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2 Using connectivity to identify climatic drivers of local adaptation

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33 **Abstract**

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36 Despite being able to conclusively demonstrate local adaptation, we are still often
37 unable to objectively determine the climatic drivers of local adaptation. Given the
38 rapid rate of global change, understanding the climatic drivers of local adaptation is
39 vital. Not only will this tell us which climate axes matter most to population fitness,
40 but such knowledge is critical to inform management strategies such as translocation
41 and targeted gene flow. While simple assessments of geographic trait variation are
42 useful, geographic variation (and its associations with environment) may represent
43 plastic, rather than evolved, differences. Additionally, the vast number of trait–
44 environment combinations makes it difficult to determine which aspects of the
45 environment populations adapt to. Here we argue that by incorporating a measure of
46 landscape connectivity as a proxy for gene flow, we can differentiate between trait–
47 environment relationships underpinned by genetic differences versus those that reflect
48 phenotypic plasticity. By doing so, we can rapidly shorten the list of trait–
49 environment combinations that may be of adaptive significance. We demonstrate how
50 this reasoning can be applied using data on geographic trait variation in a lizard
51 species from Australia’s Wet Tropics rainforest. Our analysis reveals an
52 overwhelming signal of local adaptation for the traits and environmental variables we
53 investigated. Our analysis also allows us to rank environmental variables by the
54 degree to which they appear to be driving local adaptation. Although encouraging,
55 methodological issues remain: we point to these issue in the hope that the community
56 can rapidly hone the methods we sketch here. The promise is a rapid and general
57 approach to identifying the environmental drivers of local adaptation.

59 **Introduction**

60 It is only recently that we have begun to appreciate the speed with which evolution
61 can happen; not only over relatively short timespans (e.g., 1, 2-4), but also at small
62 spatial scales (5). Rapid local adaptation has been recorded in response to a wide suite
63 of environmental drivers, including invasive species, and pollution (6). We expect
64 climate to also be a major driver of local adaptation (e.g., 7, 8), and understanding the
65 way in which species respond to climate is of increasing importance because
66 anthropogenic climate change is proceeding at such a rate that there are concerns that
67 many species will be unable to evolve rapidly enough to avoid extinction (9, 10).

68

69 Evolution typically optimizes phenotypes, but the optimum will vary through both
70 time and space (11, 12), in turn leading to populations ('demes') that have, on average,
71 higher fitness in their home environment than an immigrant would: local adaptation.
72 While adaptive optima for traits almost always vary geographically, it does not follow
73 that all geographic trait variation is due to local adaptation. Geographic trait variation
74 can arise due to other factors, such as phenotypic plasticity (including developmental
75 plasticity and maternal effects), neutral clines, and environmental factors (such as
76 geographic variation in fitness-reducing parasites). These factors can give the
77 appearance of local adaptation (10, 11), complicating our identification of climate-
78 relevant adaptive variation.

79

80 To circumvent these issues, evolutionary biologists use experimental approaches to
81 demonstrate local adaptation (12, 13). Experiments designed to detect local adaptation
82 typically utilise one of two techniques: 1) reciprocal transplants, which are done *in*
83 *situ*, and are considered the gold standard for demonstrating local adaptation; or 2)

84 common garden experiments, which are usually done in the lab where it is easier to
85 control each environmental variable (12). Both of these techniques can be difficult,
86 for reasons of time, expense, logistics, or ethics. This difficulty increases as the
87 number of separate demes and environmental variables to be tested increases and as
88 the generation time of the organism increases (12). Additionally, although reciprocal
89 transplants will detect signs of local adaptation, they are not necessarily suited to
90 identifying the environmental drivers of that local adaptation (14). This is because *in*
91 *situ* reciprocal transplants necessarily encompass all the environmental variables that
92 differ between the transplant locations. Lab-based common garden approaches may,
93 in principle, be more suited to identifying environmental drivers (because the
94 environment may be under a degree of control), but in practice it often remains
95 impossible to identify the environmental drivers of trait variation seen in the wild.
96 Thus, the best experimental tools we have for studying local adaptation are
97 demanding in terms of time and cost, and are unsuitable for assigning environmental
98 drivers (such as climate variables) to adaptive variation. If we are looking for climate-
99 driven local adaptation, this is a problem: we want to know which climate variable or
100 variables are the main drivers of adaptation, and we urgently need this information for
101 many species.

102

103 By definition, local adaptation has a genetic basis and is consequently weakened by
104 high levels of gene flow (11, 15, 16). Demes with excessive inward gene flow are
105 therefore likely to be less optimally adapted, causing an observable mismatch between
106 optimal and actual phenotypes. Some examples of this are birds dispersing and
107 producing clutch sizes that are not optimised for the habitat quality in which they are
108 now nesting (17), larval salamander colouration not matching streambed colouration

109 due to high levels of gene flow from nearby but predator-free streams (18), and stick
110 insects in smaller habitat patches having non-cryptic colouration when the
111 surrounding patches are larger and environmentally dissimilar (19). These
112 observations of "migrant load" suggest an alternative technique for identifying and
113 assessing local adaptation. First, we look across populations for relationships between
114 the environment (e.g., mean annual temperature) and traits (e.g., morphology,
115 physiology). By themselves, these relationships are not sufficient evidence of local
116 adaptation — they could also be caused by phenotypic plasticity. Second, knowing
117 that local adaptation is hindered by gene flow, we can look at whether gene flow
118 diminishes the environmental effect. With some caveats (discussed below), in cases
119 where data on gene flow are absent (which is often the case), habitat connectivity can
120 be used as a substitute for gene flow. Trait–environment relationships that are strong,
121 but which are also weakened by connectivity, are indicative of trait–environment
122 relationships that have a genetic basis. In a statistical model, this idea would be
123 represented as follows:

$$124 \quad \text{Trait}_i = A + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + \text{error}_i$$

125 Where:

126 trait_i = trait value for individual i

127 A = intercept

128 B_{env} = coefficient of the environmental variable

129 Env = environmental variable (e.g., annual mean temperature) at the
130 individual's site

131 B_{conn} = coefficient of connectivity

132 Conn = connectivity at the individual's site

133 B_{int} = coefficient of the interaction between Env and Conn

134 $error_i$ = deviation between expected value and trait value of individual i

135 Which, with slight rearrangement, can also be expressed as:

136
$$\text{Trait}_i = A + (B_{env} + B_{int} \times \text{Conn}) \times \text{Env} + B_{conn} \times \text{Conn} + error_i$$

137 showing that the slope of the relationship between the trait and the environment now
138 depends on the connectivity value. When the signs of B_{env} and B_{int} are in opposition,
139 then we have a situation in which the relationship between the trait and the
140 environment diminishes as connectivity increases.

141

142 If we now collect data on a large number of trait–environment relationships, and their
143 interaction with connectivity, we can imagine several possible patterns emerging.

144 These possibilities are depicted in Figure 1. Each panel represents a possible
145 relationship between trait–environment coefficients (along the x-axis) and the
146 interaction between environment and connectivity (y-axis). Panel A shows a set of
147 trait–environment relationships that vary in strength, but that are not influenced by
148 connectivity (i.e., no environment–connectivity interaction). This pattern is indicative
149 of a system in which trait–environment relationships are predominantly driven by
150 plastic responses of traits to their environment (i.e., traits always match the local
151 environment, regardless of the level of inward gene flow). Panel B shows a system in
152 which trait–environment relationships are eroded by connectivity: increased
153 connectivity diminishes the relationship between the environment and the trait. In this
154 situation, the interaction between the environmental variable and connectivity is
155 negative when the environmental coefficient is positive (i.e., greater connectivity
156 causes the environmental coefficient to decrease towards zero; bottom-right
157 quadrant), and positive when the environmental coefficient is negative (i.e., greater
158 connectivity causes the environmental coefficient to increase towards zero; top-left

159 quadrant). This is the pattern we would expect if there is a genetic basis to the trait–
160 environment relationship, such as is exhibited by local adaptation. Panel C shows the
161 situation where the effect of the environment tends to be enhanced by connectivity.
162 This pattern might arise in organisms that are highly mobile and can actively move to
163 their ideal environment, thus avoiding the selective pressures that would lead to local
164 adaptation.

165
166 Understanding how species respond to specific aspects of their environment is vital if
167 we are to have any hope of halting the current rapid loss of biodiversity. Climate
168 change is undoubtedly one of the biggest threats to global biodiversity (20, 21), and
169 conservation biologists are looking to a variety of techniques to assess and help
170 mitigate the impacts of climate change on vulnerable species (22-24). One technique
171 that is likely to see increasing use is targeted, or assisted, gene flow [TGF; for review,
172 see (22, 25)]. This technique involves the spatial redistribution of long-standing
173 adaptations, and acts to increase genetic diversity in recipient populations, thereby
174 bolstering capacity for evolutionary adaptation (10, 22, 24, 25). When applying TGF
175 to help species adapt to climate change, we need to find an existing location that
176 matches the future climate at our recipient site, and then translocate animals from that
177 source location. It is a simple idea, but climate is multidimensional and species will
178 not be adapting equally to each climate axis: is a difference of 0.5°C in mean
179 temperature more important than a difference of 100mm in annual rainfall? The
180 answer depends upon which aspects of climate (hereafter “climatic axes”) have the
181 strongest influence on fitness.

182

183 Here we explore the idea of using connectivity to infer local adaptation. To do this we
184 develop a case study of a lizard species from northern Australia. We use this system

185 to examine the relationship, across sites, between traits and climatic variables. We
186 assess how habitat connectivity affects these relationships and use the interaction
187 between the environmental variable and connectivity to rank trait–environment
188 combinations. In doing so, we reveal a set of trait–environment relationships that
189 appears to be dominated by local adaptation.

190

191

192 **Methods**

193 Study species and site selection

194 The Rainforest Sunskink (*Lampropholis coggeri*) is a small (snout–vent length up to
195 45 mm), diurnal scincid lizard restricted to the rainforests of the Wet Tropics region
196 of northeastern Australia (26). The rainforests of this region cover a wide range of
197 environmental conditions, spanning significant elevation (0–1600 m ASL),
198 precipitation (annual mean precipitation of 1432–8934 mm, not including input from
199 cloud stripping), and temperature (annual mean temperature of 16.3–25.8°C)
200 gradients. This heliothermic skink is active year-round, often seen basking in patches
201 of sunlight on the rainforest floor. Lizards were captured by hand from sites that were
202 selected to maximize the environmental heterogeneity sampled (Fig. 2).

203

204 Morphological measurements were obtained from 532 skinks from 32 sites.

205 Physiological measurements were obtained from a smaller subset of these lizards: 259
206 skinks from 12 sites. At each site, 8–20 skinks were caught per collecting trip.

207 Following capture, skinks were transported to James Cook University (JCU) in

208 Townsville for trait measurement. All procedures involving lizards were approved by

209 the JCU animal ethics committee (projects A1976 and A1726).

210

211 Physiological trials

212 Physiological trials commenced within seven days of skinks being collected from the
213 field; skinks being used only for morphology were measured and released back at
214 their point of capture within seven days. The following measures were taken from
215 each skink (n = 259) during laboratory trials: critical thermal minimum (CT_{min}),
216 critical thermal maximum (CT_{max}), thermal-performance breadth for sprinting
217 (breadth₈₀), maximum sprint speed (R_{max}), temperature at which sprint speed is
218 optimized (T_{opt}), active body temperature as measured in a thermal gradient
219 (T_{active}), and desiccation rate (des) (see Table S1 for further details). Details of trait
220 measurement procedures are detailed elsewhere (see 27, 28).

221

222 Morphological measurements

223 The following measurements were taken from each skink (n = 532) using digital
224 calipers: head width (HeadW); head length (HeadL); interlimb length (ILimbL);
225 hindlimb length (HindLL). Left and right measurements were averaged to obtain one
226 measurement for that trait. We also recorded snout–vent length (SVL), total length,
227 and mass (see Table S1 for further details). All measurements were taken by one
228 person (SLM) to minimize observer bias. All morphological variables were log-
229 transformed prior to regression analyses.

230

231 Climatic variables, and connectivity

232 Because our study aimed to assess adaptation to local climate, various temperature
233 and precipitation variables were extracted for each site (see Table S2 for details). We
234 considered both means and extremes. It is important to consider climatic extremes,
235 because temperature extremes may be increasing faster than mean temperatures (29),
236 and selection may often occur during extreme weather events (30). Many
237 environmental variables are highly correlated (27), so only the less-derived variables
238 were used in analyses, specifically: annual mean precipitation (AMP); seasonality of
239 precipitation (Pcov); precipitation of the driest quarter (Pdry); annual mean
240 temperature (AMT); coefficient of variation of temperature (Tcov); average minimum
241 daily temperature (Tmin); average maximum daily temperature (Tmax); average
242 variance of daily maximum temperature (TmaxVar); and average variance of daily
243 Tmin (TminVar).

244

245

246 Our connectivity index was designed to capture the flux of individuals through a
247 location and is detailed in (31). Briefly, it is a measure of habitat suitability for our
248 focal skink species, averaged over space using a species-specific estimate of dispersal
249 potential. This approach is reasonable for any species exhibiting diffusive dispersal,
250 and similar techniques (though different spatial-weighting functions) can be used for
251 species exhibiting non-diffusive dispersal. As our species is an obligate rainforest-
252 dweller, grid cells in the landscape that are rainforest and that are surrounded by
253 rainforest have high connectivity indices, while grid cells of rainforest surrounded by
254 non-rainforest matrix have low indices. See Table S2 for further details on all
255 variables, and Figure S1 for correlations between all variables.

256

257 Analysis

258 Our analysis aimed to assess: 1) the relationship, across sites, between each trait and
259 each environmental variable; and 2) how connectivity affected each of these
260 relationships (i.e., the interaction between connectivity and environment). To allow
261 comparison of coefficients across variables, and to make interaction effect-sizes
262 meaningful, all trait and environmental variables were standardized so they had a
263 mean of 0 and a standard deviation of 1. Linear models were fitted for each pair of
264 environment–trait variables, with all models including the effect of lizard body size
265 and sex, as well as the interaction between environment and connectivity:

$$266 \text{ trait}_i = A + B_{svl} \times \text{SVL}_i + B_{sex} \times \text{Sex}_i + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + \text{error}_i$$

267 Where:

268 trait_i = standardized trait value of interest for lizard i

269 A = intercept

270 B_{svl} = coefficient of SVL

271 SVL = lizard snout–vent length, to control for effect of body size

272 B_{sex} = coefficient of Sex
273 Sex = lizard sex (this species is sexually dimorphic in some morphological
274 traits)
275 B_{env} = coefficient of environmental variable
276 Env = environmental variable (e.g., annual mean temperature) at the lizard's
277 site
278 B_{conn} = coefficient of connectivity
279 Conn = connectivity index at the lizard's site
280 B_{int} = coefficient of interaction between Env and Conn
281 $error_i$ = deviation between expected value and trait value of lizard i
282

283 A score for ranking the strength of local adaptation (L) was then calculated as:

$$284 \quad L = -B_{env} \times B_{int}$$

285 If the signs of the two coefficients (B_{env} and B_{int}) are opposite (which indicates an
286 trait–environmental relationship that is diminished by increasing connectivity, i.e.,
287 evidence for local adaptation), L will be positive. If the signs are the same (which
288 indicates an environmental effect being enhanced by increased connectivity, a
289 situation not consistent with local adaptation), L will be negative. Thus, higher
290 numbers on this scale equate to stronger evidence for local adaptation in that
291 environment–trait pair. This score can, in theory, range from $-\infty$ to $+\infty$. Once many
292 environment–trait combinations have been assessed, the coefficients for all pairs can
293 be plotted (see Fig. 1). As described in the Introduction, in a system dominated by
294 local adaptation, we expect to see a negative relationship between B_{env} and B_{int} (Fig.
295 1B). All analyses were conducted in R v3.2 (32).

296

297 **Results**

298 There was substantial variation in the effect of environment (B_{env}) and its interaction
299 with connectivity (B_{int}) across climate and trait variables, with B_{env} ranging from -1.8
300 to 1.61, and B_{int} ranging from -0.73 to 0.78 (Fig. 3). Despite this variation, a clear
301 pattern is evident, with most points in Figure 3 appearing in the top-left or bottom-
302 right quadrants: the quadrants in which the two coefficients have opposing signs, and
303 where we would expect points to fall if trait–environment relationships have a genetic
304 basis. Across these trait–environment combinations there is a distinct negative linear
305 trend (slope= -0.36, $p < 0.001$). It is especially noteworthy that the trait–environment
306 pairs with the largest coefficients are in the two quadrants indicative of local
307 adaptation.

308

309 Overall, physiological traits showed substantially stronger environmental effects (i.e.,
310 larger values of B_{env}) than did morphological traits, with the largest environmental
311 effects being exhibited by CTmin (AMP: -1.80; Tmax: 1.35; Pdry: -1.55) and CTmax
312 (Pdry: 1.61; AMP: 1.21). Physiological traits also showed stronger interactions
313 between environmental effects and connectivity, again with CTmin and CTmax
314 showing the largest interactions. These trends are apparent when we examine our
315 index of local adaptation, L . Figure 4 shows a heatmap of all trait–environment pairs,
316 ranked via reciprocal averaging according to the strength of their local adaptation
317 index. The trait–environment pairs that show the strongest signature of local
318 adaptation appear at the top-left in red. There is a rough divide, with most of the
319 physiological traits on the left and most of the morphological traits on the right. The
320 exceptions are the physiological traits Topt and Rmax, which appear at the far right of
321 the figure.

322

323 The two environmental variables that produced the strongest effects (topmost rows in
324 Fig 4) were both precipitation related: annual mean precipitation (AMP) and
325 precipitation of the driest quarter (Pdry). In our system, AMP and Pdry are both
326 highly correlated with connectivity (see Fig. S1). This is expected, because our
327 connectivity index is largely a measure of where rainforest is, and the distribution of
328 rainforest in our study region is driven to a large degree by rainfall.

329

330

331 **Discussion**

332 Understanding relationships between traits and the environment will help us plan
333 management strategies, such as targeted gene flow (TGF), that can mitigate the

334 impact of climate change on vulnerable species. Numerous studies have looked for
335 (and found) trait–environment relationships (e.g., 18, 19, 33, 34-36), but the
336 interpretation of these associations is plagued with uncertainty: are they associations
337 due to local adaptation, neutral clines, habitat choice, or plasticity? By acknowledging
338 that gene flow undermines adaptation, we can incorporate connectivity (a proxy for
339 the flux of genes) into our analysis, and in doing so, separate those relationships due
340 to fixed genetic differences, from those due to plasticity or habitat choice.

341

342 Local adaptation

343 In the trait–environment combinations we assessed, physiological traits typically
344 showed a substantially stronger effect of environment (B_{env}) than did morphological
345 traits, with the largest environmental effects shown in CTmax and CTmin (Figs. 3 &
346 4). Physiological traits also generally showed stronger environment–connectivity
347 interactions (B_{int}), again with CTmin and CTmax showing the largest interactions.
348 Overall, physiological traits generally showed stronger evidence of local adaptation
349 than did morphological traits. This result is intuitive: we would expect an ectotherm’s
350 physiological traits to be under strong selection from climate (37-39), but the fitness
351 link between morphology and climate is much less clear. Had we also included some
352 environmental variables that had a clearer bearing on morphology, we might have
353 detected stronger trait–environment relationships for morphology. For example,
354 skinks that occur in rockier habitats show various morphological adaptations to that
355 environment (40). Including a measure of rockiness in our set of environmental
356 variables might have allowed us to detect a signal of local adaptation for limb length.
357 Here, however, our focus is on climatic aspects of the environment.

358

359 Of the environmental variables used, our analysis suggests that precipitation is a very
360 strong driver of local adaptation, even in thermal traits that might not seem obviously
361 related to precipitation (e.g., CTmin, CTmax). Although this may seem a surprising
362 result, precipitation has been shown to directly affect growth rate, body temperature,
363 activity patterns, and thermoregulatory opportunities in lizards (38, 41-45). Wetter
364 areas also have higher thermal inertia (and so lower cyclical thermal fluctuations
365 (46)), and changed environmental variance in temperature potentially has a strong
366 influence on thermal limits (47). Additionally, Bonebrake and Mastrandrea (48) found
367 that changes in precipitation can significantly affect modeled fitness and performance
368 curves. Finally, comparative analyses also suggest that precipitation can influence
369 thermal traits in many species (38). Thus, although the mechanisms linking
370 precipitation to thermal limits are diffuse and poorly resolved, they do exist, and our
371 analyses suggest that precipitation is a strong driver of local adaptation at thermal
372 physiological traits.

373

374 Our analysis also suggests that temperature is an important driver of local adaptation
375 in this system, but that extremes of temperature (encapsulated in minimum and
376 maximum temperatures) are at least as strongly associated with local adaptation as is
377 mean temperature. Again, this result is intuitive (natural selection from climate is
378 likely stronger during extreme events than during normal daily temperatures) and
379 agrees with results of empirical studies (38). Finally, the environmental variables with
380 the weakest signals of local adaptation are Tcov (temperature seasonality), TminVar,
381 and TmaxVar (variance of minimum and maximum daily temperatures, respectively).
382 These variables represent predictable environmental variation occurring within an
383 individual's lifespan and so are variables to which we might expect individuals to

384 develop plastic responses, rather than fixed differences; local adaptation to these
385 variables would likely be reflected in reaction norms, rather than point values for
386 traits. (49-51).

387

388 System-wide signal of local adaptation

389 The clear negative linear trend displayed in Fig. 3 is precisely what we would expect
390 in a set of trait–environment combinations dominated by local adaptation. Migrant
391 load (the negative effect of the immigration of less-locally adapted individuals) scales
392 positively with immigration as well as with the strength of selection [see equation 5 in
393 Polechová, Barton and Marion (52)]. The reason for this is that, when the strength of
394 selection is moderately high, the environment will have a large effect on relevant
395 traits, and therefore any immigrants coming from differing environments will be
396 particularly maladapted and will therefore have a large and negative impact on the
397 local phenotype. Thus, we expect trait–environment combinations with strong local
398 adaptation to show strong effects of connectivity on the trait–environment relationship
399 (52).

400

401 By setting up a statistical model in which the trait–environment relationship is altered
402 by connectivity, we have allowed the possibility that the trait–environment
403 relationship could be reversed as connectivity increases. Such an outcome is absurd
404 from a theoretical perspective. In practice, however, our interaction coefficients were
405 typically estimated to be around 0.36 times as strong as the main effect of
406 environment. In this situation, reversal would only happen when connectivity values
407 were more than 2.7 standard deviations beyond the mean (a situation that is
408 exceedingly rare). Thus, encouragingly, our system wide analysis consistently

409 provides parameter estimates that are theoretically sensible, despite there being no
410 constraint within the model for them to be so.

411

412 We used long-term climatic averages and found strong evidence that local adaptation
413 dominates over plasticity in our trait–environment set. If we had included different
414 environmental variables, such as the conditions the lizards had recently encountered,
415 signals of plasticity may have been more apparent. Clearly environmental variables
416 that are similar across generations should lead to local adaptation, while
417 environmental variables that fluctuate within generations should have a strong
418 influence on phenotypic plasticity.

419

420 Phenotypic plasticity

421 The importance of accounting for phenotypic plasticity is, however, exemplified in
422 our dataset by the relatively strong effect of precipitation of the driest quarter (P_{dry})
423 on the temperature at which maximum sprint speed is achieved (T_{opt}) and on
424 maximum sprint speed (R_{max}) itself. On their own, these strong trait–environment
425 relationships might be interpreted as evidence for local adaptation. Our analysis,
426 however, suggests that the environmental effect is largely independent of
427 connectivity, implying that variation in these traits is due to plasticity rather than
428 genetic differentiation. Other work (27) has shown little temporal variation in T_{opt}
429 (within generations) despite clear geographic variation and this, together with our
430 results, suggests that this trait undergoes developmental plasticity, but is fixed in adult
431 lizards. In principle, this non-effect of connectivity could also arise due to selection
432 that is so strong that it maintains local adaptation despite high levels of gene flow
433 [i.e., immigrants are selected against so strongly that they do not contribute to the

434 recipient population (11)]. The trait–environment relationships for T_{opt} and R_{max}
435 are, however, weaker than those for some other traits (e.g., CT_{max} and CT_{min}) that
436 show clear effects of connectivity, so extremely strong selection seems an unlikely
437 explanation for the pattern we see here.

438

439 The generally weak evidence for plasticity in our dataset should not be considered
440 weak evidence for plasticity in these traits. Indeed many of the physiological traits we
441 use (e.g., CT_{max}) are notoriously plastic, responding reversibly on timescales ranging
442 from hours to months (53, 54). That we do not see signals of plasticity in these traits
443 here reflects our choice of environmental variables: long-term climatic variables,
444 rather than short-term weather variables (such as the temperature in the week before
445 an animal was collected). We chose these long-term variables precisely because we
446 are interested in unearthing patterns of local adaptation, rather than patterns due to
447 rapid, reversible plasticity.

448

449 Caveats and challenges

450 Our intent here has been to point out the additional inference that can be drawn from
451 data on geographic trait variation if we account for the effect of gene flow on trait
452 differentiation. The idea that local adaptation is eroded by gene flow offers a novel
453 way to identify the environmental drivers of local adaptation. Such a capacity is of
454 fundamental interest, and is also sorely needed if we are to effectively manage the
455 impacts of climate change. The methods we use here are, however, embryonic, and in
456 the following we point out caveats and challenges for future work.

457

458 *Gene flow and connectivity*

459 Our approach requires a measure of gene flow across a landscape. Here we have used
460 environmental connectivity as a proxy for gene flow. We chose connectivity because
461 it can be calculated relatively easily for many species by using broad scale habitat
462 mapping datasets [e.g., vegetation mapping from DERM (55)]. Of course, these
463 measures of connectivity should be calculated at a scale relevant to the scale of
464 dispersal of the species in question [as ours was, using dispersal rate data for
465 *Lampropholis coggeri* from Singhal and Moritz (56)]. While connectivity measures
466 will often correlate with gene flow, e.g., (57)], a measure of gene flow, rather than the
467 flow of individuals, would be preferable. Such measures are increasingly becoming
468 available with the rise of landscape genomics tools (e.g., 58), but may still be cost-
469 prohibitive in many cases.

470

471 While there may be better measures of gene flow, our inference might also be
472 improved by taking into account landscape heterogeneity in the environment. Gene
473 flow, per se, does not erode local adaptation. Rather it is an influx of maladapted
474 genes that erodes local adaptation. Thus, a better index of this “migrant load” may
475 well be one in which connectivity is multiplied by a measure of environmental
476 heterogeneity, where connectivity and heterogeneity are calculated over the same
477 spatial scale (e.g., 31). An index such as this should, in principle be a better measure
478 of migrant load than our simple measure of connectivity. The cost, however, is that
479 this index would need to be calculated in a standardized manner for every
480 environmental variable under consideration.

481

482 Clearly connectivity is an imperfect measure of migrant load. By using it, we
483 implicitly assume that all migrants are equally maladapted and have equal fitness in

484 the recipient population. Nonetheless, connectivity should scale positively with
485 migrant load, and our analysis using simple connectivity generated a coherent and
486 intuitively sensible result. This is encouraging, suggesting that, in the absence of
487 precise estimates of migrant load, a readily calculable connectivity metric may suffice
488 to elucidate broad patterns.

489

490 *Linear trait–environment relationships, and covariation with connectivity*

491 Our method assumed that traits have a linear relationship to the environment (at least
492 at the environmental scale across which we are looking). In many instances, this will
493 be a reasonable null assumption: it seems unlikely, for example, that a trait such as
494 desiccation resistance would be high in dry environments, low in moderately wet
495 environments, and then high again in very wet environments. The assumption bears
496 particular mention, however, in the situation where the connectivity index is strongly
497 correlated with one or more of the other environmental variables being used. In our
498 system, for example, AMP and Pdry are correlated with connectivity (Fig. S1). Where
499 the environment–connectivity correlation is very strong, the interaction term in our
500 model (Conn×Env) could be interpreted as a quadratic term for environment (i.e.,
501 Env²). In these cases, it is possible that a strong connectivity interaction is, in fact,
502 pointing to a non-linear trait–environment relationship. Thus, for environmental
503 variables that correlate with connectivity (and there will always be some), careful
504 consideration needs to be given to the possibility of a quadratic fitness function
505 between trait and environment. In our case, it remains possible, for example, that the
506 strong influence of precipitation on local adaptation in our system is spurious, and
507 instead reflects non-linear relationships between optimal trait values and precipitation.
508 We can, however, think of no obvious reason why thermal limits should respond

509 quadratically to precipitation, nor why desiccation rates and other physiological traits
510 should also do so. Thus, in our case, we are inclined to accept the importance of this
511 environmental variable in driving local adaptation in our system.

512

513 *Covariation between explanatory variables*

514 As in any multiple regression analysis, our capacity to make precise coefficient
515 estimates diminishes if there is substantial covariation between our explanatory
516 variables. If a sampling regime is being designed *de novo*, care should be taken to
517 sample sites in such a way that covariation between environmental variables
518 (including connectivity) is avoided as far as possible. Such an aim can be achieved by,
519 for example, strategically exploiting latitudinal and altitudinal gradients.

520

521 *Multivariate traits and environments*

522 Here we examined one trait–environment combination at a time. Doing so may
523 potentially miss relationships that only appear in multivariate analyses. For example,
524 if two environmental variables are negatively correlated but both have a positive
525 effect on a trait, it is possible that these countergradients can obscure the univariate
526 relationship. Similar problems are encountered when examining response to selection
527 over time (59) and, with our approach, may lead us to underestimate the number of
528 important environmental drivers of local adaptation. Analysis incorporating multiple
529 environmental predictors is possible, but such a model will rapidly become saturated
530 with parameters. To minimize the problem of countergradients, again, care should be
531 taken to sample environmental spaces in such a way as to minimize correlations
532 between environmental variables.

533

534 An additional analytical challenge is to treat traits as multivariate. Here we have
535 treated each measured trait as independent. In reality, however, traits covary and this
536 covariance can have both genetic and environmental origins (60). As a corollary,
537 selection acts on the multivariate trait, and causes populations to move in multivariate
538 trait space (61). Consequently, local adaptation perhaps should be measured in a
539 multivariate trait space rather than on a univariate basis. Such an aim, however,
540 requires considerable theoretical development and may well require substantially
541 more data. For now, however, we should be aware that we are collapsing our trait
542 space, and each of our measured traits is not independent. For example, in our system
543 there is a strong correlation between CT_{min} and CT_{max}, thus we should be aware
544 that these two traits should not get equal weighting when we use our traits to rank
545 environmental variables by their importance to local adaptation.

546

547 *Neutral clines*

548 Finally, our approach should allow us to identify when geographic variation is a result
549 of genetic variation. That is, it can weed out relationships that are driven by plasticity
550 or habitat choice. Covariation between genotype and environment will often be the
551 result of local adaptation, but can also arise for non-adaptive reasons, the most
552 obvious being trait clines caused by the historical spread of population (62). In
553 principle, and again, with careful attention to sample design (i.e., a sample design
554 which minimizes the covariation between space and environment), it should be
555 possible to separate spatial from environmental patterns.

556

557 Conclusion

558 There is increasing urgency to identify populations that will act as suitable sources for
559 targeted gene flow efforts in the face of climate change. To identify these populations,
560 we need to know which traits influence sensitivity to climate and are locally adapted.
561 Traditional approaches to unearthing local adaptation (reciprocal transplants and
562 common garden experiments) are time consuming, and often cannot attribute
563 adaptation to any particular environmental driver. Local adaptation is, however,
564 undermined by gene flow, and we should be able to use this fact to sort patterns of
565 local adaptation from patterns with other causes. Here we have demonstrated this
566 approach: using connectivity as a proxy for gene flow, and looking for its effect on
567 trait–environment relationships. Our analysis, using a species of lizard from
568 Australia’s Wet Tropics rainforest, suggests the approach has merit: the results we
569 achieve are coherent and suggest local adaptation is the overwhelming signal in the
570 set of trait–environment relationships tested. As well as implying a strong role for
571 local adaptation, we have effectively ranked environmental drivers of local
572 adaptation, finding evidence that precipitation and temperature are important
573 environmental variables with regard to local adaptation in our system. Our analysis
574 also suggests that some traits exhibit strong plastic responses to the environment,
575 particularly in response to precipitation of the driest quarter and the seasonality of
576 temperature and precipitation. These specific results will likely apply to other species
577 that are phylogenetically or ecologically similar to our focal species, but the method
578 has the potential to apply much more broadly. Analytical and sampling challenges
579 remain, however, and we point to avenues whereby the method can be improved.
580 Given the potential of this method to provide evidence of local adaptation, and to
581 provide rapid ranking of the climatic drivers of local adaptation, assessment of the
582 method in a broader array of systems is warranted.

583

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763
764
765

766 **Figure Captions**

767

768 **Figure 1.** Graphs showing the concepts illustrated by plotting a set of trait–environment
769 coefficients (e.g., the coefficient from a linear model examining the effect of annual mean site
770 temperature on the sprint performance of organisms from that site: B_{env} , x-axis) and the
771 corresponding environment–connectivity interaction coefficients (B_{int} , y-axis). Broad grey
772 line represents the approximate area in which these points would fall. A) Phenotypic plasticity
773 is suggested when trait–environment relationships are strong, but are not influenced by
774 connectivity. B) Local adaptation is suggested when increasing connectivity diminishes the
775 relationship between the environment and the trait. C) The effect of the environment is
776 enhanced by connectivity. This latter pattern might arise in organisms that are highly mobile
777 and can actively move to their ideal environment, thus avoiding the selective pressures that
778 would lead to local adaptation.

779

780 **Figure 2.** Map of the southern Australian Wet Tropics bioregion, showing the distribution of
781 rainforest in green and the sampling locations as black dots.

782

783 **Figure 3.** Scatterplot showing the results of 99 linear models run to assess the relationship
784 between each trait–environment pair, and the environment–connectivity interaction. Trait–
785 environment coefficients (B_{env}) are on the x-axis, and environment–connectivity interaction
786 coefficients (B_{int}) are on the y-axis. Local adaptation is suggested when these two parameters
787 are opposite in sign: in trait–environment pairs in which a strong environmental effect is
788 eroded by increasing connectivity.

789

790 **Figure 4.** Heatmap showing the relative rankings of climate variables (rows) and
791 morphological and physiological traits (columns). The matrix has been sorted (by reciprocal
792 averaging) and coloured according to the strength of local adaptation, with higher values
793 coloured red and being sorted to the top/left. See Tables S1 and S2 for explanations of the
794 trait and environmental variables used.

795 $L = \text{local adaptation index: } -B_{env} \times B_{int}$

796

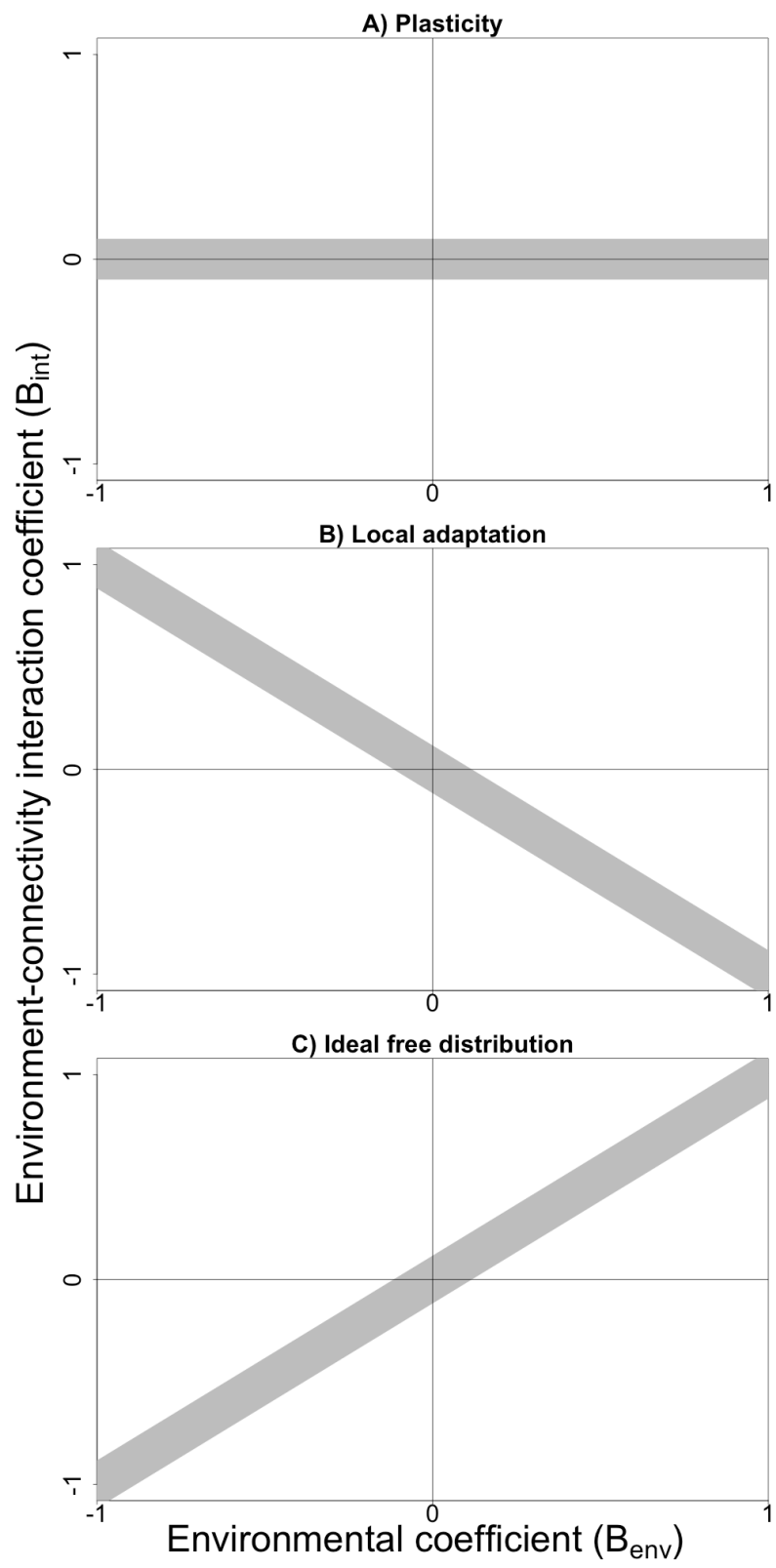
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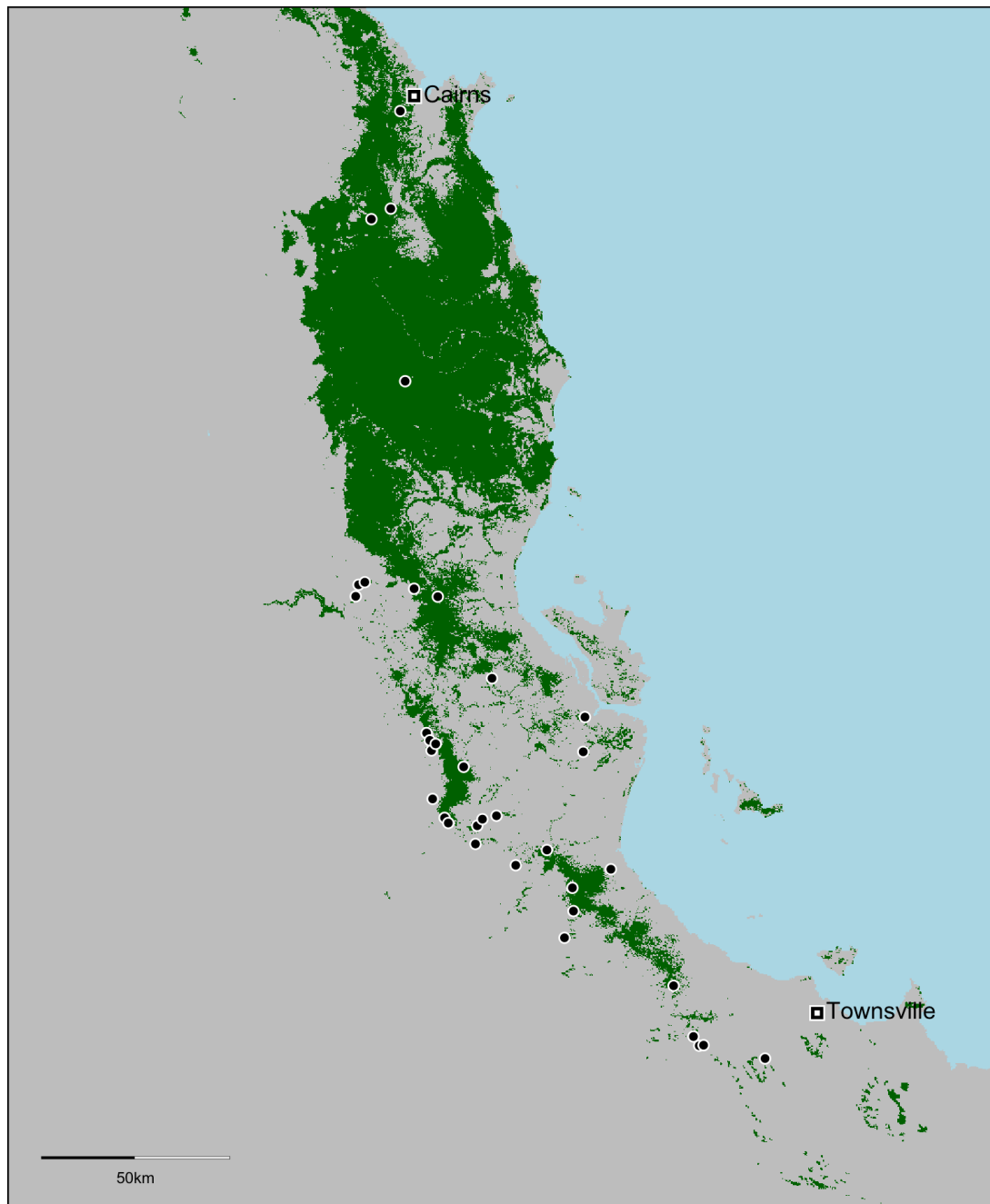
801 **Figures**



802

803 **Figure 1**

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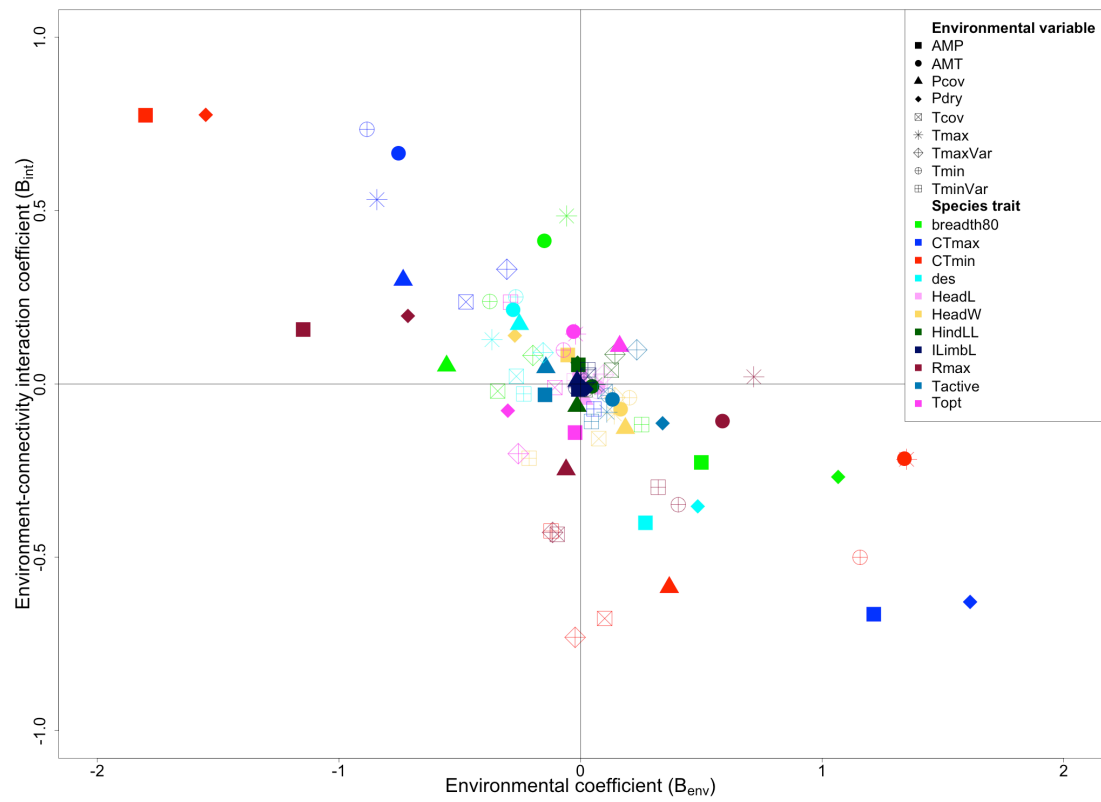
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806 **Figure 2**

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812

813

Figure 3

	CTmin	CTmax	breath80	Rmax	des	HeadW	Tactive	HindLL	HeadL	ILimBL	Topt
AMP	L = 1.3955	L = 0.8062	L = 0.1132	L = 0.1802	L = 0.1076	L = 0.0044	L = -0.0046	L = 0.0005	L = 0.0001	L = -0.0001	L = -0.0032
Pdry	L = 1.2042	L = 1.0140	L = 0.2861	L = 0.1401	L = 0.1713	L = 0.0379	L = 0.0385	L = 0.0007	L = 0.0002	L = 0.0004	L = -0.0232
Tmin	L = 0.5787	L = 0.6494	L = 0.0894	L = 0.1409	L = 0.0673	L = 0.0081	L = 0.0038	L = 0.0004	L = 0.0015	L = -0.0002	L = 0.0069
AMT	L = 0.2890	L = 0.5014	L = 0.0619	L = 0.0630	L = 0.0597	L = 0.0121	L = 0.0059	L = 0.0003	L = 0.0007	L = -0.0000	L = 0.0044
Tmax	L = 0.2932	L = 0.4480	L = 0.0281	L = -0.0148	L = 0.0467	L = 0.0121	L = 0.0089	L = 0.0001	L = 0.0003	L = -0.0000	L = 0.0029
Pcov	L = 0.2155	L = 0.2200	L = 0.0290	L = -0.0148	L = 0.0435	L = 0.0237	L = 0.0068	L = -0.0009	L = 0.0006	L = 0.0001	L = -0.0176
Tcov	L = 0.0676	L = 0.1122	L = -0.0071	L = -0.0418	L = 0.0058	L = 0.0119	L = 0.0022	L = -0.0051	L = -0.0007	L = -0.0008	L = -0.0011
TmaxVar	L = -0.0164	L = 0.1009	L = 0.0162	L = -0.0496	L = 0.0139	L = 0.0056	L = -0.0229	L = -0.0121	L = -0.0026	L = -0.0000	L = -0.0519
TminVar	L = -0.0518	L = 0.0040	L = 0.0297	L = 0.0957	L = -0.0068	L = -0.0457	L = 0.0049	L = 0.0003	L = 0.0002	L = -0.0012	L = 0.0683

814

815

816

Figure 4