Effects of Climate on *n*-Alkane Chain Length Distribution in Terrestrial Plants

Plant cuticular wax provides a protective coating on leaves and stems that has important physiological and ecological functions in plants' interactions with their environment (Eglinton et al. 1963, Jetter et al. 2006). The wax helps control water flux in and out of the plant, an essential function for plant survival, especially crucial in extremely wet or dry conditions (Eglinton et al. 1963). Furthermore, cuticular wax minimizes mechanical abrasion damage on leaf cells and some chemicals present in the waxy layer have been found to inhibit bacterial, fungal, and insect invasions (Eglinton et al. 1963, Eglinton et al. 1967). These waxes contain hydrocarbons such as *n*-alkanes, *n*-alkanols, *n*-aldehydes, and fatty acids. There are two hypotheses describing the crystal structure of wax molecules inside the cuticle and on its surface, though *n*-alkanes are an important component of the matrix either way (Dodd et al. 2003, Jetter et al. 2006).

Long chain *n*-alkanes, unlike many other hydrocarbons present in plants, remain relatively stable over geological timescales and are therefore a major component of plants' contribution to sedimentary records and the subject of many paleobiological studies (Eglinton et al. 1967, Smith et al. 2006). However, plants are also capable of adapting to changing environmental conditions through producing different relative amounts of these compounds, which have been recognized as biomarkers of climate change (Jetter et al. 2006). Therefore, *n*-alkanes have been used to model ancient climates and plant community composition changes (Smith et al. 2007). *N*-alkanes are also valuable in studying modern plants – hydrogen isotope ratios of leaf wax *n*-alkanes found in lake sediments have been shown to have a strong relationship with meteoric water values (Smith et al. 2006). Although studies such as these have created discussion of how characteristic of *n*-alkanes can be correlated to the environment and climate, determining the details of the relationship has only just begun (Smith et al. 2006). Further understanding would contribute to the reconstruction of ancient climate and ecological communities and the modeling of potential plant responses to future climate changes.

In modern plants, *n*-alkanes occur in a homologous series ranging from 21 to 34 carbons (Eglinton et al. 1963). This project will focus on the *n*-alkane chain length distribution (the relative abundance of each chain length), a characteristic that greatly affects leaf wax composition. Molecules with longer chain lengths are less soluble and are therefore expected to limit water flow across the cuticle, which conserves water within the plant. This is an essential process because the ability to maintain water balance is critical to the plant's survival. Since these crystal structures are composed of various long chain hydrocarbons, important ecological functions associated with the structures are also closely tied to chain length distribution (Jetter et al. 2006).

Some past studies have suggested a possible relationship between *n*-alkane chain length and climate factors, but have not come to any definite conclusions. For example, Dodd et al. (2003) found mean alkane chain length (N) to be related to altitude, where N is the greatest at extreme elevations (low and high) of the Pyrenees. A possible explanation is plants adapting to summer drought at low elevations and winter physiological drought at high elevations, where ground water freezes and becomes inaccessible to the plant, creating the same effects as a drought even though ground water is technically present (Rosemary Bush, personal communications). Despite this correlation, no significant relationship was found between climate data (e.g. precipitation and temperature) and N (Dodd et al. 2003). Another study focused on average chain length (ACL, same measurement as N from Dodd et al. 2003) changes along different latitudes, where they found an increase of ACL with increasing latitude

(Rommerskirchen et al. 2003). They hypothesized that this is due to raised temperatures and/or aridity at higher latitudes but did not explore this idea in another experiment (Rommerskirchen et al. 2003).

Currently, there are very few studies exploring the relationship between *n*-alkane chain length distributions and climactic variable, and the few existing experiments have inconclusive results and are based on a limited number of species. Understanding correlations between climate factors, including altitude, mean annual temperature (MAT), and mean annual precipitation (MAP), and *n*-alkane chain length distribution would advance our knowledge of plant responses to climate changes and improve our ability to reconstruct past climates.

My project to identify the possible effects of climate on *n*-alkane chain length distribution will have two components: analyzing modern leaf samples for chain length distribution as well as collecting data from primary literature. I will analyze leaf samples of sugar maple (*Acer saccharum*) and sweetgum (*Liquidambar styraciflua*) from two different transects (N-S and E-W) across the middle of the United States to examine the potential relationship between the *n*-alkane chain length distribution and climate factors within each of the species. I will use Microwave Accelerated Solvent Extraction to extract the lipid from each of the leaf samples. The total lipid extract will then be separated into polarity classes using silica gel chromatography. The hydrocarbon fraction will be eluted with hexane while the polar components of the sample will be eluted with dichloromethane and methanol. Specific *n*-alkane distribution will be identified through mass spectra, molecular ion mass, retention time, and comparison with authentic standards.

The second part of the project will be augmenting and analyzing an existing database of published literature. This vast database was compiled by graduate student Rosemary Bush and consists of 91 papers that have measured the *n*-alkane chain length distribution in a variety of gymnosperms and angiosperms from around the world. However, detailed geographic and climatic information is not yet a part of the database. Therefore, for each paper, I am recording the species name, location from which the sample was taken (i.e. country, continent, latitude, and longitude), and the degree of confidence in the location assignment. Then I will add basic climatic information (i.e. MAT and MAP) according to geographic coordinates from existing on-line databases (e.g. from the National Oceanic and Atmospheric Administration). The 2000 samples representing all five major continents and more than 15 countries and collectively contribute a huge amount of data from varying climates, giving breadth to the project.

I have worked in Dr. Francesca McInerney's lab since September 2011 and have been trained in the lipid extraction and analysis process necessary for determining *n*-alkane composition in leaf samples. Furthermore, I have taken the Chem 210, Bio 210 series, Health of the Biosphere, Conservation Biology, and Field Ecology. Therefore, I have both the technical skills to carry out the project as well as the knowledge to understand and interpret the results. In addition to receiving 2 units of 399 credits for this project, I will develop this research into a senior thesis and submit it to the biology department for honors consideration. This research will also further my own interests in pursuing a graduate degree in ecology, conservation biology, or paleobiology after graduation.

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