

Dear Ruth,

First, apologies for our slow response. I have been in the field, and Stewart has also been otherwise engaged. We have addressed both reviewer's comments. This has resulted in a clearer explanation of the basic idea (using formulae) in the Introduction, and a new section in the Discussion entitled "Caveats and Challenges". We have also updated all figures to improve integration with the text, and tightened our language throughout. Finally, we also noticed an error in our previous Figure 4, so have corrected that also.

The manuscript is, we believe, greatly improved. We thank you both for your thoughtful critique.

Following our detailed response, you should find a) the new manuscript, and b) a version of the new manuscript with all changes tracked.

Ben

decision on MacDonald et al.

by Ruth Arabelle Hufbauer, 2017-06-28 13:53

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Decision & reviews

I really like your basic idea for refining hypotheses regarding local adaptation without doing reciprocal transplants – it is clever and makes a potentially important conceptual advance, which has excellent potential for application. If you are able to address the points made in the reviews, some of which are fairly substantial issues, I would consider recommending it on PCI Evol Biol (i.e. this is a decision akin to "major revision"). The Thomas Lenormand pointed out that clines can arise from historical/biogeographic events, and not reflect local adaptation, phenotypic plasticity or behavioral tracking. In addition to the four other concerns presented, make sure not to lose track of that. I hope you find the PCI process valuable. Thank you for submitting your work for review.

Thanks for the thoughtful comments and review. We reply in detail below.

Reviewed by Ruth Arabelle Hufbauer, 2017-06-28 13:53

I really like your basic idea for refining hypotheses regarding local adaptation without doing reciprocal transplants – it is clever and makes a potentially important conceptual advance. The issue/meaning of connectivity is a challenge. Given your knowledge of the biology of this species, it seems reasonable to think that your calculation of connectivity may indicate actual movement, and thus gene flow. But what if it doesn't (for your species, or for others who would like to try this approach)? A bit of discussion about that is warranted.

This is a good point. Our approach should work well for any species that disperses in a diffusive manner. Other kernel types are, of course, very easy to use for species with different dispersal characteristics (though convolution may be trickier): we mention this in the revised manuscript.

I also find some of the ways that the main idea is described confusing. In particular, Figure 1, and the text describing it, could be made clearer, I think. Please see specific comments below. As you state, often, plastic responses to the environment are of inherent interest themselves. It might be more compelling to frame the paper as being not about identifying local adaptation (which you may not be able to actually do), but as being about distinguishing among local adaptation, plastic responses to the environment, and behavioral tracking of the environment.

The point about what the method can do is a good one. In the revised manuscript we state several times that the method allows us to weed out patterns driven by plasticity and habitat-tracking from those driven by genetic differentiation. We hint at, and then say explicitly (in the new challenges section), that we are still left with the possibility of neutral clines.

Nonetheless, we prefer to keep the original framing primarily because it was actually our original motivation for the work.

Figure 1 The axes are a bit opaque. If I understand correctly, the x-axis is the correlation between an aspect of the environment (e.g. temperature) and a trait (e.g. wing span). So, -1 would mean that as temperature increases, wing span decreases and +1 would mean that as temperature increases, so does wing span. Correct? It would be nice to have something more easily interpretable than “environmental coefficient” as a label. Even just adding trait back in so that it reads “Environment-trait correlation” would be better. In the legend, be sure to describe it fully, such as: “The correlation coefficient between an environmental variable and trait values.”

The y-axis title is also confusing to me. Line 126 makes one expect it will be simply connectivity, but it is clearly not.

We have provided more expository axis labels and figure captions.

To me, a graph of the following sentence (“Environment-trait correlations that are strong, but which are also weakened by connectivity, are indicative of environment-trait correlations that have arisen through local adaptation.”) suggests a different graph in several ways. (1) Connectivity is in this case more the explanatory variable than the envt-trait correlation, so that should be the X-axis. (e.g. see line 142 “increased connectivity diminishes the correlation” – as you yourselves say, it’s the connectivity that is potentially the causal factor) (2) Can’t the axis be simply connectivity, not ‘environment-connectivity interaction coefficient’? (I still don’t quite grasp that interaction coefficient at this point in the paper, though maybe that will come clear as I read further).

OK. It can't simply be "Connectivity"; we definitely need the axes we have used. Hopefully the need for these axes (particularly the interaction term) is clear. We have had another go at explaining this (using formulae) in the introduction.

(3) Connectivity can run from 0 (unconnected) to 1 (highly connected). No negative is needed. Simpler! (4) Similarly, I don't think that the sign of the environment-trait correlation matters. A graph with zero correlation to highly correlated (either positively or negatively) also would be more simple.

Again, we suspect these comments arise from confusion about what we are doing. We have tried to explain things more clearly in the revised introduction. The sign of the trait-environment coefficient is irrelevant. What is important is that this sign is opposed by the interaction between environment and connectivity.

I've sketched – the end result (three simple lines) is similar, but to me, much easier to interpret. To me, something like that would be much easier for the reader to interpret.

Not quite sure what this refers to. Perhaps there is an attachment we didn't get?

line 155 – the ideas in this paragraph are great.

Thanks!

line 169 – rather than 'axes' I would recommend 'aspects of the climate'

We prefer "axes" for brevity, but make the link to "aspects of climate" at this point.

line 252 – ok, here I see more what is meant by the interaction between environment and connectivity. Taking into account size (SVL) and sex, this model allows you to look at whether environment influences the trait (Benv), and whether that influence depends upon connectivity (Bint). I think that graphically, simply having 'connectivity' on the axis is still more straightforward. If there are good reasons to keep it as the Bint value, I think that this needs to be explained conceptually more thoroughly and clearly prior to presenting figure 1.

We definitely can't have connectivity on the axis, because this would imply the coefficient for the connectivity term, when we are actually graphing the coefficient for the interaction term. Again, we hope the revised introduction makes this clearer.

Is L necessary? You don't use it in any figures, or present it, just mention it briefly. Also 'L' is typically used for genetic load. If you keep this concept in, I think it should become more integral to the paper, and perhaps be changed to LA for local adaptation.

We did use LA in an earlier draft, but switched to L to minimise the potential for confusion (LA implies L times A). We use this score in the reciprocal-averaged

matrix, to rank traits and environmental variables. We attempt to make this use clearer in the revised manuscript.

311 – Does this pose a problem? – running linear models with variables that are highly correlated violates the assumption of independence. I like the discussion of the biological meaning of such potential correlations in the discussion (e.g. around line 433), but I still think that a mention of why violating the assumption of independence is not a problem is warranted.

Yes, it does pose a problem; the problem we discussed on line 433 (and which is also mentioned by Thomas). The additional problem of multicollinearity is also there, but in practice this effectively amplifies the uncertainty in our estimates of the slope: it makes our results messier than they otherwise would be. So not so much a philosophical issue as a logistical one, whose effect is to obscure the ultimate pattern we are looking for. We mention this in the new “challenges” section of the revised manuscript.

Figure 3 is interesting. I find it surprising that there are not some traits that are plastic. Is it the choice of traits? What would happen, if, for example, you evaluated size (something more likely to be plastic) as your response? Do you get the expected lack of correlation for that trait?

Interesting thought. We touch on this to some extent in mentioning that our results are sensitive to our choice of traits and environments. So if we chose a highly plastic trait and we picked up the environmental axis that drives the plastic response then, yes, we should see it falling out on the extremes of the x-axis of figure 3. We point, in the discussion, to one trait that exhibits this pattern. In the trait/environment set that we chose, however, the local adaptation signal is the stronger one.

317 – Less short hand would be more readable. E.g. Understanding correlations between the environment and traits will...

Point taken. We changed this and several other instances. We are also a bit suspect on the use of the word “correlation”, however, because it implies a specific analysis (that we don’t use). So we have switched to “relationship” throughout (whenever we refer to a regression coefficient).

Reviewed by Thomas Lenormand, 2017-06-28 13:53

General comment

This paper proposes to exploit the fact that local adaptation is eroded by gene flow to detect traits, and the causal climatic variables, involved in local adaptation. As the authors argue, this pattern of erosion can help distinguishing local plastic response (supposedly not influenced by gene flow) from that of local adaptation. In principle,

this is indeed a good idea (although plasticity also can present some degree of local adaptation). In practice, however, the methodology may be much trickier to elaborate than what the authors propose (see four major comments on the method below). Historically, phenotypic clines have always been difficult to interpret because of plasticity. However, this is not the only issue. Another difficulty is that many clinal patterns can arise due to biogeographic / historical patterns unrelated to local adaptation. Here, having a control (i.e. neutral differentiation) is necessary to make conclusive evidence. The paper should not entirely bypass this difficulty.

These are incisive observations. We largely agree with all of them and already touched on many of them in the original discussion. Despite the fact that our method is imperfect, it is encouraging that a very coherent pattern of local adaptation emerges. In the revised version we have greatly expanded and organised this discussion into a “challenges” section. Our intent is not to provide the final method in this paper but to point out the idea of incorporating gene flow into local adaptation analyses, and let the community polish it. Faster and better that way. So the revised manuscript tries to make this intent clear: this isn't the final say, and there are several avenues where the idea can be improved: primarily through careful sample design, but also through analytical innovation.

Comments on the method

The first issue is that the method proposes to measure the effect of gene flow by a measure of connectivity. Apart from the fact that connectivity may not be a good surrogate of dispersal, a more serious issue is that the effect of gene flow is not that of dispersal per se. Maladaptation only occurs when gene flow brings in genes that are not locally well adapted. Being highly connected to populations presenting the same traits/genes is not a problem. Being little connected but to very differentiated populations can lead to strong maladaptation. Connectivity does not matter that much. What matters is the genetic composition of the migrant pool compared to the local one. Hence the method proposed can be very misleading.

We agree, and we appreciate the distinction. Connectivity measures are, however, often correlated with gene flow estimates. We already mention that the connectivity measure would, ideally, be calibrated against gene flow measures. In the revised manuscript we also point out that our connectivity variable might better be replaced with a measure that incorporates connectivity as well as environmental heterogeneity. Using such an index of migrant load comes with additional analytical complexity, but would (in principle) go a substantial way towards tightening up on the idea of gene flow and maladaptation.

A second issue is that connectivity is simply measured as a local characteristic of the environment, just like any other climatic or soil variable. Finding an effect of connectivity may thus simply reflect that connectivity is partially correlated to another variable causing a plastic response (i.e. connectivity correlates with an environmental variable that influences the traits, possibly in a plastic way). Hence, with the method proposed, it is impossible to rule out that plasticity explains all the patterns.

We identify the issue of covariation between connectivity and environmental variables in the original manuscript. We didn't, however, think about the possibility adumbrated here that plastic responses to the covarying environmental variable could play into our results. But surely this would come out in the main effect of connectivity, not in the interaction between connectivity and environment? On consideration, we don't think there is a problem here.

In principle, it should be easy to check for such spurious effect. For instance, any other climatic variable could be used as a second covariate (mimicking the role of connectivity). It would be then be important to show that such analysis does not result in similar outcome. By substituting other climatic variables to connectivity (and repeat the analysis) would allow to present a baseline distribution of the effect of a second covariate (e.g. on local adapt score, or on the strength of relationship as illustrated on Fig. 3) and see whether the effect of connectivity really stands out. Finally, even if it does stand out, it could still be possible that it is caused by the structure of hidden correlations and plasticity effects alone (e.g. for variables not included in the dataset).

Again, I think the fact that we include the main effect of connectivity already covers this. We are interested in measuring the way that connectivity affects the main effect of the environment variable; that is, the interaction term. There remains a problem when connectivity is strongly correlated with aspects of the environment, and we discuss this in the manuscript.

A third issue is that the interpretation can be very unclear. For instance, trait optimum may not vary linearly with climatic variables. Further, there is a problem of interpretation in the statistical model, which depends on the choice of intercept. Comparing all the effects based on slopes requires that the mean of environmental values (which depends on the distribution of sampled locations) matches with the position where gene flow from the right and left cancel each other. This is highly unlikely to occur by chance. Another difficulty in the interpretation is that gene flow is expected to attenuate, not reverse, the trends.

We already discuss the assumption of linearity in the original manuscript and retain that discussion in the revised manuscript.

Changing the intercept will certainly change our estimates of the main effects. No doubt. But we do not see that a mean-centred estimate of the main effects requires an assumption that this is where gene flow cancels out. In fact, we are not sure that we are even interested in asymmetry in gene flow. Rather we (implicitly) assume that the environment is heterogenous in all directions (we are not assuming a cline, but more a random walk in environmental space) and that all immigrants have some mean level of maladaptation. Rather than mean-centring our variables, the only other sensible transformation would be to set the minimum connectivity value to zero. The resultant intercept would then be estimating the environment coefficient at the minimum (rather than mean) value of connectivity in the landscape, which is presumably where the environment

coefficient would be expected to have largest magnitude. But really, the choice is arbitrary, so we have left things as they are.

Shifting the intercept in this way would (partially, and inelegantly) address your final point: that our model allows environmental effects to be reversed (at large enough connectivity). Such a result is clearly silly and should never happen in the real world, but can happen in our statistical model. In practice, however, our estimates for the interaction coefficient were always substantially smaller than for the main effect, with the expected interaction being around 0.36 times the expected main effect. With such a ratio, reversals can only happen when connectivity is >2.7 standard deviations above mean connectivity (a very rare situation). So although the model permits silly results, they do not appear (at least at the gross scale). We mention this in the revised discussion. We could cook constraints into the model, but in some sense it is more encouraging that an unconstrained model gives sensible results.

A fourth issue relates to the fact that the method considers one environmental variable after another. Even considering only linear effects, there is no reason that the direction of adaptation align along any one of these axes. The linear combination of variables that are relevant for adaptation may be in a different direction for the projection in 1D. More generally, it is very difficult to see how the choice of climatic variables was guided. This is entirely arbitrary and it can completely miss the important ones (this is a general and difficult problem, not only in this paper).

Again, we largely agree with this. But we also suspect that our method will still pick out the variables with large bearing on local adaptation: those aligned most strongly with the multivariate axis. We mention this multivariate problem in the revised “challenges” section.

As for the variables chosen, yes, there is no avoiding arbitrariness here. They were chosen based on our judgement of variables that a) we could imagine might matter, and that b) other people have imagined might matter. Certainly, the set we chose are commonly used for correlative species distribution models and have also been used by others interested in adaptation of physiological traits to climate. Our choice is arbitrary, but not unhinged.

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Using connectivity to identify climatic drivers of local adaptation

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Running title: Identifying drivers of local adaptation

Statement of authorship: BLP and SLM designed research; SLM, JL, and BLP collected data; SLM & BLP performed analyses and wrote the manuscript; all authors contributed to revisions.

Keywords: local adaptation, climate, connectivity, gene flow.

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33 **Abstract**

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

56

57 **Significance Statement**

58 Despite 150 years of evolutionary research, we still do not have a good method for
59 determining the climatic drivers of local adaptation. Having such a method is critical
60 if we are to understand and mitigate the impact of climate change. Here we exploit the
61 fact that local adaptation is eroded by gene flow to develop a new method for
62 identifying the environmental drivers of local adaptation. Our method will allow
63 workers around the world, for the first time (and often using existing datasets), to
64 determine the climatic drivers of local adaptation in their system. As such, the method
65 has powerful implications not only for pure evolutionary research, but also for
66 management and conservation.

67 **Introduction**

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

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77 Evolution typically optimizes phenotypes, but the optimum will vary through both
78 time and space (11, 12), in turn leading to populations ('demes') that have, on average,
79 higher fitness in their home environment than an immigrant would: local adaptation.

80 While adaptive optima for traits almost always vary geographically, it does not follow
81 that all geographic trait variation is due to local adaptation. Geographic trait variation
82 can arise due to other factors, such as phenotypic plasticity (including developmental
83 plasticity and maternal effects), neutral clines, and environmental factors (such as
84 geographic variation in fitness-reducing parasites). These factors can give the
85 appearance of local adaptation (10, 11), complicating our identification of climate-
86 relevant adaptive variation.

87

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

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

110

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

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

$$132 \quad \text{Trait}_i = A + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + \text{error}_i$$

133 Where:

134 trait_i = trait value for individual i

135 A = intercept

136 B_{env} = coefficient of the environmental variable

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

139 B_{conn} = coefficient of connectivity

140 Conn = connectivity at the individual's site

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

142 $error_i$ = deviation between expected value and trait value of individual i

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

144
$$\text{Trait}_i = A + (B_{env} + B_{int} \times \text{Conn}) \times \text{Env} + B_{conn} \times \text{Conn} + error_i$$

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

149

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

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

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

173

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

190

191 Here we explore the idea of using connectivity to infer local adaptation. To do this we
192 develop a case study of a lizard species from northern Australia. We use this system
193 to examine the relationship, across sites, between traits and climatic variables. We
194 assess how habitat connectivity affects these relationships and use the interaction
195 between the environmental variable and connectivity to rank trait–environment
196 combinations. In doing so, we reveal a set of trait–environment relationships that
197 appears to be dominated by local adaptation.

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199

200 **Methods**

201 Study species and site selection

202 The Rainforest Sunskink (*Lampropholis coggeri*) is a small (snout–vent length up to
203 45 mm), diurnal scincid lizard restricted to the rainforests of the Wet Tropics region
204 of northeastern Australia (26). The rainforests of this region cover a wide range of
205 environmental conditions, spanning significant elevation (0–1600 m ASL),
206 precipitation (annual mean precipitation of 1432–8934 mm, not including input from
207 cloud stripping), and temperature (annual mean temperature of 16.3–25.8°C)
208 gradients. This heliothermic skink is active year-round, often seen basking in patches
209 of sunlight on the rainforest floor. Lizards were captured by hand from sites that were
210 selected to maximize the environmental heterogeneity sampled (Fig. 2).

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

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

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

216 Townsville for trait measurement. All procedures involving lizards were approved by
217 the JCU animal ethics committee (projects A1976 and A1726).

218
219 Physiological trials

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

229

230 Morphological measurements

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

238

239 Climatic variables, and connectivity

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

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

264

265 Analysis

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

$$274 \text{ trait}_i = A + B_{svl} \times \text{SVL}_i + B_{sex} \times \text{Sex}_i + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + \text{error}_i$$

275 Where:

276 trait_i = standardized trait value of interest for lizard i

277 A = intercept

278 B_{svl} = coefficient of SVL

279 SVL = lizard snout–vent length, to control for effect of body size
280 B_{sex} = coefficient of Sex
281 Sex = lizard sex (this species is sexually dimorphic in some morphological
282 traits)
283 B_{env} = coefficient of environmental variable
284 Env = environmental variable (e.g., annual mean temperature) at the lizard's
285 site
286 B_{conn} = coefficient of connectivity
287 Conn = connectivity index at the lizard's site
288 B_{int} = coefficient of interaction between Env and Conn
289 $error_i$ = deviation between expected value and trait value of lizard i
290

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

$$292 \quad L = -B_{env} \times B_{int}$$

293 If the signs of the two coefficients (B_{env} and B_{int}) are opposite (which indicates an
294 trait–environmental relationship that is diminished by increasing connectivity, i.e.,
295 evidence for local adaptation), L will be positive. If the signs are the same (which
296 indicates an environmental effect being enhanced by increased connectivity, a
297 situation not consistent with local adaptation), L will be negative. Thus, higher
298 numbers on this scale equate to stronger evidence for local adaptation in that
299 environment–trait pair. This score can, in theory, range from $-\infty$ to $+\infty$. Once many
300 environment–trait combinations have been assessed, the coefficients for all pairs can
301 be plotted (see Fig. 1). As described in the Introduction, in a system dominated by
302 local adaptation, we expect to see a negative relationship between B_{env} and B_{int} (Fig.
303 1B). All analyses were conducted in R v3.2 (32).

304

305 **Results**

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

316

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

330

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

337

338

339 **Discussion**

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

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

349

350 Local adaptation

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

366

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

381

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

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

395

396 System-wide signal of local adaptation

397 The clear negative linear trend displayed in Fig. 3 is precisely what we would expect
398 in a set of trait–environment combinations dominated by local adaptation. Migrant
399 load (the negative effect of the immigration of less-locally adapted individuals) scales
400 positively with immigration as well as with the strength of selection [see equation 5 in
401 Polechová, Barton and Marion (52)]. The reason for this is that, when the strength of
402 selection is moderately high, the environment will have a large effect on relevant
403 traits, and therefore any immigrants coming from differing environments will be
404 particularly maladapted and will therefore have a large and negative impact on the
405 local phenotype. Thus, we expect trait–environment combinations with strong local
406 adaptation to show strong effects of connectivity on the trait–environment relationship
407 (52).

408

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

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

419

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

427

428 Phenotypic plasticity

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

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

446

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

456

457 Caveats and challenges

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

465

466 *Gene flow and connectivity*

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

478

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

489

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

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

497

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

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

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

520

521 *Covariation between explanatory variables*

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

528

529 *Multivariate traits and environments*

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

541

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

554

555 *Neutral clines*

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

564

565 Conclusion

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

591

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774 **Figure Captions**

775

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

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

790

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

797

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

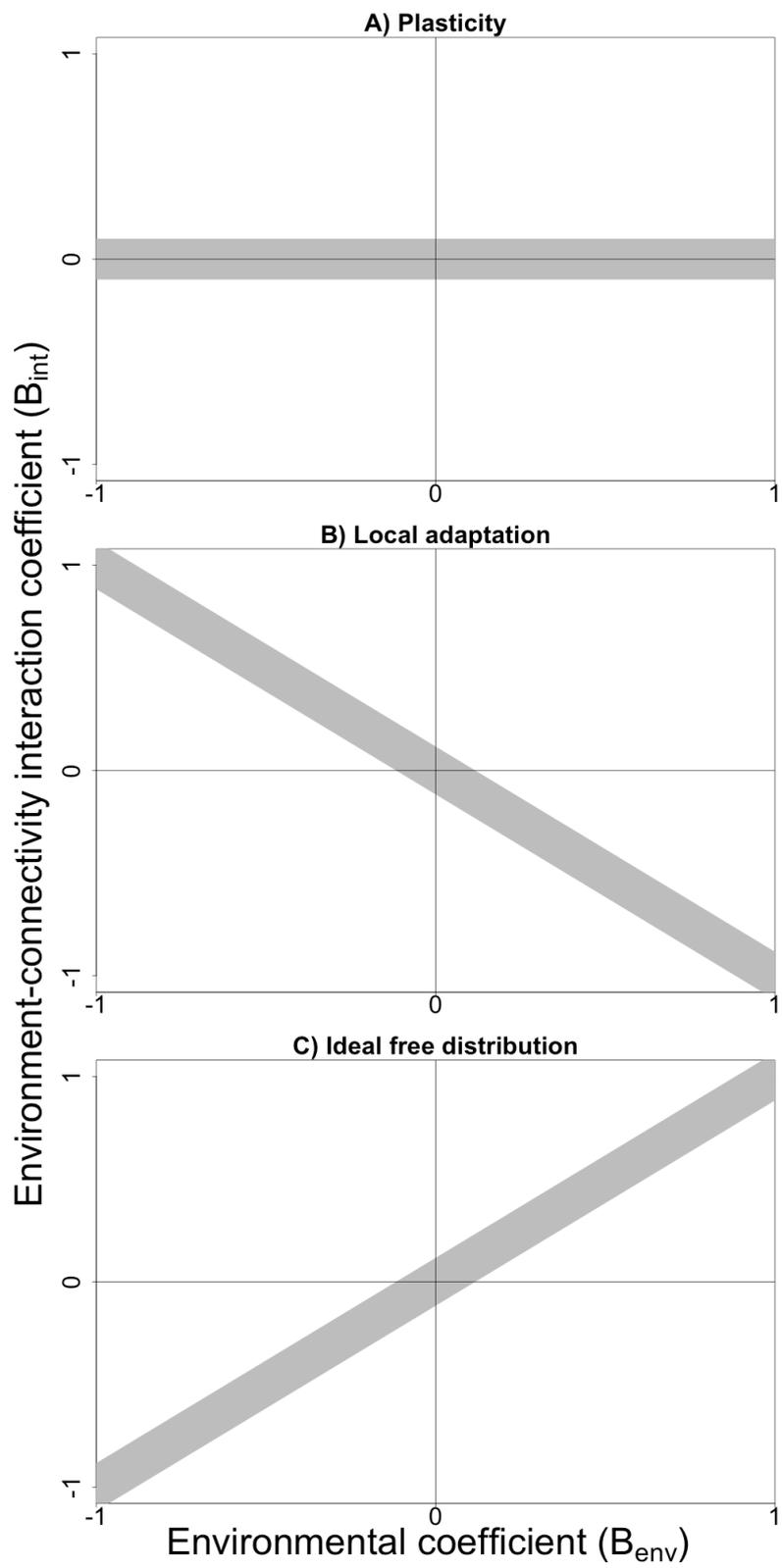
803 $L = \text{local adaptation index: } -B_{env} \times B_{int}$

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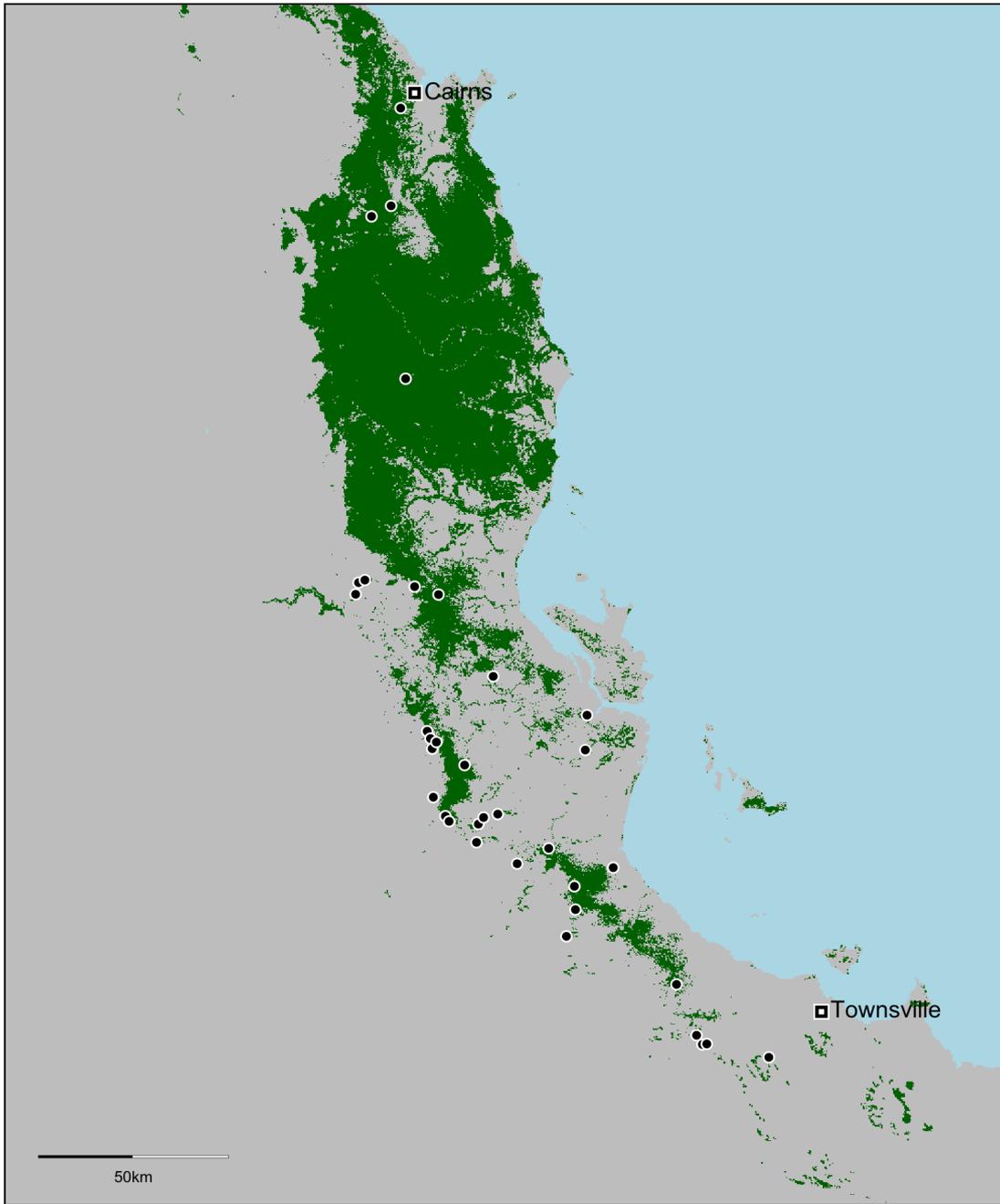
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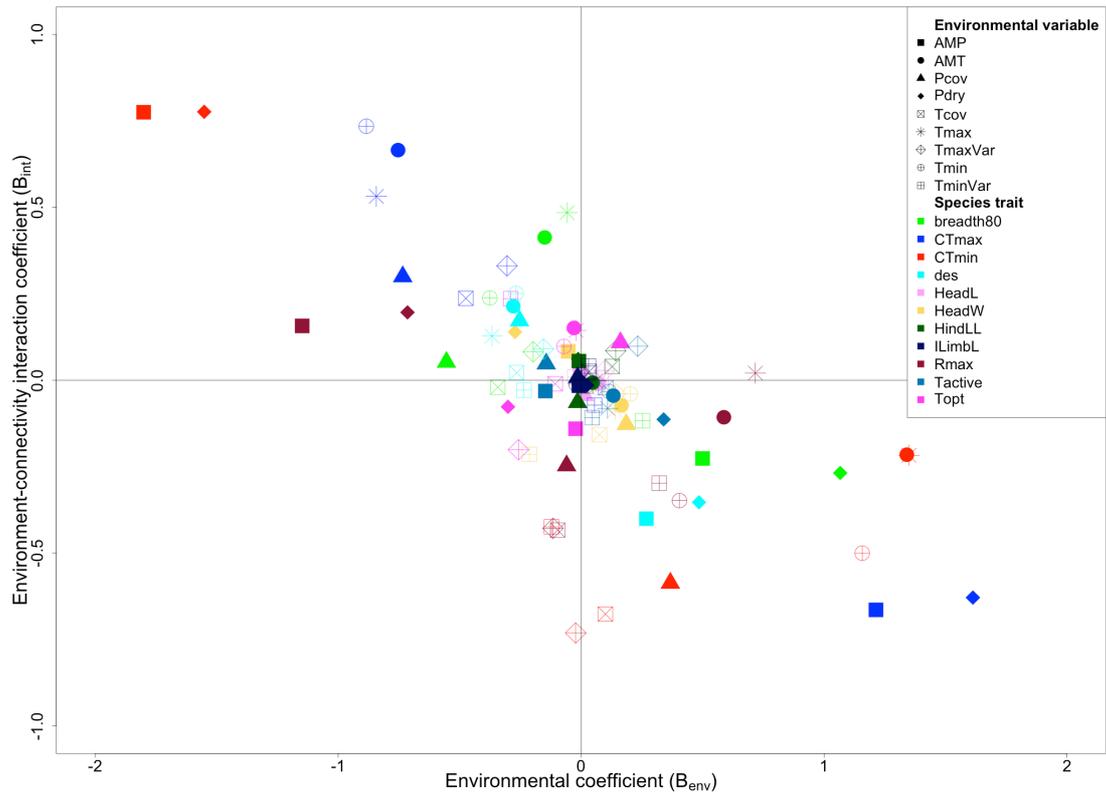
811 **Figure 1**

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Figure 2



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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

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Figure 4

1 Supplementary Material for “Using connectivity to identify
2 environmental drivers of local adaptation”

3
4 Stewart L. Macdonald, John Llewelyn, & Ben L. Phillips

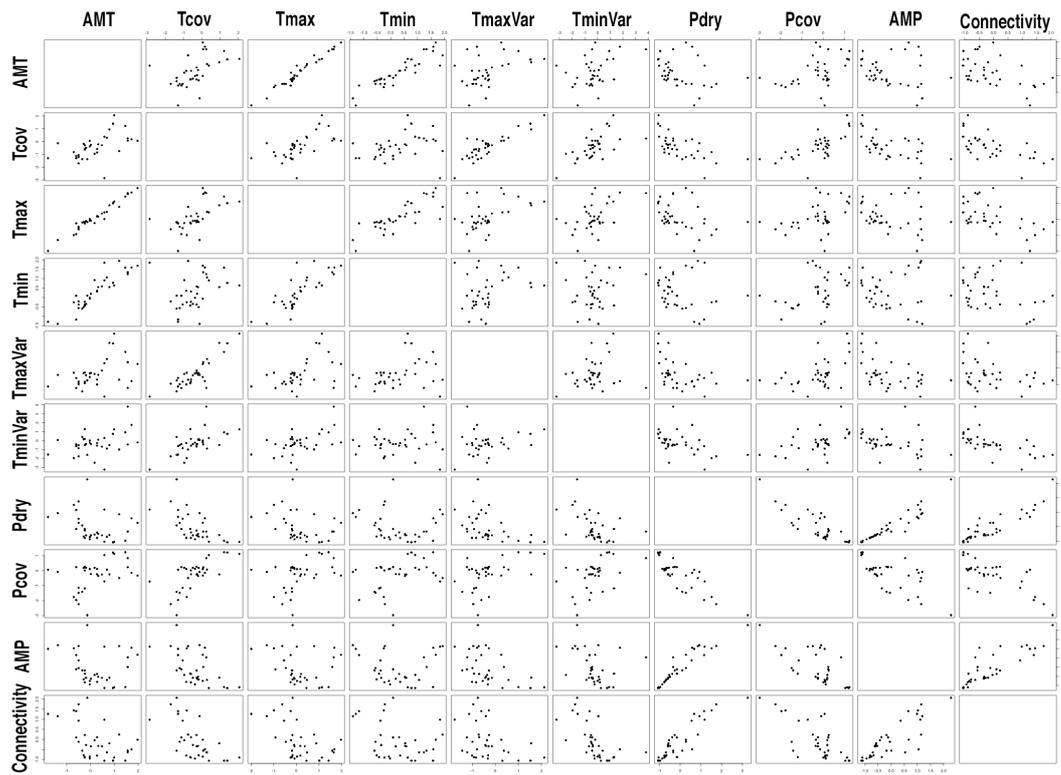
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6 **Details of variables used in the analysis**

7
8 **Table S1:** Description of lizard traits used in analysis

Measurement	Description
MORPHOLOGY	
HeadW	Head width at the widest point of the skull in mm.
HeadL	Head length in mm from tip of snout to anterior edge of ear opening. Left and right sides were measured and averaged to generate one value.
ILimBL	Interlimb length in mm from axilla to groin when fore- and hindlimbs are perpendicular to body. Left and right sides were measured and averaged to generate one value.
HindLL	Hindlimb length in mm from sole of foot to above knee, measured when femur is perpendicular to long axis of body and tibia is perpendicular to femur. Left and right sides were measured and averaged to generate one value.
SVL	Snout–vent length measured from tip of snout to anterior edge of cloaca, in mm.
Total length	From tip of snout to tail tip in mm.
Mass	In grams to three decimal places.
PHYSIOLOGY	
Measurement	Description
CTmin	Critical thermal minimum; the lower temperature at which the skink’s righting response is lost, in °C.
CTmax	Critical thermal maximum; the upper temperature at which righting response is lost, in °C.
Rmax	Rate of maximum sprint performance, in cm/sec.
Topt	The temperature at which maximum sprint performance is achieved, in °C.
Tactive	Average active temperature of skink when allowed to thermoregulate freely in a thermal gradient.
Breadth80	Thermal-performance breadth; the temperature range that encompasses the middle 80% of the performance curve, in °C.
des	Water loss rate. The rate at which mass is lost when challenged by desiccating conditions, in g/min.

10 **Table S2:** Description of the environmental variables used in analysis

Variable	Description
Annual mean precipitation (AMP)	Average annual rainfall in mm, calculated from 1976–2005 using AWAP data (56).
Seasonality of precipitation (Pcov)	The coefficient of variation of precipitation is the 30-year average (1976–2005) of standard deviation of the weekly precipitation estimates expressed as a percentage of the mean of those estimates (i.e., the annual mean).
Precipitation of the driest quarter (Pdry)	Total rainfall in mm for the quarter (90 consecutive days) that had the minimum total rainfall in a year, calculated over 1976–2005 from AWAP data (56).
Annual mean temperature (AMT)	From ANUCLIM (57).
Seasonality of temperature (Tcov)	From ANUCLIM (57).
Average minimum daily temperature (Tmin)	20-year average (1991–2010) for daily minimum temperature. From data in (58).
Average maximum daily temperature (Tmax)	20-year average (1991–2010) for daily maximum temperature. From data in (58).
Variance of Tmax (Tvar)	20-year average (1991–2010) of variance of Tmax. From data in (58).
Connectivity	A connectivity index for each grid cell of rainforest was calculated by integrating habitat suitability and proximity through space. For full details see below ('Connectivity').



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14 **Figure S1**

15 Pairwise correlation plot of the environmental variables used in analyses.

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Using connectivity to identify climatic drivers of local adaptation

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Running title: Identifying drivers of local adaptation

Statement of authorship: BLP and SLM designed research; SLM, JL, and BLP collected data; SLM & BLP performed analyses and wrote the manuscript; all authors contributed to revisions.

Keywords: local adaptation, climate, connectivity, gene flow.

Revised manuscript

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34 **Abstract**

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

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68 **Significance Statement**

69 Despite 150 years of evolutionary research, we still do not have a good method for
70 determining the climatic drivers of local adaptation. Having such a method is critical
71 if we are to understand and mitigate the impact of climate change. Here we exploit the
72 fact that local adaptation is eroded by gene flow to develop a new method for
73 identifying the environmental drivers of local adaptation. Our method will allow
74 workers around the world, for the first time (and often using existing datasets), to
75 determine the climatic drivers of local adaptation in their system. As such, the method
76 has powerful implications not only for pure evolutionary research, but also for
77 management and conservation.

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79 **Introduction**

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

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

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Deleted: , and particularly phenotypic plasticity,

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99
100 To circumvent these issues, evolutionary biologists use experimental approaches to
101 demonstrate local adaptation (12, 13). Experiments designed to detect local adaptation
102 typically utilise one of two techniques: 1) reciprocal transplants, which are done *in*
103 *situ*, and are considered the gold standard for demonstrating local adaptation; or 2)

108 common garden experiments, which are usually done in the lab where it is easier to
109 control each environmental variable (12). Both of these techniques can be difficult,
110 for reasons of time, expense, logistics, or ethics. This difficulty increases as the
111 number of separate demes and environmental variables to be tested increases and as
112 the generation time of the organism increases (12). Additionally, although reciprocal
113 transplants will detect signs of local adaptation, they are not necessarily suited to
114 identifying the environmental drivers of that local adaptation (14). This is because *in*
115 *situ* reciprocal transplants necessarily encompass all the environmental variables that
116 differ between the transplant locations. Lab-based common garden approaches may,
117 in principle, be more suited to identifying environmental drivers (because the
118 environment may be under a degree of control), but in practice it often remains
119 impossible to identify the environmental drivers of trait variation seen in the wild.
120 Thus, the best experimental tools we have for studying local adaptation are
121 demanding in terms of time and cost, and are unsuitable for assigning environmental
122 drivers (such as climate variables) to adaptive variation. If we are looking for climate-
123 driven local adaptation, this is a problem: we want to know which climate variable or
124 variables are the main drivers of adaptation, and we urgently need this information for
125 many species.

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

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

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

151 Where:

152 trait_i = trait value for individual *i*

153 A = intercept

154 B_{env} = coefficient of the environmental variable

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

157 B_{conn} = coefficient of connectivity

158 Conn = connectivity at the individual's site

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

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166 error_i = deviation between expected value and trait value of individual i

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

168
$$\text{Trait}_i = A + (B_{\text{env}} + B_{\text{int}} \times \text{Conn}) \times \text{Env} + B_{\text{conn}} \times \text{Conn} + \text{error}_i$$

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

173
174 If we now collect data on a large number of trait–environment relationships, and their
175 interaction with connectivity, we can imagine several possible patterns emerging.

176 These possibilities are depicted in Figure 1. Each panel represents a possible
177 relationship between trait–environment coefficients (along the x-axis) and the
178 interaction between environment and connectivity (y-axis). Panel A shows a set of
179 trait–environment relationships that vary in strength, but that are not influenced by
180 connectivity (i.e., no environment–connectivity interaction). This pattern is indicative

181 of a system in which trait–environment relationships are predominantly driven by
182 plastic responses of traits to their environment (i.e., traits always match the local
183 environment, regardless of the level of inward gene flow). Panel B shows a system in
184 which trait–environment relationships are eroded by connectivity: increased

185 connectivity diminishes the relationship between the environment and the trait. In this
186 situation, the interaction between the environmental variable and connectivity is
187 negative when the environmental coefficient is positive (i.e., greater connectivity
188 causes the environmental coefficient to decrease towards zero; bottom-right
189 quadrant), and positive when the environmental coefficient is negative (i.e., greater
190 connectivity causes the environmental coefficient to increase towards zero; top-left

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Deleted: Plastic responses are still of interest, and many species are likely to show such plasticity as the climate changes (20). Panel B shows a system in which environment–trait relationships are eroded by connectivity: increased connectivity diminishes the correlation between the environment and the trait.

203 quadrant). This is the pattern we would expect if there is a genetic basis to the trait–
204 environment relationship, such as is exhibited by local adaptation. Panel C shows the
205 situation where the effect of the environment tends to be enhanced by connectivity.
206 This pattern might arise in organisms that are highly mobile and can actively move to
207 their ideal environment, thus avoiding the selective pressures that would lead to local
208 adaptation.
209
210 Understanding how species respond to specific aspects of their environment is vital if
211 we are to have any hope of halting the current rapid loss of biodiversity. Climate
212 change is undoubtedly one of the biggest threats to global biodiversity (20, 21), and
213 conservation biologists are looking to a variety of techniques to assess and help
214 mitigate the impacts of climate change on vulnerable species (22–24). One technique
215 that is likely to see increasing use is targeted, or assisted, gene flow [TGF; for review,
216 see (22, 25)]. This technique involves the spatial redistribution of long-standing
217 adaptations, and acts to increase genetic diversity in recipient populations, thereby
218 bolstering capacity for evolutionary adaptation (10, 22, 24, 25). When applying TGF
219 to help species adapt to climate change, we need to find an existing location that
220 matches the future climate at our recipient site, and then translocate animals from that
221 source location. It is a simple idea, but climate is multidimensional and species will
222 not be adapting equally to each climate axis: is a difference of 0.5°C in mean
223 temperature more important than a difference of 100mm in annual rainfall? The
224 answer depends upon which aspects of climate (hereafter “climatic axes”) have the
225 strongest influence on fitness.
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234 Here we explore the idea of using connectivity to infer local adaptation. To do this we
235 develop a case study of a lizard species from northern Australia. We use this system
236 to examine the relationship, across sites, between traits and climatic variables. We
237 assess how habitat connectivity affects these relationships and use the interaction
238 between the environmental variable and connectivity to rank trait-environment
239 combinations. In doing so, we reveal a set of trait-environment relationships that
240 appears to be dominated by local adaptation.

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243 **Methods**

244 Study species and site selection

245 The Rainforest Sunskink (*Lampropholis coggeri*) is a small (snout-vent length up to
246 45 mm), diurnal scincid lizard restricted to the rainforests of the Wet Tropics region
247 of northeastern Australia (26). The rainforests of this region cover a wide range of
248 environmental conditions, spanning significant elevation (0–1600 m ASL),
249 precipitation (annual mean precipitation of 1432–8934 mm, not including input from
250 cloud stripping), and temperature (annual mean temperature of 16.3–25.8°C)
251 gradients. This heliothermic skink is active year-round, often seen basking in patches
252 of sunlight on the rainforest floor. Lizards were captured by hand from sites that were
253 selected to maximize the environmental heterogeneity sampled (Fig. 2).

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

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

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

265 Townsville for trait measurement. All procedures involving lizards were approved by
266 the JCU animal ethics committee (projects A1976 and A1726).

267
268 Physiological trials

269 Physiological trials commenced within seven days of skinks being collected from the
270 field; skinks being used only for morphology were measured and released back at
271 their point of capture within seven days. The following measures were taken from
272 each skink (n = 259) during laboratory trials: critical thermal minimum (CTmin),
273 critical thermal maximum (CTmax), thermal-performance breadth for sprinting
274 (breadth80), maximum sprint speed (Rmax), temperature at which sprint speed is
275 optimized (Topt), active body temperature as measured in a thermal gradient
276 (Tactive), and desiccation rate (des) (see Table S1 for further details). Details of trait
277 measurement procedures are detailed elsewhere (see [27, 28](#)).

278

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280 Morphological measurements

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

288

289 Climatic variables, and connectivity

290 Because our study aimed to assess adaptation to local climate, various temperature
291 and precipitation variables were extracted for each site (see Table S2 for details). We
292 considered both means and extremes. It is important to consider climatic extremes,
293 because temperature extremes may be increasing faster than mean temperatures (29),
294 and selection may often occur during extreme weather events (30). Many
295 environmental variables are highly correlated (27), so only the less-derived variables
296 were used in analyses, specifically: annual mean precipitation (AMP); seasonality of
297 precipitation (Pcov); precipitation of the driest quarter (Pd_{dry}); annual mean
298 temperature (AMT); coefficient of variation of temperature (Tcov); average minimum
299 daily temperature (T_{min}); average maximum daily temperature (T_{max}); average
300 variance of daily maximum temperature (T_{max}Var); and average variance of daily
301 T_{min} (T_{min}Var).

302

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

319 Analysis

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

$$329 \text{ trait}_i = A + B_{svl} \times \text{SVL}_i + B_{sex} \times \text{Sex}_i + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + \text{error}_i$$

330 Where:

331 trait_i = standardized trait value of interest for lizard i

332 A = intercept

333 B_{svl} = coefficient of SVL

Deleted: the Supplementary Material. Briefly, it is a measure of habitat suitability for our focal skink species, averaged over space using a species-specific estimate of dispersal potential.

338 SVL = lizard snout–vent length, to control for effect of body size
339 B_{sex} = coefficient of Sex
340 Sex = lizard sex (this species is sexually dimorphic in some morphological
341 traits)
342 B_{env} = coefficient of environmental variable
343 Env = environmental variable (e.g., annual mean temperature) at the lizard's
344 site
345 B_{conn} = coefficient of connectivity
346 Conn = connectivity index at the lizard's site
347 B_{int} = coefficient of interaction between Env and Conn
348 $error_i$ = deviation between expected value and trait value of lizard i
349

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

351
$$L = -B_{env} \times B_{int}$$

352 If the signs of the two coefficients (B_{env} and B_{int}) are opposite (which indicates an

353 ~~trait–environment relationship that is diminished by increasing~~ connectivity, i.e.,

354 evidence for local adaptation), L will be positive. If the signs are the same (which

355 indicates an environmental effect being enhanced by ~~increased~~ connectivity, a

356 situation not consistent with local adaptation), L will be negative. Thus, higher

357 numbers on this scale equate to stronger evidence for local adaptation in that

358 environment–trait pair. This score can, in theory, range from $-\infty$ to $+\infty$. Once many

359 environment–trait combinations have been assessed, the coefficients for all pairs can

360 be plotted (see Fig. 1). As described in the Introduction, in a system dominated by

361 local adaptation, we expect to see a negative relationship between B_{env} and B_{int} (Fig.

362 1B). All analyses were conducted in R v3.2 (32).

363

364 Results

365 There was substantial variation in the effect of environment (B_{env}) and its interaction

366 with connectivity (B_{int}) across climate and trait variables, with B_{env} ranging from -1.8

367 to 1.61 , and B_{int} ranging from -0.73 to 0.78 (Fig. 3). Despite this variation, a clear

368 pattern is evident, with most points in Figure 3 appearing in the top-left or bottom-

369 right quadrants: the quadrants in which the two coefficients have opposing signs, and

370 where we would expect points to fall if ~~trait–environment relationships have a genetic~~

371 ~~basis~~. Across these ~~trait–environment combinations~~ there is a distinct negative linear

372 trend (slope = -0.36 , $p < 0.001$). It is especially noteworthy that the ~~trait–environment~~

373 pairs with the largest coefficients are in the two quadrants indicative of local

374 adaptation.

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387

388 Overall, physiological traits showed substantially stronger environmental effects (i.e.,
389 larger values of B_{env}) than did morphological traits, with the largest environmental
390 effects being exhibited by CTmin (AMP: -1.80; Tmax: 1.35; Pdry: -1.55) and CTmax
391 (Pdry: 1.61; AMP: 1.21). Physiological traits also showed stronger interactions
392 between environmental effects and connectivity, again with CTmin and CTmax
393 showing the largest interactions. These trends are apparent when we examine our
394 index of local adaptation, L . Figure 4 shows a heatmap of all trait-environment pairs
395 ranked via reciprocal averaging according to the strength of their local adaptation
396 index. The trait-environment pairs that show the strongest signature of local
397 adaptation appear at the top-left in red. There is a rough divide, with most of the
398 physiological traits on the left and most of the morphological traits on the right. The
399 exceptions are the physiological traits T_{opt} and R_{max}, which appear at the far right of
400 the figure.

401

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

408

409

410 Discussion

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

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

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428 Local adaptation

429 In the trait-environment combinations we assessed, physiological traits typically
430 showed a substantially stronger effect of environment (B_{env}) than did morphological
431 traits, with the largest environmental effects shown in CTmax and CTmin (Figs. 3 &
432 4). Physiological traits also generally showed stronger environment-connectivity
433 interactions (B_{int}), again with CTmin and CTmax showing the largest interactions.
434 Overall, physiological traits generally showed stronger evidence of local adaptation
435 than did morphological traits. This result is intuitive: we would expect an ectotherm's
436 physiological traits to be under strong selection from climate (37-39), but the fitness
437 link between morphology and climate is much less clear. Had we also included some
438 environmental variables that had a clearer bearing on morphology, we might have
439 detected stronger trait-environment relationships for morphology. For example,
440 skinks that occur in rockier habitats show various morphological adaptations to that
441 environment (40). Including a measure of rockiness in our set of environmental
442 variables might have allowed us to detect a signal of local adaptation for limb length.
443 Here, however, our focus is on climatic aspects of the environment.

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453 Of the environmental variables used, our analysis suggests that precipitation is a very
454 strong driver of local adaptation, even in thermal traits that might not seem obviously
455 related to precipitation (e.g., CTmin, CTmax). Although this may seem a surprising
456 result, precipitation has been shown to directly affect growth rate, body temperature,
457 activity patterns, and thermoregulatory opportunities in lizards (38, 41-45). Wetter
458 areas also have higher thermal inertia (and so lower cyclical thermal fluctuations
459 (46)), and changed environmental variance in temperature potentially has a strong
460 influence on thermal limits (47). Additionally, Bonebrake and Mastrandrea (48) found
461 that changes in precipitation can significantly affect modeled fitness and performance
462 curves. Finally, comparative analyses also suggest that precipitation can influence
463 thermal traits in many species (38). Thus, although the mechanisms linking
464 precipitation to thermal limits are diffuse and poorly resolved, they do exist, and our
465 analyses suggest that precipitation is a strong driver of local adaptation at thermal
466 physiological traits.

467

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

478 develop plastic responses, rather than fixed differences: local adaptation to these
479 variables would likely be reflected in reaction norms, rather than point values for
480 traits. (49-51).

481

482 System-wide signal of local adaptation

483 The clear negative linear trend displayed in Fig. 3 is precisely what we would expect
484 in a set of trait–environment combinations dominated by local adaptation. Migrant
485 load (the negative effect of the immigration of less-locally adapted individuals) scales
486 positively with immigration as well as with the strength of selection [see equation 5 in
487 Polechová, Barton and Marion (52)]. The reason for this is that, when the strength of
488 selection is moderately high, the environment will have a large effect on relevant
489 traits, and therefore any immigrants coming from differing environments will be
490 particularly maladapted and will therefore have a large and negative impact on the
491 local phenotype. Thus, we expect trait–environment combinations with strong local
492 adaptation to show strong effects of connectivity on the trait–environment relationship
493 (52).

494

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

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

509

510 We used long-term climatic averages and found strong evidence that local adaptation

511 dominates over plasticity in our trait–environment set. If we had included different

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512 environmental variables, such as the conditions the lizards had recently encountered,

513 signals of plasticity may have been more apparent. Clearly environmental variables

514 that are similar across generations should lead to local adaptation, while

515 environmental variables that fluctuate within generations should have a strong

516 influence on phenotypic plasticity.

517

518 Phenotypic plasticity

519 The importance of accounting for phenotypic plasticity is, however, exemplified in

520 our dataset by the relatively strong effect of precipitation of the driest quarter (Pdry)

521 on the temperature at which maximum sprint speed is achieved (Topt) and on

522 maximum sprint speed (Rmax) itself. On their own, these strong trait–environment

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523 relationships might be interpreted as evidence for local adaptation. Our analysis,

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524 however, suggests that the environmental effect is largely independent of

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525 connectivity, implying that variation in these traits is due to plasticity rather than

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526 genetic differentiation. Other work (27) has shown little temporal variation in Topt

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527 (within generations) despite clear geographic variation and this, together with our

528 results, suggests that this trait undergoes developmental plasticity, but is fixed in adult

529 lizards. In principle, this non-effect of connectivity could also arise due to selection

530 that is so strong that it maintains local adaptation despite high levels of gene flow

531 [i.e., immigrants are selected against so strongly that they do not contribute to the

\$38 recipient population (11)]. The trait–environment relationships for T_{opt} and R_{max}
\$39 are, however, weaker than those for some other traits (e.g., CT_{max} and CT_{min}) that
\$40 show clear effects of connectivity, so extremely strong selection seems an unlikely
\$41 explanation for the pattern we see here.

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\$42
\$43 The generally weak evidence for plasticity in our dataset should not be considered
\$44 weak evidence for plasticity in these traits. Indeed many of the physiological traits we
\$45 use (e.g., CT_{max}) are notoriously plastic, responding reversibly on timescales ranging
\$46 from hours to months (53, 54). That we do not see signals of plasticity in these traits
\$47 here reflects our choice of environmental variables: long-term climatic variables,
\$48 rather than short-term weather variables (such as the temperature in the week before
\$49 an animal was collected). We chose these long-term variables precisely because we
\$50 are interested in unearthing patterns of local adaptation, rather than patterns due to
\$51 rapid, reversible plasticity.

Deleted: Assumptions

\$53 Caveats and challenges

Deleted: limitations

\$54 Our intent here has been to point out the additional inference that can be drawn from
\$55 data on geographic trait variation if we account for the effect of gene flow on trait
\$56 differentiation. The idea that local adaptation is eroded by gene flow offers a novel
\$57 way to identify the environmental drivers of local adaptation. Such a capacity is of
\$58 fundamental interest, and is also sorely needed if we are to effectively manage the
\$59 impacts of climate change. The methods we use here are, however, embryonic, and in
\$60 the following we point out caveats and challenges for future work.

Deleted: Our analysis requires an index of population connectivity across the landscape, something that can be calculated relatively easily for many species by using broad scale habitat mapping datasets [e.g., vegetation mapping from DERM (53)]. Where possible, these measures of connectivity should be calculated at a scale relevant to the scale of dispersal of the species in question [as was ours, using dispersal rate data for *Lampropholis coggeri* from Singhal and Moritz (54)]. For highly mobile species, such as plants or insects that have wind-assisted dispersal and for which calculating connectivity between populations may be difficult, it is possible our approach will not work. But for many species of animals with relatively low vagility (i.e., the species for which TGF is most needed), our approach should be generally applicable. .

... [1]

\$62 Gene flow and connectivity

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

594

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

605

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

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

614

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

616 Our method assumed that traits have a linear relationship to the environment (at least
617 at the environmental scale across which we are looking). In many instances, this will
618 be a reasonable null assumption: it seems unlikely, for example, that a trait such as
619 desiccation resistance would be high in dry environments, low in moderately wet

620 environments, and then high again in very wet environments. The assumption bears

621 particular mention, however, in the situation where the connectivity index is strongly

622 correlated with one or more of the other environmental variables being used. In our
623 system, for example, AMP and Pdry are correlated with connectivity (Fig. S1). Where

624 the environment–connectivity correlation is very strong, the interaction term in our

625 model (Conn×Env) could be interpreted as a quadratic term for environment (i.e.,

626 Env²). In these cases, it is possible that a strong connectivity interaction is, in fact,

627 pointing to a non-linear trait–environment relationship. Thus, for environmental

628 variables that correlate with connectivity (and there will always be some), careful

629 consideration needs to be given to the possibility of a quadratic fitness function

630 between trait and environment. In our case, it remains possible, for example, that the

631 strong influence of precipitation on local adaptation in our system is spurious, and

632 instead reflects non-linear relationships between optimal trait values and precipitation.

633 We can, however, think of no obvious reason why thermal limits should respond

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

645

646 Covariation between explanatory variables

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

653

654 Multivariate traits and environments

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

666

Deleted: Finally, our approach, by examining one environment–trait combination at a time,

Deleted: (59), and with our approach may lead us to underestimate the number of important environmental drivers of local adaptation. To minimize this effect, care should be taken in future work

Deleted: Such an aim can be achieved by, for example, strategically exploiting latitudinal and altitudinal gradients.

675 An additional analytical challenge is to treat traits as multivariate. Here we have
676 treated each measured trait as independent. In reality, however, traits covary and this
677 covariance can have both genetic and environmental origins (60). As a corollary,
678 selection acts on the multivariate trait, and causes populations to move in multivariate
679 trait space (61). Consequently, local adaptation perhaps should be measured in a
680 multivariate trait space rather than on a univariate basis. Such an aim, however,
681 requires considerable theoretical development and may well require substantially
682 more data. For now, however, we should be aware that we are collapsing our trait
683 space, and each of our measured traits is not independent. For example, in our system
684 there is a strong correlation between CTmin and CTmax, thus we should be aware
685 that these two traits should not get equal weighting when we use our traits to rank
686 environmental variables by their importance to local adaptation.

687

688 *Neutral clines*

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

697

698 Conclusion

699 There is increasing urgency to identify populations that will act as suitable sources for
700 targeted gene flow efforts in the face of climate change. To identify these populations,
701 we need to know which traits influence sensitivity to climate and are locally adapted.
702 Traditional approaches to unearthing local adaptation (reciprocal transplants and
703 common garden experiments) are time consuming, and often cannot attribute
704 adaptation to any particular environmental driver. Local adaptation is, however,
705 undermined by gene flow, and we should be able to use this fact to sort patterns of
706 local adaptation from patterns with other causes. Here we have demonstrated this
707 approach: using connectivity as a proxy for gene flow, and looking for its effect on
708 trait-environment relationships. Our analysis, using a species of lizard from
709 Australia's Wet Tropics rainforest, suggests the approach has merit: the results we
710 achieve are coherent and suggest local adaptation is the overwhelming signal in the
711 set of trait-environment relationships tested. As well as implying a strong role for
712 local adaptation, we have effectively ranked environmental drivers of local
713 adaptation, finding evidence that precipitation and temperature are important
714 environmental variables with regard to local adaptation in our system. Our analysis
715 also suggests that some traits exhibit strong plastic responses to the environment,
716 particularly in response to precipitation of the driest quarter and the seasonality of
717 temperature and precipitation. These specific results will likely apply to other species
718 that are phylogenetically or ecologically similar to our focal species, but the method
719 has the potential to apply much more broadly. Analytical and sampling challenges
720 remain, however, and we point to avenues whereby the method can be improved.
721 Given the potential of this method to provide evidence of local adaptation, and to
722 provide rapid ranking of the climatic drivers of local adaptation, assessment of the
723 method in a broader array of systems is warranted.

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936 **Figure Captions**

937 **Figure 1.** Graphs showing the concepts illustrated by plotting a set of trait–environment
938 coefficients (e.g., the coefficient from a linear model examining the effect of annual mean site
939 temperature on the sprint performance of organisms from that site: B_{env} , x-axis) and the
940 corresponding environment–connectivity interaction coefficients (B_{int} , y-axis). Broad grey line
941 represents the approximate area in which these points would fall. A) Phenotypic plasticity is
942 suggested when trait–environment relationships are strong, but are not influenced by
943 connectivity. B) Local adaptation is suggested when increasing connectivity diminishes the
944 relationship between the environment and the trait. C) The effect of the environment is
945 enhanced by connectivity. This latter pattern might arise in organisms that are highly mobile
946 and can actively move to their ideal environment, thus avoiding the selective pressures that
947 would lead to local adaptation.
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950 **Figure 2.** Map of the southern Australian Wet Tropics bioregion, showing the distribution of
951 rainforest in green and the sampling locations as black dots.
952

953 **Figure 3.** Scatterplot showing the results of 99 linear models run to assess the relationship
954 between each trait–environment pair, and the environment–connectivity interaction. Trait–
955 environment coefficients (B_{env}) are on the x-axis, and environment–connectivity interaction
956 coefficients (B_{int}) are on the y-axis. Local adaptation is suggested when these two parameters
957 are opposite in sign: in trait–environment pairs in which a strong environmental effect is
958 eroded by increasing connectivity.
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960 **Figure 4.** Heatmap showing the relative rankings of climate variables (rows) and
961 morphological and physiological traits (columns). The matrix has been sorted (by reciprocal
962 averaging) and coloured according to the strength of local adaptation, with higher values
963 coloured red and being sorted to the top/left. See Tables S1 and S2 for explanations of the
964 trait and environmental variables used.

965 $L = \text{local adaptation index} = -B_{env} \times B_{int}$

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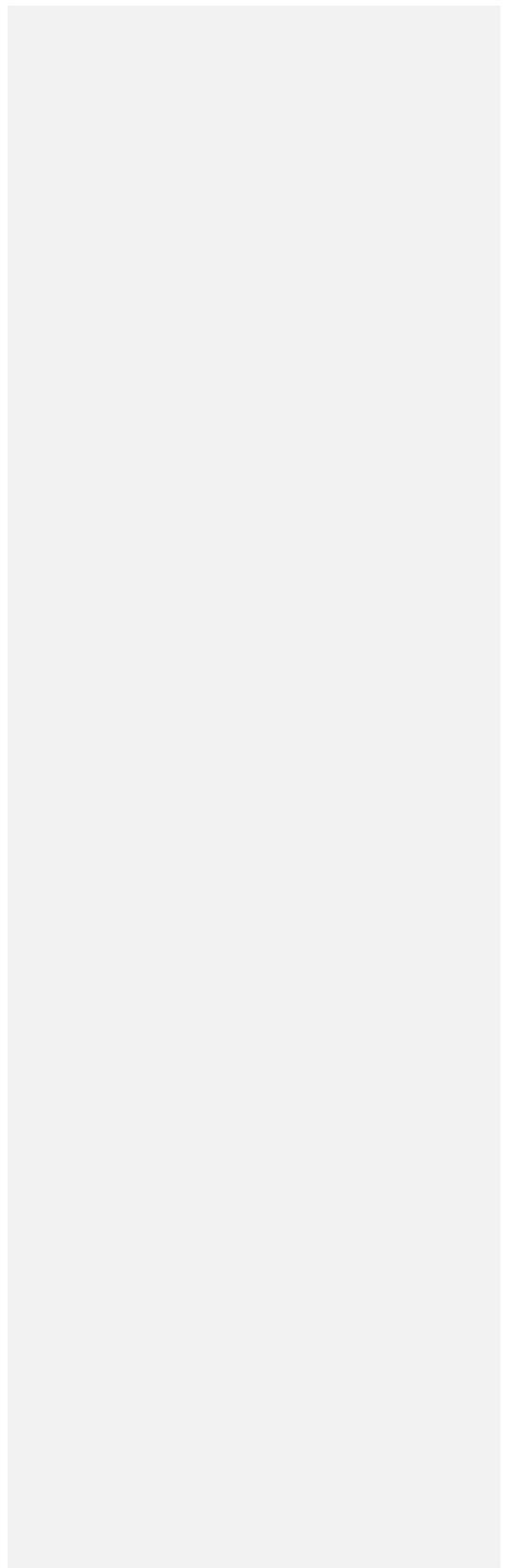
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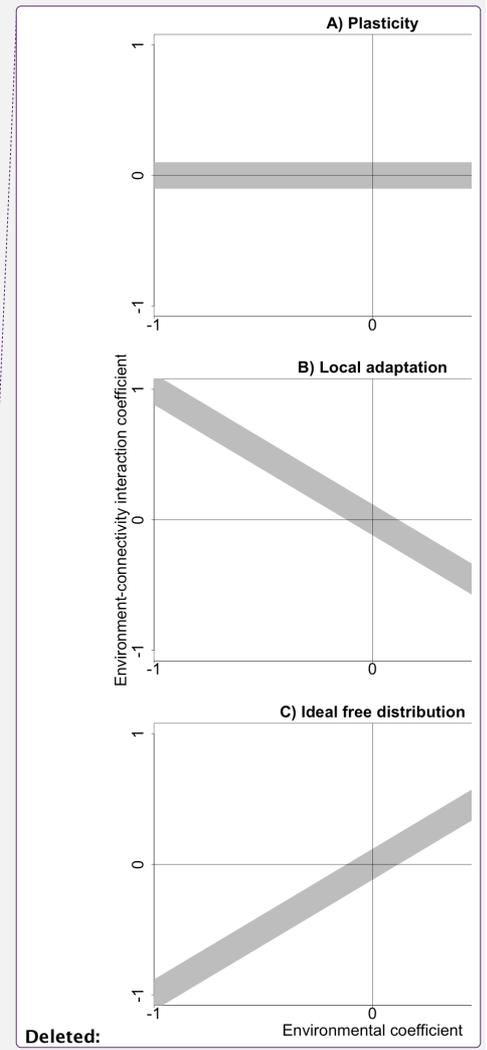
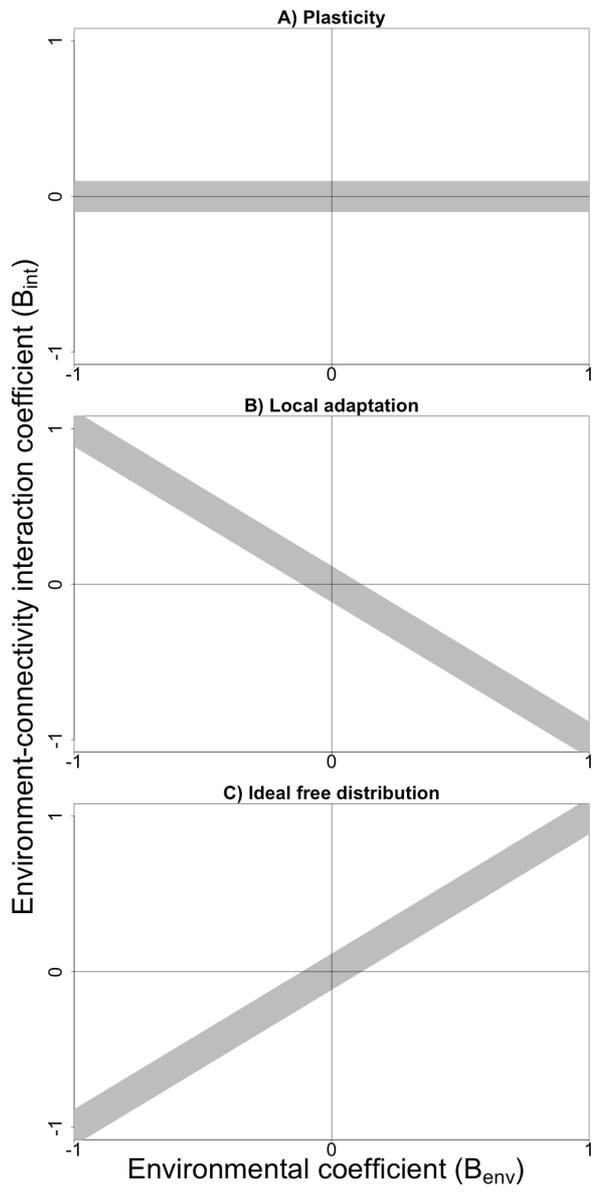
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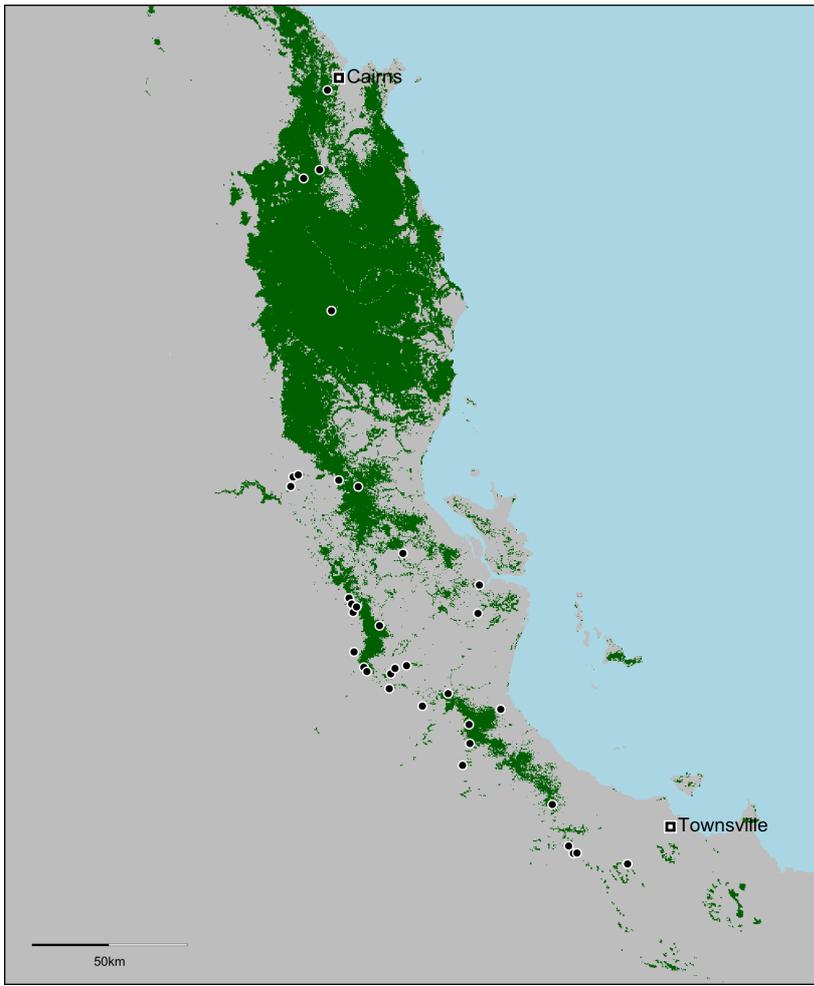
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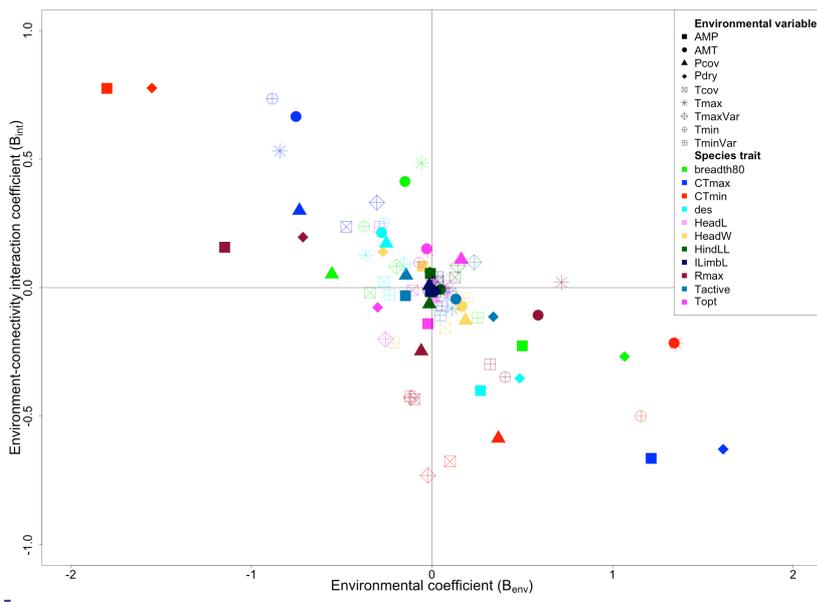


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986 **Figure 1**
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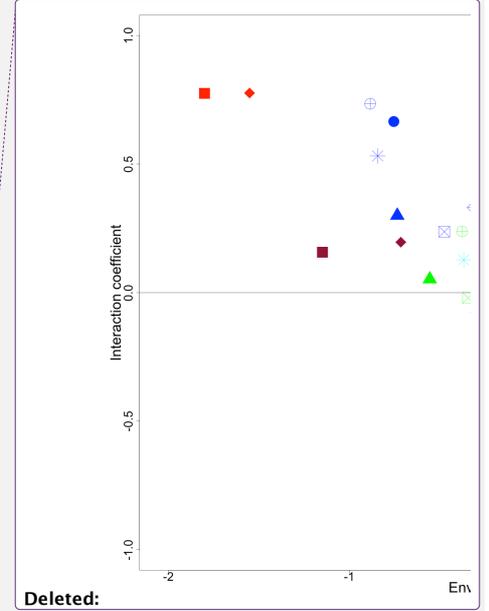


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Figure 2



995 **Figure 3**



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	CTmin	CTmax	breadth80	Rmax	des	HeadW	Tactive	HindLL	HeadL	LIimbl	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

	CTmin	CTmax	breadth80	Rmax	des
AMP	L = 0.7751	L = -0.6644	L = -0.2265	L = 0.1570	L = -0.4
Pdry	L = 0.7763	L = -0.6289	L = -0.2683	L = 0.1961	L = -0.3
Tmin	L = -0.5004	L = 0.7345	L = 0.2380	L = -0.3484	L = 0.2
AMT	L = -0.2156	L = 0.6655	L = 0.4128	L = -0.1072	L = 0.2
Tmax	L = -0.2173	L = 0.5316	L = 0.4846	L = 0.0206	L = 0.1
Pcov	L = -0.5868	L = 0.2996	L = 0.0522	L = -0.2473	L = 0.1
Tcov	L = -0.6766	L = 0.2366	L = -0.0206	L = -0.4338	L = 0.0
TmaxVar	L = -0.7312	L = 0.3308	L = 0.0820	L = -0.4284	L = 0.0
TminVar	L = -0.4241	L = -0.0721	L = -0.1174	L = -0.2979	L = -0.0

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Figure 4

Our analysis requires an index of population connectivity across the landscape, something that can be calculated relatively easily for many species by using broad scale habitat mapping datasets [e.g., vegetation mapping from DERM (53)]. Where possible, these measures of connectivity should be calculated at a scale relevant to the scale of dispersal of the species in question [as was ours, using dispersal rate data for *Lampropholis coggeri* from Singhal and Moritz (54)]. For highly mobile species, such as plants or insects that have wind-assisted dispersal and for which calculating connectivity between populations may be difficult, it is possible our approach will not work. But for many species of animals with relatively low vagility (i.e., the species for which TGF is most needed), our approach should be generally applicable.

Our approach

.
 B_{int} = coefficient of the interaction between the environment and connectivity terms.