**Testing for specialisation in ecological strategy**

In this study we describe a novel method for identifying specialisation in ecological strategy along environmental gradients. The riparian environment provides an ideal context for such a test as hydrology is such a dominant driver of community assembly, meaning significant specialisation can be expected as disturbance intensity or fluctuation in water availability increase. We used Trait Gradient Analysis (*sensu* Ackerly & Cornwell, 2007) to decompose community-level interspecific trait variation into within-site (alpha) and across-landscape (beta) components. We were then able to identify where specialisation occurs within the landscape by quantifying changes in site-wise dispersion of beta trait components (across a hydrological gradient of choice).

*Trait Gradient Analysis*

Trait Gradient Analysis was performed on wood density data following Ackerly and Cornwell (2007). All calculations were made in R, using scripts provided as Supplementary Information to Ackerly and Cornwell (2007). The analysis is fed with a matrix describing the name, abundance and trait value of species found at each site and the following parameters are generated: 1.) abundance weighted mean trait values for each site (tp), 2.) an inter-site mean trait value for each species (ts), 3.) a niche breadth (Rs) for each species 4.) an alpha trait value (alphaT) for each species, 5.) a beta trait value (betaT), for each species.

In TGA, the trait gradient is a one dimensional ordination of sites according to their abundance-weighted site mean trait values. The betaT value integrates the site mean trait values of all sites at which that species occurs, and represents the characteristic position of a species along the trait gradient. For example, a high betaT for wood density indicates that a species is typically found at sites with high mean wood density – regardless of whether the species wood density value is low or high. AlphaT and betaT values are calculated by linear decomposition of a species mean trait value (ts), defined by: ts = alphaT + betaT. The alpha trait value, then, is calculated by subtracting betaT from the mean species trait value. It provides a metric of the difference between a species’ mean trait value and the trait values of other species with which it co-occurs, and can be positive or negative. For example, a high alphaT for wood density indicates that that a species typically has higher wood density than the species it co-occurs with. Finally each species is associated with a niche breadth, Rs, which corresponds to the range of site mean trait values across which a species occurs and is measured in units of wood density (g/cm3). Both Ackerly and Cornwell (2007) and Gallagher & Leishman (2012) have provided elegant descriptions of the mechanistics of TGA, and the reader is referred to these publications for more in-depth discussion.

*betaT dispersion as a measure of niche specialisation*

We can replace raw species trait values with betaT values, and run them in a linear model against an axis where the sites are ordered according to some environmental gradient. Now we have a model that describes relationship of species trait values to the environmental gradient with noise due to intra-site variability removed (remember ts = alphaT + betaT). This model includes abundance weightings but is no more instructive than a plot of raw species trait values against the environmental gradient. We can however look at dispersion of betaT (betaTdisp) across the gradient: as betaT values of species occurring at a site converge, the likelihood that they were found at the same sites increases, indicating specialisation in ecological strategy.

An issue arises here in that betaT is generated as a mean of ts values. To give an explicit example: 0.1 + 0.9 / 0.2 = 0.5, 0.4 + 0.6 / 0.2 = 0.5. The first equation would represent a species with high niche breadth whereas the second would represent a low niche breath species. Rs values can be used to confirm whether betaTdisp indicates that species are present at the same set of sites. If both Rs.mean and betaTdisp are low at a site, species are specialised to a narrow niche. Where site Rs.mean is high, species are cosmopolitan, so tight betaTdisp does not necessarily indicate ecological strategy specialisation. By assessing correlation of betaTdisp with Rs.mean across a dataset, it is possible to determine to what extent betaTdisp is a valid measure of specialisation. If a strong correlation is apparent, we can assert that if betaTdisp decreases in a predictable manner over the gradient, this indicates an increase in specialisation in species ecological strategy.

It is also useful to consider this approach in the context of patchy sampling. In this case, species with the same real niche ranges may be assigned different betaT values. As the ratio of Rs to the number of sites used to calculate betaT (Nplots) increases, so too does the potential error associated with betaT. Therefore for species found at more than one site, this ratio Rs /Nplots can be used as a metric of error. We can plot site mean Rs/Nplots ratios against an environmental variable for kicks. The model shouldn’t be significant. Where species are only found at one site, it is not possible to determine if this measurement is representative of their true range or if it is an artefact caused by patchy sampling. Sites such as Sportsmans Creek which have a completely unique assemblage with in the datasets, giving both Rs and Nplots of zero for all species, are essentially a statistical bummer. The fact that this site sits neatly within observed trends gives the value some credence at least.

Not all field data comes from big budget programmes with saturating sampling intensity, and we shouldn’t let patchiness in ecological sampling deter us too much. We simply need to be clear on what our tests are able to say about the data we have available.

*Testing betaT dispersion over hydrological gradients*

Site-wise ranges of species betaT values were calculated. Range was used as it provides a measure of dispersion that is directly comparable to Rs. Standard deviations and coefficients of variation of betaT were also tested and produced much the same results as ranges. Ordinary least-squares regression models were then generated over gradients of the hydrological parameters shown in *Table X*. Because betaT.range distributions were not normally distributed, so we compared these models against a null model of community assembly. This null model was generated by resampling the vector of site numbers without replacement, using the R script provided by **Ackerly and Cornwell (2007).** This approach maintained site-level distributions of species diversity, *the number of occurences per species, and the intraspecific distribution of both abundance and trait values within species.* One-way ANOVA was used to test for differences between betaT.range – environment and betaT.range.null – environment models. Models that were not significantly different from the null model were discarded. The BH method was again used to account for increased type 1 error associated with multiple comparisons.

We identified the most ecologically relevant axes of variation in hydrological conditions by running a principal components analysis across hydrological metrics which showed significant relationships with betatT.range.

To determine the significance of betaT calculation error, we can compare betaT.dispersion – environment relationships with Rs – environment relationships ??

Niche breadth / number of sites species occurred in or n/Rs as measure of betaT error? – most species will throw a NaN because they were only found in one site…

Rs on its own is not a useful measure of niche specialisation at the site level because it doesn’t indicate whether different species at a particular site come from a similar region of the gradient. Whereas betaT.dispersion on its own can’t tell you whether a particular species is present at similar or different regions of a gradient (because it’s a mean).

Assuming correlation of betaT with Rs (over the environmental gradient…) the degree of dispersion of betaT values at a given site, then, indicates whether the species are specialised to a particular site or set of sites, or

Can we can use the degree of correlation between betaT.disp and Rs.mean to check for the influence of ‘broken’ betaT values (i.e. high range, low n[sites])