

Ecotypic Variation within Red Maple (*Acer rubrum*) Leaf Morphology

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ABSTRACT

Red maple (*Acer rubrum*) establishes in diverse habitats across the eastern United States and prior research suggests that ecotypic variation may occur in the shape of red maple leaves. Our first objective was to determine if shape differences were present in red maple leaves from different geographical locations and if any differences are correlated with environmental conditions found across a cline. Our second objective was to assess if red maples exhibit ecotypic differences in the leaf shape within regions. We applied geometric morphometric analyses on red maple leaves collected from locations in Pennsylvania, coastal Virginia, and Michigan to test for geographical differences. We compared leaves collected from trees on valley bottoms and upland habitat within Pennsylvania to test for ecotypic differences. We found both leaf shape differences among locations (Pennsylvania, Michigan, and Virginia) and between habitats (upland, lowland). Lobes and sinuses were most defined in leaves from Michigan and least defined from Virginia. Leaves from upland areas were broader compared to lowland areas. Since the size and shape of the leaves is influenced by ecological conditions including sunlight, water, and nutrient availability, we conclude red maples are adapted to particular habitat characteristics.

Keywords: intraspecific variation, leaf shape, relative warps analysis

Species are able exist to over large geographical ranges because local environmental conditions influence trait selection creating populations that are specifically adapted for their habitats. Hence, intraspecific variation occurs among populations of the same species in different ecosystems. When a population's set of traits is consistently correlated to particular ecological characteristics, this adaptation can be identified as an ecotype (Turrill 1946). The term ecotype was first coined in 1922 by Göte Turreson, a Swedish botanist, to clarify the genetic response of a population to habitat conditions, resulting in different trait expression among populations (Turrill 1946). Early research on plant ecotypes suggested that morphology varied between coastal and inland populations of a species in terms of leaf morphology, stem color, growth habit, flowering time, and seed size (Lowry 2012). Ecotypic variation has also been observed in

other species such as in birds, fish, and amphibians (Smith and Skulason 1996).

Plant leaves function for photosynthesis and transpiration, and the size and shape of the leaves are affected by ecological conditions including sunlight and nutrient availability, therefore, intraspecific genetic variation may strongly influence plant leaf morphology (Mauseth 2003). Leaves are composed of primary tissue, and deciduous trees shed leaves seasonally rather than growing secondary tissue on the leaves, which would add bulk and weight with annual growth of the tree. Large leaves can absorb more sunlight for primary production, but at the cost of greater water loss by transpiration (Mauseth 2003). Therefore, it may be advantageous for trees in colder regions to have larger leaf surface area for greater levels of photosynthesis during a shorter growing period. In contrast, trees in warmer climates with longer growing periods may not need to produce large leaves as sunlight is available

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for photosynthesis over a longer period of time (Royer et al. 2008). Consequently, when a plant species exists over a broad geographical range, we can expect to see ecotypic variation in leaf morphology related to broad climatic conditions.

Red maple (*Acer rubrum*) is considered a “super generalist” species due to its large range and the diversity of habitats in which it lives (Abrams 1998). Red maples are medium to large sized trees, simple-leaved, palmately lobed, and deciduous. Belonging to the family *Aceraceae*, their native range is eastern North America from Nova Scotia to Manitoba and south to Florida and eastern Texas. Within this large range, red maples establish populations in a variety of habitats, including wet, swampy lowlands and cold, dry mountain ridges (Grimm 2002). The prevalence of red maple within its range increased during the 1900s and this increase has been attributed to the fact that red maples can establish as both an early and late successional species (Abrams 1998). Some studies have identified leaf size and shape differences is suggestive of ecotypic variation. Leaf shape has been correlated with mean annual temperature, where leaves in colder climates show more definition of lobes and sinuses (Royer et al. 2008). A difference in leaf mass has also been observed for upland and lowland environments, such that mass was lower in drier conditions (Abrams and Kubiske 1990).

Since red maples are known to establish across a large temperate range, and because environmental conditions can influence variation in intraspecific leaf morphology, we have reason to believe leaf shape differences will be found across the range. Our first objective was to determine if shape differences were present in red maple leaves from different geographical locations, and if any differences were correlated with broad variation in environmental conditions. Our second objective was to assess if red maples exhibit ecotypic differences in leaf shape between upland and lowland habitats within the same region.

METHODS

We collected leaves during June and July 2013 from Michigan and Pennsylvania and during September 2013 from Virginia. Fifteen

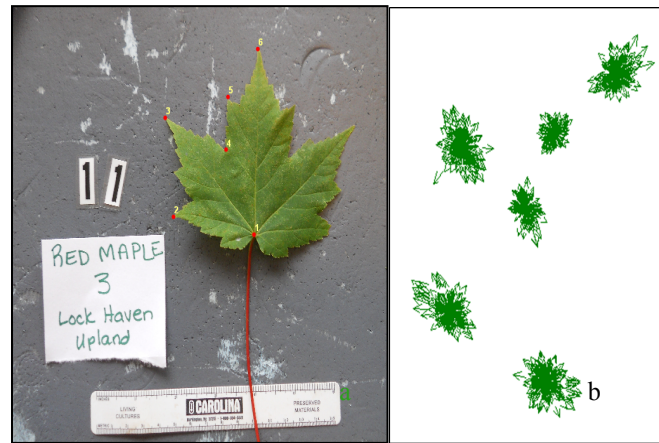


Figure 1: (a) Example photograph of a red maple leaf including scale ruler, tree number, leaf number, location, and habitat. (b) Variation in individual landmarks relative to the consensus configuration of red maple samples.

leaves were collected from each of 17 different trees for 255 total leaves. For each of the trees sampled, the leaves were removed from the outer lower branches and individually photographed against a neutral gray background. Each photograph included a unique identification label and a ruler for scale. In Pennsylvania, we sampled eight trees in the Lehigh Valley area of Northampton County and seven trees in the Lock Haven area of Clinton County. One tree was sampled from Kalamazoo County in Michigan, and one tree was sampled from Accomack County in Virginia. The habitat where each tree was sampled was broadly defined as lowland or upland. Lowland habitat indicates areas of low-lying floodplains with moist soil conditions, and upland habitat describes elevated flat or sloping areas with drier soil conditions. Three trees from Lock Haven and four trees from Lehigh Valley were in lowland environments along rivers. The other four trees from these locations were from drier upland hillsides. The tree sampled in Michigan was from upland habitat, and the tree sampled in Virginia was in a lowland habitat.

We digitized six landmarks onto the photograph of each leaf using the freeware program tpsDig (Rohlf 2010a). Landmark locations were chosen at the ends of the main veins, at the vertex of the main sinus, and the point between the leaf tip and main sinus (Figure 1a). We chose to digitize only the left half of the leaf in the image based on methods

by Viscosi and Cardini (2011). When tips or sinuses were damaged on the left side of the leaf, the image was inverted so that the intact side of the leaf was on the left. Two leaves, each from a different tree, were damaged on both the left and right side thus they were removed before morphometric analysis. A consensus configuration was created using tpsRelw (Rohlf 2010b). A consensus configuration represents the average location of each landmark across all leaves after correcting for size differences in the leaves (Figure 1b). Relative warps analysis was performed using tpsRelw (Rohlf 2010b) to summarize the total variation in leaf shape. Relative warps analysis is analogous to a principal components analysis of the shape

variables and it allows visualization of the shape variation along relative warps axes using thin plate spline diagrams (Figure 2). We investigated the first two relative warps because they summarize the most variation in shape with 36.8% on relative warp 1 and 20.7% on relative warp 2.

Our sampling method included multiple leaves from each tree. To appropriately quantify shape variation of the leaves from trees, locations, and habitats, we compressed relative warp scores from each leaf by calculating the average relative warps scores for each tree before further statistical analysis. Then we used ANOVA to test for leaf shape differences among locations (Lock Haven, Lehigh Valley, Michigan, and Virginia) and between habitats (upland and lowland).

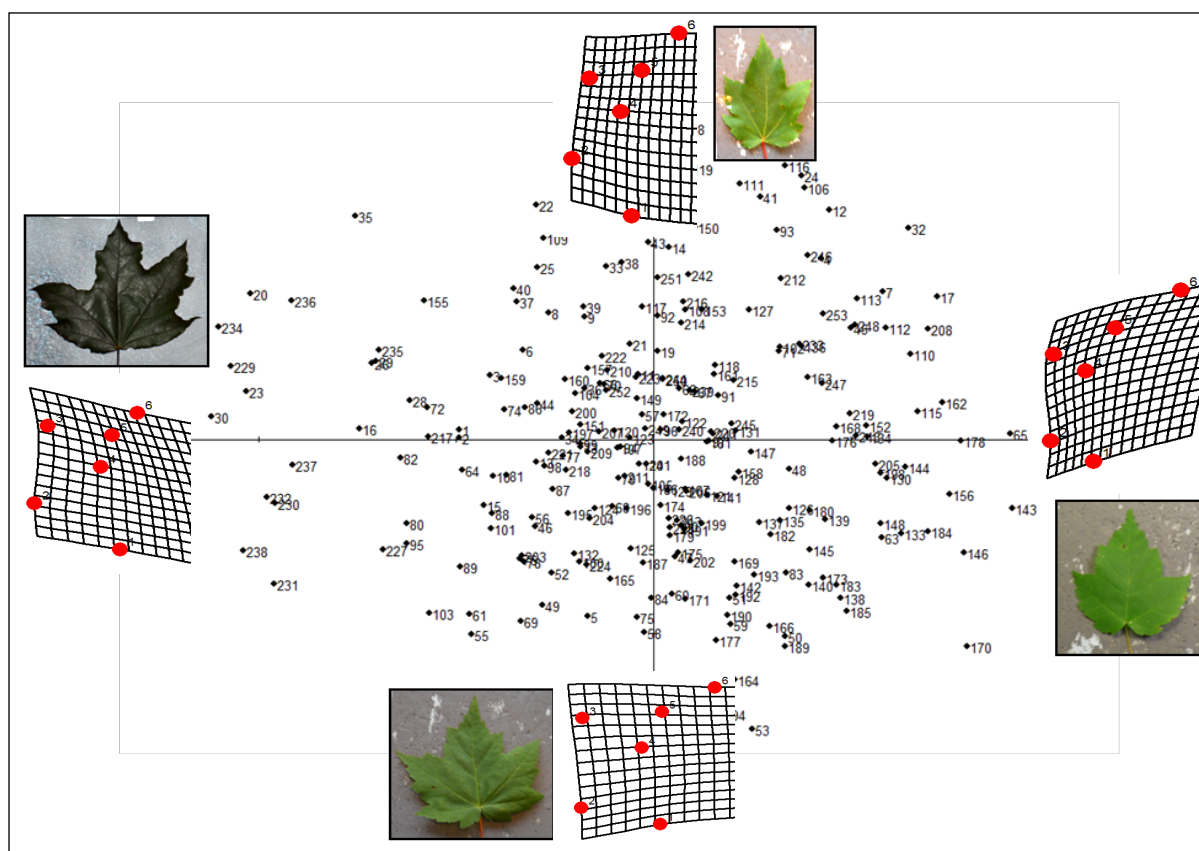


Figure 2: Relative warp diagram of red maple by individual leaves. Photographs and thin plate spline diagrams indicate the extreme shapes of the first and second relative warp.

RESULTS

The first two relative warps axes summarized over 57% of total variation in leaf shape. The first relative warp summarized 36.8%, and we found differences in leaf shape

based on location ($F_{3,16}=11.644$, $p<0.001$) but no differences based on habitat ($F_{1,16}=0.422$, $p=0.42$). The samples were distributed along relative warp 1 from inland locations to coastal locations with increasing warp scores (Figure 2). The second relative warp

summarized 20.7%, and we did not find differences in leaf shape based on location ($F_{3,16}=0.778$, $p=0.53$), but we found leaf shape differences based on habitat ($F_{1,16}=12.016$, $p=0.003$). The samples were distributed along relative warp 2 from upland to lowland habitats with increasing warp scores (Figure

2). Based on the relative warps analysis and thin plate spline diagrams, we found that leaves become broader and gain more definition of lobes and sinuses from Virginia inland to Michigan. Also, broader leaves with smaller sinuses appear to be associated with upland habitats (Figure 3).

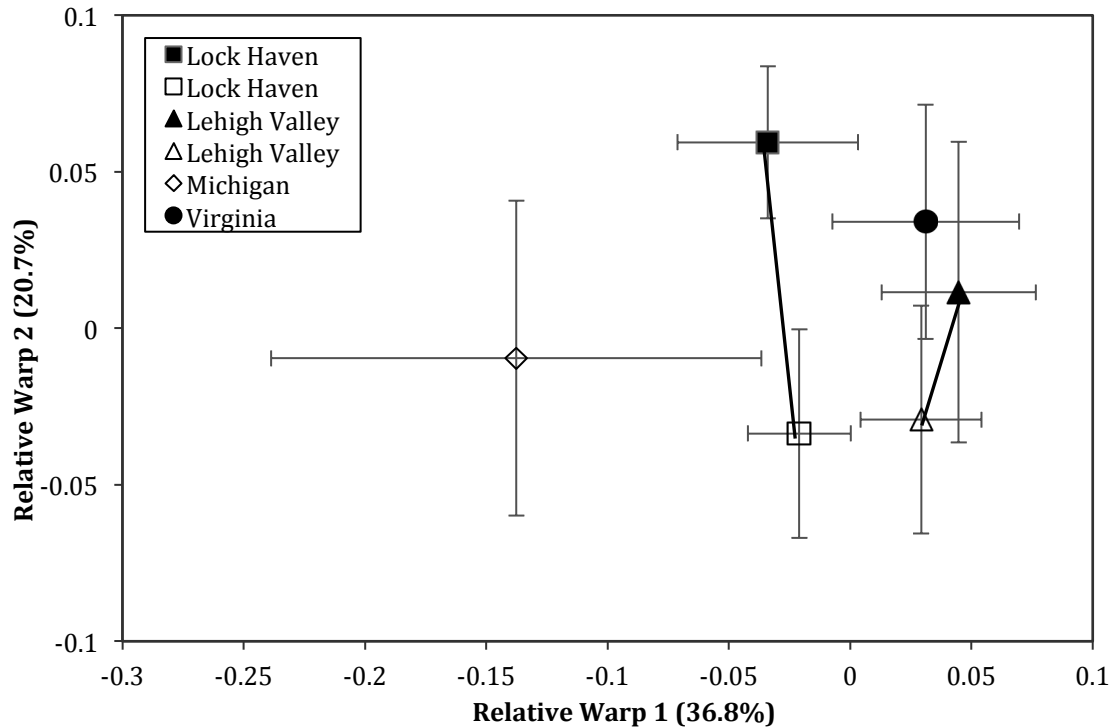


Figure 3: Relative warp averages by habitat and location for red maple. Open symbols ($\square, \triangle, \diamond$) indicate upland samples and shaded symbols ($\blacksquare, \blacktriangle, \bullet$) represent lowland samples.

DISCUSSION

Relative warps and ANOVA analysis showed significant differences in leaf shape based on location and habitat. We observed that leaf size became larger and lobes and sinuses became more defined from coastal to inland locations, and leaves were broader in upland habitats than in lowland habitats. In evolutionary terms, it is advantageous for leaves to have more surface area for photosynthesis in locations with shorter growing periods. In warmer climates, such as coastal Virginia, leaf size may need to be smaller to prevent desiccation. These results are consistent with the observed negative relationship between leaf size and mean annual temperature by Royer et al. (2008).

For lowland habitats, red maple trees are probably not limited by water, and therefore

we expected their leaves to grow larger to maximize photosynthetic rate. Conversely, we expected red maple trees in upland environments, where soil conditions are generally drier, to have smaller leaves as a consequence of limited water availability. Our observations, however were opposite of this evidence, such that leaves were broader in upland habitat and narrower in lowland habitat. McDonald et al. (2003) found that leaves of various tree species were significantly smaller in drier soils than leaves in areas of higher soil moisture; this finding could support the results of our study if we could document that soils were driest in VA and had highest moisture in MI. Additional quantitative data on our red maple leaf mass and size and seasonal hydric conditions of soils in each study area would be necessary to

understand why our results are not consistent with our predictions and other studies. Nonetheless, the consistent differences based on habitat at our two locations in Pennsylvania indicate the presence of an ecotype between upland and lowland areas within a region.

Despite applications of morphometric techniques for studies on animal species, this method is underutilized in plant research. Royer et al. (2008) demonstrated leaf shape variation in red maple leaves based on leaf surface area and number of teeth; Their study documents size and shape variation over a larger climatic range, from Vermont to Florida. Our research suggests that geometric morphometric methods are probably more powerful because we found differences suggesting ecotypes within red maple trees in a narrower geographic range and between habitats in the same geographic region.

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