CH3 Write up

Introduction

Within a community, interactions between plants for pollination form a continuum from competitive to facilitative. As fitness is the outcome of these associations, understanding them better has been of interest to ecologists for many years. The complexity of their study is compounded when moving beyond pairwise associations. All interactions are embedded in complex networks. Over the last few decades, network analysis has been use to explore these interactions at a community level. Most of these studies plant-pollinator visitation networks are generally summarized by species which gives an overview but less information about the interactions between individuals. Individual level forces drive dynamics at the species i.e. population and community level. By downscaling these species-level interaction networks, we can analyze the influence of traits that are known to influence pollination success, such as height, neighbourhood, flower number and neighbours on the network topology of the whole community of individual plants. Attributes are intrinsic characteristics of the nodes (size, floral number). Network topology are the patterns of relations.

There has been a recent interest in downscaling these networks which has aided an understanding of: trait-matching between plants and pollinators, spatial modules, conspecific density and specialization of pollinators. (Dupont 2011, Dupont 2014, Valverde 2015, Tur 2014, Rumeau 2018). [Gómez, Perfectti & Jordano, 2011](https://doi.org/10.1371%2Fjournal.pone.0016143); [Dupont, Trøjelsgaard & Olesen, 2011](https://doi.org/10.1111%2Fj.1600-0706.2010.18699.x); [Gómez & Perfectti, 2012](https://doi.org/10.1098%2Frspb.2011.2244); [Dáttilo et al., 2014](https://doi.org/10.1371%2Fjournal.pone.0099838); [Dupont et al., 2014](https://doi.org/10.1111%2Foik.01426); [Tur et al., 2014](https://doi.org/10.1111%2F1365-2656.12130); [Tur, Olesen & Traveset, 2015](https://doi.org/10.1111%2Foik.01668); [Kuppler et al., 2016](https://doi.org/10.1111%2Fnph.13858); [Valverde, Gómez & Perfectti, 2016](https://doi.org/10.1111%2Foik.02661)). These individual based networks can be analyzed as bipartite networks. . Processes can be analyzed by projecting the bipartite plant-pollinator network into a unipartite plant-plant network, commonly done in social network analysis. This has been done for single species interaction networks to explore mating-probabilites (Gomez), assortive mixing (Valverde) and to explore how populations are structured by individuals sharing similar pollinator groups (Valverde). At the community level, these networks describe competition/facilitation probabilities, pollinator preferences as well as within population structure. Down-scaling to plant individuals can help resolve questions about intra vs interspecific pollinator sharing i.e. niche breadth. This approach can shed insight into the structure of interactions within a population (Valverde), but we see no reason it cannot shed light into the structure of interactions of a community. The number of potential interacting conspecifics is a greater mating pool.

Only two papers have looked at downcaling at the whole network level. Because it is very labour intensive to characterize very species rich ecosystems, we used a natural system, an arid shrubland where the shrubs and cactus were blooming, but too late in the year for annuals to grow. Thus, the majority of blooms available for foraging species came from a relatively small set of species

Pollinator responses to flowers are density-dependent. Flowering shrubs are an interesting system because they can represent multiple scales of floral density. Each plan forms a resource concentration, however the density of these concentrations vary through space. Finally, the relative density at a large scale can matter as well. Here, a network approach lets us better understand how the network rewires over the study seasons, and how intra and interspecific variation is visitation and sharing is influences by individual traits and neighbourhood composition. Density is known to mediate competition and facilitation between plants, and interaction are what build interactions networks. Therefore, this density-dependence should be visible in the network. We used functional groups, which are groups that use resources in similar ways.

Pollinator visitation networks are a quantitative method to visualize and analyze the many interactions within a community. Species that have a disproportionate effect on a community can be identified by looking at degrees of the many nodes (Dale and Fortin). In an individual network, then individuals can be identified. Those individual have traits etc etc.

Most network indices are sensitive to size (Dormann 2009, Tur), we used a null model approach. Tur said the differences in network structure may be due to differences in pollen use due to foraging patterns and behavioural differences, as well as conspecific variability. On the plant side, differences in network structure may arise from differences in individual traits and floral offerings, pollinator availability and behaviour. Plant pollinator use can be defined with linkage density i.e. abundance of visits and diversity of visits.

Methods

Study site

The study area has an extent of x km2, and is located in the mouth of Sunset Cove on the property of the Sweeney Granite Mountains Desert Research Station within the Mojave National Preserve in California (34°46'26.5"N 115°39'31.3"W). The cove is created by tall rock formations on three sides, gently sloping and widening to the south. The diverse shrub and cactus community. Figure 1 lists the plant species.

Sampling design and experimental setup

The experiment was carried out between April 20th and May 14th, 2018 for a total of 19 sampling days. All observations were carried out between 9:00AM and 5:30 PM in the absence of strong winds. This range of dates comprised the bloom periods of most shrubs at the site. Focal plants were chosen by walking in a line starting in a different quadrant of the study site each day to avoid confounding area by time of day. To avoid oversampling the most abundant species, up to six individuals of a single species was sampled per observer per day.

Flower-visitor observations were done by one or two observations in 10-minute observations. We recorded the number and identity of potential pollinators that made contact with reproductive parts of the flowers. This approach to creating a pollinator visitation network allows visitation rates to be standardized between individuals, compared with the frequently used method of transect walks. In our case, it is the number of foraging bouts and not floral visits per se. This is because insects were sampled mid foraging. However, anytime a visitor left and came back it was counted as a new visitor.

Melyrid beetles in the subfamily Dasytinae and pollen beetles Carpophilus sp. were excluded because while abundant, they were stationary deep within the flowers. It was not possible to extract them without disturbing the flowers and also not possible from large shrubs. Very small pollinators, such as the micro-beeflys (Mythicomyiidae) were excluded in analyses as it was not possible to consistently track their visits to, very large shrubs such as L. tridentata. Species known not to be pollinators ie nectar robbers, lady bugs were excluded. Visitors were identified on the wing. Insects were identified to species, genus, or family. Species were morphotyped within these categories. Morphotyping and RTU are still useful methods for characterizing and quantifying pollinator communities (Memmott & Godfray 1993; Oliver et al). The method, despite not being species level all the way through, still provides information about the linkages between different genera and functional groups. Also, because the pollinator diversity is really high at our study site, can’t do species ID on the wing. Wanted it to be a quantitative network. We collect x number of vouchers to verify the ids. Many species are undescribed at the site.

I counted the number of flowers and measured the height of each focal plant. I recorded the abundance and identity of blooming shrubs and cactus in a 3 m radius around the focal plant. 2018 was a drought year and annual bloom density was negligible. It was not feasible to count the blooms of all neighbouring shrubs, so the surrounding shrub density is a proxy for neighbourhood floral bloom density. I also measured the distance to and identity of the focal shrub’s nearest blooming neighbour, as well of the distance to the nearest blooming *L. tridentata*.

I recorded shrub phenology and estimated the site level blooming shrub density of each species using band transects on most study days.

I also used pan traps placed in open areas to track pollinator population changes throughout the study period. I quantified the number of ‘large bodied’ pollinators to reflect the sizes of those observed during the experiments (hereafter just ‘pollinators’).

Data analysis

Pollinator-mediated interactions, density-dependence

To explain floral visitation in response to individual floral traits, number, patch density and site-level density, generalized linear mixed models were fit with species included as a random effect. Compared the model’s using AIC and likelihood ratio tests. Compared to intercept only models. When AIC < 2 and no difference in likelihood ratio test, chose the simpler of the models.

I used imputeTS to fill in densities for the days they weren’t sampled using the linear interpolation because I expect to see a trend but no seasonality.

To test for spatial autocorrelation, to look for pollination hotspots that may arise from habitat preferences rather than floral preferences, we used Moran’s I and Geary’s C to test for autocorrelation of visitation rates. *We also looked for clustering in linkage density, betweenness and Bluthgren’s D were used (n = 373). This was calculated using k = 4 nearest neighbours*

*Individual plant traits*

*To determine how individual plant traits influence network topology, the network trait was used as a response variable, and the individual traits and neighbourhood densities as predictor variables in GLMM with the shrub species as a random effect.*

Assortativity coefficients

Using the one-mode graphs, we calculated assortativity coefficients for interactions for all invidiuals at the community level (interspecific) and within each species. We calculated assortativity for species, number of flowers, height, surrounding density, conspecific density and heterospecific density, surround shrub diversity. This was calculated using igraph, and the weighted one-mode networks, with each vertex labelled with trait. To test for significance, I created 1000 null models and projected them to one-mode. I applied the same vertice labels to the random network so that the values and abundances would be the same.

and calculated assortativity for each null model and compared them to the observed values using Z-scores.

Functional group modularity

To test for how functional groups of pollinators structure the plant community. I used the individual based network with the Beckett (2016) algorithm. This is a recent algorithm for weighted networks. To test if the system is significantly modular, we compared our network to a null model.

We computed modularity for two networks: One is the individual-based, quantitative two-mode network. This explore how pollinator functional groups form modules….

To test for traits and neighbourhoods’ influence into the creation of modules, we fit GLMM with the module as a factor as a predictor, and the traits as response variables. We used the species as a random slope and intercept because the traits also vary as a response to species and we expect that the modules will be structured by species.

We projected the two-mode network into a one-mode, binary network. Each link between individuals is a shared pollinator functional group (or quantitative?). This tests for how functional groups structure the plant community into modules of interacting individuals.

Using the one-mode network, we calculated the number of interacting conspecifics and heterospecifics.

For this analysis, the one mode network is one link per pair of individuals, regardless how many visits they get, this is because we are looking at the size of the potential mating pool.

Results

Over a period of 19 days I observed 395 individuals, comprising seven species of shrub and three species of cactus for a total of 66 hours of observation. 394 observation periods, a total of 635 potentially pollination visits were recorded. Nectar robbing and visits by non-pollinating insects excluded. 430 visits were to shrubs and 205 were to cactus. The pollinators make up 10 functional groups spanning 62 RTU of visitors. All visitors, with the exception of Costa’s hummingbird Calpte costa were insects. See Appendix for full list of visitors and the functional group they were assigned to.

Visitation rates

Visitation rates increased with shrub density (Table 3). There was significant interaction between site level density and individual floral number. Plants with more flowers did much better than plants with less flowers at high site level densities, but it doesn’t matter as much as at lower site level densities. There was no influence of shrub species richness on visitation.

* This is evidence for facilitation/mutualisms between co-blooming shrubs.
* This is also consistent however with an increase in competition/a saturation effect. This is scale dependency: individual attractiveness is dependent on site level density. As the site density intensifies, so does selection between individuals based on the number of flowers they have.

Spatial autocorrelation

There was no spatial autocorrelation detected for visitation (Moran’s: -0.055108, p = 0.953, Geary: 1.0442, p = 0.8145).

* This suggests the absence of hotspots of pollinator activity, both globally through the site and more locally.

Functional group modularity

We found that q = 0.59, p <0.0001 somewhat modular. Significant when compared to random networks. No difference in flower number, height, visitation rates or shrub density between modules.

Associatitivity of the networks

Species network vs individual network characteristics

Build 1000 null networks of i-sp networks. Calculate parameters. Does the observed network fall into the 95% confidence interval for the null network derived parameter?

Alternatively, compare each observed parameter to null using z-score to describe the effect size of that parameter, and then compare the difference between each to each other.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | network.sp | network.shrub | network.cactus | network.indiv | network.indiv.shr | network.ind.cac |
| connectance | 0.195081967 | 0.245614035 | 0.538462 | 0.027464 | 0.030702 | 0.115926 |
| web asymmetry | -0.718309859 | -0.78125 | -0.625 | -0.58219 | -0.47465 | -0.69048 |
| links per species | 1.676056338 | 1.53125 | 1.3125 | 1.325342 | 1.290323 | 1.27381 |
| number of compartments | 2 | 2 | 1 | 7 | 7 | 2 |
| compartment diversity | 1.136915286 | 1.149193967 | NA | 1.298515 | 1.395488 | 1.16658 |
| cluster coefficient | 0.163934426 | 0.245614035 | 0.615385 | 0.012987 | 0.0125 | 0.028169 |
| nestedness | 21.38699296 | 31.08283247 | 13.1607 | 2.057853 | 3.0394 | 9.364582 |
| NODF | 32.13507152 | 33.93652137 | 65.12346 | 7.451157 | 7.632228 | 33.86411 |
| weighted nestedness | 0.392810818 | 0.287985288 | 0.681249 | 0.508026 | 0.425396 | 0.657818 |
| weighted NODF | 16.21011012 | 17.39278494 | 54.78395 | 2.285332 | 2.699228 | 9.034105 |
| interaction strength asymmetry | -0.227115633 | -0.27784834 | -0.35307 | -0.24618 | -0.17908 | -0.38224 |
| specialisation asymmetry | 0.214064984 | 0.331941389 | 0.250936 | 0.30968 | 0.253064 | 0.521379 |
| linkage density | 4.771531516 | 5.569392943 | 2.706056 | 10.82269 | 8.409756 | 13.90551 |
| weighted connectance | 0.067204669 | 0.087021765 | 0.169129 | 0.037064 | 0.038755 | 0.165542 |
| Fisher alpha | 43.22443798 | 39.64312224 | 5.861291 | 420.51 | 348.1018 | 90.3156 |
| Shannon diversity | 3.744086569 | 3.679034078 | 1.93221 | 5.739732 | 5.443934 | 4.411858 |
| interaction evenness | 0.583785847 | 0.614302518 | 0.527413 | 0.600812 | 0.597039 | 0.646177 |
| Alatalo interaction evenness | 0.438401524 | 0.451771594 | 0.472234 | 0.761504 | 0.785708 | 0.741201 |
| H2 | 0.615059075 | 0.592791421 | 0.208879 | 0.558604 | 0.488607 | 0.580302 |
| number.of.species.HL | 10 | 7 | 3 | 61 | 57 | 13 |
| number.of.species.LL | 61 | 57 | 13 | 231 | 160 | 71 |
| mean.number.of.shared.partners.HL | 2.311111111 | 2.761904762 | 3.333333 | 0.121858 | 0.110902 | 0.589744 |
| mean.number.of.shared.partners.LL | 0.554644809 | 0.583333333 | 1.076923 | 0.135554 | 0.144733 | 0.592757 |
| cluster.coefficient.HL | 0.27574545 | 0.352345981 | 0.767355 | 0.103876 | 0.109026 | 0.466987 |
| cluster.coefficient.LL | 0.348818898 | 0.379069767 | 0.923577 | 0.035317 | 0.04133 | 0.133208 |
| weighted.cluster.coefficient.HL | 0.267429594 | 0.225598924 | 0.074121 | 0.019346 | 0.028616 | 0.013561 |
| weighted.cluster.coefficient.LL | 0.866860742 | 0.920958787 | 0.661725 | 0.263532 | 0.233048 | 0.363956 |
| niche.overlap.HL | 0.111547857 | 0.114485722 | 0.651097 | 0.014071 | 0.016032 | 0.030068 |
| niche.overlap.LL | 0.222369044 | 0.263190085 | 0.661038 | 0.081781 | 0.08129 | 0.389658 |
| togetherness.HL | 0.083563302 | 0.083362728 | 0.212121 | 0.002968 | 0.003681 | 0.012688 |
| togetherness.LL | 0.13873224 | 0.196115288 | 0.397436 | 0.023894 | 0.025439 | 0.175509 |
| C.score.HL | 0.65153896 | 0.69366492 | 0.333333 | 0.958969 | 0.956359 | 0.809869 |
| C.score.LL | 0.590561627 | 0.548254316 | 0.181818 | 0.886015 | 0.883063 | 0.480494 |
| V.ratio.HL | 8.492468134 | 8.720338983 | 5.727273 | 13.75077 | 9.619862 | 23.69128 |
| V.ratio.LL | 1.132289628 | 0.715388858 | 1.095238 | 0.521392 | 0.56418 | 0.589789 |
| discrepancy.HL | 58 | 45 | 2 | 311 | 214 | 43 |
| discrepancy.LL | 54 | 44 | 2 | 327 | 234 | 49 |
| extinction.slope.HL | 1.623927791 | 1.555743051 | 1.566032 | 1.484163 | 1.539852 | 1.347619 |
| extinction.slope.LL | 7.572849686 | 9.346037603 | 7.720112 | 2.510613 | 2.43762 | 2.681406 |
| robustness.HL | 0.619139081 | 0.606721878 | 0.606346 | 0.595968 | 0.604494 | 0.572004 |
| robustness.LL | 0.855574176 | 0.869400332 | 0.862039 | 0.708413 | 0.702787 | 0.72304 |
| functional.complementarity.HL | 434.7017473 | 273.0027564 | 202.5422 | 239.1016 | 191.7319 | 79.31409 |
| functional.complementarity.LL | 495.216097 | 298.2138008 | 236.3746 | 303.0285 | 231.1036 | 83.24064 |
| partner.diversity.HL | 1.85015243 | 2.138600972 | 1.245114 | 2.67535 | 2.337515 | 3.042767 |
| partner.diversity.LL | 0.679704907 | 0.572614895 | 0.563119 | 0.558927 | 0.638634 | 0.391737 |
| generality.HL | 7.404265463 | 9.223208359 | 3.588922 | 19.69136 | 14.70441 | 26.19489 |
| vulnerability.LL | 2.138797569 | 1.915577527 | 1.82319 | 1.954014 | 2.115098 | 1.616131 |

Influence of density and individual traits on network topology

Additive model:

Fixed effects:

Estimate Std. Error z value Pr(>|z|)

(Intercept) 0.38125 0.41853 0.911 0.3623

shrub.density 0.08558 0.04204 2.036 0.0418 \*

N.flowers.scaled 0.40650 0.09743 4.172 3.01e-05 \*\*\*

site.density -4.53886 10.25546 -0.443 0.6581

Site density was not measured each day. There is a significant interaction, however the positive effect of individual flower number was unchanged – it just became more positive. Therefore, we feel comfortable still including it in models.

**Summary results:**

Table 3: Results of GLMM of density-dependence. P-values from type 3 wald’s chisq test

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Coeff | Chisq | p |
| Shrub.density | 0.08464 | 5.1999 | 0.022589 |
| N.flowers.scaled | -0.44179 | 1.7254 | 0.189004 |
| Im.density | -3.46973 | 0.1342 | 0.714073 |
| Nflowers:im.density | 24.61896 | 6.6971 | 0.009657 |

Appendix

Imputation: Missing values (4) of site level density for imputed using the package imputeTS (cite) to be able to use the most response data in the analysis. All species were imputed individually because different plants have different flowering strategies. We used time series because the number of flowers opened each days has temporal dependencies. “his process is a commonly used statistical method for substituting missing values in a time series with values following the same temporal or spatial pattern created by existing data (Schneider, 2001; Moritz, 2015)

“

Table 1: Imputation of site density measurements.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Mean | | Standard deviation | | % DAta |
|  | Before | After | Before | After | Before |
| LT | 0.011858974 |  | 0.00312757 |  | 76.5% |
| SD | 7.69E-05 |  | 0.00027735 |  | 76.5% |
| SM | 0.000521368 |  | 0.000874754 |  | 76.5% |
| SC | 0.002653846 |  | 0.002230327 |  | 76.5% |
| AS | 0.015478632 |  | 0.009804953 |  | 76.5% |
| EL | 0 |  | 0 |  | 76.5% |
| EC | 0.002405983 |  | 0.003057944 |  | 76.5% |
| PP | 0.000115385 |  | 0.000416025 |  | 76.5% |
| HH | 3.85E-05 |  | 0.000138675 |  | 76.5% |
| BW | 0.001 |  | 0.001607275 |  | 76.5% |

Table 2: List of shrub species, number of observation periods and blooming period

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Foundation plant species | Observation periods and length | Mean height +/- SD | Mean floral number | Blooming period |
| Acamptappapus | 96 |  |  |  |
| Buckwheat | 31 |  |  |  |
| Ericameria cooperi | 55 |  |  |  |
| Ericameria lineafolia | 4 |  |  |  |
| Larrea tridentata | 80 |  |  |  |
| S Mexicana | 12 |  |  |  |
| Salvia dorri | 13 |  |  |  |
| Hedgehog | 5 |  |  |  |
| Prickly pear | 29 |  |  |  |
| Silver cholla | 69 |  |  |  |

Appendix A

|  |  |
| --- | --- |
| Candidate Model | AIC |
| density \* N.flowers.scaled + im.site | 1337.569 |
| density + N.flowers.scaled \* im.site | 1333.017 |
| con.density + N.flowers.scaled + im.site | 1339.356 |
| con.density + N.flowers.scaled \* im.site | 1334.943 |
| con.density + het.density + N.flower | 1334.161 |
| shrub.density + N.flowers.scaled \* im.sit | 1332.177 |
| shrub.density \* N.flowers.scaled \* im.sit | 1332.609 |
| density \* N.flowers.scaled \* im.site | 1333.037 |
| shrub.density + N.flowers.scaled \* im.site + S | 1334.177 |
| S + N.flowers.scaled \* im.site | 1335.671 |

Anova on two similar models. Adding interaction term does not improve model.

Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)

m6 7 1332.2 1360.0 -659.09 1318.2

m7 10 1332.6 1372.3 -656.30 1312.6 5.5682 3 0.1346