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Tools and Techniques in Plant Ecology-A review

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Abstract: To solve ecological interference problems, ecologists are using a combination of ecological strategies and tools and are also in search of new methods. Direct gradient analysis, ordination methods, principle components analysis, cluster analysis, TWINSPAN, discriminant analysis, reciprocal averaging, canonical correspondence analysis and detrended correspondence analysis were and are most commonly used tools and techniques in plant ecology. Advanced marker techniques, ecological footprint, Laser scanning, Dirichlet allocation model and ecological engineering are advanced methods in plant ecology. Dirichlet allocation model is non parametric method for multivariate analysis. Algorithmic model can be used as an alternative to traditional regression models. Still there is need to carry out a lot of research with the recently developed techniques to improve their efficiency, reliability and validity. Articles aimed to describe methodology play a crucial role in the advancement of applied ecology.

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1. Introduction

Ecological methods in botany are used to analyze the relationship of plants and environments in various ways. These methods are also used in ethnobotanyespecially in diversity indices that are used to carry out comparison of uses of different plant populations in different environmental conditions (Begossi, 1996). Ecological models, concepts or techniques are also proved to be very useful in the study of man-environment relationship (Begossi, 1995). Basically correlative and comparative research is carried out for study of diversity. Correlation studies correlate the local diversity and environmental factors present there. Comparative studies allow comparison of floral use in different environments (Huston, 1994).

Use of advanced molecular methods in ecology gave new dimensions to ecologists and interaction between ecology and genetics has also been strengthening in this way. Now genetic markers are used to study ecological phenomena from molecular sexing of individuals and offspring percentage, species population structure and phylogenetic relationship of taxa (Baker, 2000). Multivariate analyses in ecology are used for community data because in this case we are interested in the response of many species. Simultaneously multivariate analyses are used to summarize redundancy, reduce noise, elucidate relationships, and identify outliers. Multivariate analyses can relate communities to other kinds of data (e.g., environmental, historical data). And results from multivariate analyses are designed to improve our understanding of communities, esp. community structure (Rencher, 2002).

2. Ecological tools and techniques

2.1 Ordination methods

Bias and subjectivity that is usually associated with direct gradient analysis is usually overcome by the use of ordination method. In direct gradient analysis, first of all, correlation between species and environmental factors is observed and axes of variation are specified and then species and quadrates are arranged along that axis (Chahouki, 2012). Important objectives of ordination includes making a summary of community data with the help of a three

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dimensional figure in which similarity in species is displayed by their close alignment and dissimilarity is shown by far alignment. It is also used to relate species community data with the environmental factors. Therefore appropriate matrix can be formed by using ordination.

Simple response method explains the studied species occurrence in a relation with some unknown environmental variables. Direct gradient analysis is helpful in the case of measurement of environmental variables and in direct and indirect use of environmental data. Ordination can make a starting point even if relationship is not clear (Palmar website, 2016). Ordination mainly includes principal component analysis (PCA), reciprocal averaging and detrended corresponding analysis techniques.

2.1.1. Principal component analysis

PCA was started in 1901 (Pearson, 1901); but it was not given importance until 1933 (Hotelling, 1933). In 1933, it was given full consideration when some psychologists tried to find a single intelligence measure and reduced multivariate information to 1. In this case, species are plotted in sample-dimensional space, centralization and standardization is done. A line projecting through the data represents Axis 1, Axis 2 is perpendicular to Axis 1 and so on up to Axis n.

PCA Axis can be compared with Original axes as follows;

a) Points are similarly arranged in both axes.
b) First transformation (centralization) changes the angular relations of points when viewed from original axis but second transformation does not change it.
c) Original axes have represents single specie's abundance while PCA Axis represents the combination of abundances.

PCA is used to analyze only community data (excluding environmental). Therefore further processing is needed to include environmental data (Jolliffe, 2002).

2.1.2. Direct gradient analysis

Species are distributed along environmental gradient in direct gradient analysis. Ramensky (1930) and Gause (1930) firstly devised DGA but after 1950, ecological researchers used it extensively (Whittaker, 1983). Ordination of environmental and community data helps to tell the environmental factor(s) which effect common distribution and measurement of environmental factors estimation. It also helps to recognize the principles emerging from DGA for combination of individual species into communities. Data can be easily summarized and presented by this unquestionable analysis (DGA used in Ecology). Basics of such type of data handling go back to classical ecology. Subjective data sampling in DGA is a prove of its inherited circularity. Results from this sort of sampling make basis for DGA conclusion (Whittaker, 1967).

2.2. Cluster analysis

When different data groups are specifically arranged in a numerical manner then it is called cluster analysis. This analysis gives a picture of existence of some groups but does not tell about the group structures detail. Structure or techniques used in cluster analysis make an impact on its group detection ability (Hammer, 2011; Everitt et al., 2001). Cluster analysis can be of the following four types;

2.2.1 Single-linkage clustering(syn. nearest-neighbor clustering)

This type of clustering is an oldest technique. This clustering shows that as the groups grow, space is contracted and similarity between different groups is increased which leads to the formation of chain. Thus group's properties are not considered and comparison of individual quadrats is done (Stuetzle and Nugent, 2010).

2.2.2 Complete linkage (furthest neighbor) clustering

It is opposite to single linkage clustering, as the groups grow, distance between them is dilated (dissimilarities are increased). So there is no formation of chain. Structure of groups is not considered and comparison of quadrates is done as in first type of clustering (Brian et al., 2001).

2.2.3 Centroid clustering

If Euclidean distance is smallest between two centroid groups then these are joined. In this type of clustering, along with the linkage structure information is also included. Behavior of single linkage and centroid linkage is also almost similar (Gullo and Tagarelli, 2012).

2.2.4 Minimum-variance clustering

This method helps to calculate the distance between a member of group to its centroid. This distance indicates the measurement of dispersion. Information about groups is incorporated and about individuals is ignored. This method is complex and requires a lot of time as compared to other clustering techniques (Murtagh and Legendre, 2014).

2.3.Two way indicator species analysis (TWINSPAN)

This method is different from linear methods as it is a divisive method and its results are indicated in the form of a dichotomy. Ordination of samples is carried out with respect to area. Reciprocal averaging line is used for division between two groups. On one side of line is positive and on other side is negative group.

Dichotomies made by this way are then refined and ordered. Ordination is done by minimizing the distance between groups. Firstly, higher groups are ordered. Similarities between clusters are considered for ordering of lower groups. If there is no ordering then groups are arranged arbitrarily. Relatively large groups determine the dichotomy. When quadrats classification is finished then classification of species is carried out. A two way table is made for both classifications. This table gives a brief presentation of clustering results. Quadrate data may also contain data for environmental variables (Kooch et al., 2008; Lee and Cam, 1993).

2.4. Discriminant analysis

Group allocation and group differentiation is done in this analysis. Existence of group structure is considered in this. Similar entities are grouped together. Mahalanobis distance is used to measure similarities between entities. If species are not correlated to each other then this distance is similar to Euclidean distance. Multivariate normality is important condition for discriminate analysis while univariate analysis of variance is not much effected by multivariate normality. F- test is used in discriminate analysis for the recognition of discriminative group. Although there is not much surety about the reliability of results even then discriminate analysis is widely used in ecology (Williams, 1983).

2.5. Reciprocal averaging

Hill (1973) firstly introduced reciprocal averaging in ecology and pointed out a drawback of PCA that it cannot include information of rare species in the analysis. In addition to other advantages, rare species data handling was a big advantage of reciprocal averaging. Environmental variables are used as a replacement to species and their numerical value is used as a replacement to species abundance. Correlation between ordination axis and environmental variables can be calculated by the use of algorithm.

2.6. Canonical correspondence analysis (CCA)

Canonical correspondence analysis (CCA) is used to represent the relationship of community with environment. Useful environmental gradients can be find out by this method. Gradients are used in an ordination diagram to show the niche taxa. Canonical correlation analysis, two blocks partial least square and reduction analysis are not good to relate two variable sets. So in this case, use of CCA is highly suggested (Braak and Schot, 1995).

2.7. Detrended correspondence analysis

DECORANA is the first software form of Detrended correspondence analysis. It was firstly developed in 1979 and later on in 1980 it was published in journal (Hill and Gauch, 1980). DECORANA is mostly applied on non linear data in ecology.

2.8. Diversity index

In a specific community, diversity of species can mathematically be expressed by diversity index. Species richness and abundance forms its basis. No. of species and diversity of area are proportional to each other. Diversity index is very useful measure of diversity assessment in any field of study. Such as in ecology, it is used to assess biodiversity, in demography, it is used to assess the distribution pattern of demographic population classes, in economics, for the measurement of economic activities distribution in sectors and in information science, to reveal the information complexity (Agrawal and Gopal, 2013). A set of diversity indices include the species evenness, species richness, Shannon's diversity index, Berger- Parker's index. However Simpson's diversity and Shannon's diversity indices are preferably used.

2.8.1 Simpson's Diversity index

This index presents more weight to most dominant species, so it is a dominant index. Rare species are proved to be ineffective to diversity according to this index. Formula used for Simpson's index is given in equation 1:

Simpson Index (D) =1/ $\sum_{i=1}^{s} pi^2$ [1]

Where *P* is individual proportion of species/ total species number, *Σ* is calculations sum and *s* is species number

2.8.2 Shannon's diversity index

This index gives information about assumption of presentation of all the species of a sample and about random sampling. So it is called information static index. Formula used for Shannon's index as described in equation 2:

Shannon Index (H) = - $\sum_{i=1}^{s} piI$ [2]

Where *P* is individual proportions of species/ total species number, *In* is natural logarithm and *s* is species number

2.9. Advanced marker techniques and ecology

Integration of many basic molecular techniques to increase the resolution and sensitivity for the detection of genetic problems is carried out in advanced molecular ecology techniques. New classes of DNA markers i.e. retrotransposons, microsatellite are also used so as the genomic coverage is increased, genetic variation is best revealed. cDNA based templates are used in AFLP and RAPD techniques for understanding the genetic pattern and responses (Agarwal et al., 2008).

2.10. Molecular microbial ecological methods

Ecological research techniques vary according to many factors i.e. species being studied, hypothesis and financial budgets. If we want to investigate the diversity within a eukaryotic species, then techniques applied will definitely different from those used for prokaryotic diversity study. AFLP or rep-PCR genomic finger print analyses are used for finding the subspecies relationship. DNA-DNA homology values show highly correlation methods with these typing methods, suggesting that these genomic finger printing methods truly reveal genomic relations between organisms. RAPD, AP-PCR, and DNA fingerprinting are rapid and relatively inexpensive methods but these are also less reproducible with primers and experimental conditions needing to be optimized in each application. The random or arbitrary primed PCR fingerprint profiles are useful for obtaining diversity information. The combination of several RAPD or AP-PCR profiles may yield reliable relationship information (Rastogi and Sani, 2011).

2.11. Algorithmic models

Simple linear techniques are not always good to use in ecological research because in the case of large data sets, a high level of statistical knowledge is required. The use of Bayesian approaches, use of Information-theoretic approaches for model selection and techniques to overcome problems of spurious or correlated variables are some recent advances. But despite these recent advances, in case of a large number of explanatory variables in a model based on limited observations, traditionally used regression models often fail. So use of algorithmic models to improve the explanatory power of analysis is highly recommended to ecologists. Ecologists struggle to use new analysis tool that goes beyond their original training and education. Algorithmic models have been used to investigate analysis of species range shifts, species richness patterns, identification of populations, species presence and distribution patterns and analysis of behavioral patterns. Algorithmic models are more convenient, have high speed, and are most importantly superior model fit and prediction as compared to traditional statistical models such as GLMs (Oppel et al., 2009).

2.12. Ecological footprint

Ecological footprint is used as a tool for sea and land resource accounting. It measures productivity of sea and land and tells about their safe use for humans. Global hectares are used to measure demands for land and sea use and availability of ecosystem and biocapacity. Availability of area was 11.2 billion global hectares in 2003. In the same year ecosystem services to fulfill the human demand were 14.1 billion global hectares. If this number will increase day by day with the similar rate, then ecosystem will be collapsed. Ecological footprints of humanity are related to six groups of land use, grazing grassland, cropland, forests, fishing grounds, building area and carbon absorption land. Moat significant land use category in the world is land for carbon absorption. It represents footprints of almost half of all humans in the world. Human demands should be minimized within the limits of natural resources to protect the global ecosystem (Kitzes et al., 2007).

2.13. Laser scanning

United Nations Initiative on declining Emissions keeps forest degradation under observation and tries to save them. So carbon stock and aboveground biomass information after declining emission from forest degradation is of keen interest for American nations initiative. 3D mapping of structure of the vegetation is the most efficient step of laser scanning for terrestrial systems. Because due to its accurate measurement of forest biomass and carbon stock is possible (Ponton and Disney, 2014).

2.14. Quantitative methods for ecological network analysis

Analysis of global transfertrophic network is important to have a look into dynamics of global ecosystem. Because it is very necessary for simulation modeling. A quantitative or systematic analysis of every land can be done in this regard. Linear algebra can be used to find out the indirect relationship between two taxa of different magnitude and different nature. When in trophic continuum, any individual taxa are operating and in case of web of connections, the same algebraic calculations can be carried out to organize organisms in a linear chain. If we want to recycle energy and material within a system, we can use back tracking algorithmic system. Methods of control and types of group function can also be pointed out by this method. Quantification of system performance can be done by using information theory. To differentiate organization and efficiency of performance from future recover and development, description of interaction complexity is done. Ecological bottlenecks can be obtained by the use of these techniques (Ulanowicz, 2004).

2.15. Ecological engineering

Making a linkage between economy and environment by using specific ecological pattern is referred to as ecological engineering. Well organization of ecosystem serves as a limit for ecological engineering. While the limit for environmental engineering is usually at the end of pipe. Such as when there was release of waste water on bare lands then wildlife sanctuary for coastal marsh was developed in Texas. Spatial and temporal organization can be done on the principle of energy hierarchy concept helpful for maintenance of ecological engineering techniques (Odum and Odum, 2003).

2.16. Quartered neighbor method

As quantitative data is very much important in the field of ecology so there is need of inventing new methods for density measurement. Therefore Zhu and Zhang (2009) devised a new distance method for density measurement that is called the quartered neighbor method. According to this method, centre was considered as the sampling point and four quadrates were laid down on area around the centre. Results indicate required distance which is average of these four quadrates, was considered the center, and the area around it was divided into four quadrants. Two old distance methods (point centered method and nearest neighbor method) are integrated together to make this new method. Zhu and Zhang (2009) compared the results of quartered neighbor method and point centered quarter method for calculating average spacing distance in Donglingshan forest with quadrate method. Results were almost same but quadrate method was proved to be more efficient.

2.17. Plant ecotypes: genetic differentiation in the age of ecological restoration

Ecological restoration and population genetics together form the restoration genetics. In restoration process, when there is translocation of organisms from one ecosystem to another than there are a lot of ambiguities about intraspecific hybridization between already present organisms and new organisms take place. So molecular markers are used to access the success of adaptation of new species and introduction of new genotypes. Prediction for translocation results and their effect on seed transfer zones can also be done by use of molecular markers (Hufford and Mazer, 2003).

2.18. Non-parametric multivariate analysis of variance

Univariate data can be analyzed by the use of analysis of variance (ANOVA) but for analysis of multivariate data, classical multivarious analogies are usually used. So modified non parametric methods are preferably used. A new method for non parametric multivariate analysis was devised by Anderson (2011). So it can be used as an alternative method. Square distance sum was the basis for this new method. Partition of complex model variation is possible directly by this method. So it was an improved version of previously existing non parametric method. Fischer test was used as a test statistic. Tests with the nested and factorial design were proved to be exemplary.

2.19. Dirichlet Allocation model, a probabilistic multivariate statistical method

A new method for multivariate analysis of biodiversity was devised by Valle et al., (2014). It was based on LDA (Latent Dirichlet algorithm) model. Probability was focused in this method. Arrangement of distinguished components of a community into a set was reduced by this method. Valle et al., (2014) tried to justify this method by using data from various related sources. The model proved its ability for detection of universal decrease in communities of Oak in Indiana and Minnesota. It was because of fire decline and rainfall increase. Characterization of direction of succession in species composition and factors affecting it was carried out by chronosequence analysis. This method is helpful to ecologists to track decomposition and species assemblage dynamics along spatial and temporal gradients, and effects of forest degradation and global change.

2.20. Application packages for ecological data analysis

The ecodist package (Goslee and Urban, 2007), COCKTAIL (Bruelheide, 1997; Bruelheide, 2001), TWINSPAN (Hill, 1979), PC-ORD package (McCune and Mefford, 1999), D-MAP mapping package (Morton 2005), CANOCO (Braak and Smilauer, 2002), ENMeval, mizer, MEMGENE, Rphylip, agTrend, ModestR, MeCa, E-SURGE, SDMtoolbox, BAMMtools, Synchrony, MLST@SNaP, RangeShifter, PopGenReport, *IsoMAP*, *QuaBiMo*, *asnipe*, SIDIER, PASTIS, Tea Bag Index, ITSx, RInSp, pavo, marked, nupoint, PhyloGenerator, Geiger, RobOff, EasyABC, SimAdapt, Geomorph, SURFACE, SPOTG, BEFdata, PROGRAM MARK, IPMpack, Cheddar, Treebase, GeoLight, spacecap, Gibbs sampler, FlexParamCurve, diversitree, taxonstand, betapart, paleotree, Popdemo, nadiv, simapse, jPopGen Suite, mvabund, BaSTA, abc, NichePy, RNetLogo, EB-IBM, phytools– a multifunctional phylogenetics package, RNCEP: global weather and climate data are at your fingertips and WaderMORPH, which is a user-friendly individual-based model for shore bird, are some application packages that we can use for the analysis of our ecological research work (Leps and Smilauer, 1999; Schnabel et al., 1998; Lubomir and Jason, 2006).

3. Conclusion

Variety of ecological techniques is traditionally being used by plant ecologist from many decades but now methodology in ecology is being changed and advanced. With the advent of molecular techniques, interest of ecologists seems to be converting toward molecular ecology. Molecular foot prints and molecular markers gave a new insight in study the plant diversity and ecology. And this advancement in the field of ecology is a positive sign to compete with natural ecosystem degradation processes and to fulfill the growing demand of mankind for natural resources use.

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