RESEARCH ARTICLE

System for ecological regulation of the biosynthesis of flavonoids as a strange attractor

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Abstract

Flavonoids are a large class of plant polyphenols with various adaptation functions to environmental factors. The biosynthesis of flavonoids is characterized by a high plasticity of their synthesis and multiple species differences. The HPLC spectra of flavonoid chromatograms from the populations of Juniperus sabina, Glycirrhiza korshinskyi, Achillea millefolia of the South Trans-Urals and populations of Oxycoccus palustris, Chamaedaphne calyculata, Andromeda polifolia oligotrophic bogs in Western Siberia were studied. Each chromatograms of all species differed in the number of peaks, peak areas, and peak release times (compounds).

It was shown that the system of flavonoid biosynthesis is fractal in nature. The species groups of flavonoids have properties of strange attractors. Using the Principal Component Analysis (PCA) clear differences between species and groups of species in contrasting ecosystems is shown. Thus, species-specific populations of flavonoids and compounds with similar physicochemical properties are a distinct regional product. In this regard, along with the search and selection of individual plant species based on the content of one compound, it seems appropriate to search for effective regional complexes of flavonoids.

Keywords: flavonoids, biosynthesis of flavonoids, stochastic fractals, Oxycoccus palustris, Chamaedaphne calyculata, Andromeda polifolia, Juniperus sabina, Glycirrhiza korshinskyi, Achillea millefolia, bifurcations, strange attractors

Introduction

Ecological physiology of plants is a scientific field that studies the ways and methods of plant adaptation to changing environments. In recent years, the description of habitats (mainly soils) tends to use stochastic rather than deterministic ideas. The stochastic properties of plant habitats (nutrient content and biotic environment) are backed by a significant amount of evidence (Hubbell 2001, 2006; Tilman 2004; Rosenberg 1984, 2013; Gelashvili et al. 2013; Usmanov et al. 2014, 2016, 2017).

At the same time, plant organisms were until recently deemed to be opposite to stochastic systems and defined as deterministic systems whose parameters were to be observed in experimentation. Plant physiology literature of recent years uses no such concept as stochastic processes (Lambers et al. 2008). However, more and more papers are being published that dwell upon systems featuring multiple regulators, as the biosynthesis of specific metabolism elements can be affected by exogenous and endogenous regulators of both stimulating and inhibiting effects, whether uni- or omnidirectional (Kolchanov et al. 2013; Bundy et al. 2008). An unpredictable combination of control signals can guide alternative metabolic pathways, where the metabolic pathways branch point can be defined as the point of bifurcation. When multiple control signals are in effect, i.e. multiple bifurcations take place, it is often impossible to trace all the effects in total, which is why the system behaviour may seem random. This situation
is defined as dynamic chaos, a phenomenon dictated by
deterministic laws, but has a random-looking behavioural output.

These properties of the flavonoid biosynthesis system
can be compared to those of strange attractors. Strange
attractors have the following important features: These
properties of the flavonoid biosynthesis system can
be compared to those of strange attractors. Strange
attractors have the following important features:

1. There are boundaries of the phase space (area, volume, and multidimensional spaces), where points are moved

2. There are many volatile point trajectories. The volatility of trajectories depends on how sensitive their dynamics is to external regulators. Those can force points to further move along different trajectories. The “choice” of an alternative trajectory from several options is defined as bifurcation. If there are multiple points of bifurcation, the set of trajectories manifests itself as a stochastic fractal

The purpose hereof is to analyse the general
parameters of the flavonoid biosynthesis system and
to find where the dynamics of strange attractors and flavonoid synthesis is similar

Such an analysis of the similarities and differences of systems for constructing strange attractors and
the functioning of metabolic pathways of flavonoid biosynthesis has not been carried out before

3. There are attractors within the phase space (i.e.
that of attracting points or trajectories) that guide the movement of a set of points (trajectories) in adaptation,
ontogenesis, or sundry processes appropriately for the
system’s purpose (Rozenberg, 2013)

The authors hereof earlier showed (Usmanov et al.
2014, 2015, 2016, 2017, 2019, 2020; Ivanov et al. 2016, 2019) that the entire flavonoid biosynthesis system was of a
fractal nature.

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to find where the dynamics of strange attractors and flavonoid synthesis is similar. Such an analysis of the similarities and differences of systems for constructing strange attractors and the functioning of metabolic pathways of flavonoid biosynthesis has not been carried out before.

**Materials and Methods**

Samples were collected in ecologically contrasting communities of Western Siberia and the steppes of the South Trans-Urals. Each of the areas is ecologically homogeneous. The raised bogs of Western Siberia were represented by Oxycocco-sphagnetea. The team studied the dominant species of Oxycoccus palustris, Chamaedaphne calyculata, and Andromeda polifolia. The true steppes of the South Trans-Urals were represented by the community of Festuco-Brometea, where the dominant species are Juniperus sabina, Glycyrrhiza korshinskyi, and Achillea millefolium.

Ground-level biomass was sampled from these species in the middle of vegetation (late June). Spectra of flavonoids and physico-chemically similar compounds in Juniperus sabina conifer were determined by High-Performance Liquid Chromatography (HPLC). Alcohol extracts of the conifer samples were chromatographed in the reverse-phase mode on a Luna C18 250 × 4.6 mm, 5 μm column. Standards and substances in the samples were detected at a wavelength of 360 nm.

The similarity and dissimilarity of chromatograms were evaluated by means of Koch’s biotal dispersity index:

$$jd = \frac{T - S}{n - 1} \times S$$

where, T is the sum of substances in the lists (S1+S2+S3+...+Sn); S is the total number of substances; n is the number of lists.

The index values could vary from 0 (zero similarity) to 1 (totally identical) (Koch 1957).

Fractal analysis followed the algorithm below (Tab.1.),
based on the summary by Gelashvili et al. (2013).

<table>
<thead>
<tr>
<th>Stages of analysis</th>
<th>Procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling</td>
<td>Non-formalized selection of an assumed self-similar structure</td>
<td>The minimal self-similar structure was defined as a separate single chromatogram</td>
</tr>
<tr>
<td>Skaling</td>
<td>Estimation of the range of various scales of a set of self-similar structures</td>
<td>All chromatograms from an ecologically homogeneous area (stenosis) from single samples through samples of various sizes to a total population</td>
</tr>
<tr>
<td>Estimation of self-simulation</td>
<td>$Mq(N) = \sum_{i=1}^{n} p_i$</td>
<td>The entire chosen set of chromatograms and their aggregates possess the property of self-similarity, since all the correlations between the logarithms of Mq and N are significant (p&gt;0.05), and the values of the correlation coefficients tend to 1.</td>
</tr>
<tr>
<td>Estimation of stochasticity (Akaike Information Criterion (AIC))</td>
<td>$AIC = \ln \frac{RSS}{n} + \frac{n + k}{n - k - 2}$</td>
<td>In all cases, the nonlinear model is better applicable to the observed pattern than the linear one.</td>
</tr>
</tbody>
</table>
| Estimation fractal properties of chromatogram’s pool | Complete of chromatograms are all are stochastic organized self-similar structures, forming fractal stochastic systems | }
The final step was to analyse the entirety of chromatograms of all species by the main component method in addition to the sets of low-molecular metabolites (Smolikova et al. 2015).

**Results and Discussion**

**HPLC spectra of flavonoids and physico-chemically similar substances**

Chromatograms were compared in three parameters: (1) the peak time, an indicator of the presence of this or that substance; (2) the number of peaks during chromatography, an indicator of the total amount of substances in the extract; (3) the peak area, an indicator of the relative content of a substance in the extract.

The researchers registered a high diversity of extract chromatograms for each studied species. Fig. 1. shows some chromatograms of *Juniperus sabina*. Step 1 was to count the peaks in each chromatogram, see Tab. 2.

To compare chromatograms in terms of peak time coincidence, they were superimposed on each other; see Fig. 2 and 3. On a single chromatography time axis, peak times differed significantly. Thus, peak time coincidence was low. The Koch's variability index was used to compare the chromatogram series. For all the species under analysis, the Koch's coefficient was low and varied from 0.11 to 0.18, a sign of low chromatogram similarity. After that, the researchers counted the total peaks for all the plants of the species.

**Fractal chromatogram analysis**

Fractal analysis began with sampling, i.e. selecting a structure that for further analysis would be deemed self-

![Figure 1. Diversity of chromatograms as observed for Juniperus Sabina. Axe “x”-time along the X-axis-the time of the peak emergence at HPLC analis; Y-axis: peak characteristic-area, height as an indicator of the amount of a given compound. Numbers indicate the peak time. Each peak characterizes a separate substance.](image)

<table>
<thead>
<tr>
<th>Communities/species</th>
<th>Total peaks in all chromatograms</th>
<th>Variability of the peak count in single chromatograms</th>
<th>Koch’s coefficient for the chromatogram groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic swamps, Oxicocco-Sphagnetea communities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chamaedaphne calyculata</em></td>
<td>109</td>
<td>21-41</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Oxycoccus palustris</em></td>
<td>85</td>
<td>21-30</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Andromeda polifolia</em></td>
<td>91</td>
<td>19-43</td>
<td>0.12</td>
</tr>
<tr>
<td>True steppes, Festuco-Brometea communities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Juniperus sabina</em></td>
<td>79</td>
<td>21-45</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Glycyrrhiza korshinskyi</em></td>
<td>85</td>
<td>14-39</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>40</td>
<td>12-22</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 2. Total count of species peaks and variability of chromatograms across six species.
similar; the next step was to consider a hierarchy that would preserve self-similarity (scaling).

The elementary (self-similar) unit of hierarchy was the chromatogram of a standard plant material sample. Each chromatogram had three characteristics: (1) peak time in a standard solvent system; (2) peak areas in millivolts, an indicator in linear correlation to the substance concentration; (3) peak count in the chromatogram.

Scaling was done by the staged superimposition of chromatograms, see Tab. 3. A similar chromatograms superimposition procedure was performed for every species. All species had their peak counts increasing as the samples of merged chromatograms grew larger.

Thus, the following qualitative traits were found for the tested species: (1) all chromatograms were highly heterogeneous and had low similarity; (2) the total of substances in cenopopulations always exceeded that in any recorded chromatogram; (3) total peak count in a cenopopulation differed across species. Thus, the team confirmed the self-similarity of all chromatograms and their combinations obtained by superimposition. All individual chromatograms and their combinations had

Figure 2. Chromatograms of tested plants from the South Trans-Urals. Overlapping of several chromatograms of each species indicates differences between the individual chromatograms.
Figure 3. Chromatograms of the tested plants from oligotrophic bogs of Western Siberia. Overlapping of several chromatograms of each species indicates differences between the individual chromatograms.

Table 3. Peak count hierarchy with more chromatograms merged, evidence from *Chamaedaphne calyculata*.

<table>
<thead>
<tr>
<th>Levels of combining chromatograms</th>
<th>The number of peaks in the chromatograms with different combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of peaks in individual chromatograms</td>
<td>36 41 34 33 30 34 32 28 21</td>
</tr>
<tr>
<td>Combining chromatograms of three</td>
<td>54 49 43</td>
</tr>
<tr>
<td>Combining chromatograms in 2 groups</td>
<td>75 69</td>
</tr>
<tr>
<td>Total number of peaks for all plants</td>
<td>108</td>
</tr>
</tbody>
</table>
an independent distribution of chromatogram elements, as the count and qualitative composition of peaks were distributed independently.

Another common feature was the Akaike information criterion (Gelashvili et al. 2013) that consistently pointed to the complexity of a model that would describe the flavonoid biosynthesis tree.

**Principal Component Analysis (PCA)**

The next step was to evaluate all the chromatograms (Fig. 4) by the Principal Component Method (PCA).

As shown in Fig. 4, chromatograms of all six species formed compact groups. Calculations showed that chromatograms of all species had clearly-bordered, non-intersecting phase spaces. Moreover, chromatograms did join in two regional groups; let us designate them as the Siberian group and the Ural group. Another feature was that the 2D projections of mutual positions of species groups described only 35.53 (Factor 1+Factor 2) of the entire diversity of regulators that determined their differences. Nearly 2/3 of all factors that determined the diversity of flavonoids were not interpreted in this coordinate system.

Thus, all the tested species had stochastic fractal properties. In general, the flavonoid synthesis process could be imagined as choosing this or that substance from a set of possible compounds, see Tab. 3. Such results could be analysed from the standpoint of the neutrality theories. In the molecular neutrality theory model by Kimura (1983), neutrality is a random set of the existing genes, the choice of which has a weak correlation with the external conditions. The neutrality theory assumes the existence of a “general population” that can produce various combinations of “responses”. Similar reasoning, later proven by facts, was the basis of the plant community models. In that case, neutrality is the random introduction of this or that species from the total flora list of the region to the community. The applicability of the neutrality theory was not tested on the level of metabolic pathway analysis.

Flavonoids are a large group of secondary plant metabolites that have numerous adaptive functions. Flavonoid biosynthesis is a classic tree that originates from a single precursor (naringenin chalcone) and forms multiple branching metabolic chains. Transitions between substances in plants are defined by common patterns of radical attachment or replacement, see Tab. 4. In any case, any substance must have a continuous chain of successive biosynthesis from the original naringenin chalcone molecule to the substance. Besides, the flavonoid biosynthesis system features numerous alternative pathways (shunts) that can link adjacent chains. The total number of such shunts is unknown as of today (Korulkin 2007; Tyukavkina 2008; Mierziak et al. 2013; Harborne 2013).

Each new substance of a metabolic tree is synthesized if there is an elementary conductive metabolic cell: substrate>enzyme (gene)>product. Only a mesh of such elementary cells can synthesize any substance. Metabolic chains are fundamentally continuous, as the end substance can only emerge where all the preceding links
have continuity. However, such a conductive metabolic cell is itself subject to numerous regulatory effects on the genetic, substrate, physiological, and ecological levels (Kolchanov et al. 2013; Khlestkina et al. 2014; Shuyskaya et al. 2014; Shcherbakov et al. 2011, 2012).

Conclusion

The flavonoid biosynthesis system forms a network. It can be assumed there can be multiple point-to-point routes, including alternative pathways (shunts). It can also be assumed that such alternative pathways have not yet been described in literature. There is substantial evidence that each specific biosynthesis of flavonoids has a pronounced activation and inhibition system. It is the presence of branched networks and alternative biosynthesis pathways that enables bypassing this or that unfavorable combination of activators and inhibitors for a metabolic cell via other metabolic cells, for which that combination is not critical.

Further research will seek answers to two questions of the properties of the strange flavonoid biosynthesis attractor. Phase space for a set of flavonoids has been clearly identified for all six species. The question is the meaning of this phase space in ecological and physiological terms. One may assume it is somehow related to the multidimensional space of the species’ ecological niche. The other question is whether the movement of the phase attractor centers has an adaptive value or is a product of the chaotic fluctuations in the environmental conditions.

Acknowledgments

The chromatographic analysis was carried out on the equipment of the common use center “Chemistry” of UIC of the RAS.

The work was supported by the grant from the Russian Foundation for Basic Research (RFBR).

Table 4. Strange attractors and flavonoid biosynthesis: traits of qualitative similarity in synthesis mechanisms.

<table>
<thead>
<tr>
<th>Strange attractors</th>
<th>Biosynthesis of flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase space (boundaries), within which an attractor functions</td>
<td></td>
</tr>
<tr>
<td>Point movement within the attractor is described by differential equations, hence the deterministic calculation algorithms</td>
<td></td>
</tr>
<tr>
<td>Bifurcation points emerge in equation systems, and their trajectories can diverge. In the case of an increase in bifurcation points, the attractor acquires stochastic traits while in theory, the process remains deterministic</td>
<td></td>
</tr>
<tr>
<td>The dynamics is sensitive to weak changes in the initial system conditions, hence low autocorrelations</td>
<td></td>
</tr>
<tr>
<td>There may be a variable number of violations that cause bifurcations</td>
<td></td>
</tr>
<tr>
<td>Strange attractors are fractal in nature</td>
<td></td>
</tr>
<tr>
<td>Uncertainties at points of bifurcation mean transitions can be assessed as a stochastic neutral process</td>
<td></td>
</tr>
<tr>
<td>It is difficult to project the trajectory in the attractor as even slight inaccuracy of the initial data may later result in a significant prediction vs-actual-trajectory difference.</td>
<td></td>
</tr>
<tr>
<td>The flavonoid biosynthesis system has many features of a strange attractor</td>
<td></td>
</tr>
</tbody>
</table>

References


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