• Week 5. Plant defense theory 3: Current Status:
  
  – Lecture summary:

  • **Carbon:Nutrient Balance Hypothesis:**
    – Bryant, Chapin & Klein, 1983.

  • **Growth: Differentiation Balance Hypothesis:**

  • **Screening Hypothesis:**
    – Berenbaum and Zangerl, 1996.
2. Carbon:Nutrient Balance Hypothesis:


- Many similarities to ideas of Janzen & McKey:
  - Not recognized in paper.
  - Response of plants constrained by resources (like Resource Availability Hypothesis) *(Figs. 1 & 5).*
  - Fundamental difference between woody trees and grasses.
  - Differences according to successional stage *(Table 1).*
3. Summary of Carbon:Nutrient Balance Hypothesis:

- Late successional, slow-growing, long-lived trees in low-resource environments have:
  - Carbon-based defenses at all life history stages rather than nitrogen-based defenses.
  - Light and carbon are not limiting.

- Early successional, fast-growing, shade-intolerant trees in high-resource environments have:
  - C & N resources & rapid growth rates necessary to compensate for herbivory.
  - High concentrations of chemical defenses in juveniles to protect plant when most vulnerable.
4. Effects of nutrient availability:

- Nitrogen-based defenses predominate in nutrient-rich environments.
- Carbon-based defenses predominate in nutrient-poor environments.
  - Grasses have large reserves of carbon & nutrients below ground in low- and high-resource environments and can compensate for losses from herbivory.
  - So dynamics of browsing and grazing systems are qualitatively different.
5. Growth: Differentiation Balance Hypothesis:

  - Took the physiological approach of Loomis (1932, 1954) to develop the growth-differentiation balance hypothesis:
    - Plants balance cellular growth and cellular differentiation.
    - Used this and resource availability to compare the carbon/nutrient balance hypothesis with the growth/differentiation balance hypothesis.
6. **Herms & Mattson model:**

- Trade-off between allocation to either primary or secondary metabolism and effects on competitive ability (C) and defense against herbivory (H) (Fig. 3).
7. The Screening Hypothesis:

  - Presented at a meeting of the *Royal Society* in London on 27 & 28 February 1991
    - Argued that plants counter the inherently low probability of biological activity in secondary compounds with the evolved, biochemical ability to maximize diversity of secondary metabolites.
  - Also:
8. Summary of Jones & Firn:

• “A common-sense evolutionary scenario predicts that well-defended plants should have a moderate diversity of secondary compounds with high biological activity.

• We contend that plants actually contain a very high diversity of mostly inactive secondary compounds.

• These patterns result because compounds arising via mutation have an inherently low probability of possessing any biological activity.”
9. Summary continued:

• “Only those plants that make a lot of compounds will be well defended because only high diversity confers a reasonable probability of producing active compounds. Inactive compounds are retained, not eliminated, because they increase the probability of producing new active compounds.

• Plants should therefore have predictable metabolic traits maximizing secondary chemical diversity while minimizing cost.

• Our hypothesis has important implications to the study of the evolution of plant defence.”
10. In their paper, Jones & Firn ask:

“If natural selection favours plants that are well defended but minimize cost, well-defended plants should contain a moderate diversity of highly active compounds, and few if any inactive compounds other than essential precursors. Is this what we find?”

They say no, and address this question with 3 additional questions:

– (1) Are most secondary compounds highly active?
– (2) Why are so many compounds inactive and why are they retained?
– (3) Why are there so many secondary compounds?
11. Their 3 questions:

• (1) Are most secondary compounds highly active?
  – Some are, but the number of active compounds is a small percentage of all the compounds that have been isolated

• (2) Why are so many compounds inactive and why are they retained?
  – No evidence, except to cite Coley et al. (1985) etc. - really a part of (1)

• (3) Why are there so many secondary compounds?
  – Carrot and bracken fern have 12 biosynthetically distinct pathways (not defined or explained) and bracken has at least 27 different structures of sesquiterpene indan-1-ones (same class of closely related compounds).
  – “Nevertheless, it appears that plants contain many more compounds than indicated by the common-sense scenario.”
12. The postulate of Jones and Firn:

- “we postulate that the inherent probability [of biological activity] is low.”

- Postulate justification:
  - Then they state:
    - “Although it is interesting to speculate why low activity is a fundamental toxicological property, it is not necessary to know the reasons in order to examine the consequences of plant defence.”
  - They base this statement on the low success rate of toxicological programs that screen for biological activity (as in (1) above):
    - But what does it mean for an anticancer screening program to find that only 4.3% of plant species have any activity?
13. Is their postulate justified?:

- Why would a plant evolve chemicals that kill or slow the growth of vertebrate cells?
- Why produce anticancer drugs that could benefit a vertebrate herbivore suffering from cancer?
- Surely it is purely serendipitous that any plant at all has anticancer activity?
- How is this relevant to the evolution of chemical defense in plants?
14. More questions:

– Similarly, what does it mean for 3% of plants to have activity against insects?

– What receptors were tested - probably none.
  • These are the results of screening for insecticidal activity and need to be biologically relevant before results are interpreted.

– This is an excellent example of purely inductive science and I think has little merit as an explanation for biochemical diversity in plants.

– There is also nothing that is testable in this hypothesis.
15. Resolution?:

• Given the weak basis of their contention that most secondary metabolites are biologically inactive, how justified is the argument of Jones and Firn that plants counteract this low probability by the production of an extremely high diversity of unrelated secondary metabolites?
16. Jones & Firn contend:

- “Because the probability of any compound having activity at any time is low in both cases [high vs low diversity of compounds], it is clear that plants with a high absolute diversity have a greater probability of producing one or more active compounds at any time than plants with a low diversity.

- Plants with a high diversity of compounds will 'screen' a greater number of compounds at any time and a greater variety of compounds over time than plants with low diversity.”
17. Jones & Firn continue...:

• “We argue that this is the only effective 'strategy' if the 'screening success' is inherently low.”

• It is also a 'strategy' that produces plants containing a high diversity of mostly inactive compounds and a few active compounds.

• Maintenance of high diversity therefore increases the probability of production of new active compounds in the face of consumer adaptation.”
18. Inductive reasoning:

• This is nonsense! This is one of the most blatant examples I know of an argument becoming “truth” and then continuing with that “truth” as accepted dogma.

• This is why this paper is inductive and untestable.
19. Traits that Jones and Firn argue support their postulate:

- Metabolic traits that maximize diversity and minimize cost:
  - (a) Branched pathways (exponential increase):
    - Should be more common than linear pathways (additive) - they are, but what does it mean?
    - We don't know enough about the genetics to say how steps are coded and linked and inherited - so I think their argument is premature.
20. Traits that Jones and Firn argue support their postulate:

Then it seems to me that they demolish their case by saying:

- “There are two further consequences of branched pathways.
- First, as the diversity of a pathway increases, a greater proportion of the total diversity is found in any one pathway. Thus, the diversity of compounds within a class is always likely to exceed that between classes, as is observed.
- Second, a new mutation is far more likely to add compounds to an existing pathway than start a new pathway from a primary metabolite, simply because the number of biosynthetic steps located in existing pathways is much greater.
- Consequently, there will be a high degree of canalization in pathway diversification, leading to the high degree of gross chemotaxonomic affinity that we observe in plants, despite the fact that there are clear differences in specific compound structure between related plants.”
21. Traits that Jones and Firn argue support their postulate:

- Metabolic traits that maximize diversity and minimize cost (continued):
  - (b) Matrices and grids:
    - A few enzymes convert a diversity of structurally similar precursors (the opposite of the contention) to many end products.
  - (c) Combining pathways:
    - Compounds of mixed biosynthetic origin are common (evidence??).
  - (d) Pathway regulation:
    - Control of secondary metabolite doses - i.e. "less is cheaper" shifts in concentration dominance in related metabolites according to need (could include induction).
22. Traits that Jones and Firn argue support their postulate:

- Metabolic traits that maximize diversity and minimize cost (continued):
  - (e) Hidden pathways:
    - Non-expressed pathways could be reactivated - no evidence.
  - (f) Reaction mechanisms:
    - Enzymes could show low substrate specificity:
      - 'sloppy enzymes' to make slightly different products from structurally similar substrates:
        » e.g. hydroxylation and methylation.
      - Also expect many isozymes - and polymeric assembly (repeated units being joined like terpene biosynthesis).
23. **Conclusions of Jones and Firn with regard to the evolution of plant defense:**

- (1) Relation between chemical composition of a plant and its degree of defense will be weak because of the high diversity of mostly inactive compounds - thus assumptions about causality are unreasonable.

- (2) Invalid conclusions from high correlation between consumer response and metabolite presence because of high diversity and chance statistics.

- (3) Cannot be assumed that an inactive compound serves no purpose (argues against their position!).

- (4) Cannot extrapolate from artificial experiments to natural selection.

- (5) Metabolic traits that confer diversity may have been selected very early in plant evolution - so modern patterns are not critical.
24. **Molecular parsimony?:**

- At the meeting in London, Steve Malcolm criticized the paper and these comments are published after the *Roy. Soc.* paper with the response by Clive Jones.

- Steve Malcolm argued that:
  - “it may be possible that low probability [of biological activity] will select for plants to exploit single metabolic pathways that have a high probability of producing biologically active chemicals. That taxonomically similar groups of plants rely to a large extent on single kinds of chemical defences argues for such 'molecular parsimony' and evolutionarily 'expensive' diversity to counteract low probabilities. Diversity of chemical defences can be covered by a single molecular type.”
  - The example given was for cardenolides - but also argued for alkaloids in Solanaceae, coumarins in Apiaceae, glucosinolates in crucifers.
  - Clive Jones retorted with the comment that we don't know enough to say this. I think we know even less to suggest what he has argued! But he is right that we do tend to focus on particular groups of chemicals!
25. Epilogue:


• Strongly criticized the idea of Jones & Firn on the basis of:
  – Increase in knowledge.
  – Does common sense prevail?
  – Consistency with the evidence of Jones & Firn.
26. Berenbaum & Zangerl’s criticism:

- 1. Increase in knowledge:
  - “Thus, the furanocoumarin diversity of *Pastinaca sativa* has been steadily increasing since 1948 (Fig. 1), assuredly not due to increasing selection pressures by consumers or fantastically elevated mutation rates but rather to increasing scientific interest in the phytochemicals and increasing sophistication and accuracy of chemical analytical methods.” (page 6).
27. Berenbaum & Zangerl’s criticism:

• 2. Does common sense prevail?:
  
  “Jones and Firn predicted ..... that most natural products have little or no activity against any particular organisms; that highly active compounds (compounds with broad efficacy) will be rare; and that high activity requires high receptor-site specificity. At least with respect to the distribution and abundance of furanocoumarins, most of these predictions do not appear to hold. Wild parsnip seems to be a “common-sense” plant - all of its furanocoumarins appear to be toxic, one way or another, against both adapted and non-adapted species.”

(page 16)
28. Berenbaum & Zangerl’s criticism:

3. Consistency with Jones and Firn?:

- “While empirical work done over the past two decades with wild parsnip does not disprove the theory of Jones and Firn, it is certainly inconsistent with it in almost every conceivable way. At least in terms of the interaction between the plant and its principal herbivore, the parsnip webworm, *Depressaria pastinacella*, an oligophage restricted to feeding on the reproductive structures of the plant:
  
  1) plant chemotype is consistently and predictably related to webworm densities;
  
  2) correlations between webworm abundance and concentrations of particular furanocoumarins associated with resistance are confirmed in laboratory bioassays;”
29. Berenbaum & Zangerl’s criticism:

3. Consistency with Jones and Firn? (cont’d):

- “3) compounds that, in bioassay, have little or no effect on webworm growth and development are metabolized more efficiently than those associated with field resistance and larval growth in the laboratory; and

- 4) in field populations of wild parsnips, furanocoumarin content of foliage and fruits is under genetic control, and webworm damage patterns are consistent with selection for increased concentrations of resistance-related furanocoumarins.” (pages 16 & 17)

- “....it seems premature to pronounce phytochemical diversity as redundancy, or superfluity. In fact, the remarkable conservatism of structures within a biosynthetic class among the plant families producing these compounds prompts one to ask, “Why aren’t there more natural products?” (page 19)
Figure 1: Plant characteristics according to successional status and the availability of nutrients (Bryant et al., 1983).

Fig. 1. Interacting characteristics of plant strategies that are adaptive under conditions of high and low resource availability and the changes in these strategies that occur as consequences of fire and succession. Modified from Chapin and Van Cleve (1981).
Figure 5: Effects of nutrient availability and light on plant allocation to C-based and N-based chemical defenses (Bryant et al., 1983).

*Fig. 5. Effect of suboptimal nutrient or light availability upon carbon-nutrient relationships of plants and upon concentrations of nitrogen- and carbon-based defenses. Vertical arrows within boxes indicate increase (↑) or decrease (↓) in the parameter. Arrows between boxes indicate positive effect unless otherwise indicated (→). Thickness of arrow indicates magnitude of effect. See text for explanation.*
Table 1: (Bryant et al., 1983).

<table>
<thead>
<tr>
<th></th>
<th>Evergreen Ledum palustre</th>
<th>Deciduous Salix pulchra</th>
<th>Betula nana</th>
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<tbody>
<tr>
<td>Leaf relative growth rate (mg g⁻¹ d⁻¹)</td>
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<td>29</td>
<td>77</td>
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<tr>
<td>Photosynthetic capacity (mg CO₂ h⁻¹ g⁻¹)</td>
<td>14 ± 1</td>
<td>40 ± 3</td>
<td>37 ± 4</td>
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<td>Maximum leaf nitrogen (% dry wt)</td>
<td>3.0 (n=1)</td>
<td>4.3 ± 0.1</td>
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<td>Phosphate absorption rate (µmol h⁻¹ g⁻¹)</td>
<td>0.8 ± 0.1</td>
<td>2.6 ± 0.1</td>
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<td>Below ground biomass (% of total)</td>
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<td>70 ± 4</td>
<td>80 ± 1</td>
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<td>Spring decrease in % TNC in belowground stems (Δ% TNC)</td>
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<tr>
<td>Spring decrease in %P in below ground stems (Δ% P)</td>
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