BIOLOGICAL SYSTEMS, BIODIVERSITY AND STABILITY OF PLANT COMMUNITIES

Edited by

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APPLE ACADEMIC PRESS

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Apple Academic Press, Inc
3333 Mistwell Crescent
Oakville, ON L6L 0A2
Canada

Version Date: 20150427

International Standard Book Number-13: 978-1-4822-6379-4 (eBook - PDF)

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

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CHAPTER 30

THE ANALYSIS OF ONTOGENETIC SPECTRUM OF HETEROGENEOUS POPULATION

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ABSTRACT

The distribution of discrete ontogenetic states of individuals is usually spatially and temporally different within a population. If a sample from the population sample consists of several subsamples, the comparison of their ontogenetic spectra reveals heterogeneity of samples, i.e. different subsamples cannot be described by the same polynomial distribution. Therefore, the comparison of the samples using the aggregate data is not correct and tends to result in false inferences of biological importance. The paper proposes three methods for comparison of ontogenetic spectra of heterogeneous samples: a randomized variant of ANOVA, principal components analysis and ordinal regression analysis. The following approaches are exemplified in natural populations of cowberry *Vaccinium vitis-idaea* L. and epiphytic lichens *Hypogymnia physodes* (L.) Nyl. and *Pseudevernia furfuracea* (L.) Zopf.

30.1 INTRODUCTION

Each individual of any living organism is characterized by its age: a chronological one measured in time units and a biological one defined on the basis of different morphological, physiological, biochemical, etc. characteristics of an organism. The notion of a biological age is used in population biology of plants and lichens especially if the assessment of a chronological age is impossible [1–3]. An individual development (ontogeny) of plant and lichens has successive ontogenetic periods – latent, pregenerative, generative and postgenerative ones defined on the basis of the scope of morphological markers. Each of these periods has successive discrete ontogenetic states (see Table 30.1). The virginal ontogenetic state of some plants and lichens are divided into v_1 and v_2 on account of their peculiar formation of morphological structures [4, 5].

Thus, a continuous ontogenetic process of plants and lichens is described as a set of successive ontogenetic states, and an ontogenetic state is a qualitative (not a quantitative) marker of an individual. Note that since successive ontogenetic states are arranged in time, then, inevitably, chronological and biological ages are strongly correlated, with individuals of the same ontogenetic state of a different chronological age.

Based on logistical growth curve of an individual during ontogenesis, A. Uranov introduced a weighting coefficient (numerical characteristic) of each ontogenetic state – k_i (Table 30.1).

The whole set of population individuals enables to build an ontogenetic spectrum of population. Though, it is necessary to specify that a notion of a population have two meanings in biological studies. On the one hand, population is a theoretical notion, an elementary microevolutional unit [6]. On the other hand, field researchers use a working term of population when describing demographic indicators of a group of certain individuals and definitive characters in different discontinuous

habitats. Therewith, Soviet/Russian botanical studies use the term of coenopopulation – a set of individuals of a certain plant within a single phytocoenosis [3, 5, 7]. Several types of spectra can be distinguished for different plants (Fig. 30.1). The analysis of age spectra elides seeds and germs because of fluctuations in their possible outbreak and accidental mass mortality. When a virginile ontogenetic state bisects, weight coefficients are calculated on the assumption of equal intervals on im-g_1 segment and make up accordingly v_1 – 0.0884, v_2 – 0.1589 [8].

TABLE 30.1 The Discrete Description of Plant Ontogenesis [2]

Period	Number of ontogenetic state i, its name and symbol	Weight coefficient of ontogenetic state k_i
Latent	1. Seeds, sm	0.0025
Pregenerative	2. Seedling, p	0.0067
	3. Juvenile, j	0.0180
	4. Immature, im	0.0474
	5. Virginal, v	0.1192
Generative	6. Young generative, g_1	0.2700
	7. Mature generative, g_2	0.5000
	8. Old generative, g_3	0.7310
Postgenerative	9. Subsenile, ss	0.8808
	10. Senile, s	0.9529
	11. Subcadaveric, sc	0.9819

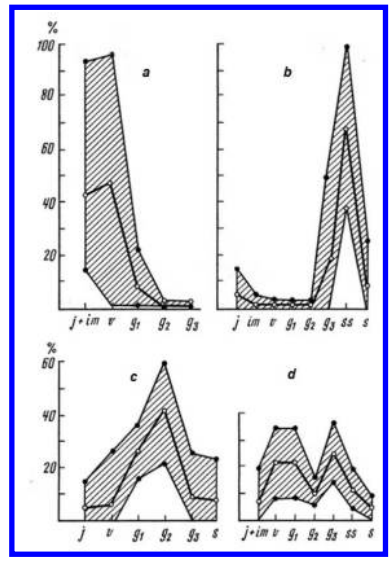

FIGURE 30.1 The types of basic coenopopulation spectra (average scores); $\pm 3\sigma$ is shaded, a – left hand spectrum, b – right hand spectrum, c – symmetric single peak, d – double-peak [7].

A. Uranov [2] introduced an *average age* Δ that became a standard parameter of an ontogenetic state of a population (coenopopulation)

$$\Delta = \frac{\sum_{i=1}^{11} k_i n_i}{\sum_{i=1}^{11} n_i},$$

n_i – the number of individuals in an ontogenetic state with *i*-number, k_i – a weight coefficient of an ontogenetic state with *i*-number.

However, different characteristics of an ontogenetic spectrum are used. Thus, N. Glotov [9] proposed to calculate *a modified recovery index* I_1:

$$I_1 = \frac{\sum_{i=3}^{5} n_i}{\sum_{i=3}^{8} n_i},$$

here a part of pregenerative individuals among pregenerative and generative ones characterizing a left part of a spectrum (Figure 1). *An aging index* I_2 [9], a part of postgenerative individuals in a population characterizes the right part of a spectrum:

$$I_2 = \frac{\sum_{i=9}^{11} n_i}{\sum_{i=3}^{11} n_i}.$$

Methodology of data collection to characterize an ontogenetic spectrum of a population consists of samples that include several subsamples collected in different parts of a population, in different years, etc. For instance, in case of herbaceous plants all plants within one square meter quadrate are collected (considered), with the ontogenetic state of each individual being defined; data on all quadrates within a transect are summed, the ontogenetic spectrum of a population (coenopopulation) is found, frequencies (percentage) of individuals of each ontogenetic state and parameters characterizing the ontogenetic spectrum of a population are calculated. Thus, a quadrant is here a measurement unit. For example, in the study of epiphytic lichens, individuals (thalli) of all ontogenetic states separately on each tree are considered, with data within a habitat or a certain phorophyte (substratum) being summed. Thus, a single tree is here a measurement unit.

However, the aggregation of subsamples (quadrants for plants, trees for lichens) and, therefore, the study of the aggregated sample (population) are only eligible if the sample is homogeneous – the distributions of different subsamples within the sample are not significantly different, i.e. can be described by the same polynomial distribution [10].

The chi-square criterion is a standard method to compare ontogenetic spectra. It is a asymptotic criterion, and it use for analyzing contingency tables is eligible only if expected values are big enough. The conditions of correct use of chi-square crite-

rion are standard: minimal anticipated value is not less than 5 or an average observable in a cell of a contingency table is not less than 5. Sokal and Roplf [10] propose to lower the requirement to the minimal expected abundance with growing degrees of freedom. Simonoff-Tsai criterion [11] is often used to determine the possibility to use chi-square criterion. Observed abundance of adjacent classes is often combined to get bigger expected values. If chi-square criterion is still inapplicable, the homogeneity analysis of RxC contingency tables uses an exact test that is a generalized Fisher's exact test for 2×2 contingency tables [12].

The present study focuses on proposing statistical methods to analyze ontogenetic structure of a population adequate to the field data collection methods taking into account heterogeneity of plant and epiphytic lichen populations in space (or in time).

30.2 MATERIALS AND METHODOLOGY

To solve this problem we propose the following three methods.

Method 1 – Estimation and comparison of the parameters Δ, I_1 and I_2, ontogenetic spectra using randomization analysis of variance (ANOVA). The values of the parameters are calculated for each subsample. In this case the assumptions of one-way ANOVA hold: levels of a factor are the k samples, which are represented by n_i (i=1, 2, –, k) subsamples. Each subsample is characterized by the value of the parameter Δ_{ij} (j=1, 2, –, n_i, the number of subsamples in the ith sample). It can similarly be done for the parameters I_1 and I_2.

Using the F-test and Scheffé's method (in order to account for multiple comparisons) in one-way ANOVA, we can compare the sample mean values of the parameter Δ ($I_1 \times I_2$, respectively). Let us denote this fixed-effect model as Model I. Using the random-effect ANOVA model (denoted by Model II), we can estimate the effect of the factor s_a^2 / s_t^2, where s_a^2 is the variance between samples, s_t^2 is the total variance. However, under ANOVA model the following assumptions should be met: equality of the variances within all groups and the residuals are normality distributed [13]. Since these requirements are often violated, various randomized procedures in ANOVA have been increasingly used in recent years. In this chapter, we use the randomized variant of ANOVA [14].

In Model I ANOVA, we carry out random permutation of the values of the parameter of subsamples between the samples without repetitions. After this randomization we calculate the value of F-test taking into account the sizes of the subsamples. Under these permutations, the subsample weight is moved together with the value of its parameter, while the total weight of the sample may vary. We repeat this procedure N=10,000 times, then we obtain the distribution of F-values (F_i). For the randomization procedure, the distribution of F-values is not important; the values are only used as quantities characterizing the differences between the samples. Then we find F-value for the initial data (F_{exp}) and count the number of F-values that

greater than or equal to the F_{exp} (that is, $F_i \geq F_{exp}$). Finally, we determine the proportion of the values such that $F_i \geq F_{exp}$ assuming that the F_{exp} has been obtained using the randomization procedure. If the results are significant, then Scheffé's method for multiple comparisons can be carried out in a similar way.

The randomized variant of Model II ANOVA is based on the bootstrap method [15]. The standard bootstrap procedure is carried out within each sample, that is, a new sample is constructed using resampling with repetitions from the initial sample. Then the effect of between-sample variability for the obtained randomized data is calculated. This procedure is repeated N=10,000 times, as a result we obtain the distribution of the effect. The median of this distribution is used as an estimate of the effect, $\alpha \times 100\%$-confidence interval is obtained using $(1-\alpha)/2$- and $(1+\alpha)/2$-quantiles (for example, $\alpha = 0.95$). When calculating the effect, the procedure of weighting the size of subsamples is also used.

Method 2 – Application of principal component analysis [16]. The initial data are the distribution of frequencies of individuals belonging to each ontogenetic state. We apply the Fisher φ-transformation for the obtained frequencies of ontogenetic states such that subsamples are treated as observations and variables (markers) are j-values of the frequencies of individuals from the same ontogenetic state. Then we obtain the correlation matrix taking into account the weights that were defined in Method 1. It can be shown that the correlation matrix will remain the same if the weights are proportionally increasing.

As a result of principal component analysis, we obtain new variables, the components, which are linear combinations of the original variables. The sum of frequencies of individuals from different ontogenetic states for each subsample is equal to 1. Therefore, the correlation matrix based on the frequencies of ontogenetic states will be singular and the variance of the last component (that is, the smallest eigenvalue of the matrix) must be 0 [16]. Formally speaking, the matrix should not be singular after the Fisher φ-transformation and, therefore, the smallest eigenvalue of the matrix is not equal to 0. However, the variances of the last components are usually very small and, taking into consideration dependence between frequencies of individuals from different ontogenetic states, it is likely that the variance of the last component under the Fisher φ-transformation will be very small. Then for each of the obtained components, we carry out the randomized variant of ANOVA taking into account the weights of the subsamples.

Method 3 – Application of cumulative link mixed models [12], also known as ordinal regression models, which belong to generalized linear models. The proposed model can be described as follows:

$$P(Y_i < j) = g(\theta_j - u(Sample) - v(Subsample)), \quad i=1, \ldots, n; \quad j=1, \ldots, J-1;$$

$$u(Sample) \sim N(0, \sigma_a^2), \quad v(Subsample) \sim N(0, \sigma_e^2),$$

where n is the total number of individuals in all samples, J is the number of ontogenetic states, j is the ontogenetic state. The random variable Y_j represents the ontogenetic state of the i^{th} individual. Then $P(Y_i \leq j)$ is the probability that the ontogenetic state of the i^{th} individual is less than or equal to j. We take the sample and the subsample effects to be random, and assume that they have normal distributions $N\left(0, \sigma_a^2\right)$ and $N\left(0, \sigma_e^2\right)$, respectively. The g is a link function usually logit or probit (although it is possible to use other functions), that is, $g(y) = \exp(y)/(1+\exp(y))$ or $g(y) = \Phi(y)$ (the cumulative normal distribution function).

This model can be interpreted in the following way. Each individual has a certain age ξ, which increases over time such that $-\infty$ indicates the birth of the individual whereas $+\infty$ represents the death of the individual. Thus, the j^{th} ontogenetic state is a time interval and θ_j is the value of the age ξ such that $\theta_{j-1} < \xi \leq \theta_j$. In this case the g is the cumulative distribution function of ξ. The θ_j, j =1, …, J–1, are known as threshold parameters or cut-points. The link function g and the values of θ_j are the same for all samples and subsamples. Distributions for different subsamples differ only by a horizontal shift of the function g, this shift is the sum of the effects in our model.

In order to test that the sample random effect is significant, we propose to use the following two nested models:

$$M_{full}: \; P(Y < j) = g\left(\theta_j - u(Sample) - v(Subsample)\right),$$

$$M_{cut}: \; P(Y < j) = g\left(\theta_j - v(Subsample)\right).$$

We use the likelihood ratio test to test the null hypothesis H_0: $\sigma_a^2 = 0$. The estimates of σ_a^2 and σ_e^2 can be used in order to assess the contribution of each predictor (Sample or Subsample).

The data were analyzed using statistical software R. We implemented the algorithms based on Methods 1 and 2, R-package "ordinal" was applied in order to analyze the data using Method 3.

Suggested methods are tested on the data from natural populations of cowberry (*Vaccinium vitis-idaea* L.) and epiphytic lichens *Hypogymnia physodes* (L.) Nyl. and *Pseudevernia furfuracea* (L.) Zopf on the territory of the Republic of Mari El.

The study of 10 coenopopulations of cowberry was carried out within the State Nature Reserve "Bolshaya Kokshaga" (№№ 1, 3–6) and in the neighborhood of the Ismentsy settlement of the Zvenigovsky district (№№ i1-i5). Coenopopulation No.1 is situated in a pine tree forest recovering from ground fire in 1995, coenopopulations No. 3, i1, i4 and i5 – in a pine tree forest with cowberries, coenopopulations No. 4, 5 and 6 – in a pine tree forest with green mosses and cowberries, coenopopulation No. i2 – in a pine tree forest with firs and cowberries, coenopopulation No. i3 – in a pine tree forest with carex, heath and cowberries. The age of forest in the habitats of different coenopopulations is 55–85 years, its normality amounts to

0.5–1. Geobotanical descriptions were processed according to D. Tsyganov's ecological scales [17, 18]. Different habitats have 12.68–13.60 scores (with 11.5% of its range for cowberry) according to Hd-scale (soil moistening), 4.55–5.56 (14.4%) according to Tr-scale (soil wealth), 4.27–5.32 (21.0%) according to Nt-scale (nitrogen abundance), 5.28–6.55 (21.2%) according to Rc-scale (soil acidity), 4.29–4.84 (6.9%) according to Lc-scale (illuminance) and 3.32–5.31 (39.8%) according to fH-scale (soil moisture variability). Habitats are different in their floristic composition: Jaccard's coefficient of community differs from 9.4% to 43.8%. Each coenopopulation had 10–30 quadrants (1 sq.m.) observed, the number of partial cowberry bushes within a quadrant is 5–778, the number of partial cowberry bushes in different coenopopulations is 1498–7117. The diagnostics of partial bushes is described in [19]. The spectrum of ontogenetic states involves the range im-sc, juvenile individuals cannot be found as partial bushes are of vegetative genesis. Some, possibly tens of partial bushes within a single quadrant belong to the same cowberry individuals [20].

The study of *Hypogymnia physodes* was carried out in two habitats within the Starozhilskoye forestry in Medvedevsky district on the thalli (up to 3 m high) of little-leaved linden (*Tilia cordata* Mill.), Siberian fir (*Abies sibirica* L.) and common pine (*Pinus sylvestris* L.). The first habitat in a flood lime-tree forest with pines, ostrich ferns and lilies-of-the-valley in a high-water bed of the Bolshaya Kokshaga river gave two samples: on 23 little-leaved lindens (the number of thalli on a tree is 42–785, the total number of thalli is 3652) and on 7 Siberian firs (the number of thalli on a tree is 4–1229, the total number of thalli is 3562). The age of forest stand is 70–80 years, the length of linden girth on the height of 1.3 m is 0.81 m, the length of fir girth is 0.92 m. The second habitat is located in a woodland park of pine tree forest with fescue and bent grass in the settlement of Starozhilsk: the sample of 16 *Hypogymnia physodes* was taken (the number of thalli on a tree is 221–691, the total number of thalli is 3562).

The second habitat is located in a woodland park of pine tree forest with fescue and bent grass in the settlement of Starozhilsk: the sample of 16 common pines was taken (the number of thalli on a tree is 221–691, the total number of thalli is 7651). The age of forest stand is 75 years, the length of linden girth is 1.04 m. The pine tree forest receives strong man's impact – stocking. Ontogenetic states of *Hypogymnia physodes* are described in [21].

Data on *Pseudevernia furfuracea* were collected in 8 habitats. In pine tree forest with green mosses and *Betula pendula* Roth. within the State Nature Reserve "Bolshaya Kokshaga," 2 samples were collected: sample No. 2a on a pine tree (the age of trees is 65 years, the length of girth is 0.6 m) and sample No. 2b on a birch tree (the age of trees is 45 years, the length of girth is 0.46 m). In the Starozhilskoye forestry in the Medvedevsky district, three samples were collected: sample No. 3 on a pine tree in a pine tree forest with blueberry (the age of trees is 70 years, the length of girth is 0.54 m); sample No. 4 on a pine tree in a low bush and bog moss pine tree

forest (the age of trees is 110 years, the length of girth is 0.63 m); sample No. 5 on a birch tree in birch tree forest with carex, calla and bog moss (the age of trees is 50–60 years, the length of girth is 0.55 m). Four samples were taken from pine trees within the Kerebelyakskoye forestry of the National Park "Mariy Chodra": on the nearly located areas of blueberry pine tree forest sample No.6 was taken (in a mesorise) and sample No.7 (in a mesofall), the age of trees is 60 years, the length of girth is 0.63 m. Sample No.4 includes 67 trees, other samples have 10–20 trees each. The number of thalli on a tree differs from some to a hundred and a half, only in a sample No.8 the number of thalli differs from 64 to 238 and sample No.9 has 93 to 359 thalli on a tree. Ontogenetic states of *Pseudevernia furfuracea* are described in [22].

Mass data collection in nature populations of lichens enables to construct ontogenetic spectra from v_1 ontogenetic state, since thalli of initial ontogenetic states are particulate and specific belonging of im individuals is sometimes difficult to determine. Note that the frequency of g_3, ss and s individuals in nature populations *Pseudevernia furfuracea* is insignificant (about 1 percent), since they are likely to remain on a tree trunk [23, 24].

30.3 RESULTS AND DISCUSSION

Coenopopulations of cowberry. For all coenopopulations, the ontogenetic distributions of subsamples within each coenopopulation differ significantly ($p < 10^{-15}$). This means that statistical analysis of the aggregated sample for coenopopulation would not be correct.

Using Method 1, we showed that the differences in all three parameters for 10 coenopopulations are statistically significant: p=0.0001 for Δ, p= 0.0001 for I_1, p=0.0012 for I_2. Fig. 30.2 shows the results of pairwise comparison of all parameters of the coenopopulations. It can be seen that 20 pairs of Δ (out of possible 45 pairs) are different at the 5% significance level, with the values of Δ for different coenopopulations are located fairly uniform. For the parameter I_1, 28 differences are significant at the 5% significance level and the values for coenopopulations №№ 1, 3, 6 are clearly shifted to the left. For the parameter I_2, only 8 differences are significant at the 5% significance level and the values of I_2 are located fairly evenly. A more detailed analysis of the values of the parameters for ontogenetic spectra of coenopopulations as well as comparison of these results with the characteristics of habitat is the objective of a special study. Note that if we were to do pairwise comparisons of ontogenetic spectra using the aggregated data for each coenopopulation, then we would get (even taking into account the Bonferroni correction) that all 45 comparisons are significantly different ($p < 10^{-12}$). The impacts of differences between the samples are 0.254 for Δ (95% confidence interval is 0.180–0.337), 0.374 for I_1 (0.306–0.443), 0.103 for I_2 (0.040–0.183).

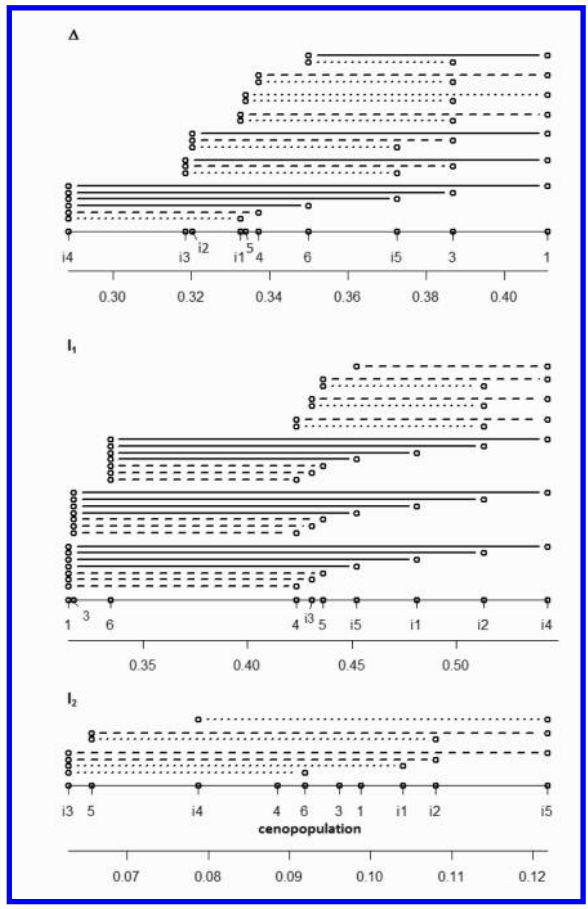

FIGURE 30.2 Significance of differences between the parameters of coenopopulations of cowberry, solid line – $p < 0.001$, dashed line – $p < 0.01$, dotted line – $p < 0.05$.

The axis (bottom line) is the scale of the values.

The results of applying principal component analysis (Method 2) are shown in Table 30.2. It can be seen that all principal components are significantly different, while the effect of variability between coenopopulations is large enough only for the first four principal components. Given that the principal components are independent (by definition), we calculated the average factor effect taking into account the weight of the corresponding principal component:

$$0.445 \times 0.454 + 0.247 \times 0.349 + 0.164 \times 0.395 + 0.081 \times 0.214 + 0.028 \times 0.087 + 0.019 \times 0.144 + 0.017 \times 0.226 = 0.379$$

TABLE 30.2 The Results of ANOVA of the Principal Components For Coenopopulations of Cowberry

Principal components	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Contribution of the principal component in the total variance	0.445	0.247	0.164	0.081	0.028	0.019	0.017
Cumulative contribution	0.445	0.692	0.855	0.937	0.965	0.983	1.000
Difference between coenopopulations, p	0.0001	0.0001	0.0001	0.0001	0.0273	0.0001	0.0001
The effect of variability between coenopopulations (95% confidence interval)	0.454 (0.392–0.516)	0.349 (0.245–0.455)	0.395 (0.323–0.468)	0.214 (0.142–0.302)	0.087 (0.038–0.171)	0.144 (0.076–0.234)	0.226 (0.152–0.312)

Using ordinal regression models (Method 3), we have demonstrated that the differences between coenopopulations are highly significant both for the probit and for logit models ($p = 10^{-9}$). The effects of variability between coenopopulations are quite close to each other: 0.213 for the probit model and 0.244 for the logit model. The meaning of these effects is clear from Fig. 30.3. Here the coenopopulations are ordered by ascending value of the effect of coenopopulation, the effects for separate quadrants within this coenopopulations are also arranged in ascending order. It can be seen that the range of variability of the coenopopulation is much smaller than the range of variability of the effect of individual quadrants.

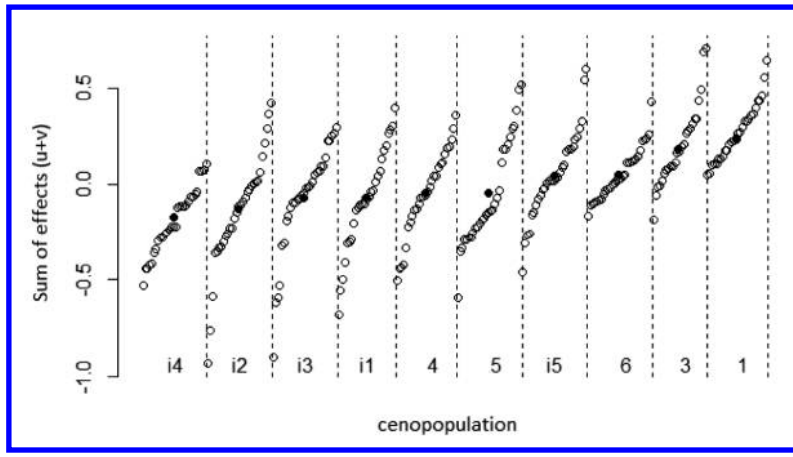

FIGURE 30.3 The results of ordinal regression analysis for ontogenetic spectra of coenopopulations of cowberry (probit model). The filled circles are the effects for the coenopopulations, the blank circles are the effects for individual quadrants.

Thus, all three methods, which take into account the heterogeneity of coenopopulations of cowberries, identify differences between ontogenetic spectra of the coenopopulations. All of the methods also reveal that the effects of differences in variability of the coenopopulations are close to each other. It can be seen that the variability between coenopopulations is relatively low (0.103–0.379), that is, variability between ontogenetic spectrum is mainly concentrated between sample quadrants within the coenopopulation. This may be due to individual characteristics of cowberry or the fact that the study area (territory of the Republic of Mari El) is the southern limit of the individual' area so that the described feature is typical for populations on the border of the area.

Hypogymnia physodes on different substrata. This example shows the need for careful preliminary examination of the experimental data as well as for an accurate statistical analysis. The sample ontogenetic spectra for all three phorophytes are heterogeneous ($p < 10^{-15}$). Method 1 detected the differences in all three parameters: $p=0.0007$ for Δ, $p=0.0025$ for I_1, $p=0.0002$ for I_2. The 95% confidence intervals for the effects between samples with different phorophytes are 0.080–0.719 for D, 0.067–0.707 for I_1 and 0.168–0.539 for I_2. Sufficiently large 95% confidence interval for I_2 may be explained by the fact that we have only three samples and the number of thalli on three firs (in three subsamples) is very small: 4, 13 and 16. The confidence intervals of the effects of between-sample differences for Δ and I_1 do not have much meaning since they cover 63.9% and 73.0% of all possible values, respectively. In order to understand this situation, we plotted the histograms of the distributions of the effects of differences between the samples (Fig. 30.4). It can easily be seen that the distributions have at least two modes. This indicates that the ontogenetic spectra of some individual trees may have systematic biases. Such bias was identified for linden trees: with high irregular variability of spectra, tree 22 appears to be an outlier, it is characterized by much greater frequency of thalli v_1, the highest frequency of thalli v_2 among all the other trees and, therefore, lower frequencies of thalli of all other ontogenetic states (Fig. 30.5). It seems reasonable to exclude linden tree 22 from the analysis. The distinctive feature of ontogenetic spectrum of thalli *H. physodes* on linden tree 22 may be explained by its position: the tree is located on the edge of a forest at the cliff of high bank of the river Bolshaya Kokshaga.

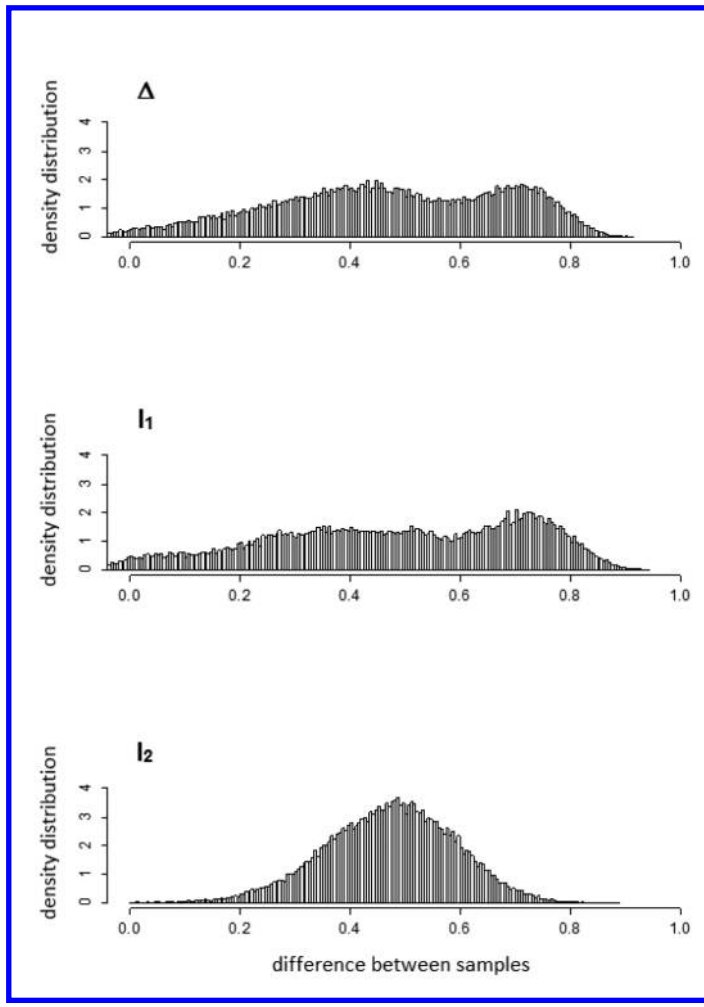

FIGURE 30.4 The histograms of the distributions of the effects for differences between samples of *H. physodes* for Δ, I_1, I_2.

Exclusion of linden tree 22 from the analysis makes the distributions of the effects for Δ and I_1 and unimodal (Fig. 30.6). The effect of differences between samples for Δ is equal to 0.603 with the 95% confidence interval 0.398–0.756, for I_1 – 0.704 (0.479–0.819), almost the same for I_2 – 0.365 (0.167–0.573). Multiple comparisons of the parameters demonstrated that Δ significantly differs for pairs linden-fir, linden-pine ($p < 0.001$), whereas the parameter does not differ for pair fir-pine ($p = 0.11$). The parameter I_1 is different for the same pairs ($p < 0.001$) and for pair fir-pine at 5% significance level ($p = 0.014$). The parameter I_2 is significantly differ-

ent for pairs linden-pine (p = 0.0002) and for linden-fir at 5% significance level (p = 0.048) and it does not differ for pair fir-pine (p = 0.80). If we ignored the heterogeneity of the samples, the ontogenetic spectra of *H. physodes* on all substrates would be significantly different ($p < 10^{-15}$).

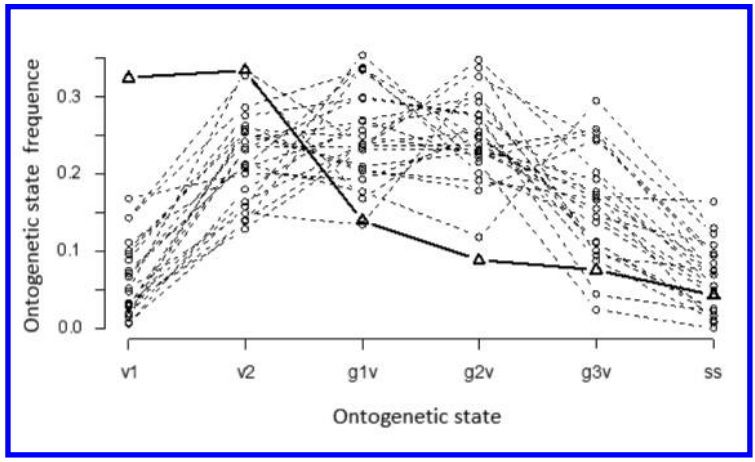

FIGURE 30.5 Ontogenetic spectra of *H. physodes* on linden trees, ontogenetic spectrum of tree 22 is highlighted by solid line.

The results of principal component analysis are shown in Table 30.3. The average effect of between-sample variability of ontogenetic spectra with the weight of the corresponding principal component is equal to 0.512.

TABLE 30.3 The Results of ANOVA of the Principal Components For Samples of *H. physodes*

Principal components	PC1	PC2	PC3	PC4	PC5
Contribution of the principal component in the total variance	0.6789	0.1288	0.1189	0.0584	0.0150
Cumulative contribution	0.6789	0.8077	0.9266	0.9850	1.0000
Difference between coenopopulations, p	0.0001	0.0636	0.0076	0.7098	0.9658
The effect of variability between coenopopulations (95% confidence interval)	0.6876 (0.4147–0.8160)	0.2020 (0.0481-0.4324)	0.1279 (0.0285–0.3231)	0.0304 (0.0009–0.1557)	0.0422 (0.0015–0.2211)

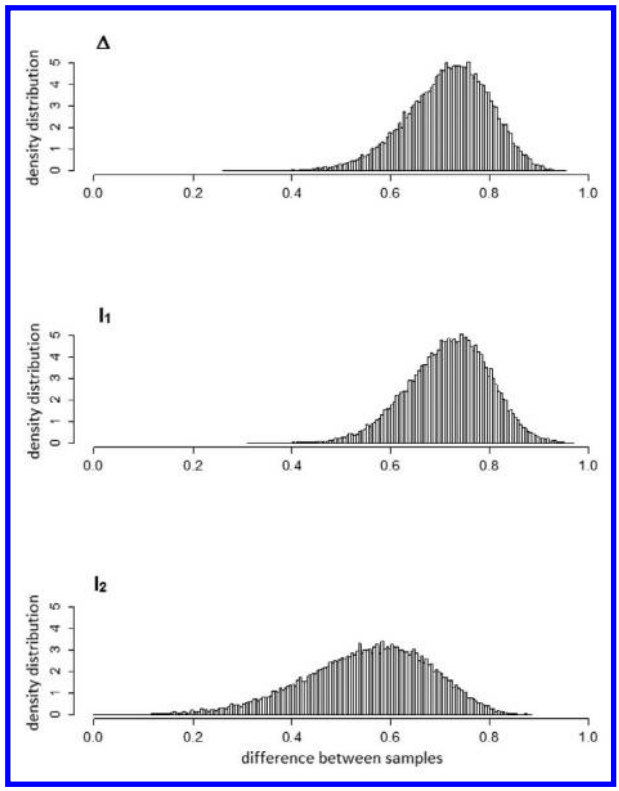

FIGURE 30.6 The histograms of the distributions of the effects of differences between samples of *H. physodes* for Δ, I_1, I_2, linden tree 22 is excluded.

The ordinal regression analysis yields similar results. Ontogenetic spectra are significantly different ($p < 10^{-6}$), the effect of between-sample differences is 0.634 for the probit model and 0.641 for the logit model.

Thus, analyzing ontogenetic spectra of *H. physodes*, all three methods produce consistent results. Unlike for coenopopulations of cowberry, the effect of between-sample variability for *H. physodes* is much higher.

Pseudevernia furfuracea in different habitats and on different substrates. We discovered that ontogenetic spectra of samples 8 and 9 were heterogeneous ($p < 0.003$), whereas spectra of sample 2a were homogenous ($p = 0.1256$). Using Method 1, we demonstrated that the differences between the sample parameters Δ and I_1 were significant ($p = 0.0001$). Pairwise comparisons of Δ show that only one pair (out of 36 possible pairs) is significant the level of 0.0001, two pairs at the level of 0.001 and 7 pairs at the level of 0.05 (Fig. 30.7). Only 5 paired differences of I_1 are significant at the level of 0.05 (Fig. 30.7). If we performed incorrect pairwise comparison of aggregated ontogenetic spectra of 9 samples, then we would make the

conclusion about the significance of 28 differences (out of possible 36) at the level << 0.001, four at the level of 0.01, one at the level of 0.05, and only 3 differences would not be statistically significant. The effects of differences between the samples are 0.339 for Δ (0.181–0.514) and 0.204 for I_1 (0.087–0.351).

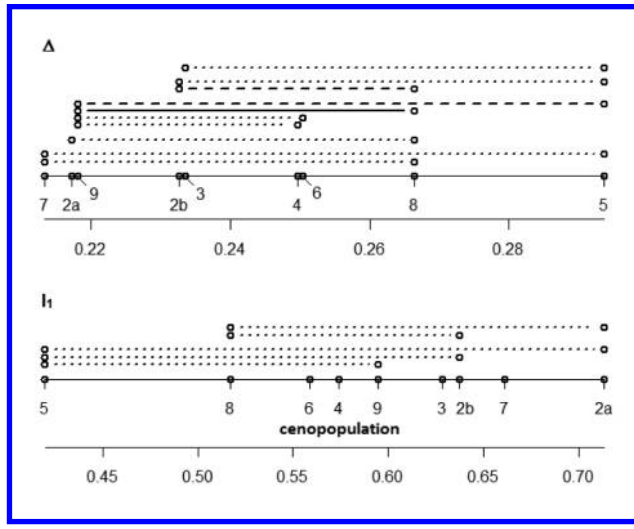

FIGURE 30.7 The significance of differences between the parameters of samples of *P. furfuracea*, solid line – $p < 0.001$, dashed line – $p < 0.01$, dotted line – $p < 0.05$. The axis (bottom line) is the scale of the values.

The results of principal component analysis (Method 2) are shown in Table 30.4. The average effect of between-sample variability of ontogenetic spectra with the weight of the corresponding principal component is equal to 0.293.

TABLE 30.4 The Results of ANOVA of the Principal Components For Samples of *P. furfuracea*

Principal components	PC1	PC2	PC3
Contribution of the principal component in the total variance	0.4232	0.3729	0.2040
Cumulative contribution	0.4232	0.7961	1.0000
Difference between coenopopulations, p	0.0003	0.0001	0.0101
The effect of variability between coenopopulations (95% confidence interval)	0.2297 (0.1080–0.3675)	0.4432 (0.2502–0.6218)	0.1487 (0.0497–0.2862)

Figure 30.8 shows the positions of all subsamples on the plane of the first and the second principal components. As an example, for four samples we encircled the regions that cover the subsamples belonging to the same sample. It can easily be seen that both the size and the position of these regions are very distinct. This type of examination should become the objective of a special study.

The ordinal regression analysis reveals significant differences between ontogenetic spectra of different samples ($<10^{-4}$). The analysis yields similar results for both models; the effect of between-sample differences is 0.404 for probit model and 0.395 for the logit model.

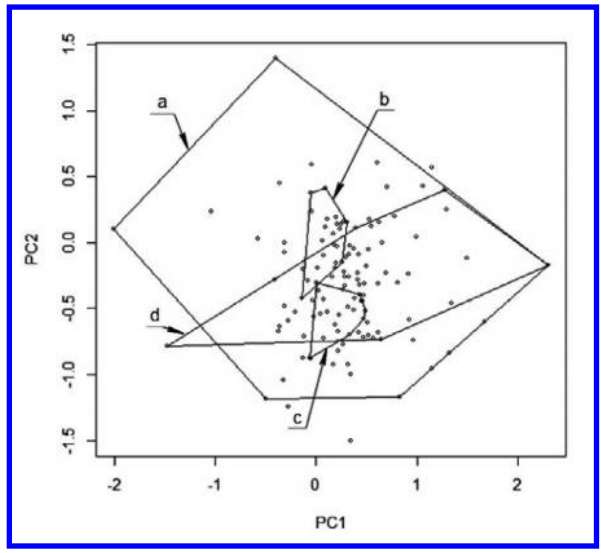

FIGURE 30.8 The positions of all subsamples (thalli of *P. furfuracea*, collected from an individual tree) on the plane of the first two principal components, PC1 and PC2. The regions covering the subsamples of the same sample are encircled: a – sample 4, b – sample 9, c – sample 8, d – sample 7.

30.4 CONCLUSIONS

The proposed methods allow correct comparisons of ontogenetic spectra of heterogeneous samples from populations of plants and epiphytic lichens and assess the effect of between-sample differences of ontogenetic spectra in the total variability. Note that principal components analysis ignores the order of ontogenetic states unlike ordinal regression and in full the comparison of Uranov's age coefficients whose calculation uses age coefficients of ontogenetic states.

It is important that the testing of these methods on data from nature populations of cowberry and epiphytic lichens raises new questions in population studies, for

example, Is small cowberry between-population variability connected with border location of populations under study? Does between-population variability correlate with the differences in ecological conditions of different habitats? Does within-population variability correlate with various conditions within a population?

ACKNOWLEDGMENT

This study was partially supported by the Russian Foundation for Basic Research (RFBR), research project No. 12–04–01251-a. The authors are grateful to M.V. Frolova for the translation of the paper into English.

KEYWORDS

- **compare of parameters of ontogenetic spectra**
- **heterogeneous samples**
- ***Hypogymnia physodes***
- **lichens**
- **ontogenetic spectrum of population**
- **ontogenetic state**
- **ordinal regression**
- **plants**
- **principal components analysis**
- ***Pseudevernia furfuracea***
- ***Vaccinium vitis-idaea***

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