Biology 304a Lab VII
Comparative Phytochemistry of Flavonoids

Introduction

Plants must produce a wide array of chemicals and substances to use for growth, maintenance and reproduction. The majority of these compounds are conserved between species and are widely found, such as chlorophyll or cellulose. However, some plants produce chemicals not essential for survival, known as secondary chemicals or metabolites, which are of great importance. Secondary chemicals can act as deterrents to herbivores or competing plants, or may serve to attract pollinators such as birds or insects (Barbour et al. 1999). They can be produced in the leaves, shoots and roots of plants, and may be dispersed through the air (volatiles) or into the soil water via root systems. 'Semiochemical' is another term for a chemical compound used by plants to send signals to their environment (Margulis et al. 2000).

Semiochemicals are grouped according to their structure. There are several main groups, including terpenoids, phenol compounds like flavonoids and nitrogen-based compounds such as alkaloids (Barbour et al. 1999). Each group has its own characteristics, but for this experiment the phenol-based flavonoids will be explored in depth. Flavonoids are usually composed of three benzene-like 6-membered carbon rings with oxygen, hydroxide and methyl groups attached (Margulis et al. 2000). Different flavonoids may be synthesized from a general precursor by altering the location and type of atoms attached to the rings.

Flavonoids are produced by scores of species, from alfalfa (Coronado et al. 1995), to grains like rye and corn (Jaehne et al. 1993) to root plants like potatoes (Lewis et al. 1998). In this experiment, the flavonoid composition of seven tree species (*Carya ovata, C. cordiformis, Quercus rubra, Q. alba, Q. macrocarpa; Juglans nigra; Fagus grandifolia*) leaf extracts will be analyzed with chromatography. Since flavonoids are highly diverse, it is possible to infer an evolutionary relationship between species with similar chromatograms. Therefore, the aim of this experiment is to determine the flavonoid constituents of each species and to determine evolutionary relationships between these species.

Materials and Methods

Procedures as per Biology 304a Laboratory Manual (Fahselt, 2002) pages 79-80. The experimental material was a prepared leaf extract of *Carya ovata*. Collective lab results were used for interspecies comparisons. S_m values were calculated with the following formula from Fahselt (2002):

$$S_m \text{ (simple matching coefficient)} = \frac{m}{(m + u)}$$

where m = matched characters (compounds found in both species)
u = unmatched characters (compounds found in only one species)

Results

Table 1. Compared S_m (simple matching coefficient) values derived from chromatograms of leaf extracts of experimental tree species. Shaded areas indicate duplicate comparisons. Asterisks (*) are used to indicate values that are higher or lower than expected for the compared species.

	J. nigra	*C. ovata*	*C. cordiformis*	*Q. rubra*	*Q. macrocarpa*	*Q. alba*	*F. grandifolia*
J. nigra	-	0.3	0.6	0.2	0	0.4 *	0.2
C. ovata		-	0.5	0.2	0	0.1	0.2
C. cordiformis			-	0.3 *	0.1	0.1	0.07
Q. rubra				-	0.7	0.5	0.3
Q. macrocarpa					-	0.2 *	0.3
Q. alba						-	0.4
F. grandifolia							-

S_m values calculated from comparing the chromatograms of various species indicated that S_m values were higher between species in the same genus. For example, the three *Quercus* species had S_m values ranging from 0.2 for *Q. alba* and *Q. macrocarpa* to 0.7 for *Q. rubra* and *Q. macrocarpa* (Table 1). Several results did not follow this pattern, however. The comparison between *Q. rubra* and *C. cordiformis* yielded a S_m value of 0.3, which is slightly higher than expected for species in two different families. A similar result (0.4) was obtained in comparing *Q. alba* and *J. nigra* (Table 1) also in different families (Figure 2).

The chromatogram for *Carya ovata* (Family Juglandaceae) showed six distinct areas of colouration. Based on their locations on the chromatogram, these compounds were likely all flavones and/or flavonols (Mabry et al. 1970). Colouring of the spots supported this inference, as most areas appeared purple or deep pink which is

indicative of flavones or flavonols. One yellow area may represent the presence of dihydroflavonols (Mabry et al. 1970).

Figure 1. Phylogenetic tree constructed using S_m values obtained from chromatograms. Species are arranged according to the degree of similarity of their constituent flavonoid compounds.

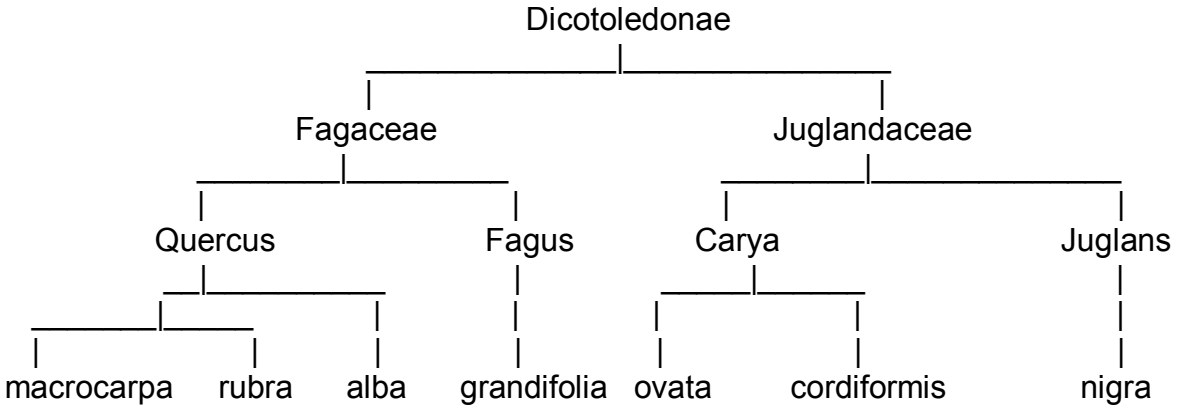
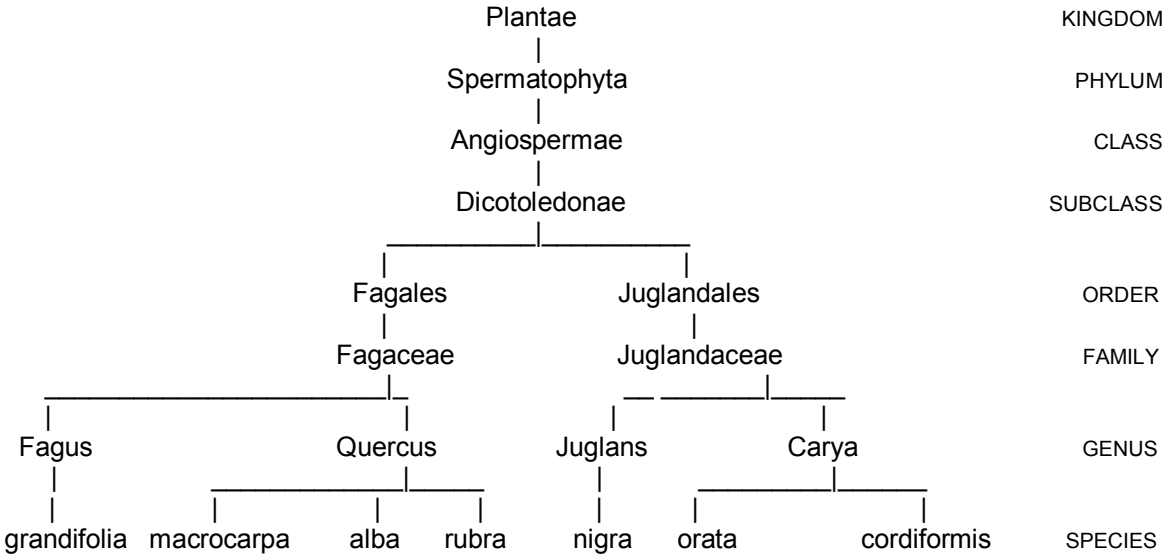


Figure 2. Phylogenetic tree adapted from Gleason (1974).



Discussion

Analysis of the experimental chromatogram results indicates that species within the same order or family tend to contain similar flavonoid compounds. This general trend is supported by the comparisons between species within the same genus which

all yielded high (≥ 0.5) S_m values, with one exception. *Q. alba* and *Q. macrocarpa* had a S_m value of only 0.2, which is much lower than the comparisons between other *Quercus* species (Table 1). Reasons for this anomaly are unknown but may include inaccurate recording of the chromatography results or different interpretation of the results between groups. Comparison of *C. ovata* with *C. cordiformis* yielded a high S_m value, showing a significant number of flavonoids were commonly produced (Table 1). Therefore, for the majority of comparisons, the phylogenetic tree assembled using the chromatography results is almost identical to those derived from other sources of comparison, ie. genetics or morphology (Fig. 1, 2). In general, the closeness between two species on the phylogenetic tree correlates with their comparative S_m value, with greater distances apart resulting in lower values. The most closely-related species were Q. macrocarpa and Q. rubra with a S_m value of 0.7.

Every chemical produced by a living organism has some purpose. Flavonoids are capable of fulfilling many functions and are widely expressed in plant extracts. Therefore, flavonoids are an important factor in plants' secondary metabolism and/or self-defense chemical repertoires. Since plants cannot actively defend themselves by moving away from stressors, they rely on compounds such a flavonoids to deter, discourage and prevent herbivory as much as possible. Flavonoids and flavones are used by crop plants particularly for insect pest resistance and as herbivore deterrents (Guevara et al. 2000). Therefore, the presence of these same compounds in the experimental species suggests that they would serve a similar and equally vital role in trees.

List of Works Cited

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