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Using connectivity to identify climatic drivers of local adaptation

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Abstract

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Understanding the climatic drivers of local adaptation is vital. Such knowledge is not only of theoretical interest but is critical to inform management actions under climate change, such as assisted translocation and targeted gene flow. Unfortunately, there is a vast number of potential trait-environment combinations, and simple relationships between trait and environment are ambiguous: representing either plastic or evolved variation. Here, we show that by incorporating connectivity as an index of gene flow we can differentiate trait-environment relationships reflecting genetic variation versus phenotypic plasticity. In this way we rapidly shorten the list of trait-environment combinations that are of significance. Our analysis of an existing dataset on geographic variation in a tropical lizard shows that we can effectively rank climatic variables by the strength of their role in local adaptation. The promise of our method is a rapid and general approach to identifying the environmental drivers of local adaptation.

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

Introduction

Understanding how species respond to their abiotic environment is a core interest of ecology and is of more than theoretical interest if we are to plan for climate change. Climate change is one of the biggest threats to global biodiversity (Meehl *et al.* 2007; IPCC 2014), and conservation biologists are considering a variety of techniques to help mitigate its impact on vulnerable species (Williams *et al.* 2008; Weeks *et al.* 2011). One technique is assisted colonization: in which populations are moved to new, climatically suitable, areas (Ricciardi & Simberloff 2009; Thomas 2011). Another technique likely to see increasing use is targeted, or assisted, gene flow (Aitken & Whitlock 2013; Kelly & Phillips 2016). This technique involves the spatial redistribution of long-standing adaptations—moving warm-adapted individuals into cool, but rapidly warming recipient populations—thereby bolstering capacity for adaptation (Hoffmann & Sgrò 2011; Weeks *et al.* 2011; Aitken & Whitlock 2013; Kelly & Phillips 2016). For both methods, we need to find a source location that matches the future climate at our recipient site, and then translocate animals. This climate matching is a simple idea, but climate is multidimensional and it is not clear how we should weight axes: is a difference of 0.5°C in mean temperature more important than a difference of 100mm in annual rainfall? The answer depends upon which aspects of climate have the strongest influence on fitness.

68
69 The challenge, then, is to identify the aspects of climate to which populations are most
70 strongly adapted. While we have strong evidence from reciprocal transplant and
71 common garden experiments that local adaptation is a real phenomenon (Hereford
72 2009), these techniques – the gold standard in local adaptation research (Reznick &
73 Travis 1996; Blanquart *et al.* 2013) – cannot identify the environmental drivers of that
74 local adaptation (Wright *et al.* 2006). This is because reciprocal transplants
75 necessarily encompass all the environmental variables that differ between the
76 transplant locations. Common garden approaches suffer from the same issue, so in
77 practice it remains impossible to identify the environmental drivers of trait variation
78 seen in the wild. This is a problem: we want to know which climate variables are the
79 main drivers of adaptation, and we urgently need this information for many species.

80
81 Another method for assessing local adaptation is to examine geographic trait variation
82 (Reznick & Travis 1996). This allows us to look for relationships between traits and
83 spatially-varying aspects of the environment (e.g., Sgrò *et al.* 2010; Llewelyn *et al.*
84 2016). Strong environment-trait relationships may be indicative of local adaptation,
85 but unfortunately not all geographic trait variation is due to local adaptation.
86 Geographic trait variation can arise for other reasons, such as phenotypic plasticity
87 (including developmental plasticity and maternal effects), and neutral clines. These
88 other mechanisms can give us the misleading appearance of local adaptation
89 (Kawecki & Ebert 2004; Hoffmann & Sgrò 2011), and so call into question the use of
90 trait-environment relationships in studying local adaptation.

91
92 By definition, local adaptation has a genetic basis and so is weakened by high levels
93 of gene flow (Wright 1931; García-Ramos & Kirkpatrick 1997; Kawecki & Ebert
94 2004). Demes with excessive inward gene flow will be less optimally adapted,
95 causing an observable mismatch between optimal and actual phenotypes. Examples
96 include birds dispersing and producing clutch sizes that are not optimised to the
97 habitat quality in which they are now nesting (Dhondt *et al.* 1990); larval salamander
98 colouration not matching streambed colouration due to high levels of gene flow from
99 nearby predator-free streams (Storfer *et al.* 1999); and stick insects in small habitat
100 patches having non-cryptic colouration when the surrounding patches are larger and
101 environmentally dissimilar (Sandoval 1994). These observations of "migrant load"

suggest an alternative technique for identifying and assessing local adaptation. First, we look across populations for relationships between the environment (e.g., mean annual temperature) and traits (e.g., morphology, physiology). By themselves, these relationships are not sufficient evidence of local adaptation. Second, knowing that local adaptation is eroded by gene flow, we can look at whether gene flow erodes the trait-environment relationships we have uncovered. Trait-environment relationships that are strong, but which are also weakened by gene flow, are indicative of trait-environment relationships that have a genetic basis. Where data on gene flow are absent (which is often the case), habitat connectivity may be a useful surrogate. In a statistical model, this idea would be represented as follows:

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

Where:

trait_i = trait value for individual i

A = intercept

B_{env} = coefficient of the environmental variable

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

B_{conn} = coefficient of connectivity

Conn = connectivity at the individual's site

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

error_i = deviation between expected and observed trait value.

With slight rearrangement, this can be expressed as:

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

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

If we now collect data on a large number of trait-environment relationships, and their interaction with connectivity, we can imagine several possible patterns emerging. These possibilities are depicted in Figure 1. Each panel represents a possible scatter of points, plotting trait-environment coefficients (B_{env} , along the x-axis) and the interaction between environment and connectivity (B_{int} , y-axis) for a large set of trait-environment pairs. Panel A shows a set of trait-environment relationships that vary in

strength, but that are not influenced by connectivity (i.e., no environment–connectivity interaction). This pattern would indicate a set in which trait–environment relationships are predominantly driven by plastic responses of traits to their environment (i.e., traits always match the local environment, regardless of the level of gene flow). Panel B shows a system in which trait–environment relationships are eroded by connectivity: increased connectivity diminishes the relationship between the environment and the trait. This is the pattern we would expect if, across our set of traits and environmental variables, there is a genetic basis to the trait–environment relationship, indicative of local adaptation. Panel C shows the situation where the effect of the environment tends to be enhanced by connectivity. This pattern might arise in organisms that are highly mobile and can actively choose their ideal environment, thus avoiding the selective pressures that would lead to local adaptation.

Here we explore the idea of using connectivity to infer local adaptation. To do this we develop a case study of a lizard species from northern Australia. We assess how habitat connectivity affects trait–environment relationships and use this information to rank trait–environment combinations by their degree of local adaptation. We reveal a set of trait–environment relationships that appears dominated by local adaptation.

Methods

Study species and site selection

The Rainforest Sunskink (*Lampropholis coggeri*) is a small (snout–vent length up to 45 mm), diurnal scincid lizard restricted to the rainforests of the Wet Tropics region of northeastern Australia (Wilson & Swan 2010). The rainforests of this region cover a wide range of environmental conditions, spanning significant elevation (0–1600 m ASL), precipitation (annual mean precipitation of 1432–8934 mm), and temperature (annual mean temperature of 16.3–25.8°C) gradients. Lizards were captured by hand from sites that were selected to maximize the environmental heterogeneity sampled (Fig. 2).

Morphological measurements were obtained from 532 skinks from 32 sites.

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

Following capture, skinks were transported to James Cook University (JCU) in Townsville for trait measurement. All procedures involving lizards were approved by the JCU animal ethics committee (projects A1976 and A1726).

Physiological trials

Physiological trials commenced within seven days of skinks being collected from the field; skinks being used only for morphology were measured and released back at their point of capture within seven days. The following measures were taken from each skink ($n = 259$) during laboratory trials: critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), thermal-performance breadth for sprinting (breadth₈₀), maximum sprint speed (R_{max}), temperature at which sprint speed is optimized (T_{opt}), active body temperature as measured in a thermal gradient (T_{active}), and desiccation rate (des) (see Table S1 for further details). Details of trait measurement procedures are reported elsewhere (see Phillips *et al.* 2014; Llewelyn *et al.* 2016).

Morphological measurements

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

Climatic variables, and connectivity

Because our study aimed to assess adaptation to local climate, various temperature and precipitation variables were extracted for each site (see Table S2 for details). We considered both means and extremes. It is important to consider climatic extremes, because temperature extremes may be increasing faster than mean temperatures (Easterling *et al.* 2000), and selection may often occur during extreme weather events (Parmesan *et al.* 2000). Many environmental variables are highly correlated (Llewelyn *et al.* 2016), so only the less-derived variables were used in analyses, specifically: annual mean precipitation (AMP); seasonality of precipitation (Pcov);

precipitation of the driest quarter (Pd_{dry}); annual mean temperature (AMT); coefficient of variation of temperature (T_{cov}); average minimum daily temperature (T_{min}); average maximum daily temperature (T_{max}); average variance of daily maximum temperature (T_{max}Var); and average variance of daily T_{min} (T_{min}Var).

Our connectivity index was designed to capture the flux of individuals through a location and is detailed in (Macdonald *et al.* 2017). Briefly, it is a measure of habitat suitability for our focal skink species, averaged over space using a species-specific estimate of dispersal potential. This approach is reasonable for any species exhibiting diffusive dispersal, and similar techniques (though different spatial-weighting functions) can be used for species exhibiting non-diffusive dispersal. As our species is an obligate rainforest-dweller, grid cells in the landscape that are rainforest and that are surrounded by rainforest have high connectivity indices, while grid cells of rainforest surrounded by non-rainforest matrix have low indices. See Table S2 for further details on all variables, and Figure S1 for correlations between all variables.

Analysis

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

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

Where:

trait_{*i*} = standardized trait value of interest for lizard *i*

A = intercept

*B*_{*svl*} = coefficient of SVL

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

*B*_{*sex*} = coefficient of Sex

238 Sex = lizard sex (this species is sexually dimorphic in some morphological
 239 traits)
 240 B_{env} = coefficient of environmental variable
 241 Env = environmental variable (e.g., annual mean temperature) at the lizard's
 242 site
 243 B_{conn} = coefficient of connectivity
 244 Conn = connectivity index at the lizard's site
 245 B_{int} = coefficient of interaction between Env and Conn
 246 $error_i$ = deviation between expected value and trait value of lizard i

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

$$248 \quad L = -B_{env} \times B_{int}$$

249 If the signs of the two coefficients (B_{env} and B_{int}) are opposite (which indicates an
 250 trait–environment relationship that is eroded by increasing connectivity, i.e.,
 251 evidence for local adaptation), L will be positive. If the signs are the same (which
 252 indicates an environmental effect being enhanced by increased connectivity, a
 253 situation not consistent with local adaptation), L will be negative. Thus, higher
 254 numbers on this scale equate to stronger evidence for local adaptation in that
 255 environment–trait pair. This score can, in theory, range from $-\infty$ to $+\infty$. Once many
 256 environment–trait combinations have been assessed, the coefficients for all pairs can
 257 be plotted (see Fig. 1). As described in the Introduction, in a system dominated by
 258 local adaptation, we expect to see a negative relationship between B_{env} and B_{int} (Fig.
 259 1B). All analyses were conducted in R v3.2 (R Core Team 2015).

260

261 Results

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

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

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

Discussion

Understanding relationships between traits and the environment will help us plan management strategies, such as targeted gene flow (TGF), that can mitigate the impact of climate change on vulnerable species. Numerous studies have looked for (and found) trait–environment relationships (Bogert 1949; Sandoval 1994; Storfer *et al.* 1999; Wegener *et al.* 2014; Klaczko *et al.* 2015; Llewelyn *et al.* 2016), but it remains unclear whether these associations are due to local adaptation, neutral clines, habitat choice, or plasticity. By acknowledging that gene flow erodes adaptation, we can incorporate connectivity (a proxy for the flux of genes) into our analysis, and in doing so, separate those relationships due to fixed genetic differences, from those due to plasticity or habitat choice.

Local adaptation

In the trait–environment combinations we assessed, physiological traits typically showed stronger evidence of local adaptation than did morphological traits, with the clearest signal apparent for CT_{max} and CT_{min} (Figs. 3 & 4). This result is intuitive: we would expect an ectotherm’s physiological traits to be under strong selection from climate (Angilletta 2009; Hoffmann 2010; Clusella-Trullas *et al.* 2011). By contrast, the fitness link between morphology and climate is much less clear. Had we also included some environmental variables that had a clearer bearing on morphology, we might have detected stronger signals of local adaptation for morphology. For example, skinks that occur in rockier habitats show various morphological adaptations to that environment (Goodman *et al.* 2008). Including a measure of rockiness in our set of environmental variables might have allowed us to detect a signal of local adaptation for limb length. Here, however, our focus was on climatic aspects of the environment.

Of the environmental variables used, our analysis suggests that precipitation is a very strong driver of local adaptation, even in thermal traits that might not seem obviously related to precipitation (i.e., CT_{min}, CT_{max}). Precipitation has been shown to directly affect growth rate, body temperature, activity patterns, and thermoregulatory opportunities in lizards (Huey & Webster 1976; Stamps & Tanaka 1981; Crowley 1987; Jones *et al.* 1987; Lorenzon *et al.* 1999; Clusella-Trullas *et al.* 2011). Wetter areas also have higher thermal inertia (and so lower cyclical thermal fluctuations (Myers & Heilman 1969)), and changed environmental variance in temperature potentially has a strong influence on thermal limits (Martin & Huey 2008). Additionally, theoretical work of Bonebrake and Mastrandrea (2010) suggests that precipitation should affect the optimum of thermal performance curves. Finally, comparative analyses also suggest that precipitation can influence thermal traits in many species (Clusella-Trullas *et al.* 2011). Thus, although the mechanisms linking precipitation to thermal limits are diffuse and poorly resolved, mechanisms do exist, and our analyses suggest that precipitation is indeed a strong driver of local adaptation at thermal physiological traits.

Our analysis also suggests that extremes of temperature (encapsulated in minimum and maximum temperatures) are at least as strongly associated with local adaptation as is mean temperature. Again, this result is intuitive—natural selection from climate is likely stronger during extreme events than during normal daily temperatures—and agrees with results of comparative studies (Clusella-Trullas *et al.* 2011). Finally, the environmental variables with the weakest signals of local adaptation are temperature seasonality (Tcov) and variance of minimum and maximum daily temperatures (TminVar, and TmaxVar). These variables represent predictable environmental variation occurring within an individual's lifespan and so are variables to which we might expect individuals to develop plastic responses, rather than fixed differences; local adaptation to these variables would likely be reflected in reaction norms, rather than point values for traits. (Gilchrist 1995; Kingsolver & Huey 1998; Phillips *et al.* 2016).

System-wide signal of local adaptation

The clear negative linear trend displayed in Fig. 3 is precisely what we would expect in a set of trait–environment combinations dominated by local adaptation. Migrant load (the fitness loss driven by immigration of less locally-adapted individuals) scales positively with immigration as well as with the strength of selection [see equation 5 in Polechová *et al.* (2009)]. This occurs because, when selection is strong, the environment has a large effect on the trait, and therefore immigrants coming from differing environments will be badly maladapted and will therefore have a large negative impact on the local phenotype. Thus, we expect trait–environment combinations with strong local adaptation to also show strong effects of connectivity on the trait–environment relationship (Polechová *et al.* 2009).

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

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

Phenotypic plasticity

The importance of accounting for phenotypic plasticity is exemplified in our dataset by the relatively strong effect of precipitation of the driest quarter (Pdry) on the temperature at which maximum sprint speed is achieved (Topt) and on maximum sprint speed (Rmax) itself. These strong trait–environment relationships might be interpreted as evidence for local adaptation. Our analysis, however, suggests that the environmental effect is unaffected by connectivity, implying that variation in these traits is due to plasticity rather than genetic differentiation. Other work (Llewelyn *et al.* 2016) has shown little temporal variation in Topt (within generations) despite clear geographic variation and this, together with our results, suggests that this trait undergoes developmental plasticity, but is fixed in adult lizards. In principle, this non-effect of connectivity could also arise due to selection that is so strong that it maintains local adaptation despite large numbers of immigrants [i.e., immigrants are selected against so strongly that they do not contribute genes to the recipient population (Kawecki & Ebert 2004)]. The trait–environment relationships for Topt and Rmax are, however, weaker than those for some other traits (e.g., CTmax and CTmin) that show clear effects of connectivity, so extremely strong selection seems unlikely in this case.

The generally weak evidence for plasticity in our dataset should not be considered weak evidence for plasticity in these traits. Indeed many of the physiological traits we use (e.g., CTmax) are notoriously plastic, responding reversibly on timescales ranging from hours to months (Seebacher 2005; Llewelyn *et al.* 2017). That we do not see signals of plasticity in these traits here reflects our choice of environmental variables: long-term climatic variables, rather than short-term weather variables (such as the temperature in the week before an animal was collected). We chose these long-term variables precisely because we are interested in unearthing patterns of local adaptation, rather than patterns due to rapid, reversible plasticity.

Caveats and challenges

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

Gene flow and connectivity

Our approach requires a measure of gene flow across a landscape. Here we have used environmental connectivity as a proxy for gene flow. We chose connectivity because it can be calculated relatively easily for many species by using broad scale habitat mapping datasets [e.g., vegetation mapping from DERM (2011)]. Of course, these measures of connectivity should be calculated at a scale relevant to the scale of dispersal of the species in question [as ours was, using dispersal estimates for *Lampropholis coggeri* from Singhal and Moritz (2012)]. While connectivity measures will often correlate with gene flow, e.g., (Palmer *et al.* 2011)], a measure of gene flow, rather than the flow of individuals, would be preferable. Such measures are increasingly becoming available with the rise of landscape genomics tools (e.g., Petkova *et al.* 2016), but may still be cost-prohibitive in many cases.

While there may be better measures of gene flow, our inference might also be improved by taking into account landscape heterogeneity in the environment. Gene flow, per se, does not erode local adaptation. Rather it is an influx of maladapted genes that erodes local adaptation. Thus, a better index of this “migrant load” may well be one in which connectivity is multiplied by a measure of environmental heterogeneity, where connectivity and heterogeneity are calculated over the same spatial scale (e.g., Macdonald *et al.* 2017). An index such as this should, in principle be a better measure of migrant load than our simple measure of connectivity.

Clearly connectivity is an imperfect measure of migrant load. Nonetheless, connectivity should scale positively with migrant load, and our analysis using simple connectivity generated a coherent and intuitively sensible result. This is encouraging,

suggesting that, in the absence of precise estimates of migrant load, a readily calculable connectivity metric may suffice to elucidate broad patterns.

Linear trait–environment relationships, and covariation with connectivity

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

Covariation between explanatory variables

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

Multivariate traits and environments

Here we estimated coefficients for one trait–environment combination at a time. Doing so may potentially miss relationships that only appear in multivariate analyses. For example, if two environmental variables are negatively correlated but both have a positive effect on a trait, it is possible that these countergradients can obscure the univariate relationship. With our approach, this may lead us to underestimate the number of important environmental drivers of local adaptation. Analysis incorporating multiple environmental predictors is possible, but such a model will rapidly become saturated with parameters. To minimize the problem of countergradients, again, care should be taken to sample environmental spaces so as to minimize covariation between environmental variables.

An additional analytical challenge is to treat traits as multivariate. Here we have treated each measured trait as independent. In reality, however, traits covary and this covariance can have both genetic and environmental origins (Lynch & Walsh 1998). As a corollary, selection acts on the multivariate trait, and causes populations to move in multivariate trait space (Blows 2007). Consequently, local adaptation perhaps should be measured in a multivariate trait space. Such an aim, however, requires considerable theoretical development and may well require substantially more data. For now, however, we should be aware that we are collapsing our trait space, and measured traits are not fully independent. For example, in our system there is a strong correlation between CTmin and CTmax, thus we should be aware that these two traits should not get equal weighting when we use our traits to rank environmental variables by their importance to local adaptation.

Neutral clines

Finally, our approach should allow us to identify when geographic variation is a result of genetic variation. That is, it can weed out relationships that are driven by plasticity or habitat choice. Covariation between genotype and environment will often be the result of local adaptation, but can also arise for non-adaptive reasons, the most obvious being trait clines caused by the historical spread of population (Phillips *et al.* 2010). In principle, and again, with careful attention to sample design (i.e., a sample design which minimizes the covariation between space and environment), it should be possible to exclude such possibilities.

507 Conclusion

508 There is increasing urgency to identify populations that will act as suitable sources for
509 assisted colonization and targeted gene flow. To identify these populations, we need
510 to know which aspects of climate drive local adaptation. Traditional approaches to
511 unearthing local adaptation (reciprocal transplants and common garden experiments)
512 cannot attribute adaptation to any particular environmental driver. Local adaptation is,
513 however, eroded by gene flow, and we can use this fact to sort patterns of local
514 adaptation from patterns with other causes. Here we have demonstrated this approach:
515 using connectivity as a proxy for gene flow, and looking for its effect on trait–
516 environment relationships. Our analysis suggests the approach has merit: the results
517 we achieve are coherent and suggest local adaptation is the overwhelming signal in
518 the set of trait–environment relationships tested. The analysis also allows us to rank
519 environmental drivers of local adaptation, and we find evidence that precipitation and
520 temperature are important climatic variables for local adaptation in our system. The
521 method has modest data requirements and so can potentially be applied to many
522 already existing datasets. Analytical and sampling challenges remain, however, and
523 we point to avenues whereby the method can be improved. Given the potential of this
524 method to provide evidence of local adaptation, and to provide rapid ranking of the
525 climatic drivers of local adaptation, its assessment in a broader array of systems is
526 warranted.

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Figure Captions

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

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

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

Figure 4. Heatmap showing the relative rankings of climate variables (rows) and morphological and physiological traits (columns). The matrix has been sorted (by reciprocal

775 averaging) and coloured according to the strength of local adaptation, with higher values
776 coloured red and being sorted to the top/left. See Tables S1 and S2 for explanations of the
777 trait and environmental variables used.

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

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784 **Figures**

Figure 1

Figure 2

Figure 3

Figure 4

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