \beta) / |\beta|^2$ , where  $^T$  means transpose and  $|\beta|^2$  is the squared strength of

354 selection; Hansen and Houle 2008). As we mentioned before, we substituted P-matrices for

355 G-matrices to estimate evolvabilities. Thus, our interest was to compare evolvability in the

356 directions of  $\beta$  before and after the hurricane to see if the P-matrices increased the amount of

357 variation in the direction of selection, and to compare evolvability in the directions of  $\beta$  and

358  $\Delta z$ . If any of these vectors were aligned with trait correlations (i.e., aligned with  $p_{\max}$ , the

359 phenotypic equivalent of  $g_{\max}$ ), we expected their evolvability to be comparably high. To

360 estimate the evolvability in the direction of selection, we then used the equation  $e(\beta) =$

361  $\beta^T P \beta$  (e.g., Assis et al. 2016; using function ‘Evolvability’ from *evolqg* R package, Melo et

362 al., 2015) with  $\beta$  normalized to unit length and covariance P-matrices. Final  $e(\beta)$  were

363 standardized using the geometric mean of all traits to account for scale differences between

364 traits and populations (e.g., Assis et al. 2016). We chose to standardize using a single value,

365 the average geometric mean of traits, instead of using the mean of each trait (e.g., Hansen and

366 Houle 2008) to preserve the structure of covariation.

367 We calculated two values of evolvability in the direction of selection for each island:

368 (1)  $e(\beta)$  before the hurricane: using pre-hurricane P-matrices and [post – pre] selection

369 gradients, and (2)  $e(\beta)$  after the hurricane: using offspring P-matrices and [post – pre]

370 selection gradients. To estimate uncertainty in evolvability, we once again used the 1,000

371 bootstrapped values of  $\beta$  and the 1,000 bootstrapped P-matrices to construct 95% CI. This

372 procedure accounts for error in estimation of both selection and P-matrices. We interpreted

373 values as significantly different when 95% of the distributions did not overlap.



374 All analyses were conducted in the R programming environment (R Core Team 2021,  
375 version 4.1). We provide R scripts showing the selection and evolvability analyses, and  
376 showing the calculations to estimate inaccuracy in evolvability and number of individuals  
377 needed to achieve a certain level of accuracy in the Supplementary Materials.

378

## 379 **Results**

### 380 *Hurricane-induced direct selection on limb traits and response to selection*

381 Selection ( $S$ ) was mostly negative on traits, however, we only found significant negative  
382 selection on the hindlimb longest toe in Pine Cay (Table 1). In contrast, we found that several  
383 selection gradients ( $\beta$ ) were significant on both island populations—mostly negative in SVL  
384 and hindlimb traits and positive for toepads. We also found positive direct selection on the  
385 humerus, but it was only significant on Water Cay (Table 1). Results were similar when using  
386  $\beta$  vectors estimated with either  $S$  or  $\Delta z$ , given that both ways of calculating  $\beta$  resulted in  
387 significantly similar vectors (i.e., vector correlations of 0.86 for Pine Cay and 0.93 for Water  
388 Cay, both above the critical value of 0.57). Moreover, selection was parallel in both islands,  
389 showing a vector correlation of 0.85. Interestingly, the mean-standardized  $\beta$  vectors are similar  
390 between-islands in magnitude, ranging from weak selection (around 0.05) to strong selection  
391 (around 0.7) when compared to the average mean-standardized  $\beta$  (0.89) found in Hereford et  
392 al. (2004) for morphological traits (see their Table 1). Response to selection ( $\Delta z$ ) showed  
393 mostly a decrease in lengths of all traits, however, significant changes were only found in Pine  
394 Cay (Table 1).  $\Delta z$  vectors were not similar between populations, showing a non-significant  
395 vector correlation of 0.49. While the response to selection was aligned with  $\beta$  in Water Cay  
396 (vector correlation of 0.84), this was not the case for Pine Cay (vector correlation of 0.48), even  
397 though both  $\beta$  vectors are not correlated with the axis of highest phenotypic variance (vector  
398 correlations of 0.0 and 0.05, respectively). These results indicate that direct selection was

399 parallel across islands, not aligned with the direction of most phenotypic variance, and the  
400 response in the offspring differed between the two islands.

401

#### 402 *Pattern of limb trait correlations and overall integration*

403 Correlation P-matrices are shown in Table S4. All correlation P-matrices show a PC1 that is  
404 predominantly isometric size, with similar magnitudes of loadings for all traits and all loadings  
405 with the same sign (Table S5). Also, PC1 explains high amounts of proportional variation in  
406 all surveys, showing that variance accumulates in the direction of size. However, the pre-  
407 hurricane population in Water Cay shows less proportional variation accumulated in PC1  
408 compared with Pine Cay. All P-matrices show a very high similarity when using Random  
409 Skewers (RS), with all values above 0.91, indicating that matrices respond similarly to random  
410 selection. Similarity of the best matched PC axes (Krzanowski correlation, Krz) is in general  
411 lower, ranging from 0.70 to 0.79, which indicates that some PCs diverge across populations  
412 (Table 2). Still, P-matrices can be considered quite similar across populations, giving  
413 confidence in our assumption that  $P$  is a good surrogate of  $G$ .

414         We found a tendency for overall integration to be higher in post-hurricane populations,  
415 but the integration distributions overlap considerably (Figure 3A). The same pattern occurs if  
416 separating forelimb (95% CI for Pine Cay: pre = 0.21, 0.50; post = 0.36, 0.61; offspring = 0.19,  
417 0.42; for Water Cay: pre = 0.12, 0.45; post = 0.25, 0.52; offspring = 0.18, 0.45; Figure 3B) and  
418 hindlimb (95% CI for Pine Cay: pre = 0.38, 0.73; post = 0.47, 0.73; offspring = 0.30, 0.60; for  
419 Water Cay: pre = 0.16, 0.5; post = 0.35, 0.65; offspring = 0.28, 0.60; Figure 3C). Results are  
420 the same if keeping all eigenvalues or just the first six eigenvalues. Therefore, mean limb trait  
421 integration did not change with the hurricane-induced selection.

422

#### 423 *Modularity of limb traits*

424 The hindlimb is the only hypothetical module that shows higher average correlations within-  
425 module than between-modules, especially for Pine Cay populations (Figure 4). However, the  
426 hindlimb showed a signal of modularity that is significant only at  $P = 0.10$  (Table S6).  
427 Therefore, forelimb, hindlimb and toepad traits can be seen as a single integrated unit. Still,  
428 we noticed that between-limb integration in pre-hurricane population was lower in Water Cay  
429 than in Pine Cay for both limbs (Figures 4B and 4C).

430         When using the CR coefficient, we found that both pre-hurricane populations have a  
431 significant CR coefficient. However, CR values are above 1.0 indicating that covariances  
432 within-modules are lower than between-modules. Moreover, we found no differences in  
433 pairwise comparisons of CR effect sizes across populations (Table S7, Figure S2). Therefore,  
434 we confirmed our results from the Mantel test that the limbs are a single integrated unit in all  
435 populations and that the pattern did not change after the hurricane.

436

#### 437 *Evolvability*

438 We found a similar trend of increased evolvability in the direction of  $\beta$  from pre-hurricane  
439 parents to offspring in both islands (Table 3). However, the simulated confidence intervals  
440 are quite large, making these differences non-significant. This lack of significance may be  
441 due to low sample size to detect an evolvability difference. To determine the level of  
442 accuracy in our evolvability values, we used the analytical framework developed by  
443 Grabowski and Porto (2017), in which we could calculate the inaccuracy of evolvability  
444 given our sample size (lowest in pre-hurricane populations, around 35 individuals) and the  
445 overall integration of P-matrices (from 0.5 to 0.6). By looking at Figure S3A, our inaccuracy  
446 in evolvability ranged from 0.05 to 0.06. We also determined how many individuals we  
447 would need to find a significant difference (accuracy of 0.03 or lower) given the overall  
448 integration of P-matrices: we would need at least 65 to 75 individuals (Figure S3B).

449 **Table 1. Selection differentials ( $S$ ), selection gradients ( $\beta$ ), and response to selection ( $\Delta z$ )**  
 450 **separated by island.** We show empirical values and the bootstrapped 95% CI. CI in bold are  
 451 significant.

Selection and Response to Selection							
Pine Cay	Traits	S (post -pre)	95% CI	$\beta$ (post - pre)	95% CI	$\Delta z$ (off - pre)	95% CI
<b>Total size</b>	SVL	-0.028	-0.088, 0.036	-0.187	<b>-0.269, -0.099</b>	-0.060	-0.124, 0.005
<b>Hindlimb</b>	femur	-0.068	-0.133, 0.002	-0.179	<b>-0.253, -0.099</b>	-0.081	<b>-0.149, -0.010</b>
	tibia	-0.033	-0.095, 0.034	-0.080	-0.160, 0.002	-0.054	-0.119, 0.0112
	metatarsal	-0.033	-0.089, 0.028	0.003	-0.062, 0.071	-0.062	<b>-0.123, -0.002</b>
	longest toe	-0.073	<b>-0.134, -0.008</b>	-0.303	<b>-0.413, -0.200</b>	-0.074	<b>-0.140, -0.008</b>
	toepad area	0.057	-0.110, 0.230	0.186	<b>0.103, 0.265</b>	-0.068	-0.237, 0.100
<b>Forelimb</b>	humerus	-0.017	-0.085, 0.057	0.062	-0.030, 0.161	-0.021	-0.087, 0.048
	radius	-0.034	-0.093, 0.032	-0.095	<b>-0.160, -0.030</b>	-0.065	<b>-0.125, -0.007</b>
	metacarp	-0.024	-0.080, 0.036	-0.048	-0.102, 0.008	0.012	-0.049, 0.071
	longest finger	-0.040	-0.101, 0.030	-0.021	-0.085, 0.044	-0.040	-0.113, 0.031
	toepad area	0.083	-0.075, 0.216	0.094	<b>0.035, 0.150</b>	-0.026	-0.195, 0.143
<b>Water Cay</b>	<b>Traits</b>	<b>S (post -pre)</b>	<b>95% CI</b>	<b><math>\beta</math> (post - pre)</b>	<b>95% CI</b>	<b><math>\Delta z</math> (off - pre)</b>	<b>95% CI</b>
<b>Total size</b>	SVL	-0.010	-0.065, 0.047	-0.101	<b>-0.146, -0.053</b>	-0.028	-0.085, 0.030
<b>Hindlimb</b>	femur	-0.067	-0.137, 0.004	-0.290	<b>-0.394, -0.187</b>	-0.064	-0.130, 0.006
	tibia	-0.009	-0.068, 0.051	-0.051	-0.129, 0.025	-0.024	-0.087, 0.037
	metatarsal	0.000	-0.055, 0.059	-0.014	-0.067, 0.042	-0.035	-0.094, 0.022
	longest toe	-0.030	-0.089, 0.033	-0.172	<b>-0.247, -0.101</b>	-0.023	-0.085, 0.040
	toepad area	0.086	-0.074, 0.248	0.069	<b>0.010, 0.129</b>	0.046	-0.120, 0.218
<b>Forelimb</b>	humerus	0.034	-0.028, 0.100	0.132	<b>0.067, 0.200</b>	0.009	-0.057, 0.075,
	radius	-0.005	-0.061, 0.054	-0.045	-0.090, 0.005	-0.026	-0.087, 0.034
	metacarp	0.031	-0.025, 0.093	-0.004	-0.065, 0.060	-0.003	-0.067, 0.062
	longest finger	0.021	-0.050, 0.091	0.023	-0.055, 0.099	-0.011	-0.079, 0.058
	toepad area	0.098	-0.061, 0.256	0.119	<b>0.050, 0.182</b>	0.038	-0.123, 0.205

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461 **Table 2. Matrix similarity between all P-matrices.** We used two methods to compare P-  
 462 matrices. Random Skewers (RS) indicates the similarity in the response to random selection,  
 463 whereas Krzanowski correlation (Krz) indicates similarity based on the best matched pairs of  
 464 eigenvectors. All values are significant at  $\alpha = 0.05$ .

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<b>Matrix similarity</b>		
<b>Pine Cay</b>	<b>RS</b>	<b>Krz</b>
pre x post	0.96	0.76
pre x offspring	0.95	0.73
post x offspring	0.94	0.74
<b>Water Cay</b>	<b>RS</b>	<b>Krz</b>
pre x post	0.92	0.71
pre x offspring	0.92	0.79
post x offspring	0.96	0.75
<b>Pine Cay x Water Cay</b>	<b>RS</b>	<b>Krz</b>
pre	0.91	0.7
post	0.97	0.74
offspring	0.94	0.79

**Table 3. Empirical evolvabilities and associated 95% confidence intervals.**

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<b>Evolvabilities</b>	<b>pre <math>e(\beta)</math></b>	<b>offspring <math>e(\beta)</math></b>
Pine Cay	0.15	0.20
95% CI (simulations)	0.11, 0.19	0.14, 0.25
Water Cay	0.11	0.19
95% CI (simulations)	0.08, 0.22	0.14, 0.39

## 483 **Discussion**

484 Incorporating multivariate adaptive responses of populations to climate change is a necessary  
485 step to better predict how and which species may overcome these challenges (Peschel et al.  
486 2020). However, if climate-induced selection acts on multiple traits simultaneously and if  
487 traits are highly integrated, the response to selection may be constrained, not closely  
488 following the direction of selection (Etterson and Shaw 2001, Hellmann and Pineda-Krch  
489 2007, Chevin 2012). In this scenario, populations may not be able to cope with climate  
490 change even when responding to selection because the response would lead to a lower  
491 increase in mean fitness given that some traits will respond non-adaptively. This is especially  
492 likely if genetic correlations between positively selected traits are negative (e.g., Etterson and  
493 Shaw 2001), but also if selection is not aligned with axes accumulating the most variance in  
494 the G-matrix (Arnold et al. 2001, Blows and Walsh 2009). Yet, if selection also changes the  
495 pattern of trait integration and increases the amount of genetic variance aligned with  
496 selection, future response to this same selection may be facilitated (Pavlicev et al. 2011).  
497 These alternative scenarios ultimately depend on the extent to which trait integration results  
498 in only a few directions accumulating most of the genetic variance, and how much selection  
499 is aligned with these directions (showing high evolvability; scenario B in Figure 1) or  
500 whether selection changes trait integration to increase evolvability.

501         We investigated these possibilities in a case study of an extreme climatic event,  
502 hurricane-induced selection on lizard limb traits (Donihue et al. 2018, 2020), in which we had  
503 samples of survivors and their offspring for two different populations. Given that selection  
504 was inferred to be strong on some of the limb traits (Donihue et al. 2018), we hypothesized  
505 that selection would change the pattern of limb integration and re-distribute genetic variation  
506 in a way that would increase the chance of adaptation to hurricanes in the future. Because we  
507 could not estimate G-matrices for the populations, our results are only suggestive, but

508 indicate that both populations had higher evolvability in the direction of selection after the  
509 hurricane, although this result was not significant. This lack of significance may be more  
510 related to a low sample size than to a genuinely non-significant effect, as we calculated that a  
511 sample size of around 70 individuals with the same evolvability values would lead to enough  
512 accuracy to find significant difference in evolvability before and after the hurricane.  
513 Interestingly, our results raise the possibility that evolvability may increase after a single  
514 episode of selection and even if the direction of selection is not aligned with size (or PC1, the  
515 direction accumulating most genetic variance). Still, using P-matrices as surrogates of G-  
516 matrices likely may have overestimated evolvability values if environmental variation  
517 contributes to total variation in the direction of selection. Alternatively, environmental  
518 variation may be much higher along  $p_{max}$  than  $g_{max}$  given that this direction is related to  
519 size, and several environmental factors can affect growth. In this case, evolvabilities can be  
520 underestimated by using P-matrices instead of G-matrices.

521         The model of directional selection influencing evolvability predicts changes in the  
522 pattern of correlation even with fluctuating selection, but the change depends on the level of  
523 epistasis between rQTLs and other genes (Pavlicev et al. 2011). Hence, it may be that only  
524 sustained or frequent directional selection, such as maintaining the same direction for several  
525 generations, would be able to increase evolvability in the direction of selection. Although we  
526 do not expect hurricane-induced selection to be this persistent because of its infrequency,  
527 lizard populations experiencing periodic hurricanes (up to four hurricanes over a 70-year  
528 period) show larger toepads than populations exposed to fewer hurricanes (Donihue et al.  
529 2020), indicating that hurricanes can leave persistent marks on phenotypes. Moreover,  
530 hurricane frequency and magnitude are predicted to increase in the future due to climate  
531 change (Bender et al. 2010, Knutson et al. 2010).

532

533 *Direction of selection, locomotor performance and variation in responses to selection*  
534 Direct selection on limb traits in Pine Cay and Water Cay populations was parallel, giving  
535 some confidence in their estimation with the sampling noise control approach when inverting  
536 P-matrices (Marroig et al. 2012). Parallel selection was also found in other lizard systems  
537 inferred to have suffered hurricane-induced selection (e.g., Rabe et al. 2020). Direct selection  
538 was strong in several limb traits, but not so strong on toepad area, although selection  
539 gradients were towards increased surface area, and this is likely related to increased clinging  
540 capacity in *A. scriptus* (Crandell et al. 2014, Donihue et al. 2018). However, shifts in toepad  
541 area do not always lead to higher clinging capacity in lizards (e.g., Dufour et al. 2019) and  
542 other traits beyond toepad area also change clinging capacity (such as setae length and limb  
543 length; Hagey et al. 2014, Kolbe 2015). Direct selection to reduce femur length was stronger,  
544 supporting the evidence in favor of reduced aerodynamic drag in lizards that have smaller  
545 hindlimbs and consequently exposing less of the pelvic region to the strong winds induced by  
546 hurricanes (Debaere et al. 2021). Thus, selection induced by hurricanes, and possibly  
547 selection induced by other climatic events, may vary in direction and strength on different  
548 traits, showing the relevance of using a multivariate approach to estimate selection.

549 Another source of variation induced by climatic events is how much the responses are  
550 constrained by trait integration. Fore- and hindlimbs compose a single integrated unit in both  
551 populations. This high between-limb integration was expected from previous studies (Kolbe  
552 et al. 2011, McGlothlin et al. 2018) and likely results from the need to coordinate both limbs  
553 in locomotion (Aerts et al. 2000). However, populations may still differ in the strength of  
554 between-limb integration. For instance, the modularity analysis showed that between-limb  
555 correlations were lower in Water Cay than in Pine Cay, and the former had a morphological  
556 response that followed much more closely the direction of selection than Pine Cay (vector  
557 correlation of 0.84 compared with 0.48, respectively). Because direct selection was not



558 aligned with limb integration in either of the populations (i.e., selection did not increase or  
559 decrease all limb traits, as evidenced by the lower correlation between  $\beta$  and pmax), the  
560 lower correlations between limbs may have resulted in less correlated responses of limb  
561 elements in Water Cay, and therefore, a lower constraint in the direction of the response to  
562 selection. Finally, we must also consider that populations may differ due to chance and  
563 evolutionary forces unrelated to selection and having more than two populations in future  
564 studies will aid in evaluating the consistency of patterns of selection and responses to  
565 selection induced by hurricanes.

566         Therefore, although extreme climatic events like hurricanes are predicted to become  
567 more frequent and intense with future climate change (Bender et al. 2010, Knutson et al.  
568 2010), the responses of lizard populations may vary because of differences in strength of  
569 selection, and differences in trait integration resulting in different degrees of constrained  
570 responses to selection. A broader implication of all these sources of variation for responses to  
571 climate-driven selective pressures is the resultant variation in how long these changes last in  
572 terms of evolutionary time. For traits experiencing stronger selection and having lower  
573 genetic correlations, a longer-lasting evolutionary response to extreme climatic events may be  
574 more common, as shown for populations and species more often hit by hurricanes showing  
575 larger toepads (Donihue et al. 2020). On the other hand, for traits that show a more  
576 constrained response because of high trait integration, the evolutionary responses may be  
577 more ephemeral.

578

#### 579 *Selection on trait integration patterns and evolvability*

580         Even though we found evidence for strong selection on some limb traits in both  
581 populations, integration patterns were not changed by this selection, suggesting that only very  
582 strong selection (or lesser magnitude of selection sustained over multiple generations) can

583 redistribute variation in the G-matrix. For instance, in two different species of chipmunks that  
584 experienced selection induced by 100 years of climate change, only the species under  
585 stronger selection showed increased evolvability after selection (Assis et al. 2016). Whether  
586 strong selection changes trait integration, and perhaps also evolvability, may be related to a  
587 conflict with multivariate stabilizing selection (Cheverud 1984, Roff and Fairbain 2012)  
588 associated with maintenance of a pattern of higher correlations among traits that perform the  
589 same function. In this scenario, the G-matrix is kept evolutionary conserved by a stable  
590 pattern of correlational selection, that in the case of lizard limbs would be associated with  
591 locomotor tasks normally performed in the environment (e.g., sprinting, jumping, climbing;  
592 Irschick and Losos 1998, Perry et al. 2004, Foster and Higham 2012). These performances  
593 demand a coordination between fore- and hindlimbs, and thus selection to enhance these  
594 performances would favor high integration between limbs (McGlothlin et al. 2018). This  
595 conflict between episodes of directional selection causing short-term, fast changes in  $G$ , and  
596 multivariate stabilizing selection promoting long-term evolutionary stability in  $G$ , must be  
597 common in nature given that different types of selection may act on populations (Arnold et  
598 al., 2008). Selective forces and other factors, such as mutation, all influence simultaneously  
599 the G-matrix and the balance between these factors ultimately shape the evolutionary  
600 potential of populations. Therefore, hurricane-induced selection on *A. scriptus* limbs in the  
601 Turks and Caicos appears to conflict with selection associated with normal environmental  
602 conditions with the latter being much more persistent. Hence, the P- and G-matrices of *A.*  
603 *scriptus* are likely shaped by multivariate selection related to normal limb functioning to a  
604 much greater extent than by hurricane-induced selection, even if these extreme selection  
605 events are much stronger than selection in normal ecological conditions. Even for populations  
606 subjected to hurricanes in a higher frequency, selection in normal ecological conditions may

607 erase any potential long-lasting signal of lower limb integration due to hurricane-induced  
608 selection.

609

### 610 *Conclusion*

611 We showed how the hypothesis of directional selection leading to increased evolvability can  
612 be studied in the context of population-wide responses to extreme climatic events. Ideally,  
613 this hypothesis should be tested with G-matrices with appropriate sample sizes before and  
614 after the extreme event, which are likely above 70 individuals if studying around 10 traits.

615 Although we expected strong hurricane-induced selection to reduce limb trait integration, this  
616 did not happen in *A. scriptus* populations, potentially because the episodic selection caused  
617 by this hurricane was not persistent enough to contrast normal ecological conditions, despite  
618 hurricanes being a relatively strong selective force. Even so, our results indicate that  
619 evolutionary responses follow more closely the direction of selection if between-limb  
620 integration is lower. Therefore, persistence of populations facing strong environmental  
621 changes may be enhanced by lower trait integration, even if individuals perform normal  
622 functions at a lower level than more highly integrated individuals in normal ecological  
623 conditions.

624

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838

### 839 **Figure legends**

840 **Figure 1. Visual representation of the G-matrix and its interaction with directional**  
841 **selection ( $\beta$ ) to produce an evolutionary response ( $\Delta z$ ).** The G-matrix is generally  
842 represented by the directions of its first and second axes of variation (PC1 or pmax, and PC2,  
843 respectively). Selection is represented as a selection surface (or fitness landscape, blue contour  
844 lines) and the direction of selection (blue arrows). In scenario **A**, the G-matrix has low  
845 integration between traits, showing a *ball-shaped representation*. Because the genetic  
846 integration is low, the evolutionary response (red arrows) follows the direction of selection  
847 independent of the position of populations in relation to the selection surface. In scenario **B**,  
848 the G-matrix has high integration between traits, showing a *cigar-shaped representation*, with

849 most variance accumulating in the direction of  $g_{max}$ . In this scenario, the evolutionary  
850 response may not follow the direction of selection, representing a case of genetic constraint, as  
851 shown for population 1 (Pop 1). However, if selection is aligned with  $g_{max}$  (Pop 2) or with  
852 PC2 (Pop 3), the evolutionary response will follow the direction of selection even with high  
853 genetic integration between traits. Figure adapted from Arnold et al. 2001.

854

855 **Figure 2. Conceptual framework of directional selection shifting evolvability.** **A)** A  
856 population under strong sustained selection gradually changes its G-matrix (ellipse) to first  
857 become less integrated and then aligning completely with the direction of selection, leading to  
858 increased evolvability. Adapted from Assis et al. (2016). **B)** Under weak sustained selection,  
859 we expect the G-matrix to show lower integration only after some generations (50 generations  
860 in this example). **C)** Under very strong selection, such as the one induced by extreme climatic  
861 events, the G-matrix may show lower integration in just a single generation, also increasing  
862 evolvability in the direction of selection, despite not being completely aligned with the  
863 direction of selection.

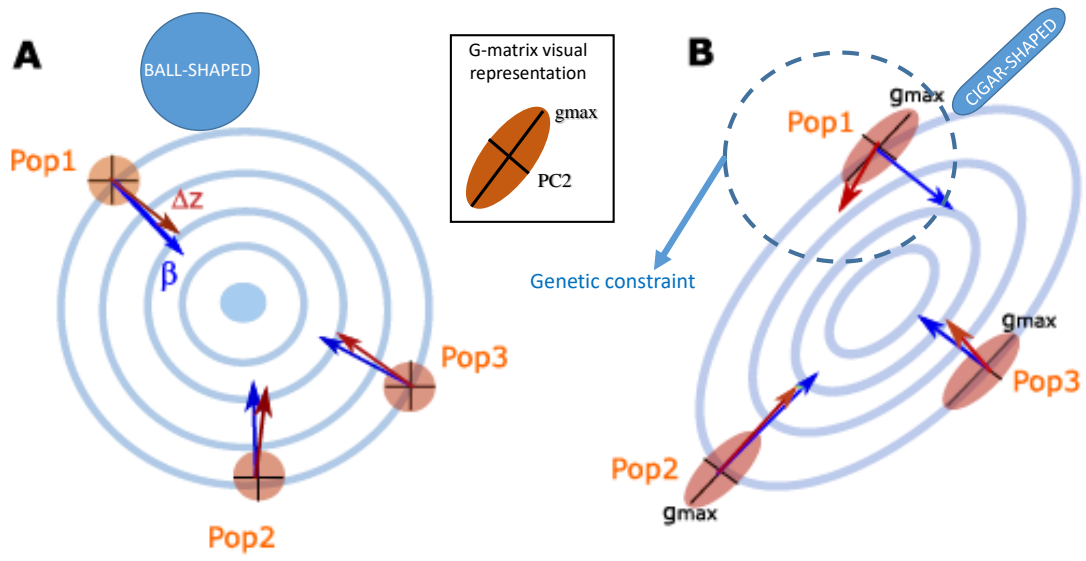
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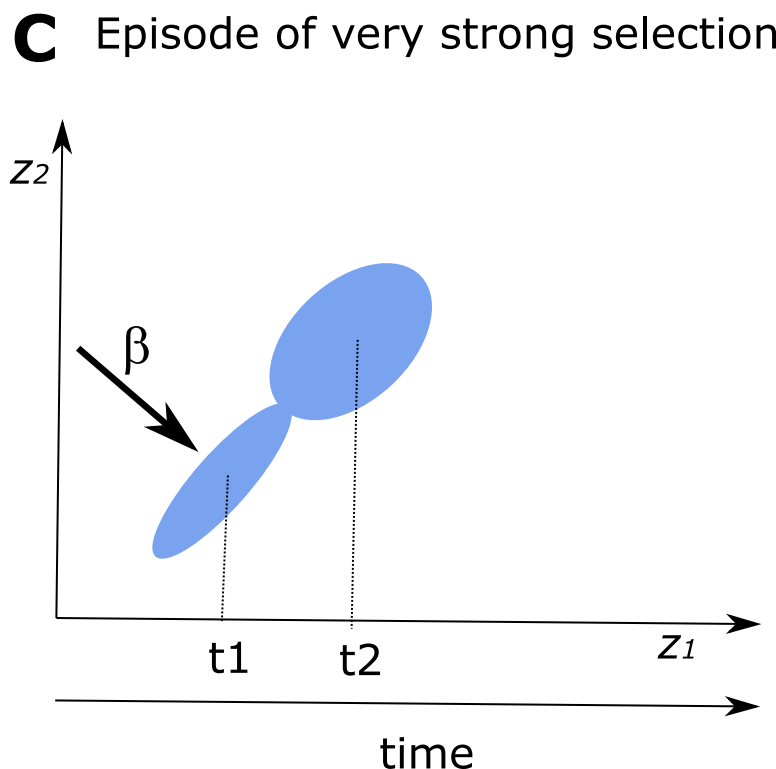
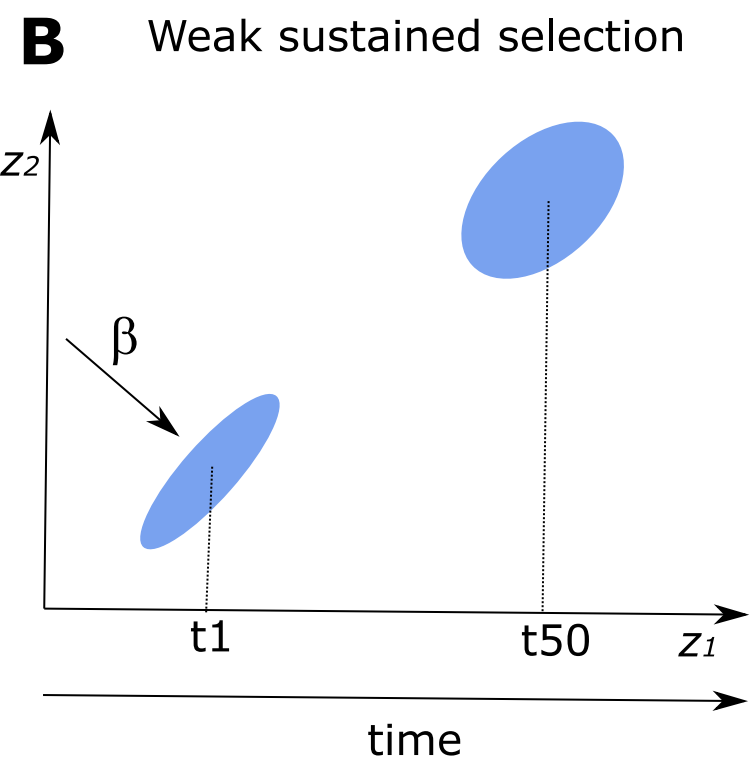
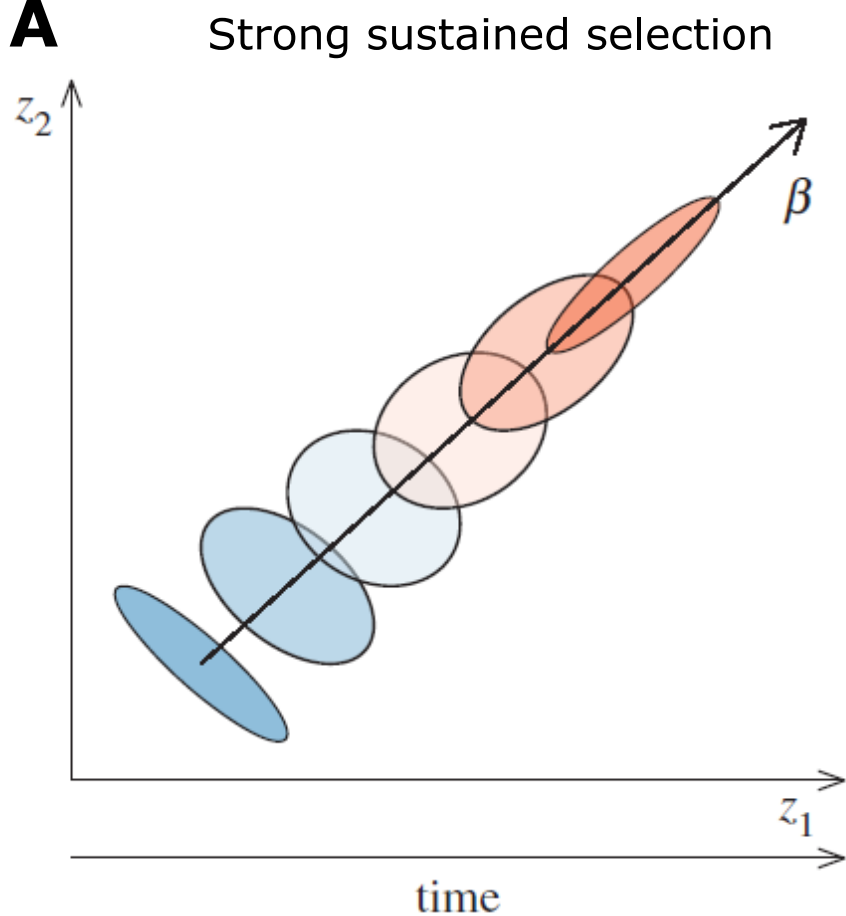
865 **Figure 3. Mean overall integration of limb traits and toepad area.** The values are  
866 eigenvalue variance of correlation P-matrices. **A)** All traits. **B)** Forelimb traits. **C)** Hindlimb  
867 traits. The distributions were constructed by bootstrap resampling with 1,000 iterations.

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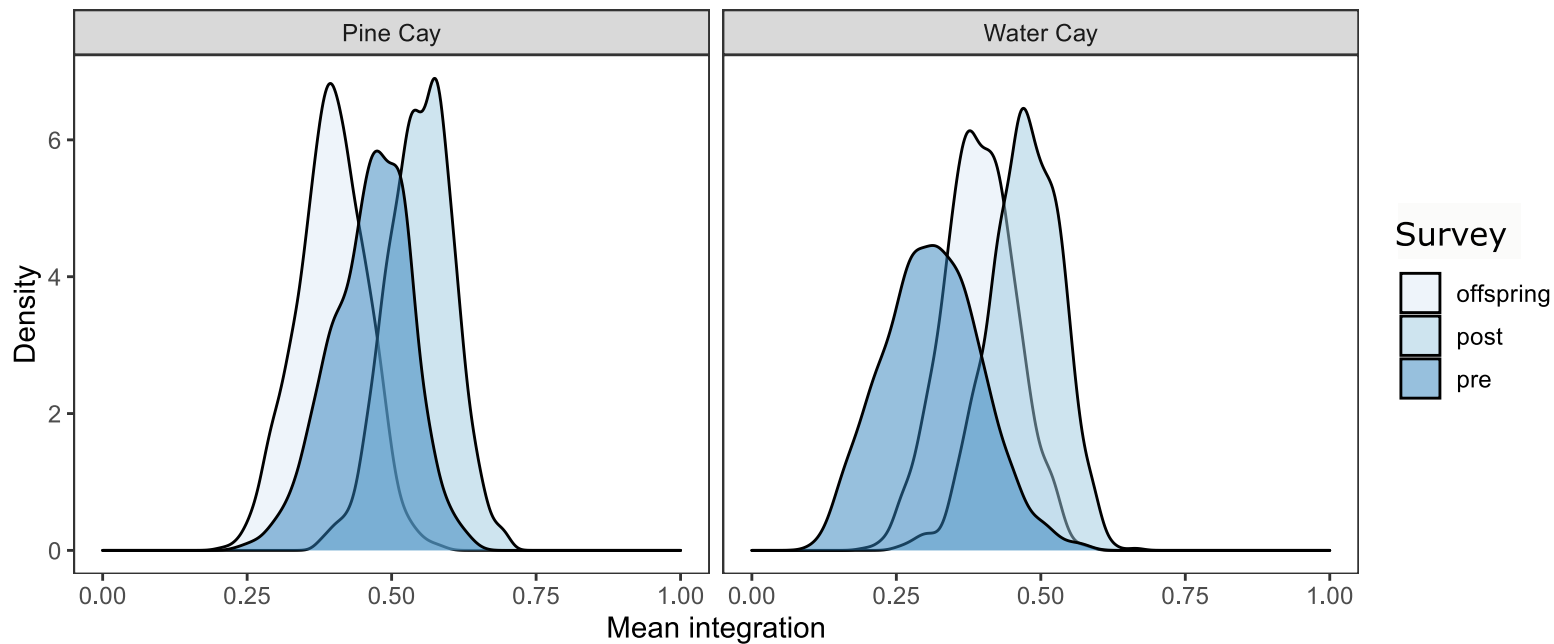
869 **Figure 4. Average correlations within (w) and between (b) the three hypothetical modules.**  
870 **A)** Pine Cay. **B)** Water Cay. Boxplots are showing the average correlation as a horizontal line.  
871 A set of traits is considered a module when within-set average correlation is higher than  
872 between-set average correlation. See table S2 for the results of the modularity tests.

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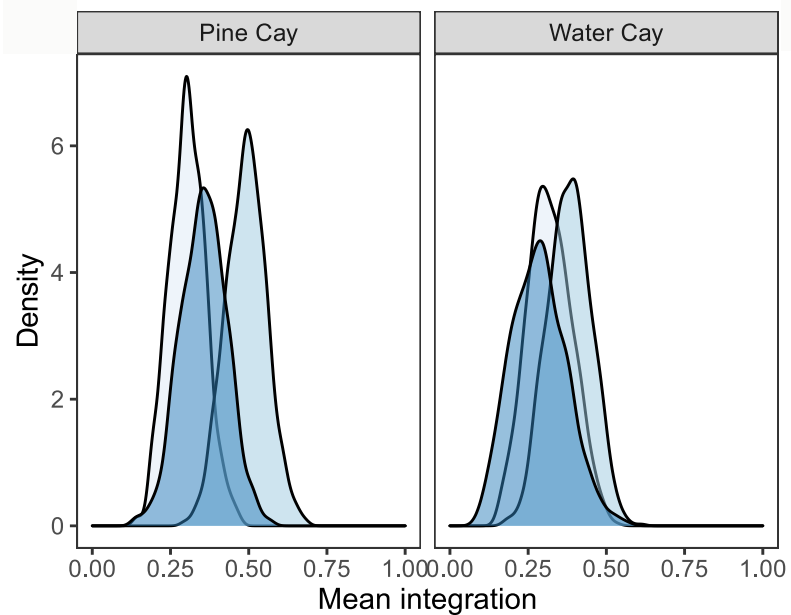




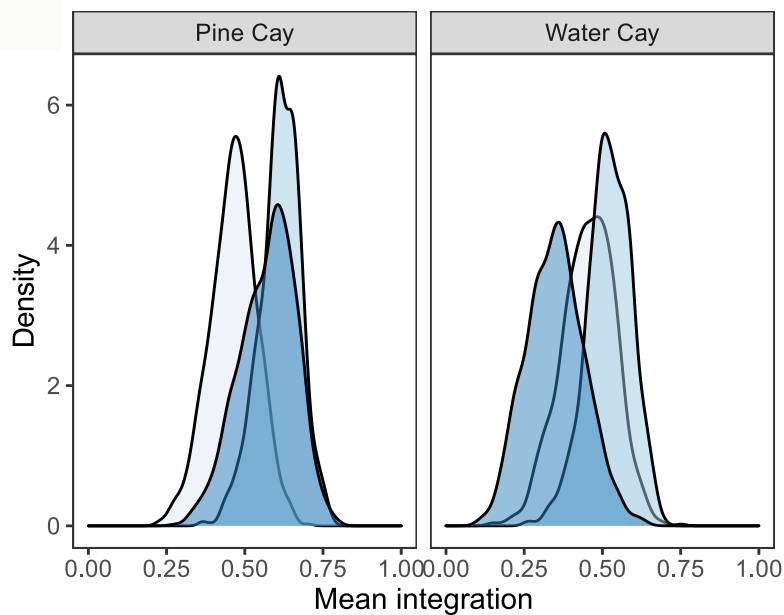
## A. Total Integration



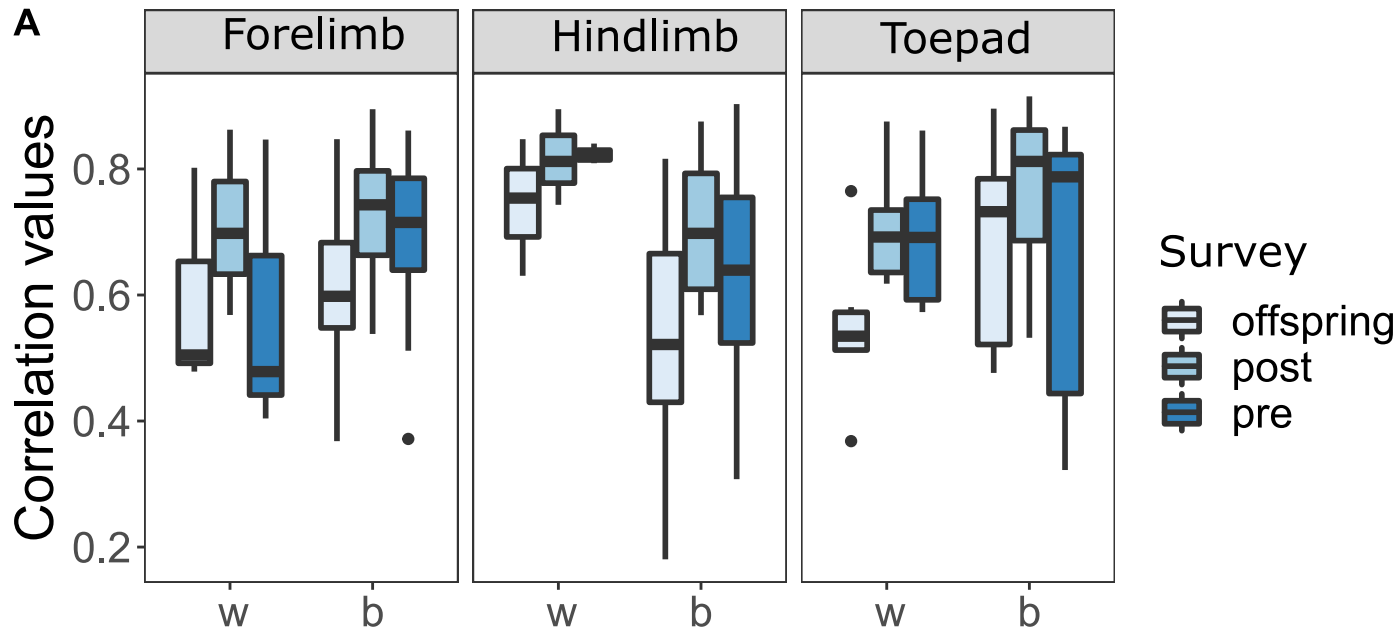
## B. Forelimb Integration



## C. Hindlimb Integration



## Pine Cay

**A**

## Water Cay

**B**