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TEMPORAL AND ENVIRONMENTAL DIMENSIONS OF  
VARIABLE SEX EXPRESSION  
IN STRIPED MAPLE, *ACER PENSYLVANICUM* (SAPINDACEAE)

BY

JENNIFER BLAKE-MAHMUD

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Written under the direction of

Lena Struwe

And approved by

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## ABSTRACT OF THE DISSERTATION

Temporal and environmental dimensions of variable sex expression

in striped maple, *Acer pensylvanicum* (Sapindaceae)

by JENNIFER BLAKE-MAHMUD

Dissertation director:

Lena Struwe

Plant sex is expressed via flowers and is usually a fixed characteristic present throughout a plant's reproductive lifespan. In rare cases, sex expression is flexible and may be influenced by size or environmental factors. A better understanding of the mechanisms that influence shifts in sex is essential to our understanding of life history theory regarding trade-offs in sex allocation, sex ratios, and differential mortality. My objective is to elucidate how complex environmental signals and individual stress affect labile sex determination in plants, using striped maple (*Acer pensylvanicum*; Sapindaceae) as a study system. Striped maple is often considered an example of environmental sex determination (ESD), although doubt has been raised about the extent of its plasticity and whether it should truly be classified as exhibiting environmental sex determination. The timing of the changes in sex expression and the correlations with environmental cues remain unexplored.

During the course of my research I investigated sex ratios and mortality, the patterns of sex expression and flowering, the correlates of sex expression, and the impact of trauma on sex expression in striped maple. I found that populations were highly male-skewed and female mortality greatly exceeded male mortality. During any two-year period, approximately two-thirds of trees will not change sex; however over half of trees changed sex during the 2014-2017 time period. Of trees that changed sex expression, 25% changed sex at least twice. Even the relatively small percentage of trees that had both male and female flowers in consecutive years could change their percentage of female flowering by up to 95%. In contrast to general theory predicting femaleness for trees at larger sizes and in better condition, I found that femaleness in this species did not depend on size and correlated instead with reduced health. When assessing the relationship between sex expression and non-structural carbohydrates (NSC, stored sugar resources) I found that changes from male to female correlated with higher NSC concentration than trees remaining male. Larger trees did not have larger NSC concentrations. Furthermore, female trees that were dying did not draw down NSCs as they approached death. In manipulative experiments I found that severe damage such as full defoliation or pruning increased odds of changing to female, while less severe physical trauma did not have an effect. Striped maple trees have the potential to change sex expression within three weeks of flowering, with the excising of branches being the instigating cue for sex change in that branch. This work demonstrates that striped maple does exhibit ESD and responds to cues in manners and at timescales previously unknown.

## Acknowledgements

When I first considered leaving teaching to pursue my doctorate two things attracted me: not only would I get to be a student myself for another six years, but I would be wholly and completely responsible for my successes and would not need to depend on so many other people or systems. Now I realize that idea was a ridiculous one. As I look back I am amazed by the sheer number of people, systems, and institutions who have supported me along the way, many of them decades prior to my decision to do doctoral work in ecology and evolution. Many of these blessings result from the dedicated efforts of myself, my parents, my family, and my community; other supports have come to me completely unearned: an accident of my race, nationality, and social class. The origins of individual success are complex, often with roots in historic and current systemic injustices. While nothing can be achieved without hard work, there are countless examples of hard-working people who have been excluded from educational and scientific opportunities for reasons beyond their control. To pretend that I've pulled my dissertation up by its own bootstraps would foolishly ignore the tremendous privileges and opportunities I have inherited, in many cases at the expense of others unbeknownst to me. I count myself supremely lucky to have enjoyed, these last six years, the sweetest bounty of what modern civilization may offer: the chance to have *as my job* the task of exploring deeply questions I find interesting; to pursue both dead-ends and promising lines of thought, to direct my intellectual fate. It seems amazing to me that I have been employed, not to grow food, or produce widgets, or sweep streets, but to figure out something about the world that we did not know before and share it with others via classes, papers, posters, or presentations.

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## **Dedication**

This thesis is dedicated to those in my childhood who  
helped me explore worms, and clover, and roly-poly bugs  
and nurtured curiosity over fear.

and to

Simeen Mahmud

a fierce and loving woman who worked with tenacity and hope  
to shape the world she wanted

1950 - 2018

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## Introduction

### Main research questions by chapter

#### Chapter 2: Gender demographics:

Multi-season sex expression monitoring in striped maple: patterns, sex ratios, and correlations with size, growth, mortality and condition.

*How frequently do trees change sex expression?*

*How do sex ratios compare across years?*

*Do trees change directly from wholly female to wholly male, or are the observed sex changes mediated through monoecious individuals?*

*Do trees who flower monoeciously in consecutive years maintain the same relative percentage of sex expression?*

*What is the distribution of sex among different sized individuals?*

*Is there a relationship between sex and mortality?*

*Is there a relationship between sex and growth rate?*

*Is there a relationship between sex, condition, and size?*

#### Chapter 3: Internal resources and sex expression

The correlations of non-structural carbohydrates with sex expression and mortality

*To what extent does sex expression and change in sex expression correlate with concentration of non-structural carbohydrate (NSCs) present in individual trees?*

*Are NSC concentrations different across different tree sizes?*

*How does NSC concentration correlate with mortality?*

*At what time scale does reduction in NSCs correlate with death?*

**Chapter 4:** The impact of physiological stress on sex expression:

The effects of different kinds and intensities of stress, created by injury, on sex expression

*What are the effects of extreme stress (via pruning) on sex expression the following year and two years out?*

*Does extreme stress (via pruning) have similar effects on trees starting as male, female, or monoecious?*

*Does stress created by complete defoliation mid-summer affect future flowering or mortality in male and non-reproductive trees differently?*

*Does stress created by partial defoliation late-summer affect future flowering or mortality in male and female trees differently?*

*Does stress caused by 50% removal of xylem and phloem tissue affect sex expression in the following year?*

*Does stress caused by 50% removal of phloem tissue affect sex expression in the following year?*

**Chapter 5:** Phenology of sex expression in striped maple:

Timing of sex expression in flower development

*When does sex determination occur during flower development?*

*What cues trigger sex expression in cut branches?*

## Background and rationale

Life is a series of trade-offs. The decisions an organism makes regarding the timing and allocation of resources to important life events result in a wide variety of ecological strategies. Over time, the variety of ways organisms prioritize competing needs and limited resources lead to diverse evolutionary pathways. Life history theory seeks to quantify the trade-offs between competing tasks at the organismal level and to envision evolutionary trajectories under varying ecological conditions (Stearns 1976, 1977). The most fundamental trade-offs concern reproduction, which competes with other critical processes such as maintenance of somatic tissues. The maintenance – reproduction dynamic has been noted by naturalists for millennia (Jönsson and Tuomi 1994, Obeso 2002). One of the earlier observations of this trade-off is found in Aristotle's "On the Generation of Animals" when he writes:

*"For most trees, if they bear too much fruit, wither away after the crop when nutriment is not reserved for themselves...For they consume all their nutriment to make seed [and] become exhausted" (Aristotle, 350 BC).*

Trade-offs in reproduction become more complex when we consider the sex of the organism. While we expect mean reproductive success to be equivalent for males and females over time (Snyder et al. 2012), an organism's gender and reproductive schedule have important implications for its own growth and longevity (Lloyd and Webb 1977, Agren 1988, Austad 1993, Cipollini and Whigham 1994). The distribution of sexes between and within individuals affects sex ratios and mating opportunities within populations which may impact the health, persistence, and evolution of species and the communities of which they are a part (Kokko and Rankin 2006, Kasumovic et al. 2008, Dreiss et al. 2010, Sinclair et al. 2012, Hultine et al. 2016). Particularly for modular sessile species such as plants, the



ability to modify sex expression in relation to environmental cues may represent an adaptive strategy that enables persistence and growth (Freeman et al. 1980, Schlessman 1986, Korpelainen 1998, Ehlers and Bataillon 2007) by allowing more flexible prioritization of limited resources.

### *Reproductive systems and sex ratios in plants*

Over the course of one growing season, most flowering plants express both male and female reproductive organs (stamens and pistils, respectively) in their flowers. When both male and female parts reside in the same (i.e. “perfect”) flower, the sexual system is called hermaphroditism. If staminate and pistillate parts are in different flowers within the same plant, this system is termed monoecy (Sakai and Weller 1999). Other terminology referring to plant sex takes into account both the timing and expression of sex during the flowering period. The diversity of plant sexes and breeding systems is due to various methods of plant sex determination, which include sex chromosomes, autosomes, and various environmental factors (Meagher 1988, Charlesworth 2002, Pannell 2017).

Dioecy at the level of the individual, in which a single tree or herb expresses only one sex throughout its lifetime, is relatively rare, and occurs in approximately 6% of angiosperm species. Separate sexes have arisen at least 871 times and are found in 7% of angiosperm genera and 43% of families (Renner and Ricklefs 1995, Renner 2014). Even in dioecious populations, single-sexed “inconstant” individuals are known to occasionally produce flowers of the opposite sex (Anderson et al. 2015, Barrett et al. 2010). In subdioecy, populations contain primarily single-sexed individuals, with a few monoecious or hermaphroditic individuals (Lloyd 1974, 1980a, Ehlers and Bataillon 2007).

Sex ratios result from differences in sex allocation to male and female function and

are computed by comparing the number of male and female individuals in a population. Although theory predicts 1:1 sex ratios, we find that sex ratios in natural populations are frequently not at equilibrium values (Field et al. 2012, Sinclair et al. 2012, Anderson and Levine 1982, Anderson 1979). The causes for the deviation of sex-ratios from the predicted equilibrium are still uncertain, particularly in the field of botany, due to the underrepresentation of plants in research on sex ratios (Barrett et al. 2010, Field et al. 2012, 2013). One potential explanation for biased population sex-ratios might be due to environmental sex determination (Charnov and Bull 1977, Schlessman 1988, Barrett et al. 2010, Field et al. 2012) but the role of ESD in sex ratios is poorly understood (Hultine et al. 2016).

Differential mortality is another possible cause of biased sex ratios. If one gender shows differential death rates, it may be advantageous for an individual to begin its reproductive life as the sex with lower mortality (Warner 1988, Day and Aarssen 1997). The importance of mortality to the evolutionary maintenance of sexual plasticity is largely unexplored (Day and Aarssen 1997, de Jong and Klinkhamer 2005). Only one study exists examining the correlation of differential mortality and sexual plasticity. In *Acer rufrinerve*, Nanami found that 62% of sex-changing trees flowered completely female before dying (Nanami et al. 2004).

#### *Sex allocation and environmental sex determination*

Sex allocation reflects the amount of energy devoted to male or female function per plant (Charlesworth and Charlesworth 1981, Lloyd 1983). Sex allocation may reflect trade-offs between staminate and pistillate flowers in a single season, across a plant's lifetime, or between growth and reproduction within a single sex (Lloyd 1980b, 1983, Charnov 1986,

Zhang and Jiang 2002, Case and Ashman 2005). While it is common for monoecious plants to adjust sex allocation from year to year (Lloyd 1980b), the ability to adjust sex allocation drastically, from a fully male phase one year to a fully female phase the next year, is rare (Lloyd and Bawa 1984, Renner 2014).

The ability to fully change sex expression during one's lifetime has been called by a variety of names including sequential hermaphroditism, diphasy, sex choice, sexual plasticity, sexual lability, or environmental sex determination (Warner et al. 1975, Charnov and Bull 1977a, Schlessman 1986, Meagher 1988, Korpelainen 1998; here the last three terms will be used interchangeably). Sexual plasticity might be selected for if the reproductive success of males and females is differentially affected by environmental (e.g. non-genetic) factors that are patchily distributed in time or space (Charnov and Bull 1977, Schlessman 1986). These factors may be specific to the individual or the result of the ambient environment.

When sex is labile and influenced by size, the change in nature is usually unidirectional. The most well-known example of this unidirectional sex shift associated with size-dependent sex allocation is found in *Arisaema* spp., Jack in the Pulpit, in which increasing size (particularly of the underground storage corms) manifests as increasing female sex expression (Schaffner 1922, Maekawa 1924, Bierzychudek 1984). Studies such as the ones regarding *Arisaema*, as well as other animal studies, have led to a theory of size-dependent sex allocation which predicts that the sex that gains the most fitness with size will be expressed by larger individuals (Ghiselin 1969, Charnov 1982, Warner 1988, Day and Aarssen 1997, Cadet et al. 2004, de Jong and Klinkhamer 2005). This may be due to direct effects of size (e.g. large, tall males can disperse pollen further) or due to budget

effects (e.g. large females have more resources to devote to gamete production; Klinkhamer et al. 1997, Cadet et al. 2004, Vega-Frutis et al. 2014). For insect-pollinated plant species in which the energetic demands of being female (e.g. seed and fruit development) are substantial, females are usually found to be larger (de Jong and Klinkhamer 2005). Examples in which females manifest a larger size include *Lilium apertum* (Zhang et al. 2014), *Coptis laciniata* (Lindh 2017), *Panax trifolium* (Schlessman 1991) and others. Under size-dependent sex allocation, populations are expected to contain a larger proportion of smaller plants, resulting in male-skewed sex-ratios (Schlessman 1988).

The patchy environmental model of sex allocation theorizes that ambient environmental conditions may also affect allocation to male and female function. These include environmental variables such as precipitation, light levels, general stress, trauma, soil nutrients, photoperiod, temperature, or hormone application (Heslop-Harrison 1957, Freeman et al. 1980). It seems that physical stress such as drought, lack of nutrients, or low light levels may induce maleness in sexually plastic species (Heslop-Harrison 1957, Freeman et al. 1980, Korpelainen 1998). Examples of this include *Atriplex canescens*, which exhibited changes to male following drought, extreme cold, and heavy fruiting, and *Castanetum viridflavum*, which showed increased male flowering in response to reduced light levels (Freeman et al. 1984; Zimmerman 1991).

The role of physical injury, in particular, is unresolved. Most research involving physical injury to plants has been done on species without documented ESD. In some monoecious species, trauma causes shifts towards male sex expression, such as in *Pinus edulis*, while it encourages femaleness in *Zea mays* (Heslop-Harrison 1957). In dioecious

species with genetic sex determination, such as *Carica papaya* (Heslop-Harrison 1957) or *Mercurialis annua*, injury may influence the “leakiness” of dioecious state (John Pannell, pers. comm.) by promoting brief shifts in sex expression in otherwise stable sexual systems. In other species, such as *Lindera beonzoin*, physical or physiological trauma apparently causes death, without sex switches (GJ Anderson, pers comm). The sole example of trauma on a species with ESD is documented in *Arisaema triphyllum*, where damage causes shift to maleness (Heslop-Harrison 1957; Maekawa 1924). Injury, however, is frequently confounded with the reduction of biomass, making it impossible to determine which variable directly affects sex expression.

While the patchy environment model has been used to examine some ecological parameters, the variability within the hyper-local environment of the individual may also shed light on key factors for environmental sex determination (ESD). For example, Nanami suggested that sex expression may be related to the internal status (stored energy reserves) of an individual in *Acer rufinerve* (2004) rather than simply external resources. This idea was echoed, though not tested, as a potential cause of sex-switching in *Coptis laciniata* (Lindh 2017). Although many studies have investigated the small and large scale segregation of sexes along ecological parameters (Bierzychudek and Eckhart 1988, Stark et al. 2005, Nuñez et al. 2008), only one study has investigated the correlation of resources internal to a plant with expressed sex in a sexually plastic species (Maekawa 1924).

Although most hypotheses for ESD in plants conform to the size-dependent or patchy-environment models, there exists other theory regarding when and why organisms should change sex. Iwasa (1991) developed two complimentary models based on the

importance of growth rate and mortality. These models were worked out for animal systems and have been investigated multiple times in fish, but have been largely ignored in plant sex-determination research. In the mortality-advantage and growth-rate advantage models, an individual may have multiple viable “reproductive” states, including a resting (or non-reproductive) option. In these models, costliness is related to slower growth or higher mortality; larger organisms are assumed to have higher fitness. The model predicts that the evolutionarily stable strategy for individuals will be to minimize costs by switching amongst reproductive states so that growth-rate is maximized and mortality-risk minimized. While the specific reproductive schedule will vary within and among species based on numerous factors, these models potentially support repeated switching between sexes and prolonged or iterative bouts of non-reproduction (Iwasa 1991).

There remain unanswered questions regarding the importance of resources, mortality, stress, and trauma on environmental sex determination and the resulting population sex ratios. The timing of the change in sex expression and correlation with environmental cues remains largely unexplored (Sinclair et al. 2012), as does the degree to which ecological factors influence patterns in flowering (Barrett and Hough 2013). These unanswered questions and the underrepresentation of plants in research on sex-ratios (Barrett et al. 2010) and sexual plasticity warrant further study of this phenomena (Vega-Frutis et al. 2014). Furthermore, the potential of an individual to prioritize both issues of sex expression and issues of maintenance – reproduction provide an interesting lens through which to investigate life history trade-offs.

Historically, most studies of environmental sex determination in sexually plastic species assigned individuals to one of three gender classes: male, female, or monoecious. This, however, may give an incomplete snapshot of gender within a population by obscuring the relative sex allocations among all individuals, particularly monoecious individuals (Lloyd and Bawa 1984, Schlessman 1986). This has raised doubt over the classification of several species as sexually plastic due to a lack of quantitative data (Schlessman 1986). To understand the true distribution of sexual phenotypes across a population we need to know the exact proportion of male and female flowers, especially for the monoecious individuals. (Lloyd and Bawa 1984, Zhang et al. 2010).

In order to understand the sex expression dynamics of natural populations, it is critical to track individuals over multiple years (Spencer Barrett, pers. comm., Sato 2012). Only by extended studies (three or more years, Primack and McCall 1986, Schlessman 1986) of natural populations can we infer whether a species exhibiting sexual plasticity is monomorphic for gender (all individuals belong to one genetic class and over their lifetimes gain equal fitness from both genders) or polymorphic for gender (most individuals derive their primary fitness from being either male or female but also exhibit “inconstancies”) (Lloyd 1980, Lloyd and Bawa 1984, but see Matsui 1995). Lloyd and Bawa (1984) named the confusion over this seemingly insignificant distinction as “one of the major barriers to a better understanding of plant sexuality”, perhaps because of the impediment it causes in untangling the route to dioecy, a particularly vexing problem in plant reproductive biology (Delph 2003, Delph and Wolf 2005).

### **Study system**

In order to address some of these exciting questions, I collected quantitative sex-

expression data on multiple populations of *Acer pensylvanicum*, a species with unconfirmed ESD, from 2014 -2017.

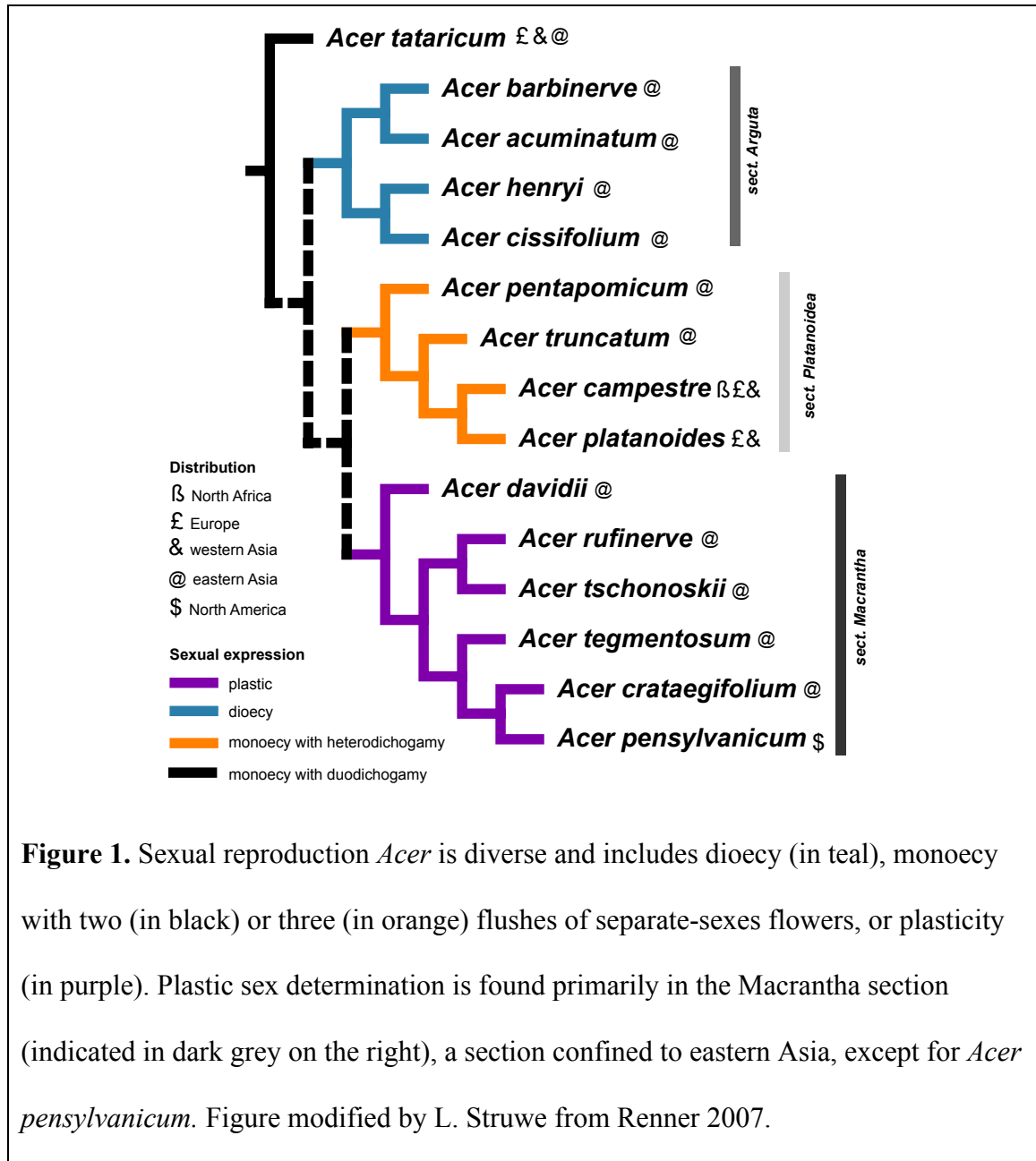
The genus *Acer* (Sapindaceae) is a group of large shrubs and trees distributed in temperate areas of North America, Europe, Asia, and tropical areas of southeast Asia (Gelderen et al., 1994). The genus contains 16 taxonomic sections (Gelderen et al., 1994). *Acer* is the second most speciose tree genus in the northern hemisphere (after the Oaks, *Quercus*) and is part of the family Sapindaceae (Renner et al., 2008). *Acer* is reproductively diverse. Four sexual systems are represented within this group, including dioecy, ESD, and monoecy separated by time (two batches of separate-sexed flowers = dichogamous flowering; three batches of separate sexed flowers = duodichogamous flowering, **figure 1**).

*Acer pensylvanicum*, or striped maple, is the sole member of the macrantha clade (Hillhouse and Michaux 1819, Renner et al. 2007, Zhang et al. 2010) found in North America; the other 13 species grow in Japan and China (Gelderen et al., 1994). The isolation of striped maple from its sister taxa occurred in the miocene (Renner et al., 2008) and exhibits a geographic pattern characteristic of the North American – East Asian disjunct (Wen, 1999). Renner estimated that the macrantha clade split off from the rest of the maples approximately 28 (16-46) mya (see **figure 1**, Renner et al., 2007). More recent estimates indicate that *A. pensylvanicum* may have split off from its East Asian cousins even earlier in the miocene, approximately  $35 \pm 9.4$  mya, due to the formation of the Sea of Japan (Zhang et al. 2010).

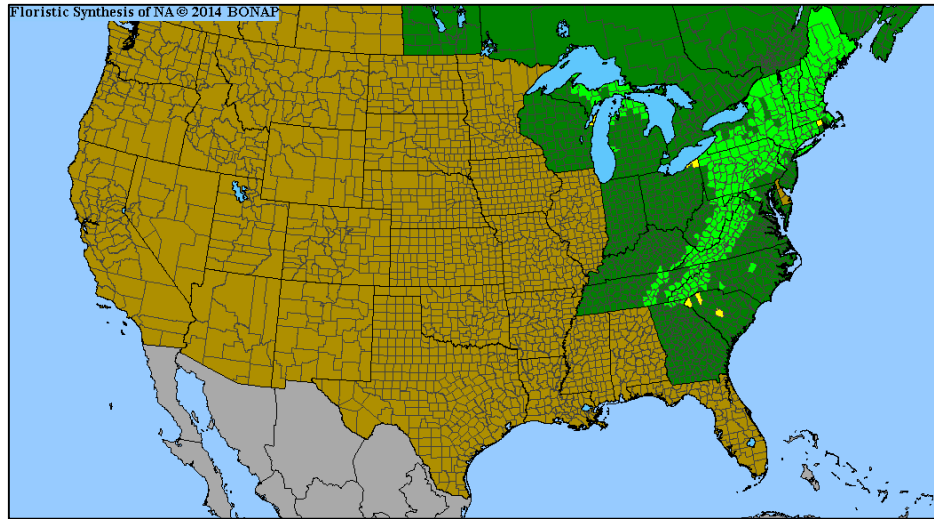
Several biogeographical scenarios could lead to the current distribution pattern within the macrantha clade and others. It is most likely that the earlier distribution of



macrantha predecessors had a widespread distribution along the North Atlantic coastal bridge during the Tertiary, which was much later disrupted by glaciations, resulting in the current separation in macrantha group taxa (Wen, 1999). The consequence of this isolation is that striped maple is the only species of the originally Asian clade to be found in North America.



The macrantha group is especially interesting because it contains a number of species that are sexually plastic, a reproductive mode that has evolved four times in the *Acer* genus. Overall sexual plasticity in dioecious and subdioecious species is a rare life history strategy (Renner 2014) that is present in approximately 6% of all *Acer* species (Renner et al. 2007).



**Figure 2.** Striped maple grows at higher elevations along the Appalachian Mountain range. Light green indicates presence of striped maple in county, dark green indicates presence in state, yellow signifies rare presence of species. Map created by the Biota of North America and reprinted with permission (Kartesz 2017).

In present day, striped maple grows in mountainous areas (400-1700 m; **figure 2**) of northeastern North America, including New Jersey, where it occurs in mesic rocky soils (Hibbs et al. 1980). As a subdioecious species, the majority of individuals are either male or female any given year, with a small proportion (<5% of individuals) expressing both male and female genders (Hibbs and Fischer 1979). Flowers are functionally either female or male (de Jong 1976, Sullivan 1983). Separate flowers occur combined in pendulous

racemose inflorescences. The flowers are yellowish green, with free, linear-lanceolate to obovate calyx lobes and a campanulate corolla up to 5 mm long and 8(-11) mm wide with obovate corolla lobes (see **figure 3**). In the rare cases of monoecious trees, Hibbs and Fisher found that male and female flowers are born on separate branches, with male branches predominating. Female flowers were usually restricted to damaged lower branches (1979), in contrast to other plant species in which injury sometimes correlates with maleness (Heslop-Harrison 1957, Vega-Frutis et al. 2014). However, in monoecious trees, there are occasionally inflorescences that also bear both staminate and pistillate flowers. These bisexual inflorescences are usually found in a transition area between male and female flowering zones. These do not occur on all monoecious trees, nor do they appear to occur on trees without both male and female unisexual inflorescences. Although de Jong observed some male flowers to contain a highly reduced abortive pistil (1976), this has been found to be non-receptive, therefore these flowers are functionally male (Sullivan 1983). De Jong also reported rare fully-developed morphologically perfect flowers on cultivated trees (1976), but subsequent observations in the field have failed to find any morphologically or functionally perfect flowers (Hibbs and Fischer 1979; Sullivan 1983).

Striped maple is often cited as an example of a species with environmental sex determination (Hibbs and Fischer 1979), but many questions remain, including the foundational questions about whether this species truly exhibits sex change or is instead displaying sexual inconstancies (Lloyd and Bawa 1984, Schlessman 1988). Other outstanding questions include the timing of changes or modifications in sex expression, the

role of monoecy, the cues for sex change, and why this species seems to contradict the trends found in other species exhibiting ESD (Lloyd and Bawa 1984, Schlessman 1988).



**Figure 3.** *Acer pensylvanicum* inflorescences. From left to right, starting at the top: male inflorescence, bisexual inflorescence, (no perfect flowers), female inflorescence. Individual male (left) and female (right) flowers are shown in cross-section below. Drawings by Robert E. Blake.

Complex ecosystems, such as forests, often have considerable time lags between abiotic stimuli and the resulting biotic responses. In some cases, long-lived trees may take many years to respond to disturbances, stress, or extreme climatic events (Brubaker 1986, Foster et al. 1997, 1998, Holmes and Likens 2016). Biotic responses such as the timing of

gender changes remain largely unexplored (Sinclair et al. 2012). Research on other allied *Acer* species suggests that some sexually labile maple are capable of changing the sex of flowers within one month of normal phenological flowering time (Haas 1933, de Jong 1976), but no one has investigated the timeline of flower development in striped maple. Such late-season sex determination would imply that striped maple is responsive to sex changing cues (Barker et al. 1982) at a speed that other trees (e.g., *A. saccharum*) may not be due to time lags (Gelderen et al. 1994). In order to understand the time scale at which sex change occurs in striped maple and the magnitude and immediacy of environmental disturbance necessary to trigger a change, we must understand the floral developmental timeline and when the sex for the following year becomes determined in flower primordia.

In striped maple, anecdotal evidence attributes the switch from male to female to crown closure (Hibbs and Fischer 1979), and the internal resource status of the tree in a sister species (Nanami et al. 2004), but manipulative work regarding the impact of these factors on sex change has not yet been conducted. Furthermore, previous work on striped maple indicated that size did not correlate with sex expression (Hibbs and Fischer 1979). Trees in various size classes may switch gender several times throughout their lives. In any given year, both small, sickly trees and large, healthy trees can be female. The lack of a strong observed correlation between sex and size highlights the potential importance of other factors such as environmental stressors or physiology in determining sex expression in striped maple.

### **Significance and Broader impacts**

My research regarding sex expression in striped maple has implications for our understanding of plant reproduction, sex ratios, life-history trade-offs, resource allocation,

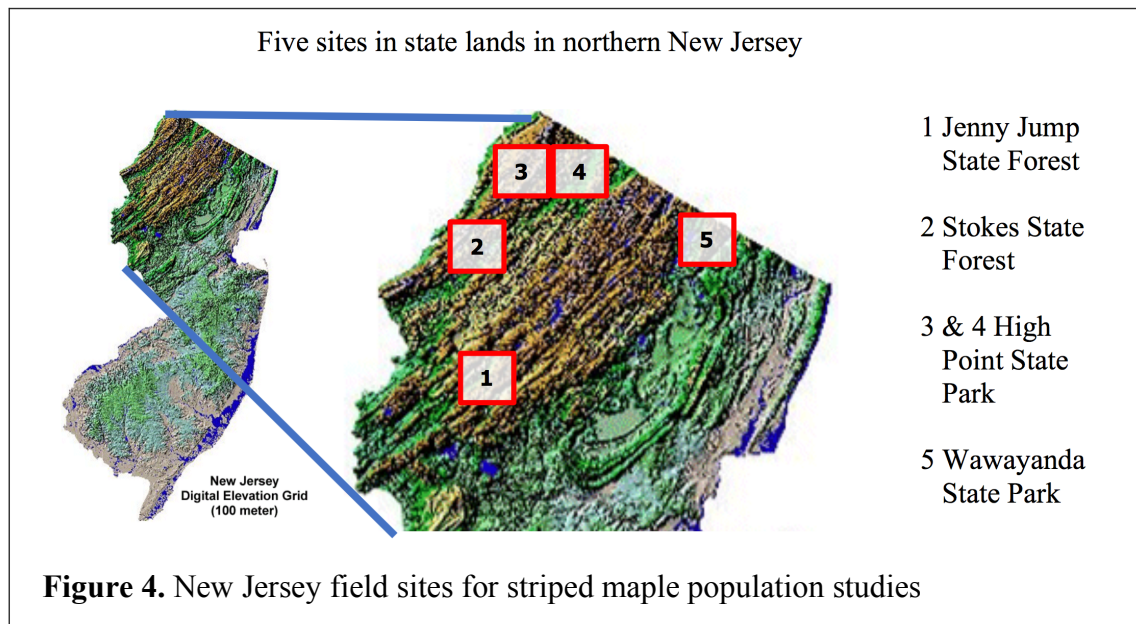
and applied forestry. Current theory would predict that striped maple trees would change sex expression to female with increasing size, or change to male with increasing physical stress. However, preliminary data suggested that neither of these hypotheses were true for striped maple (Hibbs and Fisher 1979). To have a better understanding of theory regarding sex-determination in plants we need to untangle the sex expression triggers for this species. The maintenance of subdioecy via ESD is of particular interest due to its hypothesized place in the evolutionary pathway from monoecy to dioecy, an area of ongoing research in botany (Barrett et al. 2010).

Furthermore, as a member of northern hardwood forest understory communities, striped maple has attracted the attention of forest managers due to its ability to take over large swaths of forest following clear-cutting (Horsley and Bjorkbom 1983, Sipe and Bazzaz 1995, Stalter et al. 1997), tree die-offs, or extensive storm damage (Shirer and Zimmerman 2010). Given the projected increase in severe weather events (Planton et al. 2008, Whytlaw et al. 2017, USGCRP 2017), it is important to understand how stress (due to increased physical damage) may affect these trees and impact the relative frequency of females within populations. Furthermore, the developmental timeline of flower primordia into male and female flower parts affects the time scale at which the results of sex determining cues, such as damage, will manifest in populations. This has implications for seed set, recruitment, and persistence, not only of maples, but also of shade-intolerant hardwood species. Increases in striped maple populations will affect the composition, sustainability, and regeneration of the eastern hardwood forests (Horsley and Bjorkbom 1983), which provide important ecosystem services to the most densely populated region of the United States. *Understanding the environmental factors influencing sex expression is*

valuable for both basic and applied science because it furthers our knowledge of sex allocation theory and offers practical insight into forest sustainability, regeneration, and management in the wake of changing climate patterns.

### Research Sites

I conducted my research on striped maple in five study populations in northern New Jersey. All populations grow in state parks and forests in Sussex and Passaic counties (see **Figure 4**).



I chose these sites based on the presence of striped maples, selecting sites with large numbers of different size classes. I also looked for sites that were accessible via roads or trails, but not highly visible in order to prevent tampering by park visitors. I preferentially picked sites along environmental gradients such as light or elevation. The canopy trees include *Acer rubrum*, *A. saccharum*, *A. platanoides*, *Betula nigra*, *B. lenta*, *B. alleghaniensis*, *Carya ovata*, *Castanea dentata*, *Quercus rubra*, *Q. velutina*, and *Ostrya virginiana*. The substrates are rocky and have a history of disturbance by recent

rockslides or via tree thinning to enhance the view by parkgoers.

Within each site, I tagged and collected data on all reproductive individuals within a demarcated plot. The first set of tagged trees from 2014 numbered 370 individuals. The net number of initial study trees decreased to 241 due to removal of consistently non-flowering trees from the data set and the felling of study trees by the New Jersey Park Service, among other things. The following year I expanded my plot sizes and consequently added 190 new trees to my unmanipulated tree populations. At that time I also started marking trees outside the boundaries for study plots populations to be used in future manipulative studies. New study plot trees added in 2016 and 2017 were trees that became reproductive during the time of the study.

I collected the following data at the individual and site level.

<b>Independent variables (within sites)</b>	<b>Independent variables (across individuals)</b>
Soil nutrients Aspect Elevation Ambient light levels	Size (diameter at breast height, dbh) Mortality Condition, Damage Resources (non-structural carbohydrates)* Growth (internode distance)* Sex expression
<b>Calculated variables (within individuals)</b>	<b>Calculated variables (across individuals)</b>
sex ratio phenotypic sex (ie – percent female sex expression)	Frequency of sex-change

**Table 1.** Data collected during the course of the study at the individual and site level. Asterisks indicate data collected on a subset of study trees.



Not all data receive a full treatment here. Preliminary data are available in the appendices. These include:

- I. Analysis of accuracy of sex expression methods
- II. Sex ratios by site
- III. Soil analysis data
- IV. Preliminary forced flowering results from 2014
- V. Preliminary phosphorus analyses
- VI. Preliminary photosynthetic rate analyses
- VII. Boosted classification tree notes
- VIII. Flowering intensity by sex

### **Scope and Objectives**

The objective of this doctoral work is to understand the causes and consequences of environmental sex determination and its potential responses to a changing climatic regime, using striped maple, *A. pensylvanicum*, as a study species. To confirm striped maple as a sexually plastic species and address concerns regarding sex expression I collected data over four years on the sex of approximately 900 individuals in five sites in northern New Jersey, USA.

In order to understand which ecological and tree-specific factors are associated with sex-switching behavior and how this relates to our understanding of sex-allocation theory, I tested for correlations between expressed sex, tree size, mortality, growth, and condition in approximately 500 trees. I used these models to look at patterns and pathways in sex expression and the extent they could be used to predict sex in the future.

I collected data on the consistency of sex expression within monoecious trees, examined differential mortality, and quantified sex ratios across populations and years (Chapter 2).

Drawing first on the theory of the patchy environment model of sex allocation, I investigated the underlying assumption that expression of the more energetically-costly sex (usually female) correlates with access to more resources. I investigated this by experimentally assessing the concentrations of non-structural carbohydrates (NSC) in male trees switching sex expression to female. I quantified the stored NSC concentrations of male and female trees along a size gradient. In order to ascertain the relationship of NSC concentration and mortality in females, I tested sugar resources in senescing trees (Chapter 3).

To better understand the intensity of proximity of sex determining stress cues needed to influence sex, I experimentally tested the effects of various kinds and intensities of physical damage on sex expression. At the lowest intensity I used partial defoliation of trees (in 2016). At mid-intensity levels of stress I evaluated the effect of partial removal of conductive tissue via ringing on sex the following year. At the highest intensity of physical stress I examined the effect of full defoliation of trees (in 2015) and severe crown pruning on expressed sex (Chapter 4).

In order to elucidate the timescales at which sex may respond to expression determining cues, I investigated the timing of floral development and the proximate triggers for changes in sex expression. Using forced flowering greenhouse techniques, I first explored the speed at which flowering sex may change in relation to natural flowering in the field. I then investigated the impact of potential sex-determining cues on the flowering of branches kept in the greenhouse and the field (Chapter 5).

I conclude by synthesizing the data from chapters 2-5 and hypothesizing potential evolutionary causes and consequences of this unique life history trait, with attention to the effects of changing climatic norms and implications for forest communities (Chapter 6).

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## Chapter 2

### **Time for a change: patterns of sex expression, health, and mortality in striped maple (*Acer pensylvanicum*)**

Jennifer Blake-Mahmud<sup>1\*</sup>, Lena Struwe<sup>2,3</sup>

<sup>1</sup> Graduate Program in Ecology & Evolution, Rutgers University, 14 College Farm Rd.,  
New Brunswick, NJ 08901

<sup>2</sup> Department of Ecology, Evolution, & Natural Resources, 14 College Farm Rd., Rutgers  
University, New Brunswick, NJ 08901

<sup>3</sup> Department of Plant Biology, 59 Dudley Road, Rutgers University, New Brunswick, NJ  
08901

\* Corresponding author [Jennifer.n.blake@rutgers.edu](mailto:Jennifer.n.blake@rutgers.edu)

Blake-Mahmud ORCID: 0000-0002-6737-2824

Struwe ORCID: 0000-0001-6074-5758

Running title: patterns of sex expression, health, and mortality in *Acer pensylvanicum*

**Abstract:**

- *Background and Aims:* The ability of individuals to change sex during their lifetime is known as environmental sex determination (ESD). This represents a unique life history trait, allowing plants to allocate resources differentially to male and female functions across lifetimes, potentially maximizing fitness in response to changing environmental or internal cues. We investigated an often-cited example of ESD, *Acer pensylvanicum*, to see whether it conformed to theoretical predictions that females are larger and in better condition. We also explored whether sex correlates with growth and mortality.
- *Methods:* We documented patterns of sex expression over four years in populations located in New Jersey, USA and collected data on size, mortality, health, and growth. Using a machine-learning algorithm known as a boosted classification tree, we developed a model to predict the sex of a tree based on its previous sex, condition and size.
- *Results:* In our study, more than 50% of the trees switched sex expression during a four-year period, with 26% of those trees switching sex at least twice. Consistently monoecious trees could change sex expression by as much as 95%. Size and condition were both important predictors of sex, with condition exerting three times more relative influence than size on expressed sex. Healthy trees are more likely to be male; female sex expression increases with decreasing health. Growth rate negatively correlates with multiple years of female sex expression. Populations maintain similar male-skewed sex ratios across years and locations and

may result from differential mortality: 75% of dead trees flowered female immediately before death.

- Conclusions: Our study shows for the first time that *A. pensylvanicum* strongly exhibits ESD and that femaleness correlates with individual-level factors in previously unsuspected ways. The mortality findings advance our understanding of puzzling non-equilibrium sex ratios and life history trade-offs resulting from male and female sex expression.

Key words: angiosperms, *Acer pensylvanicum*, environmental sex determination, health, mortality, Sapindaceae, sex expression, dioecy, hardwood forests, plastic sex determination



## Introduction

Sex is a fixed trait in most angiosperms (Renner 2014). While individuals may adjust proportions of male and female flowers in a flowering year, large changes from one sex to another remain rare (Schlessman 1986; Korpelainen 1998; Renner 2014). Approximately 250 species exhibit labile sex determination (Renner 2014), eight of them within *Acer* (Renner *et al.* 2007).

Labile sex determination, also known as environmental sex determination (ESD), sexual plasticity, or diphasy, is a form of ontogenic sex change where individuals change sex expression from year to year (Charnov and Bull 1977; Schlessman 1986; Korpelainen 1998). In most cases sex change is uni-directional but sex may be reversed under experimental conditions (Maekawa 1924, Bierzychudek 1984). ESD is thought to be adaptive by allowing individuals to assess local cues that differentially impact the sexes (Charnov and Bull 1977; de Jong and Klinkhamer 2005).

There are many environmental cues that affect sex expression in different species (see reviews in Heslop-Harrison 1957; Freeman *et al.* 1980; Korpelainen 1998; and Bachtrog *et al.* 2014). These include abiotic factors such as the availability of sunlight, water, or nutrients. Factors particular to an individual such as size, energy reserves, or suffering some physical trauma may also affect sex expression (Heslop-Harrison 1957; Freeman *et al.* 1980; Freeman *et al.* 1984; Korpelainen 1998). The general rule is that femaleness usually manifests at a larger size, in better microhabitats, or for plants in better condition. Male individuals, on the other hand, are often in poorer condition and found in areas of lower light, water, and nutrients (Heslop-Harrison 1957; Freeman *et al.* 1980; Meagher 1988; Korpelainen 1998). What constitutes good or poor condition may

vary slightly among species. In trees, poor health is generally characterized by one or more of the following: discolored or dead foliage during the growing season, structural cracks, decay and dieback of branches, cankers or visible fungal fruiting bodies, or damaged and diseased roots (Schwarz *et al.* 2000; Angwin *et al.* 2012). Healthy trees, on the other hand, exhibit bright foliage, vigorous growth, and abundant flowering.

Here we examine the influence of size and condition on sex expression in the sexually labile tree known as striped maple, *Acer pensylvanicum* (Sapindaceae). This species grows in rocky, mesic soils at higher elevations along the Appalachian Mountains (Hibbs *et al.* 1980). As an understory tree it is well adapted to shaded environments and its stature means it frequently incurs damage from white-tailed deer (through browsing and antler rubbing) and from falling canopy trees and their branches. It is a subdioecious species and most individuals bear either staminate or pistillate inflorescences in a given flowering season with males outnumbering females. Individuals expressing both male and female inflorescences on the same tree represent less than 5% of a population (Hibbs and Fischer 1979). The flowers in an inflorescence are all the same sex, except for a small fraction of the monoecious trees, where male and female flowers can be found in the same inflorescence (de Jong 1976; J. Blake-Mahmud unpub. results). Interestingly, and importantly, the final sex determination in this species may occur as late as three weeks prior to flowering (Blake-Mahmud and Struwe 2018).

Using *A. pensylvanicum* as a study system, we investigated the following questions:

1. Patterns in sex expression

- a. How frequently do individual trees change sex expression?

- b. Are sex ratios stable across years?
- c. What patterns of sex expression exist across time?
- d. Does sex expression correlate with growth rate?

## 2. Predictors of sex expression

To what extent do tree size, condition, previous sex, and growing location predict expressed sex the following year?

## 3. Mortality

Is mortality equally distributed amongst sexes?

## Methods

Study sites are located in state forests and state park lands in New Jersey, USA: Jenny Jump State Forest (40.913, -74.922, Warren County), Stokes State Forest (41.218, -74.720, Sussex County), High Point State Park (41.321, -74.662, Sussex County), and Wawayanda State Park (41.217, -74.451, Passaic and Sussex Counties). Sites were selected in 2014 based on the presence of striped maple populations. Study trees were those that fell within the boundaries of the randomly delimited plots. Sexes grow intermixed. Following high mortality the summer of 2014 due to unexpected tree thinning by the park service (and, thus, canopy trees being felled on the understory striped maples), study plot sizes were expanded in 2015 to include additional *A. pensylvanicum* trees. From 2015-2017, all trees added were due to recruitment events, that is, previously non-flowering trees flowered and therefore became study trees. *Acer pensylvanicum* is known to sprout from stumps or roots, therefore we considered multi-stemmed clumps to be genets, i.e., branches of a single individual (Stalter *et al.* 1997). We removed soil from bases of tree trunks growing in close proximity (within 6 inches)

to confirm whether they were joined by roots. Due to the presumed importance of hormonal signals in affecting sex expression, which may proceed either from the bud or the root, plants with common vascular systems were classified as one individual.

The following data were collected on all study trees within plots: size (measured as diameter 1.35 m from the ground; i.e. at breast height - dbh), condition, and flowering sex. There is not yet discipline-wide consensus on methods for quantifying the size of multi-stemmed trees, thus the dbh of the largest stem – as recommended by the U.S. Forest Service (Powell 2005) – was used. Condition was assessed visually by looking for external physical damage to the tree such as broken or dead branches, open cankers, discoloration of the green-photosynthetic bark, or split trunk bark due to rapid spring warming, impact damage, or antler-rubbing by deer. The U.S. Forest Service uses a visual assessment of street tree condition to bin trees into numbered categories according to health (or damage condition based on risk analysis; Angwin *et al.* 2012). We modified this protocol to measure condition on a scale of 0-5 and adjusted for smaller tree sizes, so that a 1 m flowering tree missing three of four branches was scored as being in worse condition than a 5 m tree missing three of ten branches. The full condition assessment protocol is available in **supplementary information**. Trees receiving scores of 0-1 were considered to be in excellent condition, scores of 2 good condition, scores of 3 fair condition, and scores of 4 poor condition. Trees receiving scores of 5 were in very poor condition and were 50% or more dead. Trees were considered fully dead when their bark turned black and they failed to produce and expand leaves in the spring or when their bark turned black, leaves shriveled and turned brown on the tree, and all twigs and branches became dry and brittle prior to leaf drop in the fall.

In 2014, flowering and sex were evaluated by visually scoring the sex of a minimum of 20 inflorescences per tree. The inflorescences were distributed from the tip of branches to the trunk on multiple branches and at multiple heights. The sex of the tree was then recorded as male, female, or monoecious. Starting in 2015, sex assessment was enhanced by including counts of the total number of inflorescences per tree. Although *A. pennsylvanicum* is a small, understory tree, the sexual structures of inflorescences growing high in large specimens are not always visible to the unaided eye. In these cases, approximately 20 inflorescences from various regions were then spot-checked with binoculars (Pentax Papilio 8.5x21mm). Assessment of 27 trees of dbh 1.5 – 5.25 cm had indicated that our visual assessment of 15 inflorescences from multiple areas of different branches at different heights achieved >95% accuracy in sex assessment. When all inflorescences were counted, accuracy reached 100%. For large monoecious trees, all inflorescences were viewed with binoculars (where necessary) and tallied as male or female to compute relative percent female sex expression. Trees that did not flower in a given year were included in analyses if they flowered at least once over the course of the study. Because of logistical difficulties in marking and re-locating non-reproductive individuals in the smallest size classes, consistently non-flowering trees over 2014-2017 were not included in the analysis.

Growth rate was assessed on trees that were consistent in unisexual sex expression during the years of this study. The lowest branches in the south, southwest, or west exposures were selected. Growth rate was scored by measuring the distance between terminal bud scars moving distally from branch tip to capture at least three years of shoot extension.

## Statistics

To address patterns of sex expression and the relative influence of predictors the data were analyzed using a combined classification and machine learning approach known as boosted classification trees (BCTs). Compared to other analysis methods such as generalized linear models, BCTs have several benefits. Boosted classification trees can be used easily for categorical variables, have no assumptions regarding observation independence, are robust to measurement error, and have superior predictive power compared to other modeling methods (Elith *et al.* 2008). We performed the BCT analysis in R (version 3.4.1, R Core Team 2015) using the caret (Kuhn 2017) and gbm packages (Ridgeway 2016). Remaining analyses were carried out in JMP Pro (version 13). Figures were created using ggplot2 (Wickham 2009) and SankeyMatic (Bogart 2016).

For building a model using the boosted classification tree (BCT) method, data were combined across four years for all live study trees. Trees that were initially tagged in 2014 and remained alive throughout the study period are represented three times in the data set, while trees that started to flower in 2017 are only represented once in the analysis. The resulting 1082 records were randomly divided into training (75% of data,  $n = 813$  flowering bouts) and testing (25% of data,  $n = 269$  flowering bouts) data sets. To ensure that stochasticity arising from random data partitioning did not influence the model training and testing, the frequencies of sexual transitions were compared between datasets and found to be within 1.5% of each other.

The BCT model was fit using the training dataset, while the testing dataset provided data unseen during model construction for an independent evaluation of the model's predictive capacity. The algorithm searches for model parameters that maximize

predictive ability (as measured by Cohen's kappa) using a 10-fold cross-validation approach of the training data. The following parameters were found to produce the optimal classification tree: learning rate=0.001, interaction depth=3, number of trees=1000, and bag fraction=0.5 with multinomial error. The full range of tested model parameters are available online [**supplementary information**].

Model predictive performance may be assessed via accuracy using Cohen's kappa. In situations where outcome categories are unevenly distributed, kappa is preferred because it compares predicted outcomes to real outcomes by controlling for expected accuracy. In *A. pensylvanicum*, populations are male skewed. Kappa accounts for the fact that if model simply predicted "male" every time, it would have relatively good accuracy in predicting the future sex of a tree without any biological understanding of what is going on via other predictors.

Using the model parameters above, a model was trained for a categorical outcome (sex) and both categorical (sex the previous year, site) and continuous (size, condition) predictors. Because BCTs do not provide p-values, we included continuous and categorical random variables against which to compare the relative importance of predictors. A classification matrix is also used to assess model fit. This matrix provides information regarding correct and incorrect classifications of the testing dataset by the model by comparing the predicted and actual sexual states of trees.

The BCT model was trained to allow for an interaction depth of 2-3, which means that both two and three-way interactions are possible. In generalized linear model approaches, the presence of an interaction reduces the interpretability of main effects to simple effects for involved predictors. An added benefit of BCTs is that the relative

importance of predictors and their marginal effects remain easily interpretable in the presence of interactions. Like other BCT model outcomes, p-values are not produced. Instead, interactions are quantified via Friedman's H statistics, which vary from 0 (weak interaction) to 1 (strong interaction).

To address questions regarding differential mortality, we used Fisher's exact test to compare observed and expected outcomes. Fisher's exact test extends a Chi-squared test to multiple categories and works well for small sample sizes (Fisher 1935). Growth via stem elongation was compared using a t-test for unequal variances.

## Results

Our results show that starting sex, condition, and size are important predictors of sex the following year in *A. pensylvanicum*. We found that males are often in better condition. Female mortality is high, with three-quarters of dead trees flowering fully female the year before death.

Male-skewed sex ratios across study sites and years were consistent over the four years sex expression was monitored (see **Fig. 1**). In any single two-year period, trees were most likely to remain the same sex. Approximately one-third of trees changed during any one two-year period (2014-2015 - 25.7%; 2015-2016 - 31.5%; 2016-2017 - 43.9%). The five most common transitions were, in descending order: from non-reproductive to male, male to full or partial female flowering, female to dead, and from partial to full female flowering. (see **Fig. 2**)

During the study period, 54% of trees changed sex at least once. Of those that changed expression, 74% switched sex one time and 25% switched sex twice. Among trees exhibiting one change, over half (39% of changing trees) of them switched from



male, female, or monoecious to a different flowering or non-flowering state. The others (34% of changing trees) switched from non-flowering to flowering. While many of these may have simply reached sexual maturity for the first time, others will have returned to reproduction after several years of not flowering. Given the time limits of the study, it is not possible to say, with certainty, which trees belong to which reproductive schedule. Of the trees classified as monoecious in consecutive years, individual trees changed their sex expression (i.e., the relative percentage of male and female flowers) as much as 95% or as little as 1%, with a mean change of 38%.

Mortality correlates strongly with female sex expression the previous year. The majority of deaths (53 of the 71), happened to trees that flowered fully female the year prior to dying [see **supplementary information**]. If all recorded deaths were evenly distributed among flowering states (male, female, monoecious, or non-reproductive), we would expect approximately 18 deaths per state. Even with this highly conservative expectation (given that populations are approximately 60% male) Fisher's exact test yields p values of  $<.0001$  for the probability of getting these observed mortality data or more extreme data by chance alone. Only about 1% of male-flowering trees died on average. Between 2-5% of monoecious trees died during any given year. A similar small percentage (between 0-10%) of non-flowering trees died. Females experienced 13% mortality in the 2014-2015 time period, 15% mortality in 2015-2016, and 39% mortality in 2016-2017, following a dry, hot 2016 summer in the northeast and a generally mild 2016/2017 winter with several hard freezes.

Growth, as measured by branch elongation per year, was different between the sexes. Trees flowering consistently female over a 3-4 year period had lower mean rates

of stem elongation ( $18.5\text{mm} \pm 9.7$  per year) than did trees flowering consistently male ( $51.5\text{mm} \pm 29.4$  per year) during this time period (t-test for unequal variances  $n = 14$  (7 male, 7 female),  $df = 7$ ,  $p = .0257$ ).

We evaluated a model that looked at the influence of starting sex, condition, size, and site on sex the following year. The relative influences of the predictors were compared against a random continuous and random categorical variable (shown in see **Fig. 3**, and given in numerical format in **supplementary information**). Higher relative influences indicate that with this model, the predictor provides more information for correctly categorizing observations and is therefore more strongly correlated with the predicted variable (in this case, sex). The relative influence of the random variables provides a baseline by answering the following question: how well does a randomly generated set of data help us predict sex?

In addition to comparisons against random predictors, model fit may also be assessed using other means. The accuracy of this model is 0.758 (where 1.0 is perfect predictability) indicating that the predictive ability of the model is good. Cohen's kappa is 0.452 and indicates moderate to good agreement in model predictions and actual data (Landis and Koch 1977). A classification matrix may be used to compare the model-predicted data values with the actual observed values and is available online [**supplementary information**].

The BCT model showed that the flowering sex in the previous year heavily influences the flowering sex the next year. This is not surprising as roughly two thirds of trees do not change sex in any one year. Among flowering trees, femaleness has the most influence on sex the next year, followed by monoecy (**Fig. 3**). The non-flowering state

exerts slight influence on sex the following year and is more informative than a random categorical variable. As in other types of analyses such as logistic regression, one group is necessarily withheld as the comparison group; in this BCT model, males are the group to which others are compared.

Condition is approximately three times as important (17.4%) as size (6.2%) in determining sex; both variables are better predictors of sex than a randomly generated continuous predictor (1.4%). Site is relatively unimportant in determining sex the following year (0.6%, all sites combined) though still more informative than a random categorical variable (0.3%).

By holding other variables constant, we can examine the marginal effect of changing one predictor, such as initial sexual state or “starting sex”. Based on our model, flowering trees are most likely to remain their current flowering sex in the following year. Of non-reproducing trees that flowered at least once during the study time, most were likely to switch to male the following year. Trees that did not flower at all during the study period were excluded from the model (**Fig. 4**).

When condition and size parameters are held constant, the model predicts that males and females have approximately equal baseline probabilities of changing to a different sexual state (**Fig. 4**). Males and females have high predicted probabilities (58% and 61%, respectively) of remaining in that sexual state from year to year. However, when population sex ratios are taken into account, the transitions from male to partial or full female flowering are much more frequent than reverse transitions (**Fig. 2**). Monoecious trees have approximately equal probabilities (37% and 35%, respectively) of remaining monoecious or changing to female.

We examined the marginal effect of tree health on flowering sex (see **Fig. 5**). The correlation between size and condition was low. High correlations would have resulted if condition scores were assigned by assessing absolute tree damage so that only very large trees would have high condition scores. The marginal effect plot indicates that healthy trees are most likely to be male (blue line, **Fig. 5**). For trees in generally good health (condition levels 0-2; see **supplemental information**) the worsening of condition does not change the marginal probability of various expressed sexes much. Worsening condition for trees in moderate to poor health (levels 2-5) changes the marginal probability of being male or female quite a lot. With increasingly poor condition, the predicted probability of being male decreases from approximately 0.65 to 0.35, while the probability of being female increases from 0.14 to 0.35. The probability of not flowering increases slightly with poorer condition from 0.09 to 0.13. The probability of monoecious flowering starts at 0.13 for healthy trees and peaks at 0.2 for intermediate levels of condition. The model predicts equal representation of males and females at worst levels of health. Because population sex ratios are male-skewed in striped maple populations, this means that females are more over-represented in trees of poor condition than would be expected.

When assessing the marginal effect of size (by controlling for starting sex, condition, and site) we see that its effect remains similar across size classes (see **Fig. 6**). While the predicted probability of flowering male is always high, male flowering peaks at sizes of 2 cm dbh and then decreases slightly with increasing size. Probability of being female remains constant over all documented size classes, while the predicted probability of flowering monoeciously doubles from approximately 0.1 to 0.2. With increasing size,

the predicted probabilities of being non-flowering are highest at very small sizes (less than 1 cm dbh), then remain low across size classes.

Tests of the strength of two and three-way interactions were conducted using a Friedman's H statistic (vary from 0 - weak interaction, to 1 - strong interaction). Interactions with condition are strongest for males (0.420), followed by females (0.181), and monoecious trees (0.115). Non-flowering *A. pensylvanicum* trees or trees that contained both male and female inflorescences exhibit a slightly different response to size. Smaller trees are over-represented in the non-flowering state, while larger trees are over-represented in the monoecious state (**Fig. 6**; green and yellow lines). This is quantified in the interactions between starting sex and size for both monoecious and non-reproductive trees (0.296 and 0.286 respectively; **Table 1**). Although our best-fit model indicated a tree depth of three [see **supplementary information**], allowing for three-way interactions, the interaction between starting sex, size, and condition are negligible.

## Discussion

Here we confirm that *A. pensylvanicum* individuals not only change sex but may do so repeatedly (**Fig. 2**). Results show that initial sex, condition, and size are important predictors for sex the following year in this sexually plastic species (**Fig. 3**). In contrast with previous studies on dioecious species with ESD showing females are usually in better health, we found that in *A. pensylvanicum* females are proportionally over-represented in trees in poor condition (**Fig. 5**). Female mortality is highest among the four categories of sexual expression [**supplementary information**]. Monoecy, previously hypothesized to be an expression of inconstancy (Schlessman 1986), is a viable and variable state in the sexual system of this species (**Fig. 2**).

The importance of body size has been highlighted in the theory of size-dependent sex-allocation in both plants and animals. Indeed, in dioecious plants, females are often larger (Heslop-Harrison 1957; Freeman *et al.* 1984; Korpelainen 1998), particularly in insect-pollinated species (de Jong and Klinkhamer 2005). For example, in herbaceous *Arisaema triphyllum* (Araceae) females usually have larger leaf area and (Bierzychudek 1984); while in *Lilium apertum* (Liliaceae), greater plant height correlates with increased prevalence of female sex expression (Zhang *et al.* 2014). Previous work on *A. pensylvanicum* had suggested that sex expression was not correlated with size (Hibbs and Fischer 1979). We did find that size was a better-than-random predictor of sexual state (**Fig. 3**); specifically that the probability of flowering monoeciously increased with size, while non-flowering plants were more likely to be small. The marginal effect plots depict a relatively constant relationship with size for trees flowering exclusively male or female (**Fig. 6**; blue and pink lines); most populations have a male-biased sex ratio, so at any given size, a tree has a higher overall marginal probability of being male.

Flowering plants, like many animals, may differ greatly in the resource allocation to sexual reproduction in male versus female individuals (Case and Ashman 2005). In female angiosperms, the pistil is fully developed before pollination, but the nutritious endosperm is only developed if the ovule is fertilized, yielding a diploid embryo. The male plants provide pollen through stamens that usually wither after anthesis (Case and Ashman 2005). In contrast, the female plant will have a heavy resource investment in developing seeds and fruits specialized for dispersal that requires additional resources (e.g., fleshy or thickened fruit walls, arils, wings, thick seed walls, bright colors, etc.). This imbalance has led to the hypothesis that advantageous conditions such as large body

size or overall health select for female reproduction, while maleness is more feasible under harsh conditions (Charnov and Bull 1977; Freeman *et al.* 1980; Schlessman 1986; Korpelainen 1998; de Jong and Klinkhamer 2005).

While we did not find that larger body size increases the predicted probability of female sex expression, we did find evidence of other trade-offs and support for the growth-rate / mortality advantage model (Iwasa 1991). This model hypothesizes that when growth-rate and mortality-risk vary between sexes, sexual plasticity may be selected for. The evolutionarily stable strategy is to express the less-costly sex first (as measured by higher growth rate or lower mortality), followed by periods of non-reproduction, before expressing the more-costly sex (Iwasa 1991). Our findings of decreased growth and increased mortality among females, coupled with the higher prevalence of male transitions to partial or full female flowering rather than the reverse, support this model. Although studies on sex-differential mortality are rare, particularly in species exhibiting ESD, our results are in line with research done on *Acer rufinerve* in which females experienced significantly higher mortality than males (Nanami *et al.* 2004)

Evidence of reduced growth rates in females is also consistent with sex-specific costs of reproduction (e.g. devoting energy to provisioning seeds rather than growth; Obeso 2002). Research on sex-specific costs of reproduction yields mixed results. Work on perennial plants with stable sex expression, such as *Populus* or *Asparagus* species, indicates that males often exhibit increased growth rates (reviewed in Lloyd and Webb 1977). Other work has found the opposite, with growth of female clones of *P. tremuloides* exceeding that of males (Sakai and Burris 1985). Dioecious *A. negundo* showed no sex-based differences in shoot elongation (Willson 1986). In sexually stable

species, flowers are often larger in males or hermaphrodites compared to females (reviewed in Eckhart 1999) or are more numerous, constituting more flowering biomass (Anderson and Symon 1989, Ramp and Stephenson 1988).

For other plants exhibiting ESD, it appears that males are typically individuals found in poorer condition, or following environmental stress as in *Atriplex canescens* (Freeman *et al.* 1980; Freeman *et al.* 1984; Korpelainen 1998). However in *A. pensylvanicum* as individual health decreases and trees exhibit moderate damage, the incidence of partial and full female flowering increases. At the worst levels of condition where trees have multiple kinds of damage to vascular tissue and branches, including sections of crown dieback, a tree is equally likely to be male or female. In contrast, a female striped maple is twice as likely to be in poor condition than it is to be healthy. This is particularly interesting due to the overall male skew of *A. pensylvanicum* populations. The influence of poor condition raises the prediction of female flowering well above observed population sex ratios (of approximately 3.5 males to 1 female) to almost 1:1.

Poor physical condition in trees due to dead or lost branches, open cankers, infection, or bark injuries can affect various physiological processes. Wounds and infections are sealed off from surrounding tissues by the deposition of chemical and anatomical barrier (Shigo 1984, Dujessiefken and Liese 2015). The resulting compartmentalization limits the direct extent of injuries and infections and allows the tree to continue to live and grow. The tree-produced boundaries, while protective, are common starting points for structural failures (Shigo 1984, Schwarze *et al.* 2000). Over time, one might expect the repeated compartmentalization to impede the vascular



connections between leaves and roots that are necessary for transportation of water, nutrients, non-structural carbohydrates, and regulatory hormones. This could both reduce the health of individuals and change the hormonal balance responsible for sex-specific flowering. The mechanistic links between wounding, decreased health, and sex expression have yet to be uncovered.

There are no hard and fast guidelines for interpreting predictive capacity via Cohen's kappa values, though an often-cited rule of thumb in the field of medicine indicates that agreement values from 0.4 - .6 signify moderate agreement (Landis and Koch 1977). The use of kappa is not widespread in ecology, though a metanalysis of 34 studies indicated that the majority of ecological research with presence-absence data had kappa values less than 0.4 for their explanatory models (Manel *et al.* 2001), though recent work in ecological fisheries had kappa values as high as 0.55 (Free *et al.* 2017). The interpretation guidelines are "clearly arbitrary" (Landis and Koch 1977) and certainly context-dependent. Due to the complexity and environmental stochasticity inherent in ecological studies, revised benchmarks and ecologically based test-statistics could be useful (Manel *et al.* 2001). The current guidelines, coupled with comparisons with other ecological studies, indicate that our model sufficiently encapsulates the effects of the independent variables to adequately predict future flowering behavior.

While truly untangling the underlying ecological controls over sex expression in this species will take decades of monitoring, complex environmental data sets, and sophisticated statistical analyses, the generalized pathway we suggest in figure 7 is consistent with observations and results from our 4-year study. In this hypothesized schematic, increases in body size generally cause individuals to move from non-

reproductive to male-flowering phases. Trees leaving the male phase often do so by one or two primary paths. They may begin flowering monoeciously (which appears to be due to increased size). Males may also skip the in-between stage and transition directly to complete female flowering if their health is deteriorating. Individuals may remain in any flowering state for a number of years, with trees more likely to maintain female or male flowering than they are monoecious flowering. This observation further supports the idea that monoecy is a transitory state. Most trees enter the dead state from female flowering. While reverse transitions happen in *A. pensylvanicum*, current data are not yet sufficient to hypothesize causes for these transitions.

In conclusion, we show that in the sexually plastic tree *A. pensylvanicum*, expressed sex is influenced by a variety of factors. Chief among them are the previous sex and the condition of an individual. Although the general theory regarding environmental sex determination in dioecious plants has indicated that females are often found in relatively better condition and at larger sizes, we find the opposite pattern in this species. These conclusions suggest that factors involved in ESD are less generalizable than previously thought. Furthermore, we show that mortality is disproportionately high in females, which potentially explains the overrepresentation of males in these populations. Studies like this are rare due to their long-term scale and intense field efforts, but are valuable to examine the viability of theoretical predictions in wild populations.

The population and forest-level implications of these findings could be substantial but are not yet fully clear. The climate is changing, with multiple effects, including warming, and an increased incidence of strong meteorological incidents (IPCC 2014).

We might expect the number of females in populations to increase due to reduced tree health following storm damage or increased prevalence of fungal or other infections – both risks associated with the effects of climate change in temperate regions (Barker *et al.* 2007; Whytlaw *et al.* 2017). While increased shift to femaleness could increase seed set in the short term, the larger number of females will likely also result in increased mortality across populations. At its extreme, this scenario endangers the long-term recruitment and persistence of *A. pennsylvanicum* populations. We also show here that generalized assumptions of sex-ratios and sexual plasticity should be applied with caution to species that previously have not been investigated. Further understanding of the correlated patterns and causative effects of body size, health, damage levels, and other individual variation on sex expression is needed to fully evaluate potential interacting effects of emerging diseases, climate change and weather, and forest dynamics on species with plastic sex determination.

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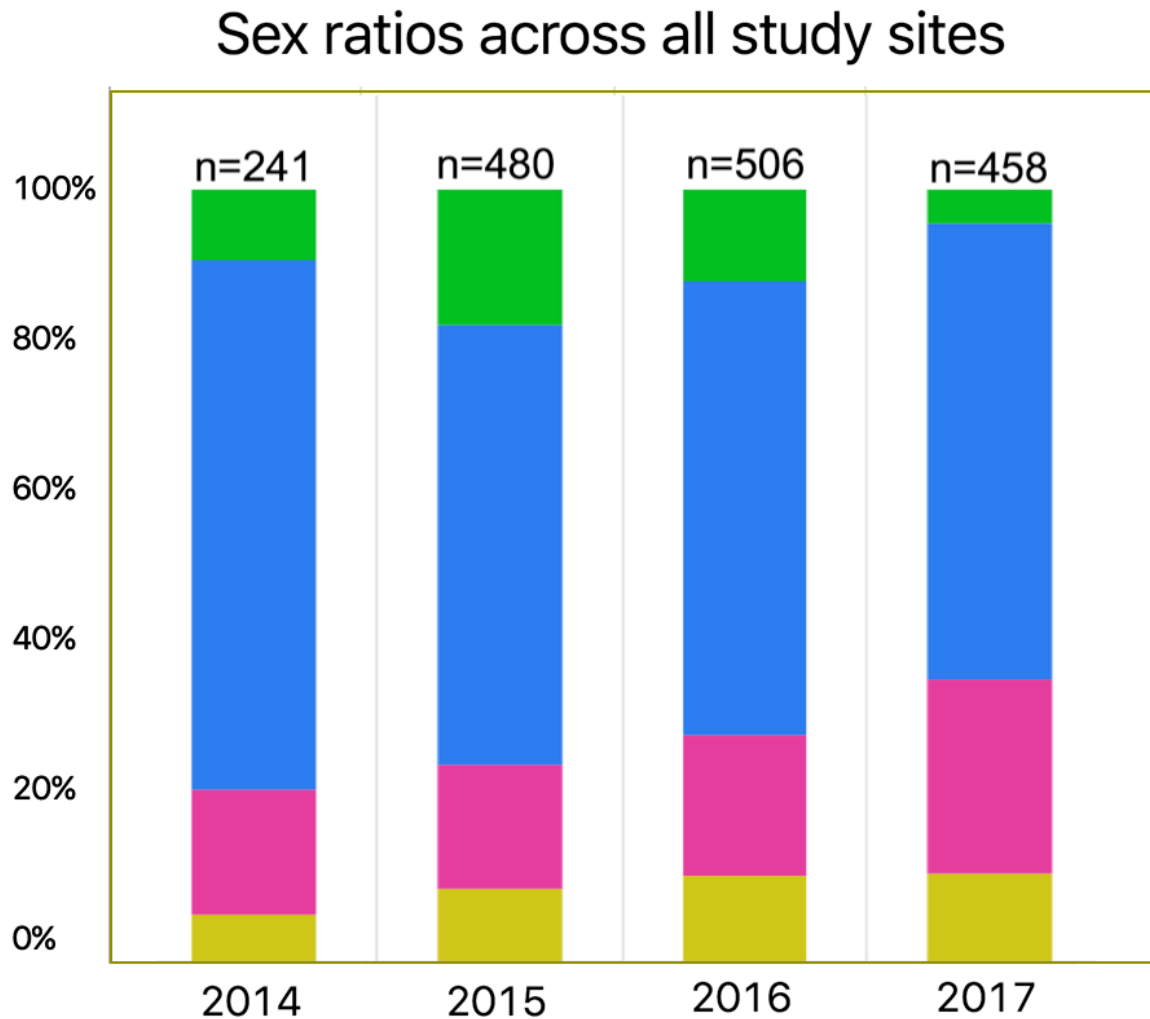
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**Table 1.** Interaction strengths of two and three-way interactions on flowering state in *Acer pensylvanicum*. Largest interactions exist between male sex and condition and for monoecious and non-reproductive trees and size.

	Male	Monoecious	Female	Non-reproductive
Sex*Condition	0.420	0.115	0.181	0.052
Sex*Size	0.059	0.296	0.037	0.286
Condition*Size	0.023	0.093	0.014	0.165
Condition*Size*Sex	0.009	0.008	0.001	0.008

## Figures

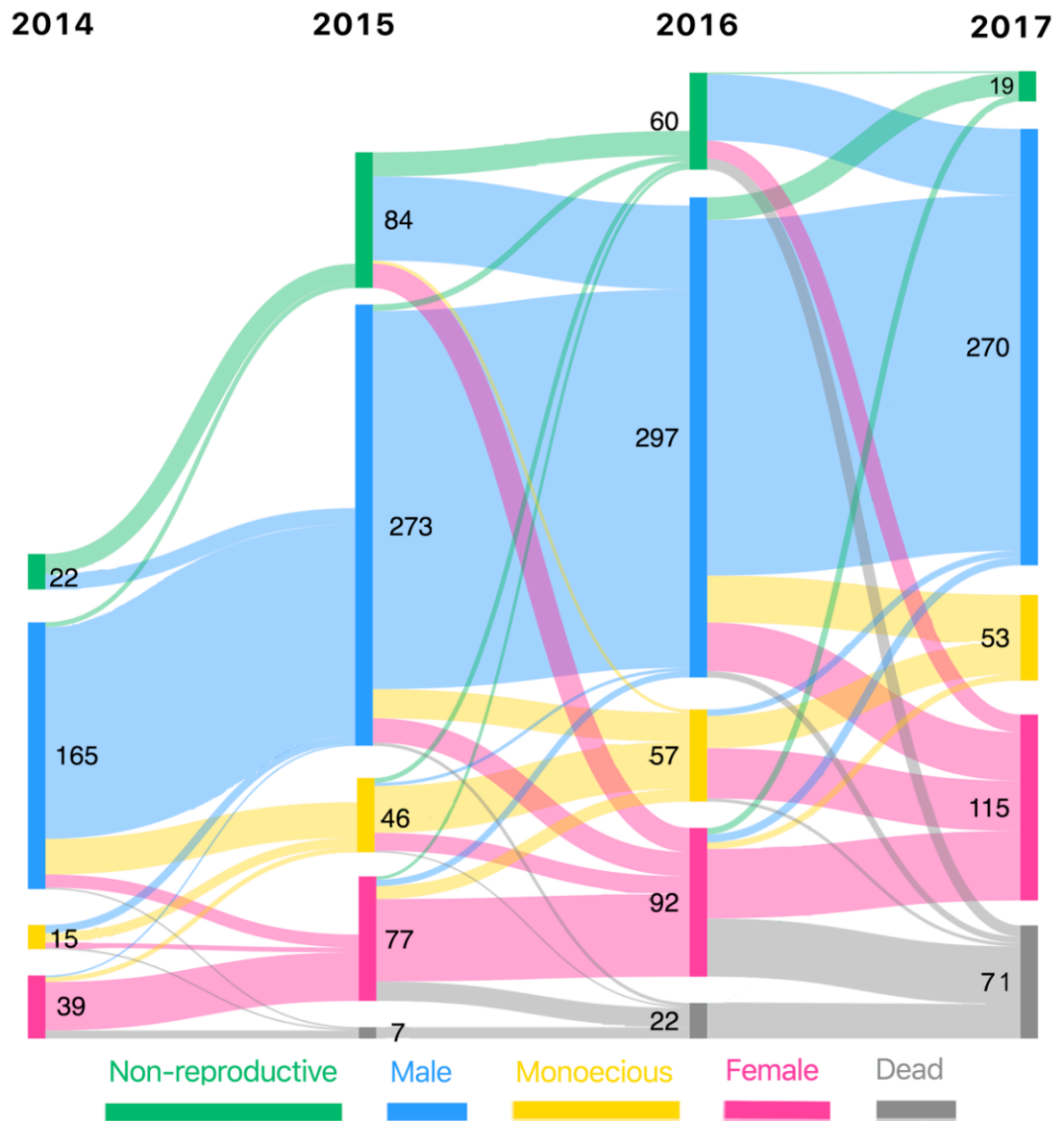
**Figure 1**



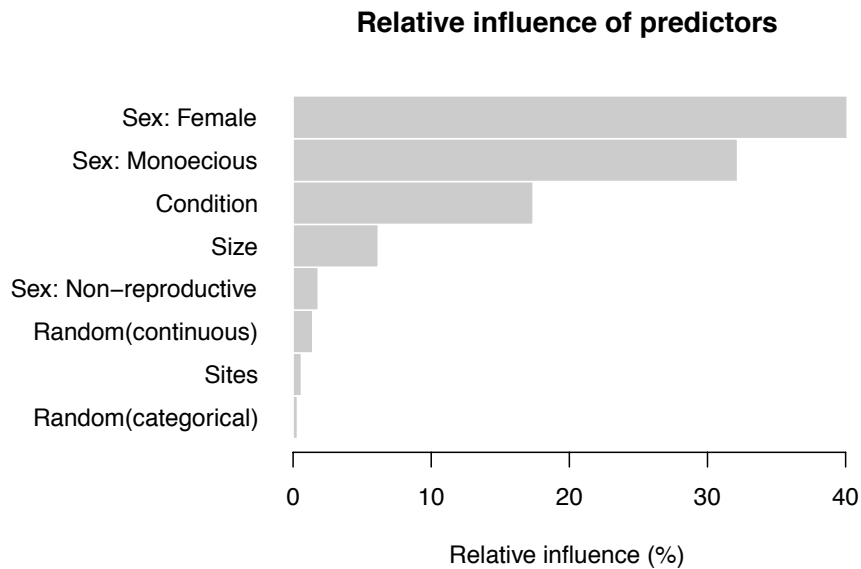
**Figure 1.** Sex ratios of living *Acer pensylvanicum* trees across all sites by year. Blue represents male trees, pink female trees, yellow monoecious, green non-reproductive trees (i.e = not flowering that year). Sample size changed based on expansion of study area (2014-2015), tree mortality, and recruitment to reproductive state over the study period.

Figure 2

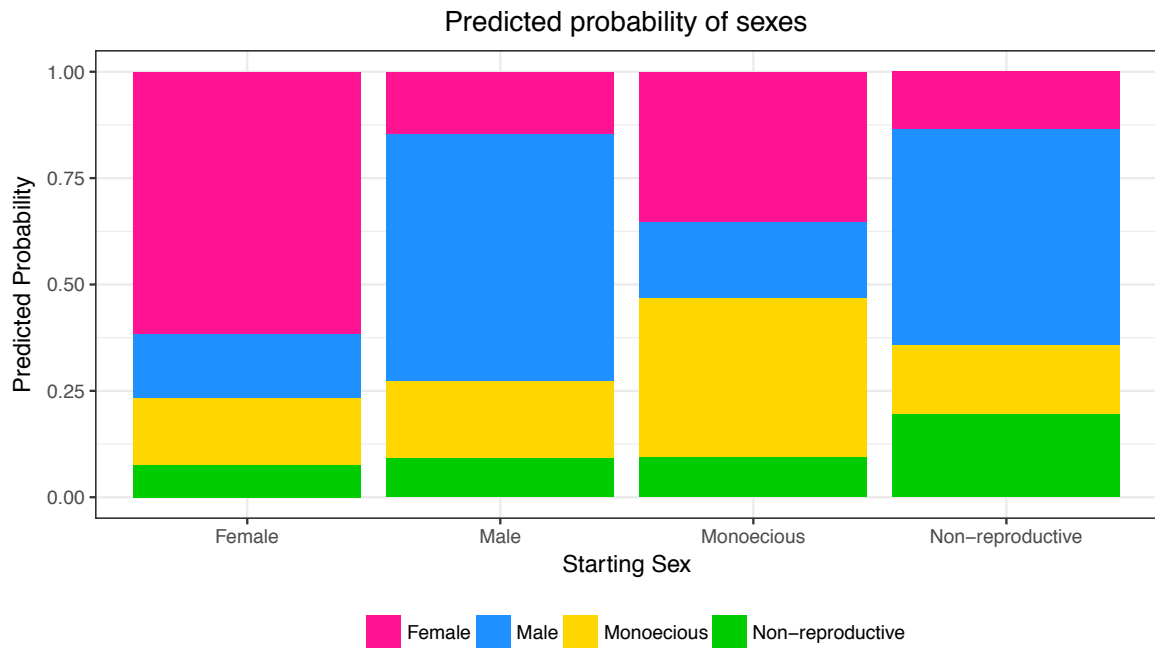
## Sex expression across years



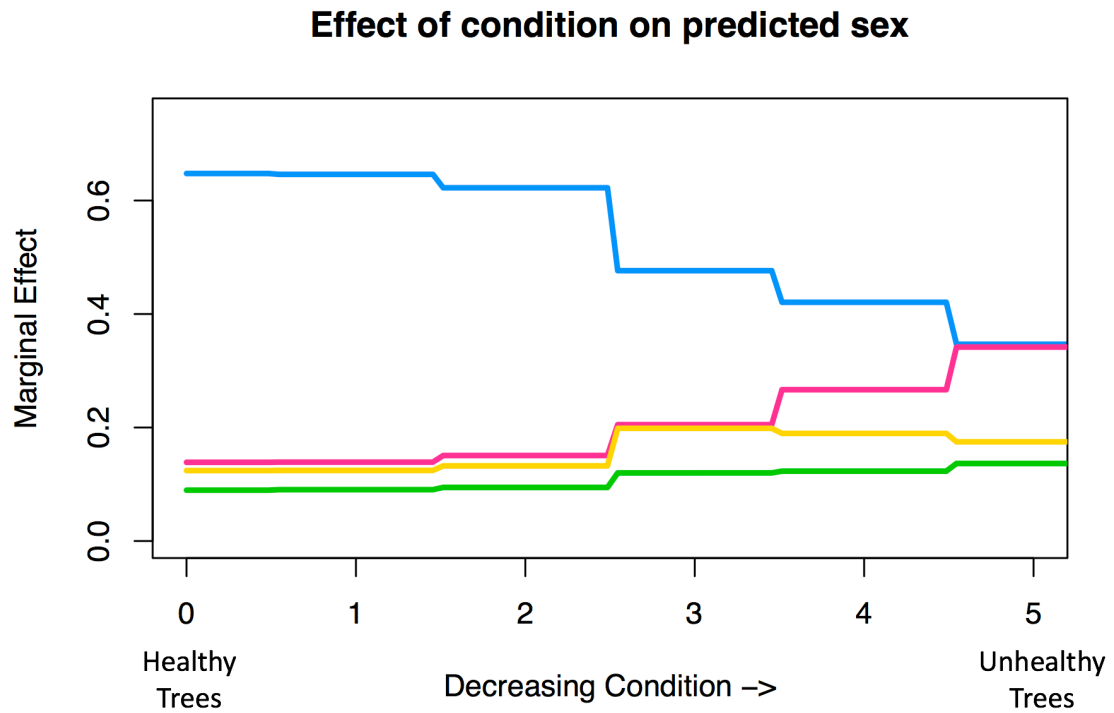
**Figure 2.** Sankey diagram of sexual transition in *Acer pensylvanicum* showing male trees in blue, female in pink, monoecious in yellow, non-reproductive in green, dead in grey. Flowering years proceed left to right from 2014 to 2017. Darker colored vertical bars under the years represent sex expression at that time, numbers in black correspond to number of individuals expressing that sex at that time. Curves between bars of the same color represent the fraction of trees that maintained that same sex expression. Curves take on color of second bar representing the sexual state that subset of trees are changing to. Sample size grows from 2014-2015 due to increases in study area. Sample size grows between 2015-2017 due to recruitment to the reproductive phase from previously non-flowering trees. Trees that did not flower during 2014-2017 are excluded from this analysis.

**Figure 3**

**Figure 3.** Bar chart showing relative influence of predictors in model for expressed sex in *Acer pensylvanicum*. Sexual state in year 1 has high influence in determining sex the following year. Reproductive states (female, monoecious) are more influential than non-flowering (non-reproductive). Condition is approximately three times more influential than size, both of which are more influential than a random predictor. Site is not influential.

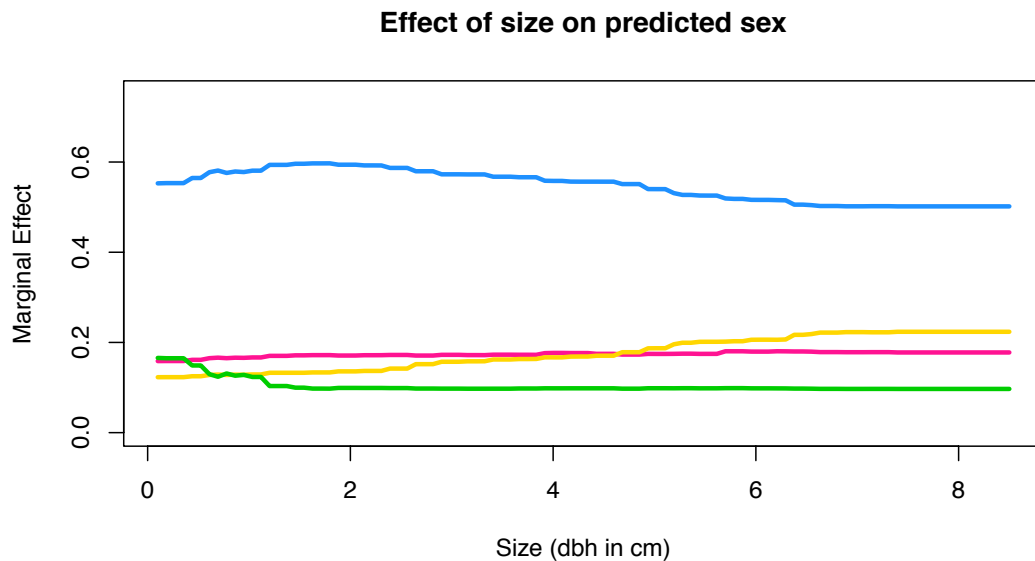
**Figure 4**

**Figure 4.** Stacked bar chart showing the predicted probability of a sex change in *Acer pensylvanicum*, by considering sex alone; that is, by holding constant the parameters of size, condition, and site. The sexual state in the initial year is on the x axis, the marginal predicted probability of a particular sex change is on the y axis. Colors represent the sex changed to. Females are represented in pink, males in blue, monoecious individuals in yellow, and non-reproductive trees in green. Flowering sex in one year strongly influences flowering sex in the next year.

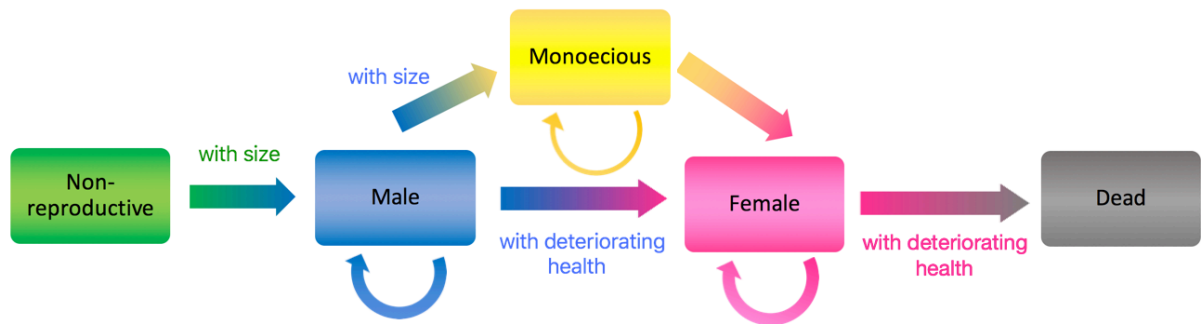
**Figure 5**

**Figure 5.** Showing the effect of poor condition (x axis) on the marginal effect of predicted sex (y axis) in *Acer pensylvanicum* with size, site, and starting sex held constant. Colors indicate predicted sexual state: females are represented in pink, males in blue, monoecious individuals in yellow, and non-reproductive trees in green. Females are over-represented in trees of poor condition.



**Figure 6**

**Figure 6.** The effect of increasing dbh size (x axis) on the marginal effect of predicted sex in *Acer pensylvanicum* by holding the parameters of condition, site, and starting sex constant (y axis). Colors indicate predicted sexual state: females are represented in pink, males in blue, monoecious individuals in yellow, and non-reproductive trees in green. At the smallest dbh sizes the model predicts approximately 55% male, 13% monoecious, 16% female, and 16% non-flowering trees, while at largest dbh sizes, the model predicts 50% male, 18% female, 22% monoecious, and 10% non-flowering.

**Figure 7**

**Figure 7.** A generalized pathway for sex expression in *Acer pensylvanicum* for the most common transitions in flowering. For specific changes, including reverse transitions, see **Fig. 2.**

### **Supplementary information**

**Table S1.** We evaluated tree condition using a modified tree assessment based on the forest services' hazard tree assessment protocol (Angwin *et al.* 2012). Low numbers (0-1) indicate trees in good condition with no or a minimal relative amount of damage; mid-range (2-3) scores indicate trees with moderate relative amounts of damage, high numbers (4-5) indicate trees with multiple kinds of heavy damage including large sections of standing dead biomass.

#### **Scoring of Condition**

<b>Score</b>	<b>Condition Description</b>
0	no visible damage: no branches missing, no discoloration of bark or wounds
1	slight damage of one kind: <10% of branches missing (only small); or small open cankers <.5cm in diameter (e.g. 5-10 for a medium sized tree, totaling <5cm open wounds); discoloration of bark due to black spotting
2	light damage of one or two kinds: 10-20% of branches missing (multiple small or 1-2 medium sized); small open cankers <1cm in diameter (e.g. 5-10 for a medium sized tree of 3 cm dbh, totaling 5-10 cm open wounds); small areas of splits or dead bark on trunk (e.g. <5 cm for a medium sized tree); discoloration of bark due to black spotting or other infection
3	moderate damage of multiple kinds: 20-30% of branches missing (multiple medium or one large); frequent open cankers (e.g. 10-20 medium and large cankers for a medium sized tree, totaling 10-25 cm open wounds); moderate areas of split or dead bark on trunk (e.g. 5-15 cm for a medium sized tree); discoloration of bark due to black spotting or other infection
4	heavy damage of multiple kinds: 30-50% of branches missing (multiple medium and large branches), frequent open continuous cankers (e.g. totaling 25-50 cm open wounds on a medium tree); substantial areas of split or dead bark on trunk (e.g. 5-40 cm for a medium sized tree); discoloration of bark due to black spotting or other infection
5	extreme damage and partial death: over 50% of branches missing of all sizes resulting in large portions of the tree being dead; substantial open continuous cankers covering >1/3 of tree area; heavy and frequent areas of slit or dead bark on trunk (e.g. >40 cm for a medium sized tree); discoloration of bark due to black spotting or other infection

**Table S2.** Sex of study trees prior to dying. Previously female trees experience mortality at much higher rates than expected given their prevalence in the population.

**Mortality by sex**

	Male	Monoecious	Female	Non-reproductive	Total
Observed mortality	7	4	53	7	71

**Table S3.** We evaluated the standard parameters used in computing BCTs – interaction depth, learning rate, number of trees, as well as bag fraction. Parameters used in the best fitting model are in bold.

**Boosted classification tree (BCT) model parameters and tuning values.**

BCT model parameter	Values evaluated
Interaction depth (i.e., tree complexity)	1, 2, <b>3</b>
Learning rate (i.e., shrinkage)	<b>0.001</b> , 0.005
Number of trees	<b>1000</b> to 7000, by 1000
Bag fraction	<b>0.5</b> to 0.9, by 0.1

**Table S4.** Model-predicted probability of sex in year 2, while holding the parameters of size, condition, and site constant. Data are represented graphically in text in **Fig 4**.

**Predicted marginal probabilities for sex the previous year as a predictor**

<b>Predicted sex</b> <b>Starting sex</b>	Male	Female	Monoecious	Non-reproductive
Starting Male	58.14%	14.54%	17.96%	9.36%
Starting Female	15.04%	61.49%	15.85%	7.62%
Starting Monoecious	17.91%	35.20%	37.37%	9.52%
Starting Non-reproductive	50.60%	13.45%	16.29%	19.65%

**Table S5.** Relative influence of predictors. Influence of categorical predictors (sex, site, and categorical random) are reported by category and may be combined to show the overall influence of the variable. In the text we combined uninfluential categorical variables (site and categorical random) into single variables for the sake of clarity (**Fig 3**).

**Relative influence of predictors**

<b>Predictor</b>	<b>Relative influence</b>
Sex (Female)	40.11%
Sex (Monoecious)	32.18%
Condition	17.38%
Size	6.17%
Sex (Non-reproductive)	1.82%
Continuous random variable	1.42%
Site (JJ)	0.38%
Categorical random variable (1)	0.22%
Site (WW)	0.11%
Site (STK)	0.10%
Categorical random variable (2)	0.07%
Categorical random variable (3)	0.03%
Site (HPR)	0.01%

**Table S6.** Classification matrix for predicted and observed data on sexual state of individual *Acer pensylvanicum* trees. Correct predictions are in diagonal grey cells.

Model testing data: n = 269; Model training data: n = 813 (not shown) A classification matrix compares the model-predicted data values with the actual observed values. Our model is most prone to over-predict “male” as an outcome when the actual observed sexual state is female, monoecious, or non-reproductive. The model was the least successful in predicting the sexual state of non-reproductive individuals.

**Classification matrix**

Predicted \ Data	Male	Monoecious	Female	Non-reproductive	<i>totals</i>
Male	170	17	19	11	217
Monoecious	1	9	4	0	14
Female	5	7	25	1	38
Non-reproductive	0	0	0	0	0
<i>totals</i>	176	33	48	12	269

### Chapter 3

#### **Death, sugars, and sex change: the relation of sexual plasticity and non-structural carbohydrate concentration in an understory tree**

Jennifer Blake-Mahmud<sup>1</sup>, Lena Struwe<sup>2,3</sup>

<sup>1</sup> Graduate Program in Ecology & Evolution, Rutgers University, 14 College Farm Rd.,  
New Brunswick, NJ 08901

<sup>2</sup> Department of Ecology, Evolution, & Natural Resources, Rutgers University, New  
Brunswick, NJ 08901

<sup>3</sup> Department of Plant Biology, Rutgers University, New Brunswick, NJ 08901

\* Corresponding author [Jennifer.n.blake@rutgers.edu](mailto:Jennifer.n.blake@rutgers.edu)

Blake-Mahmud ORCID: 0000-0002-6737-2824

Struwe ORCID: 0000-0001-6074-5758

Running head title: Non-structural carbohydrates and sexual plasticity

**Abstract:**

Sexual plasticity is a rare sex determination system in which individuals may switch sex expression throughout their lifetimes in response to environmental or developmental factors. It is common for individuals to bear more (or only) female flowers if the plants are larger, have greater access to limiting resources, or are in better condition. While theory predicts that resources internal to an individual should also affect expressed sex, few studies have sought to quantify this. Using a sexually plastic understory tree, *Acer pensylvanicum*, we analyzed the resource status of individuals in the form of stored non-structural carbohydrates (NSCs). We collected twig samples in 2014 – 2016 in December and May, and compared this with expressed sex. We found that females had higher sugar concentrations than males. Furthermore, males changing expression to female had higher sugar concentrations during the prior winter than did males that stayed male. This suggests that sex switching may be a consequence of resource availability. We also found that size was not a key predictor of sugar concentration: neither male or female-flowering individuals increased NSCs with size. Dying female trees had high concentrations of NSCs throughout the dying process and only manifested reduced NSC concentrations once completely dead. This suggests that the high mortality of females found in *A. pensylvanicum* populations is likely not a direct result of carbon starvation.

Keywords: *Acer*, dioecy, environmental sex determination, life-history, mortality, non-structural carbohydrates, physiology, hardwood forests



## Introduction

Life history theory seeks to quantify the trade-offs inherent in allocating resources between competing functions (Stearns, 1977). One of the most fundamental trade-offs in plants is between male and female function due to the energetic costs inherent in provisioning large numbers of fruits. Dioecious species have been used to quantify the costs of sexual dimorphism (Obeso, 2002), but may be problematic due to spatial segregation or differences due to secondary sex characteristics (Lloyd & Webb, 1977; Bierzychudek & Eckhart, 1988). Ideally, we might use a species that, while either male or female at any given time, is monoecious over the course of its lifetime, reducing the spatial and physiological differences that could influence resource availability or procurement.

This rare sex determination system, known as sexual plasticity, sex-choice, or environmental sex determination (ESD), is one in which environmental factors may influence the expressed sex of individuals during the course of their lifetime (Freeman, Harper, & Charnov 1980; Schlessman 1986; Korpelainen 1998; Renner 2014). Factors affecting sex expression may include changing local environmental conditions or individual-specific factors such as size, condition, or resource status (Charnov & Bull, 1977; Freeman, Harper, & Charnov 1980; Korpelainen 1998). Generally in species with ESD, females are found at larger sizes, in better microhabitats, or in better condition, due to the presumed higher cost of making fruits (Charnov & Bull, 1977; Freeman, Harper, & Charnov 1980; Korpelainen 1998).

While there are several potential currencies with which we can investigate reproduction and offspring provisioning (Obeso 2002); carbon is a useful currency due to

its importance in multiple areas of plant life including growth, reproduction, and the uptake of other resources (Harper 1985; Chapin, Schulze, & Mooney 1990; Kozlowski 1992). In long-lived tree species, carbohydrates are classified by their relative mobility and purpose. Non-structural carbohydrates (NSCs) consist chiefly of sugars (sucrose and fructose) and starch (Chapin, Schulze, & Mooney 1990; Kozlowski 1992). Understanding how trees allocate carbon resources between growth, reproduction, and survival is still intensively studied (Sala, Woodruff, & Meinzer 2012; Martinez-Vilalta et al., 2016; Hartmann & Trumbore, 2016). In particular, understanding why potentially long-lived organisms, such as trees, die has garnered much recent interest (McDowell 2011; O'Brien, Leuzinger, Philipson, Tay, & Hector 2014; Dietze et al., 2014; Sevanto et al 2014, Adams et al., 2017).

Main hypotheses for mortality include the carbon starvation hypothesis, the hydraulic failure hypothesis, or some combination of the two (McDowell et al., 2008). The carbon starvation hypothesis argues that death results when a tree has used up sufficient NSCs to impede metabolism, defense, and other processes necessary for survival and is unable to recover (Anderegg et al., 2012; N. McDowell et al., 2008). Decline following 'starvation' manifests itself as a slow decline of NSCs until death (McDowell et al., 2008; McDowell & Sevanto, 2010). The hydraulic failure hypothesis suggests that death is a consequence of severe, irreparable breaks in the continuous water column that normally extends from root to leaf, resulting from cavitation due to extended stomatal closure, infection resulting in blocked xylem, or physical damage of vascular tissue (Tyree & Sperry, 1989; McDowell et al., 2008; McDowell & Sevanto 2010). Under hydraulic failure we might see lower NSCs due to reduced opportunity to

photosynthesize, but also expect to see NSC concentrations dropping off precipitously in comparison to a drawn-out starvation (Sala, Piper, & Hoch 2010). More recent work has pointed out that these theories are not necessarily mutually exclusive and that mortality may result from a combination of the two (McDowell & Sevanto, 2010; Adams et al., 2017).

Despite observations that in species with ESD females are often larger than males, few studies have sought to quantify the internal resources available for individuals and the impact this has on expressed sex. In some species with ESD, internal resources might provide clues to understand changes in sex and how individuals allocate resources to male and female function over their lifetimes. Non-structural carbohydrate concentrations might also provide insight into sex-differential mortality, which is a hypothesized but untested potential cause of the puzzling non-equilibrium sex ratios prevalent in plant populations (Obeso 2002; de Jong & Klinkhamer, 2005; Sinclair, Emlen, & Freeman 2012).

In this study, we investigated the NSC concentrations found in a sexually plastic tree species, *Acer pensylvanicum*, e.g., or striped maple. *Acer pensylvanicum* is a small understory tree native to the Appalachian mountain range of North America (Hibbs, Wilson, & Fischer 1980). In contrast with other documented species with ESD, individuals may change sex throughout their lives (Hibbs & Fischer, 1979), making the switch within three weeks of flowering time (Blake-Mahmud & Struwe, 2018). Little support has been found for the size-advantage hypothesis in this species, with both large and small trees expressing the female sex (Hibbs & Fischer, 1979). Female mortality is quite high in *A. pensylvanicum*. Following a New Jersey (USA) population over the

course of three years showed that 73% of dead trees were female the year prior to their death, although females made up only 15-20% of the population. Male mortality is very rare (Blake-Mahmud & Struwe, 2016).

We set out to address the following questions.

1. To what extent does sex expression and change in sex expression correlate with concentrations of NSCs present in individual trees?
2. Are NSC concentrations different across different tree sizes, measured as diameter at breast height (dbh)?
3. How does NSC concentration correlate with mortality?
4. At what time scale does reduction in NSCs correlate with death?

Our predictions were:

1. Male trees changing to female would have higher NSC concentrations than those remaining male, and female trees maintaining female flowering would have higher concentrations of NSCs than non-changing male trees.
2. Within trees maintaining the same sex expression, concentrations of NSCs would increase with size.
3. Female trees that died would have lower concentrations of NSCs in the winter prior to death than surviving females, and female trees in the process of dying would have lower concentrations of NSCs in the spring compared to concentrations sampled from dying trees in the winter.

## Materials and Methods

### *Sampling*

Study sites were located in the following state forests and state parks in northern New Jersey, USA: Jenny Jump State Forest (40.913, -74.922, Warren County), Stokes State Forest (41.218, -74.720, Sussex County), High Point State Park (41.321, -74.662, Sussex County), and Wawayanda State Park (41.217, -74.451, Passaic and Sussex Counties). Previous work on *Acer rubrum* and *A. saccharum* has indicated that twig NSCs increase in concentration in the late fall and early winter (Hoch, Richter, & Körner 2003; Wong, Baggett, & Rye 2003). In this species, preliminary data suggests that medium to significant injury might also be a potential correlate of sex switching. *Acer pensylvanicum* trees are small; the collection of samples via coring would severely injure the trees, as could repeated sampling from multiple tissues. Root collection was not possible due to the rocky substrate. Because of this, we limited our sample collection to twigs to assess concentrations of NSCs and not overall pools.

Over a period of two weeks in mid-December of 2014 and 2015, we collected ten-centimeter long twig segments containing one bud from all *A. pensylvanicum* trees across study sites<sup>1</sup>. We selected apical stems without disease or visible injury such as cankers. Samples were placed in plastic vials and kept frozen in a cooler with dry ice for up to three days before being stored in a -80 C degree freezer. We used samples collected from

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<sup>1</sup> Because we could not foresee which trees might change sex and which trees would maintain previous sex expression, we collected samples from every possible tree in the study sites in December of 2014 and 2015. Trees that had no live twigs with apical buds within reach were excluded. In some cases, such as 2014 M→F trees, every collected sample for that transition was analyzed. For other groups, such as M→M trees, there were many samples to pick from, so the samples were stratified by size and site to match the M→F trees, then selected via random number.

different trees in each of the two winter periods to compare constant males with sex-changing males and to compare living females with dying females. To compare constant males and females and the effects of size on NSCs we used only samples collected in 2015.

During the flowering period in late April and May of 2017, we collected one ten-centimeter sample from each tree that was in the process of dying. Near death was indicated by lack of budding, lack of leaf out, and color changes in bark from green to deep purple. Samples were classified as being from dying trees at the time of cutting if they were still internally moist and retained a green-purple exterior hue on at least part of the sample. Samples were classified as dead if the twigs were internally dry with grey bark. All samples collected in May 2017 came from trees that were completely female and alive in August 2016. These sampled trees did not flower and were completely dead by August 2017. Mortality samples are independent: no individual is represented in more than one group.

#### *Non-structural carbohydrate analysis*

We quantified carbohydrate concentration by digesting starch and fructans enzymatically and using a colorimetric procedure to simultaneously quantify the total non-structural carbohydrate (digested starch and fructans; soluble glucose, fructose, and sucrose; Song, Yu, & Huang 2014). Samples were chosen for analysis post hoc based on expressed sex or mortality in the second year. In groups with excess numbers of samples, individuals were chosen via a random number generator. These selected twig samples were dried at 80 degrees before being pulverized with liquid nitrogen and sifted through a 425 $\mu$ m (#40) mesh.

We divided one sample from each individual into two subsamples and brought these through the colorimetric analysis separately using the procedure described by S. V. Ting (1956). Fifty-milligram ground twig samples were incubated in glass tubes with 2.5 ml 5% amylase at 37°C. After 24 hours we added 0.5 ml of 0.6 N HCL and continued incubation at 37°C for another 18 hours before adding 0.31 ml of 10N NaOH to adjust pH to between 5 and 7. We transferred the solution to round-bottomed flasks, brought the volume up to 50 ml with deionized water, then filtered the solution. One milliliter of solution was added to 1.5 ml alkaline ferricyanide and heated in boiling water bath for 10 minutes. After cooling in an ice water bath we added 3 ml of 2N H<sub>2</sub>SO<sub>4</sub>. to partially neutralize the solution. We added 1.2 ml arsenomolybdate and then deionized water to bring the volume to 25 ml. We measured absorbance at 515 nm using a spectrometer (Thermoscientific Evolution 201 UV-visible spectrophotometer; Thermo Fisher Scientific, Waltham, MA, USA). Total non-structural carbohydrate is measured in mg as the combined levels of glucose, fructose, and sucrose present, which includes both the soluble sugars and the enzymatically digested starch and fructans, per amount of sample in grams. Total non-structural carbohydrate analysis is a process sensitive to small fluctuations; but Quentin et al. found that the colorimetric approach was the most robust and least sensitive to experimental variation (2015). Although total NSC tests performed within a single laboratory can be compared, caution should be exercised when comparing concentrations ascertained from varying NSC analytic methods or laboratories (Quentin et al., 2015).

We conducted all analyses (T-tests, regression, and Anova) in JMP 13, graphs were created in R (version 3.3.1, Wickham 2009; R Core Team 2015).

## Results

A t-test comparison for male trees remaining male and male trees switching to female sex expression indicated that switching trees had significantly higher concentrations of non-structural carbohydrate present in their stems in the December prior to spring flowering (Figure 1a,  $N=24$ ,  $df=14$ ,  $p < .05$ , Cohen's  $d = .96$ ). Furthermore, there were differences in NSCs in trees maintaining their sex, with those maintaining female expression having higher concentrations of NSCs in December (Figure 1b,  $N= 28$ ,  $df= 26$ ,  $p < .05$ , Cohen's  $d = .81$ ).

We found that larger trees did not have statistically higher concentrations of NSCs, regardless of sex. Both male trees (Figure 2, linear regression,  $N=13$ ,  $df=7$ ,  $p > .05$ ), and female trees exhibited similar concentrations of NSCs across all size classes (Figure 2, linear regression,  $N=15$ ,  $df = 8$ ,  $p > .05$ ).

Regarding the question of NSC concentrations in trees prior to dying, we compared NSCs from samples collected in December of female trees that remained female (group:  $F \rightarrow F$ ) with female trees that were dead by the following May (group:  $F \rightarrow D$ ). We also compared NSCs from samples collected in May for dead and dying trees. Some trees were actively dying at that time (group: Dying), while others had died in the previous five months (group: Dead). All of these trees had flowered fully female in 2016 (Figure 3). Samples were also collected in May as trees were dying (Dying,  $n = 7$ ) or after trees were dead (Dead,  $n= 4$ ). Individuals were only sampled once during the collection period of 2014-2016.

We found that living female trees, and samples from dying trees collected both in December and in May had similar NSC concentrations (groups:  $F \rightarrow F$ ,  $F \rightarrow D$ , Dying).



Only samples collected from trees that were completely dead and dry had significantly lower NSC concentrations (Dead, ANOVA  $N=34$ ,  $df=3$ ,  $p < .01$ ). This group of trees that had been dead for 1-5 months (Dead) had significantly different values of NSCs from every other group (pairwise comparison,  $N=34$ ,  $df=3$ ,  $p < .01$ , Cohen's  $d$ :  $F \rightarrow F = 1.67$ ,  $F \rightarrow D = 1.47$ ,  $Dying = 2.11$ ).

## Discussion

We found that male trees later changing to female as well as female trees maintaining their sex expression from one season to the next had higher concentrations of non-structural carbohydrates than consistently male trees. While correlative, these data are consistent with the idea that trees require more carbon resources when initiating and maintaining female sex expression. Contrary to expectations, we found that total non-structural carbohydrate concentrations did not increase with size. Furthermore, NSCs did not decrease as individuals approached death.

Previous work on striped maples speculated on the causes for changes in sex expression. Both large and small individuals may express the female sex in any given year (Hibbs & Fischer, 1979; Blake-Mahmud & Struwe, 2016). Although the long-standing assumption was that NSCs decrease with tree height, more recent work has not confirmed that pattern (reviewed in Sala, Fouts, & Hoch 2011). The concentration of NSCs increases overall with tree size and age in *Pinus ponderosa* (Sala & Hoch, 2009), *Pseudotsuga menziesii* (Woodruff & Meinzer, 2011), and *Fagus sylvatica* (Genet, Bréda, & Dufrêne 2009). Other studies on *Quercus petraea* (Genet, Bréda, & Dufrêne 2009), or in saplings of *Abies balsamea* and *Acer rubrum* (Machado & Reich, 2006) indicated that whole plant concentration of NSCs may stay the same across different sized trees. Our

findings on *A. pensylvanicum* along a range of sizes (measured as dbh) coincides with these latter findings. We found that, like female sex expression (Hibbs & Fischer, 1979), carbohydrate concentrations do not correlate with tree size.

Studies linking ESD and internal resources are rare. Work on *Acer rufrinerve* and *Coptis laciniata* suggested that internal resources such as NSCs might be partially responsible for changes in sex expression, but did not investigate NSC concentration directly (Nanami et al. 2004, Lindh 2017). In *Arisaema triphyllum* and *A. japonica*, switches from male to female correlate with increased plant leaf area and corm size (Bierzychudek, 1982; Maekawa, 1924; Schaffner, 1922). Early experimental work found that the reduction of corm size of females (which does not naturally happen in the wild) led to reversals to male sex expression (Maekawa, 1924; Schaffner, 1926). Changes in sex expression also correlate with seed set, plant size, and defoliation (Bierzychudek, 1982), adding further inferential evidence for the importance of carbohydrates.

Our data show that the maintenance of higher carbohydrate concentrations in *Acer pensylvanicum* correlate with the maintenance of female flowering similar to larger corm size in *Arisaema* spp. However, we advise caution in drawing conclusions regarding the causative relationship between increased carbohydrate concentrations and female flowering. Whether increased non-structural carbohydrates trigger female flowering in *A. pensylvanicum* or whether both phenomena result from another environmental cue will require manipulative experiments to elucidate. Preliminary data from other studies indicate that declining health (poor condition) also correlate with female sex expression in this species (Blake & Struwe, 2017). It may be that injury impedes NSC mobilization which then cues changes in sex expression. It is also possible that increased NSC

concentrations lead to increased infection and herbivory which in turn leads to decreased physical condition. Further work is necessary to untangle the mechanistic and physiological pathways of sex expression.

Our findings support the idea that the high mortality associated with being female is not a direct result of resource depletion the months preceding death. Because of the role of NSCs in osmoregulation, it is possible that these carbohydrates must be maintained for other purposes, even though they cannot be used in metabolism and growth (McDowell & Sevanto, 2010; Sala, Fouts, & Hoch 2011). However, the minimum level of NSCs needed for survival is still up for debate (McDowell & Sevanto, 2010; Sala, Piper, & Hoch 2010; Martinez-Vilalta et al., 2016). In contrast to the gradual reduction of NSCs, a change that would support the carbon starvation hypothesis, it has been shown that NSC levels may increase for a while prior to death due to physiological and water stress (Sala, Piper, & Hoch 2010). In some cases it is only immediately prior to death that carbohydrates decrease to a level apparently incompatible with life (McDowell, 2011). Our results show that NSC concentrations are commensurate across living and dying trees, and do not decrease until after a tree is apparently dead, that is, with no leaves or photosynthetic pigments present in formally green bark. A logical hypothesis is then that hydraulic failure is responsible for triggering senescence in this species and precedes final carbon depletion. Of course, there are other potential causes for mortality (e.g., insect-related death, bacterial, viral, or fungal infection, all of which potentially impact hydraulic functioning in trees) that are not associated with NSCs per se (Noodén, 2004; Schwarze, Engels, & Mattheck, 2000). While we did not see evidence of extreme defoliation due herbivory, we cannot rule out mortality from infection or root

death.

In summary, as far as we are aware this is the first study that links non-structural carbohydrates with sex expression in a plant species with ESD. Changes in sex expression to femaleness and maintenance of female flowering clearly correlate with higher concentrations of sucrose and fructose, which is consistent with sex-allocation theory and the hypothesis that females need more resources. We found no support for the idea that using up these carbohydrate resources led to death, adding to mounting evidence that starvation is rarely the cause of tree mortality (Adams et al., 2017). The causes of increased female mortality in *Acer pensylvanicum* are still unknown, but hydraulic failure due to drought, infection, or root damage are potential candidates. Given the high mortality of females in this species, the long-term viability of populations depends heavily on reducing the frequency of death in females. More work will be done to understand the end-of-life population dynamics and how that affects the forest communities of which *A. pensylvanicum* is a part.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author contribution statement: J. Blake-Mahmud designed and conducted the experiments, collected and analyzed data, and wrote the manuscript as part of a doctoral dissertation. L. Struwe serves as the doctoral dissertation advisor.

Data accessibility: Data available from RUCore.

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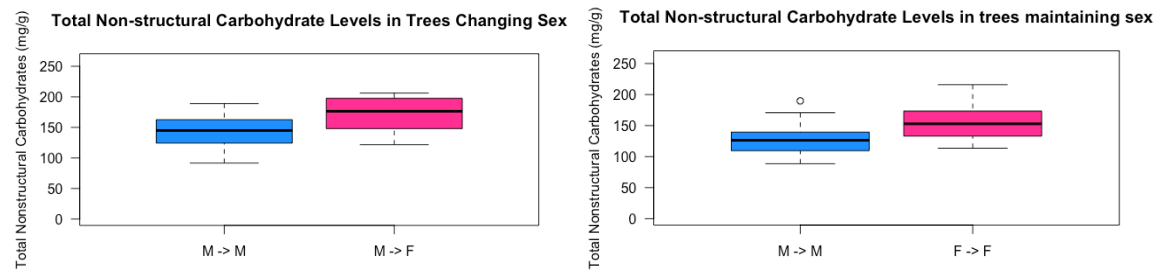


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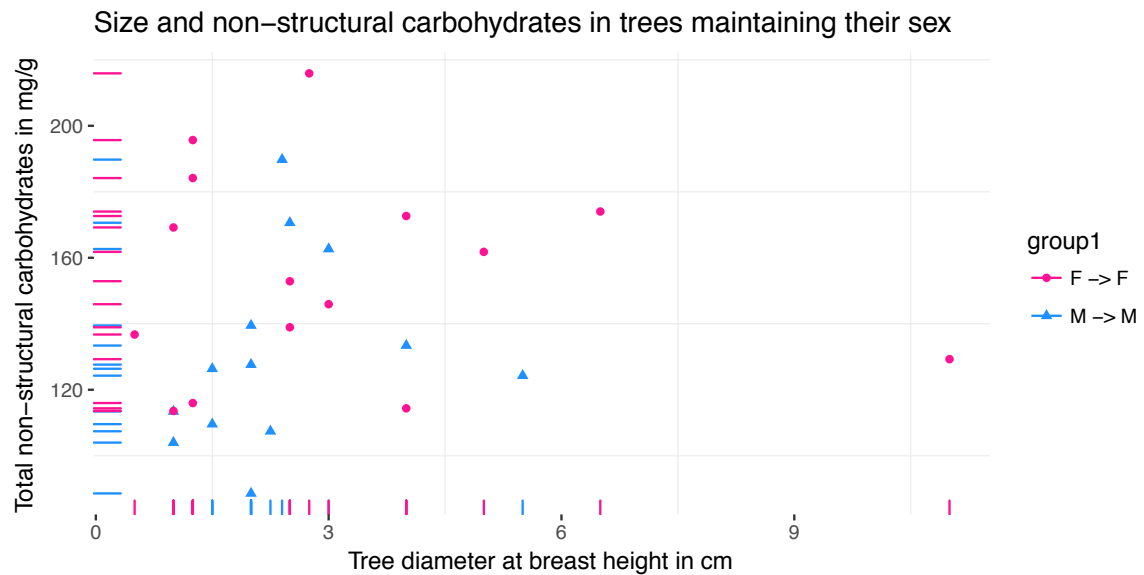
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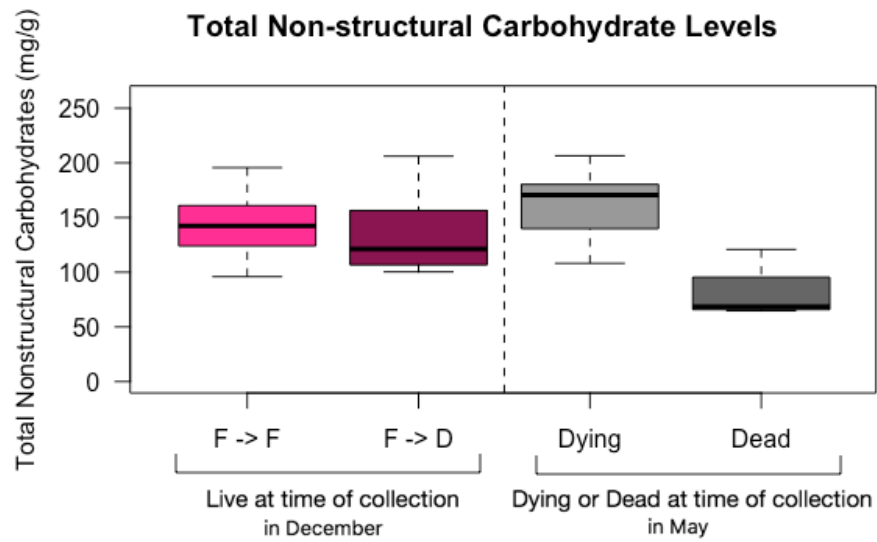
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**Figure 1 a and b**

**Figure 1a, left.** Comparing non-structural carbohydrate concentration (in mg sugar / g of plant tissue) the winter before flowering in male trees that remained male (n = 14) with male trees that switched to female (n=10). Samples were collected in 2014 and 2015. **1b, right.** Comparing non-structural carbohydrates the winter between flowering in consistent males (n =13) and consistent females (n =15). Samples were collected in 2015.

**Figure 2**

**Figure 2.** There is no relationship between size and concentration of non-structural carbohydrates (in mg / g), sampled the winter prior to flowering for trees flowering consistently male (blue triangles;  $R^2 = .007$ ,  $df = 12$ ,  $p = .40$ ,  $n = 13$ ) or for those flowering consistently female (pink circles;  $R^2 = .001$ ,  $df = 14$ ,  $p = .75$ ,  $n = 15$ ). Shaded areas represent the 95% confidence intervals; rugs along x and y axes indicate the distribution of points. Non-significant relationship for females is maintained even when two larger females are removed from analysis.

**Figure 3**

**Figure 3.** Showing non-structural carbohydrate concentration (in mg/g) for female trees that remained living and female (F → F, n = 16) and for female trees that died in spring and in late winter (F→D, n = 7). These samples were collected in December prior to any signs of death.

## Chapter 4

Title: When the going gets tough, the tough turn female: Injury and sex expression in *Acer pensylvanicum* (Sapindaceae)

Environmental sex determination (ESD) is the ability of individuals of a species to switch sex during the course of their lifetimes (Charnov and Bull 1977; Schlessman 1986; Korpelainen 1998; Pannell 2017). Sex is usually a consistently expressed characteristic of an individual and ESD is rare among plants, although found across taxa (Renner 2014). Environmental sex determination, also known as sexual lability, diphasy, sex choice, sexual plasticity, or sequential hermaphroditism, usually results in a one-time change in sex expression during the lifetime of the individual (Charnov and Bull 1977; Freeman *et al.* 1980; Schlessman 1986; Korpelainen 1998; Vega-Frutis *et al.* 2014). Two main models seek to explain why nature might select for sex-changing in individuals.

The size-dependent sex allocation model, first hypothesized in animal systems, posits how body size could affect the sex of an individual (Charnov 1982; Warner 1988). For species in which fitness is higher for one sex when an individual is small and another sex when the individual is large, selection should favor sex change at an intermediate size. For animal-pollinated plants, this often results in larger females (Charnov 1982; Klinkhamer *et al.* 1997; Korpelainen 1998; Cadet *et al.* 2004). Size-dependent sex allocation is a frequently cited trigger for ESD, but it does not apply in all species. For example, in *Acer pensylvanicum*, little support has been found for transitions in sex expression based on changes in body size (Hibbs and Fischer 1979; chapter 2).

The second “patchy-environment” model, hypothesizes another way of differential sex expression resulting from environmental variables that are unevenly distributed in time or space. This is often interpreted to mean that the increased availability of nutrients, light, or water, usually result in increased female sex expression (see Heslop-Harrison 1957; Freeman *et al.* 1980; Korpelainen 1998; Bachtrog *et al.* 2014 for reviews). The patchy-environment model may also be more generally interpreted to include factors specific to an organism such as individual energy reserves or the experience of physical trauma. These hyper-local environments, while crucial to understanding ESD in species with little spatial variation in sex expression, are understudied as proximate triggers of sexual plasticity.

It is possible to have cues that induce one sex in one species promote the opposite sex in another species. For example, while poor plant health generally correlates with maleness, the experience of physical trauma is more complex. In some species with generally constant sex expression, the removal of branches, stems, flowers or storage tissues promotes maleness; in other species similar actions promote femaleness, at least temporarily (Heslop-Harrison 1957). Research on the effects of trauma on sex expression in species with ESD is limited.

Here I examine the influence of physical trauma on sex expression in the sexually labile tree, *Acer pensylvanicum* (Sapindaceae) or striped maple. This species grows in the understories of hardwood forests along the Appalachian Mountain range. It prefers rocky soils and is well-adapted to shaded conditions (Hibbs *et al.* 1980). Individuals frequently incur damage from deer (through browsing and antler rubbing) and from falling canopy branches or whole trees. Striped maple populations are composed almost entirely of



plants who are either fully male or fully female, with a small percentage of trees bearing both male and female flowers either in separate or combined inflorescences (i.e., monoecy; de Jong 1976; Hibbs and Fischer 1979). Males outnumber females, possibly as a consequence of high female mortality (chapter 2, Appendix II). Previous studies have shown that in striped maple, deteriorating health correlates with increased female sex expression in individuals, and that branch excision from male trees leads to female-flowering branches (chapter 2 and chapter 5). However, there are no published causative links between physical trauma and sex expression at the individual level in striped maple or in related *Acer* species.

Through a series of damage manipulations I sought to address the following questions:

1. What are the effects of differing levels of physiological stress (caused by severe crown pruning, complete or partial defoliation, or hydraulic disruption) on sex expression in individuals?
2. Does physiological stress caused by defoliation or pruning have similar effects on trees of different starting sexes?

## **Methods**

### **Experimental**

My study sites are located in state forests and state park lands in New Jersey, USA :

Jenny Jump State Forest (40.913, -74.922, Warren County), Stokes State Forest (41.218, -74.720, Sussex County), High Point State Park (41.321, -74.662, Sussex County), and Wawayanda State Park (41.217, -74.451, Passaic and Sussex Counties). From 2015-2016, I conducted four experimental manipulations on separate individuals along a

gradient of intensity to elucidate the effects of physical damage and reduced carbohydrates on sex expression in *A. pensylvanicum*. These manipulations were: 1) crown pruning in May 2015, 2) full defoliation in July 2015, 3) hydraulic restriction through xylem and phloem removal in March 2016 and August 2016, and 4) partial defoliation in August 2016. I monitored the sex expression of manipulated and control trees in the flowering seasons of 2015, 2016, and 2017 using methods described in Appendix I and chapter 2.

### *Pruning*

In April and May of 2015, I selected 26 striped maple trees of 1.5-6.0 cm diameter at breast height (dbh), that were flowering male (10 trees), female (7 trees), or monoeciously (9 trees). These trees were confined to two of my sites: Jenny Jump State Forest and High Point State Park. These sites were selected due to the availability of larger trees and the relative flatness of terrain enabling safe use of pruning equipment. I used pole-pruners to remove every inflorescence and its associated pair of leaves. This resulted in extreme damage to the tree's crowns and left trees with very few, if any, leaves. These were compared to 26 control trees of similar dbh and starting sex from the same sites. Starting sex was calculated as the percent female sex expression by dividing the number of female inflorescences by the total number of inflorescences on the tree. This resulted in sex as a continuous predictor, with females as 1, males as 0, and monoeciously flowering trees taking values between 0 and 1. I chose to compute percent female rather than a phenotypic gender score (Lloyd and Bawa 1984) because the latter takes into account the sex expression of other trees in the population and is more useful in determining relative maternal and paternal contribution to the next generation than it is to

assessing relative allocation in flowering.

I monitored the sex expression and fate of these trees throughout the growing season of 2015, 2016, and 2017. Of the 26 pruned trees almost all of them put out new growth by the September following pruning, except for one female that died during those five months. I assessed the health, sex expression, and mortality of individuals in the springs of 2016 and 2017.

#### *Full defoliation*

In the summer of 2015 I selected 44 trees between 0.5 cm and 2.5 cm dbh from three sites (High Point State Park, Wawayanda State forest, Stokes State Forest). I selected only healthy trees that were either non-reproductive (did not flower) or flowered male in the previous spring. I excluded females from this manipulation because females of this small size are generally in poor condition. There were consequently not enough small, healthy females for a balanced study. Study trees were compared to 44 control trees of similar dbh and starting sex from the same sites.

Using scissors, I removed every leaf from each tree at the petiole. The fully defoliated trees were then monitored for the rest of the summer and fall. Of the 44 individuals, surprisingly, only 14 put out new leaves between July and leaf drop. Using branch samples collected the winter before and after defoliation, I conducted a non-structural carbohydrate assay using the method described in chapter 3. In the springs of 2016 and 2017 I recorded the health and sex expression of these trees.

#### *Hydraulic restriction*

In March of 2016 I selected male trees in one site (Jenny Jump State Forest) to examine the effects of experimentally reducing vascular connection on flowering via controlled

ringbarking (Pepper 2008). I chose one group of 14 trees with strong trunk forking. These Y-shaped trees allowed me to partially ring one branch while allowing the other branch to remain as control for that tree. Using a razor, I excised two strips of bark approximately 3 mm in width from 50% of the trunk diameter. The 50% of remaining bark was distributed in two patches, 25% on one side, 25% on the opposite side. The wound was covered in masking tape to inhibit infection. In August of 2016 I refreshed the wounds and recovered with masking tape. I assessed sex in spring of 2016 and 2017 using the protocol described in Appendix I.

In March of 2016 I also selected 36 unforked male trees to compare the effects of experimentally reducing vascular connection via 50% phloem removal and 50% xylem and phloem removal. I excised phloem as described above on eight trees. For xylem removal I excised an additional strip of woody tissue below the cambium before sealing with masking tape on ten trees. The removal of these outer rings restricts nutrient and water flow above and below the ringed area.

### *Partial defoliation*

Following the heavy mortality of trees fully defoliated in July of 2016, I performed a less drastic defoliation later in the 2016 growing season during August. I selected a new set of 22 single-sexed trees (12 female, 10 male) divided amongst two sites (High Point and Jenny Jump). Because of the logistics of defoliation (leaves are removed with scissors at the petiole) all trees were small, with dbhs between 0.5 and 2.0 cm. In striped maple, leaves emerge in opposite pairs along the branch; I removed one leaf from each pair across the entire tree for approximately 50% defoliation. If one leaf was noticeably less healthy (due to tissue necrosis or herbivory), that leaf was removed.

In cases where only single leaves remained, the leaf was left intact. The leaves were packed in paper sacks and dried in a drying oven at 50°C for three days for use in a different experiment (see Appendix V). In the spring of 2017 I assessed the sex of individuals using the protocol described in chapter 2.

## **Statistical**

For the pruning and defoliation analyses, I analyzed the data using multivariate binomial and multinomial logistic regression models. Analysis was conducted in R (version 3.4.1, R Core Team 2015) using the NNET package (version 7.3-12, Venables and Ripley 2002); the paired t-test and mosaic plots were analyzed and created in JMP pro 13 and sankey diagrams using SankeyMatic (Bogart 2016).

To examine the effects of full and partial defoliation on sex expression the following year, I conducted multinomial multivariate logistic regressions looking at the effect of site, size, treatment, and starting sex in 2015 on sex in 2017. For the pruning analysis, I first conducted binomial multiple logistic regressions to determine the effects of site, size, treatment, starting sex in 2015, and the interaction of sex and treatment on mortality in 2017. I then conducted a multinomial multivariate logistic regression looking at the effect of the same set of predictors on sex in 2017.

To examine the effects of reduced phloem connectivity, I used McNemar's test to test for consistency in sex expression in ringed and unringed branches. A McNemar's test is similar to a chi-square analysis and may be used for paired categorical data (McCrum-Gardner 2008). McNemar's test statistic with a Yates' continuity correction (Yates, 1934) follows a chi-square distribution and is computed as follows:

$$X^2 = \frac{(|b - c| - 1)^2}{b + c}$$

In this formula,  $b$  represents the number of individuals in state one in the control but state two in the treatment and  $c$  represents the number of individuals in state one in the treatment but state two in control. Those individuals with branches that express the same sex for both ringed and unringed states are not included in the computation of the test statistic (McNemar 1947).

I used Fisher's exact test (Fisher 1935) to compare the expressed sex of trees with phloem reduction, xylem and phloem reduction, and control trees.

## **Results**

### *Pruning Results*

I investigated whether the pruning treatment, starting sex in 2015, site, size, or the interaction of sex and treatment were significant predictors for survival to 2017 or sex in 2017. Size and site were excluded as they were shown to be non-significant in both the binomial model predicting mortality and the multinomial model predicting sex.

Mortality was high in the study group. Of the 52 trees, nine died between 2015 and 2016, an additional five died between 2016 and 2017. I first analyzed whether the odds of dying were related to the pruning treatment using a binomial multivariate logistic regression. I found that, both starting sex in 2015 and the pruning treatment had statistically significant effects on the odds of dying (see table 1,  $n = 52$ ,  $df = 49$ , residual deviance = 41.61).

The first analysis showed that pruned trees had odds 14.3 times higher of dying than did unpruned trees. Furthermore, for every one point increase in female sex expression, an individual had 1.03 times higher odds of dying. Effectively, a 25% increase in female flowering doubles the odds of dying. Fifty percent female flowering

increases odds of dying to 4.25, while a tree flowering 100% female has increased odds of dying of 18 times more likely to die compared to males flowering plants.

### The effect of pruning and sex on mortality

	Coefficient	Odds ratio (OR)	Lower Coef CI (2.5%)	Upper Coef CI (97.5%)	P-value
Intercept	-3.94	0.02	-6.18	-2.22	8.53 e-5 ***
Pruning treatment	2.66	14.29	1.09	4.60	.0024 **
Starting sex (percent female)	.029	1.03	.01	.05	.0018 **

**Table 1.** Control group is comparison group for treatment, live trees are comparison group for dead trees.

I then analyzed whether the pruning treatment, the starting sex in 2015 (measured as percent female), and their interaction had an effect on sex expression in 2017 using multinomial multiple logistic regression. I found that two years out, that all three variables had statistically significant effects on sex in 2017 (see Table 2,  $n = 35$ ,  $df = 12$ , residual deviance = 58.23). The effect of the pruning treatment completely separated the data along the non-flowering outcome, resulting in very small odds ratios. Of all the trees in the study, none of the pruned trees flowered completely male two years out (seven were female, two monoecious), and all of the non-reproductive trees in 2017 were from the pruned group (three non-flowering; see figure 1). For the interaction, differences arise in the upper and lower bounds of the confidence intervals only at 5<sup>th</sup> decimal place.

### The effect of pruning and (previous) sex on sex expression

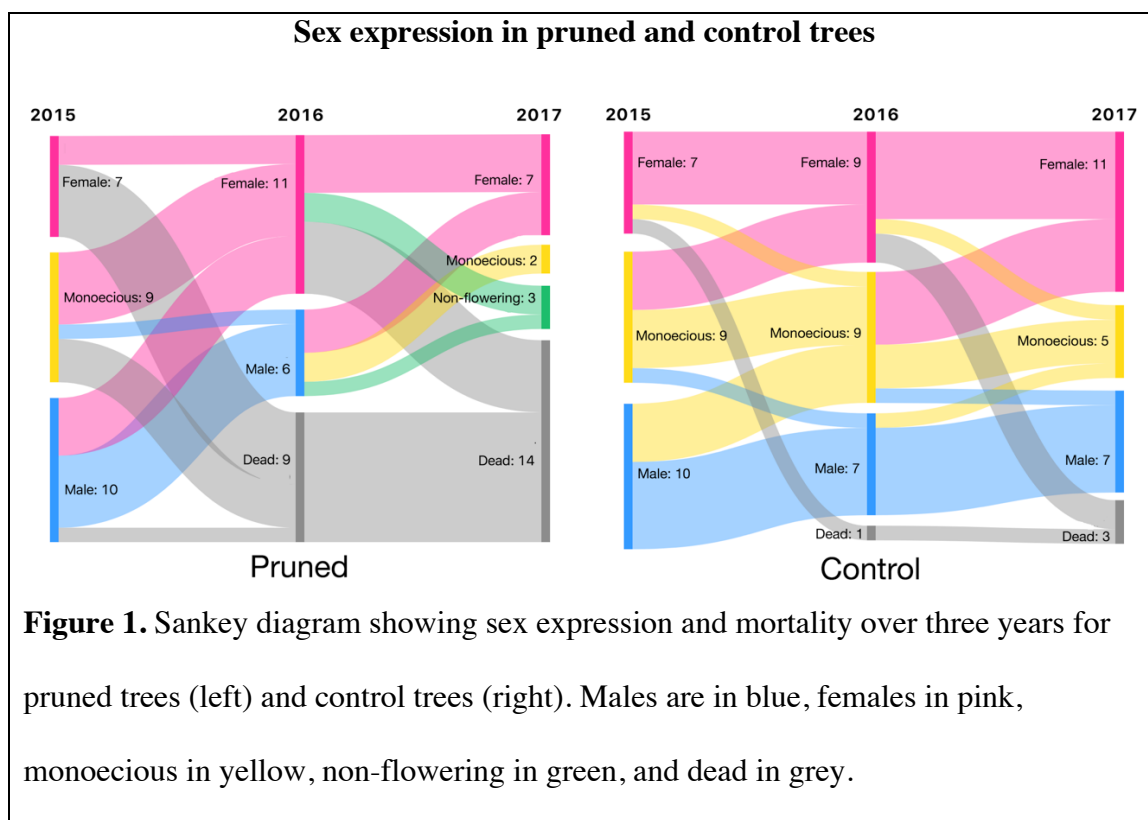
2017 sex	Coefficient	Odds ratio	Lower Coef CI (2.5%)	Upper Coef CI (97.5%)	P-value
Intercept					
Monoecious	-0.47	0.63	-1.80	0.86	P = 0.49
Female	-0.73	0.48	-2.13	0.67	P = 0.31
Non-flowering	-75.30	1.99 e-33	-76.03	-74.56	P= 0 ***
Pruning treatment					
Monoecious	84.28	4.01 e 36	83.03	85.53	P = 0 ***
Female	84.94	7.80 e 36	83.73	86.16	P = 0 ***
Non-flowering	159.51	1.89 e 69	158.78	160.25	P = 0 ***
Sex in 2015 (continuous; 0-1 scale)					
Monoecious	7.60 e-3	1.01	3.02 e-2	4.5 e-2	P = .694
Female	3.35 e-2	1.03	1.54 e-3	6.55 e-2	P = .0399 *
Non-flowering	-.24	.79	-.24	-.24	P = 0 ***
Interaction of treatment and sex					
Monoecious	-5.77	3.1 e-3	-5.77	-5.77	P = 0 ***
Female	4.38	80.1	4.38	4.38	P = 0 ***
Non-flowering	-4.5	1.1 e-2	-4.51	-4.51	P = 0 ***

**Table 2.** Male is comparison category for sex 2017; control is comparison category for treatment. The significant intercept parameter for non-flowering trees reflects that the non-reproductive outcome for unpruned males did not occur, yielding the extremely negative coefficient.

The pruning treatment had a significant impact on flowering sex in 2017 of female and non-reproductive trees compared to males (figure 1). All of these outcomes are much more likely than being male if a tree was pruned. Because the pruning treatment completely separates the data, odds ratios are extreme.



Sex in 2015 is also important (figure 1). For every 1 point increase in female flowering, odds of being non-flowering in 2017 decreased to 0.79. For every one point increase in female flowering, trees have increased odds of 1.03 of being female in 2017. Functionally, a 25% increase in female flowering corresponds to increased odds of being female in 2017 by 2.3. Fifty percent female flowering in 2015 increases odds of being female compared to male to 5.3, while a tree flowering 100% female has odds 28.6 times higher of being female than male in 2017. Effectively, any tree flowering even 1% female in 2015 had zero odds of being non-reproductive in 2017.



There is a significant interaction between sex and treatment. Compared to unpruned males, for pruned trees, every one point increase in female sex expression in 2015 decreases the odds of being monoecious or not flowering in 2017 to effectively

zero. All trees that were monoecious and non-flowering in 2017 had started off as male in 2015. For pruned trees, even a one point increase in female sex expression in 2015 ensures females flowering two years out.

### *Full defoliation*

I investigated whether the defoliation treatment, starting sex in 2015, site, size, or the interaction of sex and treatment were significant predictors for sex in 2017. Both size and site were shown to be non-significant; the model with lowest AIC contained only starting sex and treatment as predictors (see table 3).

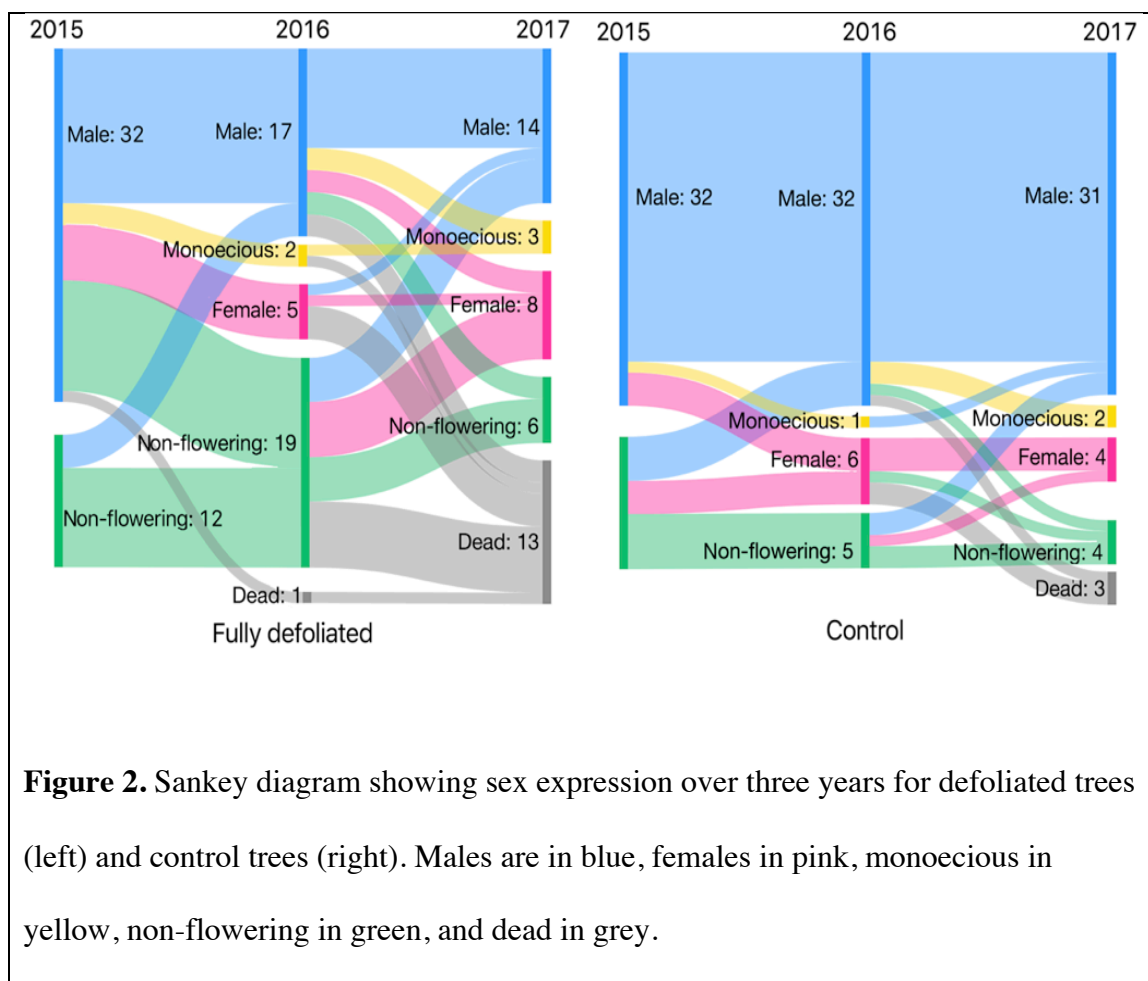
Compared to control trees two years after defoliation, treated trees had odds 9.6 times higher of dying than of being male and 4.5 times higher odds of being female than being male. Compared to male trees, trees that were non-reproductive have much higher odds (39 times higher) of remaining non-reproductive than changing to male (figure 2). In the non-structural carbohydrate assay I found the NSC concentrations significantly decreased following leaf removal (one-tailed paired T-test,  $N = 4$ ,  $df = 3$ ,  $p = 0.028$ ).

## **The effect of defoliation and (previous) sex on sex expression**

	Coefficient	Odds ratio (OR)	Lower Coef CI (2.5%)	Upper Coef CI (97.5%)	P-value
Intercept					
Monoecious	-2.502	0.082	-3.942	-1.063	P<.001***
Female	-2.139	0.118	-3.264	-1.015	P<.001***
Non-reproductive	-4.249	0.014	-6.504	-1.994	P<.001***
Dead	-2.345	0.096	-3.580	-1.111	P<.001***
Defoliation treatment					
Monoecious	1.153	3.167	-0.758	3.063	P>.05
Female	1.504	4.499	0.143	2.865	P<.05*
Non-reproductive	1.374	3.950	-0.292	3.040	P>.05
Dead	2.263	9.616	0.857	3.670	P<.005**
Starting sex (non-flowering)					
Monoecious	-10.759	1.041 e-5	-403.987	382.469	P>.05
Female	0.375	9.333	-1.187	1.937	P>.05
Non-reproductive	3.663	38.964	1.426	5.900	P<.005**
Dead	0.043	1.580 e-4	-1.538	1.624	P>.05

**Table 3.** Control trees are baseline comparison group for treatment, male trees are baseline group for starting sex. Intercept parameters reflect that the male outcome across both groups is significantly more frequent, with non-defoliated males most likely to remain male.

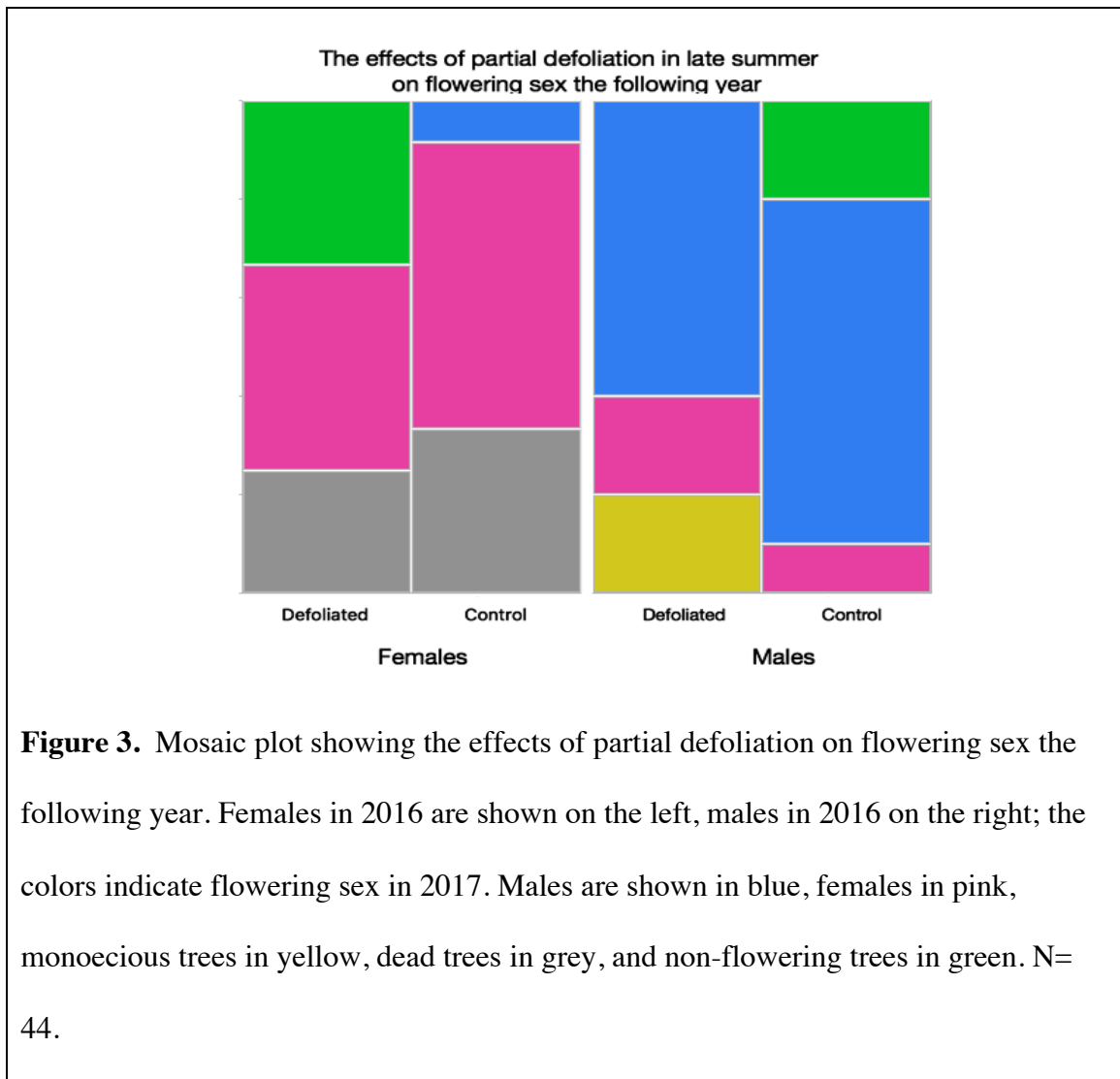
#### Sex expression in defoliated and control trees



### *Partial defoliation*

Of the models examining the effect of partial defoliation on sex, the best fitting model as determined by AIC, included sex in year 1 as the sole predictor. The model including treatment group had a higher AIC (+ 3.9 AIC) and non-significant p-values for the effect of treatment. Even with this much-reduced sample size, the effects of previous sex on subsequent sex follow patterns similar to those documented in chapter 2. Females have much higher odds of being female (52 times,  $p < .005$ ) or non-reproductive (26 times,  $p < .05$ ) than being male. Males are more likely to remain male. They have much lower

odds of flowering monoeciously (.15 times,  $p < .05$ ), completely female (.23 times,  $p < .05$ ), or not flowering (.15 times,  $P < .05$ ) compared to flowering male (figure 3).



### *Hydraulic disruption*

There was no effect of the initial 50% phloem removal in March of 2016. Thirteen trees flowered consistently male, one tree flowered monoeciously, with male and female inflorescences dispersed on both ringed and un-ringed branches. There was

no statistically significant effect of renewing the 50% phloem removal in August 2016 on flowering in 2017. Ten trees flowered consistently male on both control and treated branches, two flowered consistently female on both branches. Only two trees flowered monoeciously, with female flowers expressed on ringed branches, male on unringed ( $n = 14$ ,  $X^2$  with Yates correction = 1.135,  $p = .289$ ). There sex expression was not affected by vascular disconnection in trees that had only phloem removed or those with both xylem and phloem removed ( $n = 36$ ,  $df = 4$ ,  $p = 1$ ).

### **Discussion**

I conducted a series of damage manipulations along a gradient of intensity, from mild (hydraulic disruption and partial defoliation in late summer) through extreme (crown removal and full defoliation in mid summer) in striped maple trees. Only the more intense damage manipulations (full defoliation in high summer and crown pruning) impacted sex expression in subsequent years. In the latter two experiments, damage resulted in an increased prevalence of female sex expression and increased mortality, when compared to control groups.

Given the high mortality that resulted from the severe and extreme damage manipulations, it was surprising that the less severe treatments induced little response in individuals. These data point to the possibility of a threshold level of stress, below which the tree does not respond. Although reducing hydraulic connections in the tree by half has no effect, other studies have shown that complete hydraulic interruption does change the expressed sex of severed branches (Blake-Mahmud and Struwe 2018). Furthermore, although individuals in this species have the potential to respond to damage cues very quickly (Blake-Mahmud and Struwe 2018), these data show that the legacy of damage

persists and continues to affect sex expression in trees for two or more years.

The increased odds of female expression and non-flowering in trees that underwent severe crown pruning suggest that this kind of severe injury constitutes an important cue. The two trees classified as monoecious in 2017 provide interesting anecdotes that support this hypothesis. These two trees started as male in 2015. In 2016, all pruned sections of the tree were male; small shoots coming off the root had leaves, but no flowers. By 2017, the main trees that experienced pruning were entirely female; but the new basal shoots that had grown over the last two years flowered male. Looking at two years of sex expression data for pruned trees seems to indicate that severe damage pushes monoecious trees towards complete female sex expression. This does not prohibit trees from basal sprouting and adding male flowers in the future, but it does suggest one cause of sexual transitions between monoecy and femaleness. With complete defoliation, trees also had higher odds of being female.

Defoliation and pruning presumably have multiple physiological impacts likely involving the production of a hormonal cue signaling damage and the reduction in concentrations of NSCs, among other outcomes. Because of the effects of physical trauma in plants, damage is often confounded with biomass (and therefore resource) reduction. It can be difficult to separate the effects of these two potentially differing processes. In most treatments involving pruning, defoliation, and flower removal, the reduction in non-structural carbohydrates cannot be separated from the wounding process. However the results from this study show that females maintain *higher* concentrations of NSCs (chapter 3), lending support to the hypothesis that it is the damage cue, and not the NSC reduction, that is an important trigger for femaleness in

striped maple.

The damage response that affects flowering is likely ultimately triggered by hormone production. The effects of similar hormones vary drastically among taxa, so it is difficult to speculate on which hormones might be responsible without testing hormones explicitly. Several possibilities exist when thinking of these manipulations and the branch severance data in chapter 5. There could be a wounding hormone, produced at the location of the injury that moves up to buds or down to roots. Alternatively, there could be a bud hormone building up in the branch tips and cueing female flower development either through active promotion or lack of inhibition. It could also be a root hormone for maleness that is prevented from reaching the developing buds and either does not promote maleness or does not inhibit femaleness. Further work might investigate hormonal responses to injury and hormonal cues for

Other examples of the effects of trauma in plants show conflicting results. In some taxa, trauma causes shifts towards male expression. For instance, in *Arisaema triphyllum*, reduction of the underground storage corm or of leaf tissue causes increased numbers of plants to switch to male sex expression (Maekawa 1924; Bierzychudek 1984). Chronic herbivory in monoecious *Pinus edulis* leads to increased male sex expression (Cobb *et al.* 2002). Removal of female flower heads leads to increased male flowering in dioecious *Cannabis sativa* but increased female flowering in monoecious *Cleome spinose* (reviewed in Heslop-Harrison 1957). In *Zea mays*, trauma encourages female flowering and in *Carica papaya*, a dioecious species with sex chromosomes and without documented ESD, severe pruning of males can trigger female flowering. In *Mercurialis annua*, a subdioecous annual herb with sex chromosomes (Pannell 2017), pruning of males leads to temporary femaleness



before returning to male sex expression (reviewed in Heslop-Harrison 1957). Most of the aforementioned results derive from work done in species without demonstrated ESD and therefore shows the potential importance of trauma in *modifying* sex allocation between sexes or within hermaphrodites (Lloyd and Bawa 1984). Even when sex is known to be the result of sex chromosomes, as is *C. papaya* and *M. annua*, environment can play a modulating role and is perhaps responsible for the “leakiness” present in some plant sexual systems. The effects of damage in largescale changes between sexual phases (that is, in known species with ESD) has been limited to studies in *Arisaema* until now. This is the first experimental study of the effects of damage on sex expression in a woody species.

Over evolutionary time, the cueing of female sex expression by damage might provide a fitness benefit in male-skewed populations. With high numbers of males and low pollen limitation, a tree flowering female should have higher relative parentage of the next generation than if the same tree flowered male. If females indeed produce more offspring, it would make sense for trees that are past some health threshold to devote their resources to flowering female, even if it kills them. Shifting to female sex expression might provide increased reproductive fitness for trees with uncertain futures. Such differential mortality is a key player in the mortality-advantage model developed by Iwasa (1991), which predicts that the sex with lower mortality rates should be expressed initially, followed by intermittent periods of non-reproduction before switching to the higher-mortality sex. While under frequent investigation in animal systems, the influence of mortality on sex expression in plants has not been studied in plants (Iwasa 1991; de Jong and Klinkhamer 2005, but see Nanami et al. 2004) )

In conclusion, I have shown here that the hyper-local environment of an individual can inform understanding of sex expression and that severe damage is an important sex-determining factor in striped maple. These results support the idea that individuals experience a threshold-effect, with only the most severe levels of trauma triggering changes to complete female sex expression in males and monoecious trees. In the coming years we expect a globally warming climate to affect the incidence of extreme weather events and disease outbreaks (Rosenzweig *et al.* 2001; Dale *et al.* 2001; Whytlaw *et al.* 2017), both of which may increase the incidence of insect herbivory and physical damage in understory tree populations like striped maple. In the short term this may increase female expression in the population. However, given the high female mortality (see chapter 2) it is questionable whether striped maple populations will remain demographically stable over extended periods of time.

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## Chapter 5

### **Down to the wire: Late season changes in sex expression in a sexually labile tree species, *Acer pensylvanicum* (Sapindaceae)**

Jennifer Blake-Mahmud<sup>1</sup>, Lena Struwe<sup>2,3</sup>

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<sup>1</sup> Graduate Program in Ecology & Evolution, Rutgers University, New Brunswick, NJ  
08901

<sup>2</sup> Department of Ecology, Evolution, & Natural Resources, Rutgers University, New  
Brunswick, NJ 08901

<sup>3</sup> Department of Plant Biology, Rutgers University, New Brunswick, NJ 08901

\* Corresponding author [Jennifer.n.blake@rutgers.edu](mailto:Jennifer.n.blake@rutgers.edu)

Blake-Mahmud ORCID: 0000-0002-6737-2824

Struwe ORCID: 0000-0001-6074-5758

Running head title: floral development in *Acer pensylvanicum*

## Abstract

Species with environmental sex determination are rare amongst angiosperms but widely distributed across taxa. The timing of floral development in species that change sex based on environmental cues is unexplored. We investigated the timing of differentiation of sexual organs in buds of *Acer pensylvanicum*, an understory tree in eastern North America with environmental sex determination. We collected branches from individuals at three collection times in the early spring of 2016 and kept them in a warm greenhouse until anthesis. All individuals exhibited complete or partial female inflorescences in the greenhouse in one or more collection. However, none of these same individuals produced only female flowers in the field. Unlike many other woody species that differentiate bud sexual primordia nine to ten months prior to flowering, *A. pensylvanicum* may differentiate the sexual organs in its flower buds as late as three weeks prior to anthesis. In a separate series of branch collections, we found that the stress response to cutting leads to increased female sex expression in branches, while earlier warm temperatures (e.g., those caused by growing in a protected greenhouse environment) or increased carbohydrate availability does not. Given the labile sex determination system of *A. pensylvanicum*, the ability to delay differentiation of buds into male or female until shortly before spring flowering would allow individual trees to respond to sex determining damage cues as late as mid-spring. This supports the hypothesis that *Acer pensylvanicum* may not exhibit the lag times characteristic of temperate spring and early-summer flowering woody species and may change sex expression in response to stress.

**Keywords:** *Acer* - dioecy - environmental sex determination - flowering - phenology - sex expression



**Key message:** In sexually plastic *Acer pensylvanicum*, determination of sex can occur extremely late, within three weeks of spring flowering. Physical damage causing complete vascular tissue severance results in increased female expression.

## Introduction

Sex expression in perennial plants is usually a fixed characteristic over the lifetime of an individual, however, in some rare cases sex expression may be labile and change from year to year (Schlessman 1986; Korpelainen 1998). Although environmental sex determination is found within various angiosperm groups, few studies document the time scale on which changes in sex occur in the absence of applied hormones. Elucidating the phenology of floral sex expression, particularly in sexually labile species, provides foundational data for understanding the necessary temporal proximity of cues for triggering sex change. Changes in sex expression can have immediate effects on sex ratios (Charnov and Bull 1989), and longer-term effects on population viability (Sinclair et al. 2012).

Labile sex determination in plants (also known as environmental sex determination or ESD) may be affected by many environmental cues (Charnov and Bull 1977; Schlessman 1986; Korpelainen 1998; Bachtrog et al. 2014). These include abiotic factors such as nutrient availability or factors particular to a specific individual such as size, energy reserves, or the experience of physical trauma (Heslop-Harrison 1957; Freeman et al. 1980; Korpelainen 1998). As a general rule, when compared to male plants, female plants grow in the more advantageous microhabitats, are in better condition, have more access to resources, and manifest a larger size (Heslop-Harrison 1957; Charnov and Bull 1977; Freeman et al. 1980; Korpelainen 1998). In order to understand the proximity and magnitude of environmental cues that trigger changes in sex expression it is crucial to know when reproductive primordia in the flower buds

differentiate and complete development. Our study addresses this aspect as it is exhibited in *Acer pensylvanicum* (Sapindaceae) from eastern North America.

Floral development includes the whole process from bud initiation through differentiation of floral organ primordia and eventually anthesis, i.e. flowering (Sedgley and Griffin 1989). Here we define differentiation as determination of floral primordia identity into their eventual parts such as sepals, petals, stamens, or carpels (Fabbri and Benelli 2000) and final fate. While differentiation occurs for numerous flower parts, we are primarily interested in the development and fate of reproductive structures. In both sexually plastic and sexually fixed species, hormones induce meristematic tissues and trigger differentiation of sex in flower primordia and later development into sexual organs (Bernier 1988). Earlier work on physiology and phenology in northern temperate deciduous trees that flower in spring and summer seasons shows that bud induction (initiation of development) and differentiation (determination of floral primordia identity and subsequent development) often occur 9-12 months prior to flowering (Sedgley 1989; Fabbri and Benelli 2000; Wilkie et al. 2008).

Woody species are known for their lagged responses to environmental cues so that in some situations the effects of disturbances are not manifested for years (Davis, 1986; Kozlowski et al., 1991; Hogg 1997; Mohan et al., 2009; Svenning and Sandel, 2013). If the result is to be seen in a particular year, a cue must occur prior to the end of a sensitive period. For individuals to respond to sex-determining triggers, stimuli must occur prior to sexual organ differentiation in flower buds. If sexually labile woody plants differentiate flower buds the summer prior to flowering, only environmental triggers *prior* to the differentiation period in the previous season would be reflected in the next

flowering year, leading to response lag-times of one to two years for trees with ESD.

Conversely, if sexually labile species differentiate the sexual organs of flower buds closer to anthesis, then the lag in response to sex-determining cues will be much reduced.

To address the question of bud development in sexually labile woody perennials, we used the sexually plastic species *A. pensylvanicum* (Sapindaceae) (Hibbs and Fischer 1979). This species, commonly known as striped maple in the United States, is a small understory maple tree found in rocky soils at higher elevations along the Appalachian Mountains (Hibbs et al. 1980). It is a subdioecious species, with most of the individuals bearing only staminate or pistillate inflorescences in a given season. Less than five percent of a population may express both male and female inflorescences on the same tree (Hibbs and Fischer 1979) and, very rarely, male and female flowers in the same inflorescence (de Jong 1976; Blake-Mahmud and Struwe 2016). Male flowering individuals outnumber female individuals by five to one (Hibbs and Fischer 1979). Environmental cues for sex switching are currently under investigation. Anecdotal evidence attributes the switch in expressed sex to crown closure in canopy trees (Hibbs and Fischer 1979), or to resource status in a related *Acer* species native to eastern Asia (Nanami et al. 2004).

In *A. pensylvanicum*, functionally unisexual flowers are arranged in pendulous, racemose inflorescences. The flowers are yellowish green, with free, linear-lanceolate to obovate calyx lobes and a campanulate corolla up to 5 mm long and 8(-11) mm wide with obovate corolla lobes. In the small fraction of individuals flowering monoeciously (i.e., bearing both staminate and pistillate inflorescences), there are occasionally inflorescences that also bear both staminate and pistillate flowers. These bisexual inflorescences are

usually found in a transition area between male and female flowering zones. These do not occur on all monoecious trees, nor do they appear to occur on trees without both male and female unisexual inflorescences. Although some male flowers are observed to contain a highly reduced abortive pistil (de Jong 1976), this has been found to be non-receptive, therefore these flowers are functionally male (Sullivan 1983). While de Jong (1976) reported rare fully-developed morphologically perfect flowers on cultivated trees, subsequent observations in the field have failed to find any morphologically or functionally perfect flowers (Hibbs and Fischer 1979; Sullivan 1983, Blake-Mahmud and Struwe 2016).

Over the course of two years we investigated the following questions in a combined forced flowering greenhouse and field study:

1. What is the timing of sex differentiation in *A. pennsylvanicum* buds? Do branches excised from trees consistently express the same sex throughout flower development in a given year?
2. What is the impact of environmental and physiological cues on flowering sex, specifically earlier warm temperatures, damage via cutting, and increased sugar availability?

## **Materials and Methods**

To assess the timing of sex determination, we used trees from our study site located in Jenny Jump State Forest (40.913, -74.922 Warren County, New Jersey, USA). Over a period of seven weeks in the late spring of 2016, we collected branches from 35 *A. pennsylvanicum* trees of unknown sex, with diameters at breast height (DBH) of 1-3 cm.

Sampled trees were in good condition, had not lost branches, and displayed no visible infection, cankers, or other wounds. We collected two branches from each tree every two weeks on March 8<sup>th</sup>, March 23<sup>rd</sup>, and April 6<sup>th</sup> (group one, two, and three, respectively). Based on previous pilot studies conducted in 2015, we had narrowed the window of bud differentiation to less than six weeks prior to blooming (Blake and Struwe 2015). Collected branches were tagged with individual numbers and collection dates and kept in a cooler with ice until they were brought to a research greenhouse in New Brunswick, NJ. In the greenhouse, we recut the stems under water and placed them in a mildly acidic water solution, randomly assigned into one of two replicates. The water solution contained tap water, 1.5 ml 3% bleach, and .32 g granulated citric acid per liter of solution for a pH of 3.5 (16.7 mM citric acid). Water was kept in translucent brown glass bottles to reduce microbial growth and was not changed during the seven weeks of the experiment. Bottles were kept in a random design (blocked by collection time) away from artificial greenhouse lights. Temperatures in the greenhouse approximated ambient warm spring temperatures of the study site (10-12 °C at night, 20- 22 °C during the day). Branches remained under full shade and flowered approximately 14-28 days after collection. Branches that did not develop flowers were excluded from the study. Trees at Jenny Jump State Park began to flower the week of April 26<sup>th</sup> 2016. We scored the sex of the trees from which we collected branches and counted the number of female and male inflorescences per individual. As the individual trees were relatively small, all inflorescences were visible without binoculars.

To address the question of triggering cues we used trees located in our other study site at Wawayanda State Park (41.210, -74.464, Passaic and Sussex Counties, New

Jersey, USA). In early April of 2017 we collected branches from 40 *A. pensylvanicum* trees of unknown sex, 1.0-5.5 cm DBH. We collected six branches from each tree between April 5<sup>th</sup> and April 7<sup>th</sup>, during a time when we would expect to see variation in flowering sex, based on previous data (Blake and Struwe 2015). In 2017 anthesis in the field was slightly later than anticipated, due to cold periods in April. Collected branches were put in one of three treatments: plain water in the field, sugar water in the field, and plain water in the greenhouse. “Plain” water solutions consisted of the same water mixture used in 2016 (i.e., water, 1.5 ml 3% bleach, and .32 g granulated citric acid per liter of solution for a pH of 3.5). The sugar water solution consisted of water, citric acid, bleach, plus sucrose (40g granulated sucrose, 1.5 ml 3% bleach, and .32 g granulated citric acid per liter water, 117 mM sucrose). Every plastic bottle contained two branches from each tree and was painted black to inhibit microbial growth. Field bottles were covered with parafilm to prevent unintended additions from rainwater or leaf litter and tied securely to the base of the tree from which the branches were taken. Two branches per tree were tagged and kept in a cooler with ice until they were brought to a research greenhouse in New Brunswick, NJ. In the greenhouse, we recut the stems under water and placed them in the plain water solution in black plastic bottles. Temperatures in the greenhouse approximated the ambient warm spring temperatures of the study site (10-12 °C at night, 20- 22 °C during the day). Branches remained under full shade and flowered 14 to 24 days after collection. Branches that did not develop flowers were excluded from the study. Trees at Wawayanda State park began to flower the week of May 1<sup>st</sup> 2017. We scored the sex of the trees from which we collected branches and counted the number of female and male inflorescences per individual.

We used McNemur's test to test for consistency in sex expression in treatment and control groups as well as consistency in flowering sex expressed in the greenhouse and field. Unlike a chi-square analysis, McNemur's test may be used for paired categorical data (McCrum-Gardner 2008). McNemur's test statistic with a Yates' continuity correction (Yates, 1934) follows a chi-square distribution and is computed as follows:

$$X^2 = \frac{(|b - c| - 1)^2}{b + c}$$

In this formula,  $b$  represents the number of individuals in state one in the control but state two in the treatment and  $c$  represents the number of individuals in state one in the treatment but state two in control. Those individuals that are in the same state (in this case, expressed sex) in both control and treatment are not included in the computation of the test statistic (McNemar 1947).

Voucher specimens are deposited in the Chrysler Herbarium (CHRB) at Rutgers University in New Brunswick, NJ, USA.

## Results

### *Timing of flower development - experiments in 2016*

Of 35 trees, seven did not flower in the field or had collected branches that produced only leaves and were excluded from the study. Each of the remaining 28 trees had at least one collected branch that bloomed fully or partially female in the greenhouse, while all of the trees bloomed partially or completely male in the field. Collected branches showed only 50% fully male sex expression when collected as late as three weeks prior to anthesis.

Of the 28 study trees, sampled branches from 19 individuals produced flowers



following the first collection of branch segments (group 1) in March (Table 1). All 19 flowered female on one or both of the two branches collected in this initial sample; one individual (tree 26) had one branch that bloomed male and one that bloomed female. Of the branches collected as part of the second sampling in March (Group 2), branches from 23 trees (out of 28) produced flowers. From this collection, 74% of those individuals produced only female inflorescences and 17% produced only male flowers. Two individuals flowered differently, with male flowers and female flowers produced on different branches in different replicates. From the third and last collection in early April, 43% of individuals flowered female, 48% male, and 9% of individuals produced inflorescences including both male and female (but no perfect) flowers. Interestingly, the two individuals producing inflorescences with both staminate and pistillate flowers in the greenhouse (trees 17 and 21) were not among the three trees flowering monoeciously in the field. When study trees flowered in the field, 25 of 28 flowered exclusively male, while three trees flowered monoeciously, bearing male inflorescences, female inflorescences, and inflorescences with both male and female flowers.

We did not detect a significant difference between the two replicates in any collection group (group 1: McNemur's  $X^2 = 0$ ,  $p > .05$ ,  $df = 1$ ; group 2: McNemur's  $X^2 = 0.5$ ,  $p > .05$ ,  $df = 1$ ; group 3: McNemur's  $X^2 = 0$ ,  $p > .05$ ,  $df = 1$ ). Because of this we combined data across replicates (Table 1) to give a more complete picture of floral development. Data divided into replicate groups are available online (see online resource 1).

We found that branches kept in the greenhouse environment began by presenting female inflorescences before trees reached anthesis in the field. Later and closer to their

natural flowering time in the field, approximately half exhibited inflorescences with male reproductive structures (see Table 1). Furthermore, all trees that had early-flowering female inflorescences in the greenhouse flowered male (partly or exclusively) in their natural forest habitats. Of the 28 trees that flowered, nine differentiated sex as late as three weeks before flowering, while 32% of trees differentiated their buds less than three weeks before flowering. The mean time for trees determining sex expression was approximately four and half weeks prior to anthesis (Table 1). Results from McNemur's test showed that collected branches bloomed differently in the greenhouse than they did in the field at each collection (group 1: McNemur's  $X^2 = 15.06$ ,  $p < .001$ ,  $df = 1$ ; group 2: McNemur's  $X^2 = 12.07$ ,  $p < .001$ ,  $df = 1$ ; group 3: McNemur's  $X^2 = 7.01$ ,  $p < .01$ ,  $df = 1$ ).

*Cues for flower development – experiments in 2017*

In 2017 we collected branches from 40 different trees from a different site to investigate cues for changes in sex expression. Of 240 branches collected, 160 were kept in the field until anthesis. A large proportion of these field-bottles were disturbed by wildlife and destroyed. The data resulting from the fraction of branches that remained in bottles until anthesis is presented here. Of the 40 trees, 24 flowered male in the field, eight flowered female in the field, five exhibited both male and female flowers, and three did not flower. While populations were still overwhelmingly male, the increased proportion of female trees in 2017 is consistent with sex ratios across a larger sample of multiple populations. Responses of excised branches are summarized in Table 2.

To examine the effect of earlier warmer temperatures, we made a pair-wise comparison of flowering specimens kept in the greenhouse with surviving branches kept in the plain water in the field ( $n = 15$  pairs available for comparison, see Table 2). All of

these co-flowering specimens expressed the same sex (female). These results support the conclusion that earlier, warmer temperatures do not affect flowering sex.

To examine the effect of increased carbohydrates, we compared flowering specimens kept in the field in plain water and in sugar water (n=14 comparisons, see Table 2). Of these, branches from 13 individuals flowered completely female in both treatments, one individual flowered female in plain water and monoeciously in sugar water, and one individual flowered female in the plain water and male in the sugar water. This lends no statistically significant support to the theory that increased carbohydrates affect flowering (McNemur's  $X^2 = 0$ ,  $p > .05$ ,  $df = 1$ ).

To examine the effect of the shock administered by cutting, we compared intact trees with flowering specimens kept in the plain water in the field (n=17 comparisons, see Table 2). Nine trees flowered fully or partially female in the field (five female, four monoecious) while all of their branches kept *in situ* flowered female; eight male-flowering trees had branches in the field that flowered female. This supports the conclusion that removing branches from a tree through cutting had a significant effect on flowering sex of the branches (McNemur's  $X^2 = 6.125$ ,  $p < .05$ ,  $df = 1$ ) and was the crucial cue triggering early female flowering of branches collected over a six-week period in 2016.

## Discussion

Our results show that the determination of sex expression in flower buds can occur within three weeks of flowering and is strongly influenced by physical damage in *A. pensylvanicum*. The 2017 findings on sex determining cues are consistent with the hypothesis that physical damage triggered the female sex expression seen both in cut

branches in the field in 2017 and in the greenhouse in 2016. The 2016 experiment indicates the speed at which these cues can be processed by the tree. The late timing of sex determination in *A. pensylvanicum* is in stark contrast to longer timescales found in many other woody species with constant sex expression, and some other species in *Acer*. When combined with the labile sex determination system of this species, the late differentiation of buds suggests that sex expression in *A. pensylvanicum* may be influenced by injury caused by storms and extreme weather events at a speed unusual for woody plants.

#### *Timing of flower development - experiments in 2016*

Ideally, studies addressing flower development use histological methods, but sometimes this is not possible. For example, in *Acer pensylvanicum*, tough hairs present within the inflorescence buds prevent traditional sectioning via conventional anatomical histology procedures such as paraffin embedding, preventing the investigation of sexual development prior to flowering (J. Blake-Mahmud, unpubl. data). Furthermore, Sullivan found that flower bud sex could not be determined using dissection techniques (Sullivan 1983). In these types of situations, plant phenology and flower development may be addressed via twig studies in which dormant twigs or small branches are collected and allowed to flower in a greenhouse. In sexually stable species (no ESD), the flowering behavior of cut branches parallels the behavior of trees in the field (Vitasse and Basler 2014) and may be used to explore changing plant phenology in response to particular climatic drivers such as earlier warmer temperatures or reduced chilling (Primack *et al.*, 2015). De Jong addressed the response of floral sex and timing to various plant hormones of selected *Acer* species by collecting branches over the winter and forcing them into

flower via applied hormones (gibberellins). He associated the changes in flowering in the greenhouse with corresponding development of floral primordia. Samples that bloomed consistently as one sex were assumed to have differentiated their buds prior to the first collection, while those that changed flowering sex were differentiating their sexual structures during the collection period. While his study used only one to two individuals per species, it provided anecdotal evidence regarding the complexity of flower development in maples and showed that development spans a wide temporal range. In cultivated trees in the Netherlands, *Acer* flower buds are generally initiated in June or July, approximately eight to nine months before flowering (de Jong, 1976). For example, in *A. rubrum*, buds begin development as perfect (bisexual) flowers for the first two months, before differentiating into male and female buds at the end of August (de Jong 1976). In *Pistacia vera* (*Anacardiaceae*, also classified in Sapindales like *Acer*), unisexual buds differentiate reproductive primordia at approximately the same time, roughly 10 months prior to flowering (Hormaza and Polito 1996).

There are some previous observations of late flower differentiation in *Acer* and related species. For example, sexual determination in buds of *A. platanoides* occurs four weeks before blooming (Haas 1933). *Acer platanoides* is monoecious with heterodichogamous flowering (Renner et al., 2007), meaning that every tree bears both male and female flowers and does not change sex expression. Given the occurrence of both staminate and pistillate flowers on every individual in a single flowering year in *A. platanoides*, it is not unexpected that sex could remain flexible until shortly before anthesis as this is a way to adjust maternal investment and energy expenditure in monoecious species (Lloyd 1980). De Jong (1976) found a similar response with a single

individual of the sexually labile *A. davidii* subsp. *grosseri*, with sampled branches blooming first female in the presence of added gibberellic acid, then male in a greenhouse before blooming monoeciously in the field. In *Kirkia wilsonii* (Kirkiaceae: Sapindales), trees flower in successive unisexual flushes so that a tree is functionally dioecious at any one time but monoecious over a 40 day flowering season (Immelman 1984). Observations of herbarium specimens indicated flower buds were the opposite sex of the preserved open flowers. The author did not investigate when trees initiated buds nor when buds differentiated sexual primordia (Immelman 1984).

In *Acer pensylvanicum*, however, approximately 95% of trees growing in their natural habitats flower with unisexual inflorescences of a single sex in a given year (Blake-Mahmud and Struwe 2016; Hibbs and Fischer 1979). The plasticity of flower sex not induced by addition of artificial hormones so close to blooming time is highly unusual for a functionally dioecious, sexually plastic species, and thus notable in comparison to results observed in other woody species with separate sexes. Future work might employ other methods, such as a scanning electron microscopy, to address the timing of initiation of bud primordia, which cannot be ascertained via branch studies.

In other temperate taxa such as *Malus*, buds for the next year differentiate approximately six weeks after the current-year's flowering (Buban and Faust 1982, see Figure 1). Other trees also begin bud development and differentiation the previous year and well in advance of flowering: *Diospyros kaki* in early June (Yonemori et al. 1993) and *Prunus avium* in July to August (Guimond et al. 1998). In temperate trees with separate unisexual male and female inflorescences, buds will often differentiate at different times, usually with staminate buds preceding the development of pistillate buds

(Longman and Coutts 1974). For example, *Quercus alba* differentiates staminate buds in late May through July; pistillate buds develop in August of the previous year (Longman and Coutts 1974; Lavender 1986). In dioecious *Carya illinoensis*, all female and male buds differentiate in May for the following flowering year, but flowers develop at different times. Following sex differentiation in *C. illinoensis*, male buds continue to develop that year while female trees arrest bud development until late winter. Buds complete the development of female reproductive structures during the last week of February (Woodroof and Chapman Woodroof 1926; Wetzstein and Sparks 1986; Wetzstein 1989, See Figure 1). In *Juglans regia*, male primordia initiate in May the year prior to flowering, while female primordia initiate in August. Both male and female buds are dormant during the winter before completing development in March and flowering in April (R. Lin, personal communication).

Results from studies of *Carya*, *Juglans*, and previous work on *A. pensylvanicum* (deJong 1976) might suggest that male flowers develop first due to earlier initiation of stamen primordia compared to gynoecial primordia. This, however, was not the case. Our findings also illustrate that buds maintain a totipotency to express either sex until shortly before flowering. Even though almost all individuals express only one sex in the field, trees maintain the capacity to express both sexes sequentially under the experimental condition of earlier anthesis.

#### *Cues for flower development – experiments in 2017*

The influence of branch excision on female sex expression is the first experimental evidence linking damage and expressed sex in *A. pensylvanicum*. Interestingly, other experimental manipulations in which phloem and xylem conductivity

were reduced via 50% removal did not result in changes in sex expression significantly different from background rates (J. Blake-Mahmud, unpub. data). This suggests that a complete (or near complete) severance of conductive tissue is required to change the sex of an individual branch. Whether the severance in conductive tissues results in a lack of root-produced hormones (such as cytokinins), an overabundance of bud-produced hormones (such as auxins), or something else (not associated with a growth-regulator) warrants further investigation. Taken with the results from 2016 involving branch collection over a six-week time period, it appears that while complete severance of conductive tissue triggers femaleness, its effectiveness as a cue for female sex expression in the current year decreases slightly as flowers approach anthesis and does not continue to override male primordia development (as evidenced by the approximately 50% male flowering cut branches three weeks prior to anthesis in the 2016 studies). We are still elucidating the proximate environmental triggers for changes in sex expression in entire individuals, though preliminary unpublished data indicate that diminished plant health (due to extreme physical damage or infection) correlates with changes to female sex expression (Blake-Mahmud and Struwe 2016).

Other studies have indicated that higher nonstructural carbohydrates are present in female flowering individuals in the winter prior to flowering (Blake and Struwe 2017), but we did not find a significant influence of increased carbohydrates (i.e., the sugar added in 2017) on sex expression in our studies. This may mean that sugar does not trigger female sex expression, that the amount of added sugar was below the threshold level needed, or that sugar was not taken up into the developing buds. This may also be due to limits in study design. The number of co-flowering specimens was low due to



animal disturbance (i.e., only 14 comparisons). Earlier spring temperatures as simulated in the greenhouse did not affect the sex expression of individual branches. This supports the hypothesis that the earlier onset of spring we have seen in recent decades (Cleland et al. 2007) and the progression of this phenomenon we will expect in the future will affect timing of flowering in *A. pensylvanicum*, but will likely not affect the sex ratios present in natural populations.

Because of the nature of ESD, the late determination of sex may allow *A. pensylvanicum* to remain potentially receptive to sex determining cues for a longer time period. This may have important ramifications for sex ratios in populations and subsequently fruit set. Depending on the timing and nature of a disturbance, most forest trees exhibit a one to two-year lag in their responses to stimuli (Holmes and Likens, 2016). However, in the case of *A. pensylvanicum*, the sensitive period for sex determination lasts until April of the flowering year, translating into a time lag potentially shorter than three weeks prior to anthesis. While the impacts of large storms on forests remain complex, the potential lack of lag-time in changing sex expression for *A. pensylvanicum* may allow this species to respond reproductively to storm damage at previously unanticipated rates.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author contribution statement: J. Blake-Mahmud designed and conducted the experiments, collected and analyzed data, and wrote the manuscript as part of a doctoral dissertation. L. Struwe co-led the embedding and sectioning investigation and serves as the doctoral dissertation advisor.

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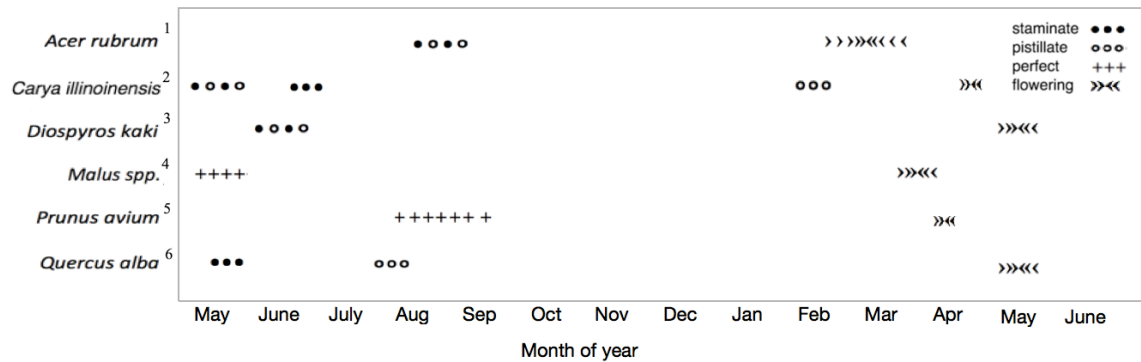
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## Figures



**Figure 1**

**Title: Differentiation of bud sexual structures in relation to flowering**

Most woody species differentiate the primordial reproductive structures within buds the year prior to flowering. In species with separate male and female flowers, staminate buds are often determined first (as in *Quercus alba*) or simultaneously (such as in *Carya illinoensis*). Differentiation of sexual structures may occur over the course of two to six weeks and will vary according to location, population, climate, and current weather.

1 - Wageningen, the Netherlands (de Jong, 1976), 2 - Georgia, USA (Woodroof & Chapman Woodroof, 1926), 3 - Kyoto, Japan (Yonemori *et al.*, 1993), 4 - Northern Hemisphere, flower buds initiated 2-5 weeks following anthesis (Buban & Faust, 1982), 5 - Prosser, Washington, USA (Guimond *et al.*, 1998), 6 - Cambridgeshire, England (Longman & Coutts, 1974)



**Table 1****Title:** Timing of sex expression of *Acer pensylvanicum***Table caption:**

Sex-expression data from all branches. Empty circles denote inflorescences with female flowers, filled circles indicate inflorescences with male flowers, asterisks indicate both male and female flowers within a single inflorescence (monoecious), blank boxes represent non-flowering branches. We collected branches in group 1 seven weeks prior to flowering, group 2 at five weeks prior, and group 3 at three weeks prior to anthesis in the field. In group three, Tree 17 had one branch that bloomed with male inflorescences and female inflorescences and another branch with male inflorescences and monoecious inflorescences. In the same group, Tree 21 had one branch with male inflorescences only and another with male and monoecious inflorescences. Trees 25 and 26 had one branch that bloomed female and one branch that bloomed male in collections one and two. Trees 4-28 bloomed entirely male in the field. Trees 1-3 had male, female, and monoecious inflorescences in the field. Differentiation was assumed to occur in the window between changes in sex expression. In most cases this is a two-week period, but may extend upwards to 7 week in cases where intermittent branches did not flower in the greenhouse such as tree 5, 6, or 24. In this situation, the most conservative time to differentiation (ie – the longest) was used to calculate mean time to sexual differentiation. In trees 1-3, we observed different-sexed inflorescences dispersed throughout the tree and not concentrated on some branches, therefore differentiation for these individuals most likely occurred within three weeks. However, the small possibility that 4-6 randomly chosen branches were all destined to be female prior to the start of collection cannot be ruled out.

Individuals	Group 1	Group 2	Group 3	In Field	Differentiation occurred
Tree 1	○	○	○	○ * ●	within 3 weeks prior to flowering (most likely)
Tree 2		○	○	○ * ●	within 3 weeks prior to flowering (most likely)
Tree 3		○	○	○ * ●	within 3 weeks prior to flowering (most likely)
Tree 4			○	●	within 3 weeks prior to flowering
Tree 5	○			●	within 7 weeks prior to flowering
Tree 6	○			●	within 7 weeks prior to flowering
Tree 7	○	○	○	●	within 3 weeks prior to flowering
Tree 8	○	○	○	●	within 3 weeks prior to flowering
Tree 9	○	○	○	●	within 3 weeks prior to flowering
Tree 10	○	○	○	●	within 3 weeks prior to flowering
Tree 11	○	○	○	●	within 3 weeks prior to flowering
Tree 12	○	○	○	●	within 3 weeks prior to flowering
Tree 13	○	○	●	●	3-5 weeks prior to flowering
Tree 14	○	○	●	●	3-5 weeks prior to flowering
Tree 15	○	○	●	●	3-5 weeks prior to flowering
Tree 16		○	●	●	3-5 weeks prior to flowering
Tree 17		○	*	●	within 3 weeks prior to flowering
Tree 18	○		●	●	3- 7 weeks prior to flowering
Tree 19		○	●	●	3-5 weeks prior to flowering
Tree 20		○	●	●	3-5 weeks prior to flowering
Tree 21		●	* ●	●	within 3 weeks prior to flowering
Tree 22	○	●	●	●	5-7 weeks prior to flowering
Tree 23	○	●		●	5-7 weeks prior to flowering
Tree 24	○			●	within 7 weeks prior to flowering
Tree 25		○ ●	●	●	3-5 weeks prior to flowering
Tree 26	○ ●	○ ●	●	●	3-5 weeks prior to flowering
Tree 27	○	○	●	●	3-5 weeks prior to flowering
Tree 28	○	●	●	●	5-7 weeks prior to flowering
Female trees	19	17	10	0	trees differentiating between 5 and 7 weeks before = 7 trees differentiating between 3 and 5 weeks before =9 trees differentiating between 0 and 3 weeks before = 12 Average time to differentiation = 4.6 weeks, based on most conservative time estimate
Male trees	0	4	12	25	
Monoecious trees	1	2	2	3	

**Table 2**

**Title:** Flowering in *Acer pensylvanicum* across experimental treatments

**Table caption:**

Sex was monitored in whole study trees (A) and from branches collected from study trees kept in three different treatments (B-D). Approximately half of branches kept in bottles in the field were destroyed by animals and did not flower. We conducted analyses on individuals that co-flowered in two treatments. To assess the effects of temperature we compared treatment B and D. To assess the effect of sugar we compared B and C. To assess the effect of cutting we compared A and B.

Location <i>Unit</i> Treatment	A: Field <i>Whole tree</i>	B: Field <i>Branch</i> Plain water	C: Field <i>Branch</i> Sugar water	D: Greenhouse <i>Branch</i> Plain water
Males	24	0	2	0
Females	8	17	18	29
Monoecious	5	0	1	1
Non-reproductive / bottle destroyed	3	23	19	10

**Supplementary information****Title:** Flowering in *Acer pensylvanicum*

**Supplemental Table 1:** Sex-expression data from branches held in water (control, left) and in sugar water (treatment, right) groups. Empty circles indicate the production of female inflorescences, filled circles indicate male inflorescences, asterisks indicate both male and female flowers on a single inflorescence (monecious). The only trees that bloomed in both control and treatment groups *and* bloomed differently in these two groups were trees 25 and 26. Total numbers of males, female, and moneciously flowering trees are included at the bottom.

Water only				
Individuals	Group 1	Group 2	Group 3	In Field
Tree 1	○	○	○	○ * ●
Tree 2		○	○	○ * ●
Tree 3			○	○ * ●
Tree 4			○	●
Tree 5	○			●
Tree 6	○			●
Tree 7		○		●
Tree 8	○	○	○	●
Tree 9	○	○	○	●
Tree 10	○	○	○	●
Tree 11		○	○	●
Tree 12	○	○	○	●
Tree 13	○	○		●
Tree 14	○		●	●
Tree 15	○	○	●	●
Tree 16		○	●	●
Tree 17			○ * ●	●
Tree 18	○		●	●
Tree 19		○	●	●
Tree 20		○	●	●
Tree 21			* ●	●
Tree 22	○	●	●	●
Tree 23	○	●		●
Tree 24				●
Tree 25		●	●	●
Tree 26	●	●	●	●
Tree 27			●	●
Tree 28			●	●
TOTALS				
Female trees	13	13	9	0
Male trees	1	4	11	25
Monecious trees	0	0	2	3

Sugar only			
Group 1	Group 2	Group 3	In Field
○	○	○	○ * ●
	○	○	○ * ●
	○	○	○ * ●
		○	●
○			●
○			●
○		○	●
	○	○	●
○	○		●
	○	○	●
○		○	●
○		●	●
○	○	●	●
○	○	●	●
	○	●	●
	○	○ * ●	●
		●	●
		●	●
		●	●
	●	●	●
		●	●
			●
○			●
	○	●	●
○	○	●	●
○	○	●	●
○	●		●
14	15	9	0
0	2	12	25
0	0	1	3

## **Chapter 6**

### **Conclusions**

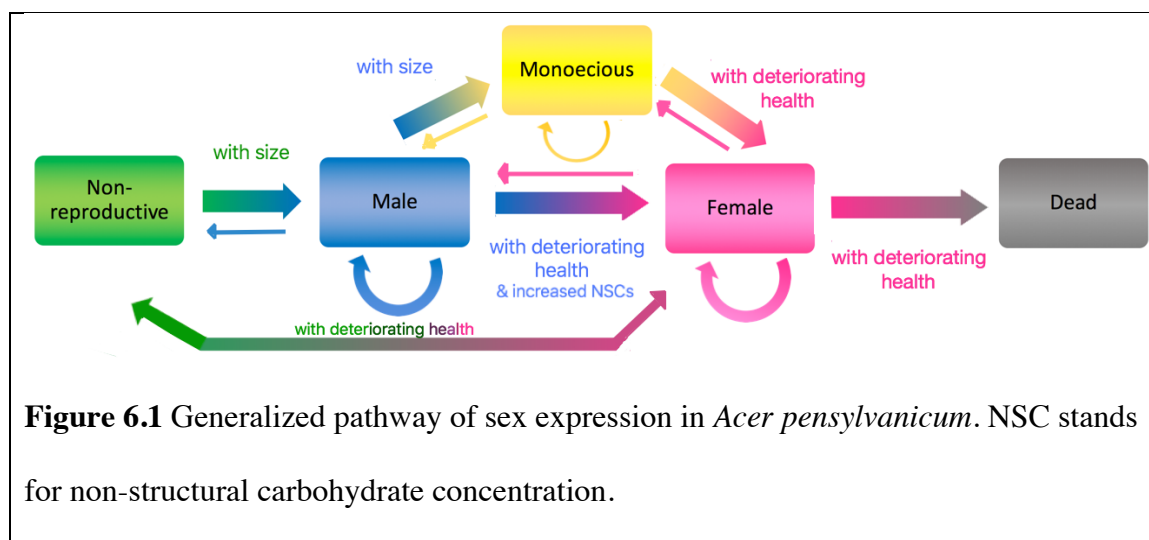
The general goal of my dissertation research was to explore life-history consequences of sex expression in natural settings. I did this by examining the predictions of theory regarding environmental sex determination in a model species in native upland forests in the woods of New Jersey from 2014 - 2017. During the course of this work I monitored approximately 900 striped maple trees in five sites and collected data at the individual and population level. I used a variety of field, lab, and greenhouse techniques to test hypotheses and construct predictive models. Although there remain elements of the demography of this species and the resulting consequences on the forest community to be worked out, I use my extensive four-year multi-approach study to offer hypotheses related to the ecological functioning of the current populations/communities, and the evolutionary trajectory of this species.

Environmental sex determination depends on several factors including the patchiness of an environment in time or space and the ability of an individual to assess, but not change, these factors (Charnov and Bull 1977). An underlying assumption for modular organisms such as plants, is that, in dioecious taxa, males and females use the patchily distributed resources (e.g, environmental nutrients, light, or plant size) differently (Charnov and Bull 1977). Without this last assumption, it would be as easy for a modular plant to simply change allocation when the environment changes; changing sex expression would not be necessary to optimize resources or to prevent premature mortality. More specifically, if resources or health decline, we might predict a general reduction in flowering or reproduction, regardless of sex expression. The outstanding

question is, given a set resource budget or health status for a tree over a given year, what is the threshold between reducing flowering versus changing sex expression?

Striped maple has been a botanical puzzle because it seems not to follow the theory that we imagine should guide it (Lloyd and Bawa 1984). For instance, why are the populations male-skewed? Why can both small and large individuals bloom female? Why do some trees switch sex directly while others spend many years flowering moneciously? Is femaleness a cause or consequence of mortality?

Although any given tree may change sexual state in a multitude of ways (see Chapter 2, figure 2 for details), I hypothesize here a generalized pathway, modified from Chapter 2, figure 7.



In this generalized pathway, following germination, trees start as non-reproductive and stay in that state for many years, devoting time and resources to growth. If they experience good conditions, as most do, they grow, reach sexual maturity, and flower male. Other non-reproductive trees may be in poor condition. In this case, they might reach sexual maturity and start flowering immediately as female. For trees in the

male state, individuals may spend many years flowering as males, and may add female function with increased size if they are generally in good health. Sex expression in plants that are monoecious is the least stable state of sex expression. Monoeciously flowering plants may return to flowering male or change to fully female, the latter usually with deteriorating health. Male to female transitions correlate with both decreased health and increased non-structural carbohydrate (NSC) concentration. Females may maintain female flowering for some years, occasionally not flowering for a year or two. Almost all individuals that die do so after flowering female.

If this generalized conception is accurate, this would mean there are essentially two pathways leading to female flowering: (1) be healthy and grow into it gradually, (2) be unhealthy and become female quickly. The first trajectory would make sense over evolutionary time if femaleness does require more resources. Although I noticed no differences in soil or light associated with the ecological conditions under which male or female-flowering plants live, the observed higher concentrations of non-structural carbohydrates in females are consistent with the idea that femaleness requires more resources. This also supports the second assumption needed for ESD – that males and females use resources differently.

The second pathway leading to female flowering is less straightforward and relates directly to differential mortality rates. If death is a consequence of the sheer resource depletion resulting from female expression, then becoming female could be perceived as maladaptive for an individual. In the “maladaptive-switching” case, for any individual tree, the best life history strategy would be better to stay male, even in a male-dominated population, and distribute reduced parentage over multiple years, rather than



to change to female and die quickly. Over time, one might expect the evolutionary stable strategy to yield generally male-skewed populations, with a frequency-dependent maintenance of a minimal numbers of females. Furthermore, I would expect the resource-exhaustion resulting from female fruit development to manifest itself in reduced NSC concentration for females prior to death.

On the other hand, female sex expression might be a consequence of impending death. In this “adaptive-switching” scenario, trees die at a variety of ages and sizes for reasons other than NSC depletion. Femaleness is assumed to be the more intensive reproductive state, with the costs of motherhood exceeding those of fatherhood. Mother- and fatherhood are equally effective from a genetic standpoint, therefore we’d predict that less-costly male function would result in skewed sex ratios. This would mean that in striped maple populations in any one year, individuals might have more reproductive assurance as females. In the rare cases of impending death, the costs of female function would be negligible. Therefore over long time periods, evolution might select for a life-history strategy in which trees that could switch to femaleness with the onset of illness, injury, or other death-is-coming cue. In this situation, we might predict that NSC concentration to be maintained up to the time of death. Male-skewed sex ratios would result from the continual siphoning off of females from populations. In both scenarios, a male-biased sex ratio would result from increased female mortality.

While I cannot disprove either of these hypotheses, my finding in Chapter 3 that NSCs are maintained until death supports the “adaptive-switching” scenario. In this situation, the sex-switching behavior is adaptive and relies on a poor-health induced injury cue to trigger and maintain femaleness at low frequencies. The lack of prescience

in evolution means that what once was potentially adaptive may now result in drastically different outcomes in the face of changing climatic regimes.

While I do not yet have enough data to examine the less frequent transitions (such as monecious to male and female to male) one could imagine the “adaptive-switching” scenario being applicable to “reverse” transitions as well. The sealing of wounds, growing of new tissues, or change in external conditions might improve health and stop the production of stress hormones, thereby facilitating a transition to the less energy-demanding male state, as predicted in the mortality and growth rate advantage models (Iwasa 1991).

The proposed hypotheses are not without complications. Femaleness is universally touted as more resource-intensive. While intuitively appealing, no studies have attempted to quantify the relative energy expenditure per offspring of separate plant sexes. Also problematic is the surprising lack of understanding regarding mortality in trees. While we know that drought, insect infestations, and fungal infections may increase mortality, the more proximate physiological mechanisms are still hotly disputed, particularly when it comes to the impact of carbon budgets (Galen 2000; McDowell *et al.* 2008; McDowell 2011; Sevanto *et al.* 2014; Allen *et al.* 2015; Anderegg *et al.* 2015). Because trees seal wounds, rather than heal them, it is also difficult to accurately assess improvement in overall condition.

In a broader life-history context, this work contributes to understanding of the trade-offs between growth, reproduction, mortality, and sex expression. Both sexes must balance resource allocation between growth and reproduction. In striped maple, both males and females produce inflorescences consisting of approximately the same size and

same number of flowers. Females have a higher mean number of flowers, but when corrected for body size, females and males had similar flowering intensities in two out of three years (Appendix VIII). In this species, males devote more energy to growth than do females, on average, even though males are not generally larger (Chapter 2). Even with approximately equal flower production among sexes, females continue to devote substantial energy to the provisioning of fruits during the summer months.

Although pollen limitation has not been examined specifically in the genus *Acer*, studies in other species indicate that trees often receive adequate pollen to fertilize available ovules (Stephenson 1981). In striped maple populations, the larger numbers of males and the insect-pollination system (Sullivan 1983) further support the idea that pollen quantity is not a limitation to successful reproduction (i.e., fruit production), but rather that ovule availability limits the number of potential fertilizations. Consequently, a tree producing female gametes would potentially have higher fitness than a tree producing male gametes in a given year, *if energy reserves were not an issue*. We therefore expect trees to potentially have higher mean lifetime fitness as mothers than as fathers.

In this striped maple, females, compared to males, are in poorer health (chapter 2), grow less (chapter 2), have higher resource concentrations (chapter 3), die more frequently (chapter 2), or more likely to have sustained serious damage (chapter 4), and seem to spend more energy on reproducing. Taken all together, these conclusions might suggest that the environment is selecting for complex life history traits by optimizing not only along the growth – reproduction axis, but also along the continuum of male - female sex expression over time. This would lead trees to be male while healthy and growing

and to switch to female once health decreases, essentially a “now or never” strategy (Lloyd and Bawa 1984, Iwasa 1991). In light of the mortality findings, it appears that striped maple may be converging on a curious life history strategy that incorporates both semelparity and iteroparity: reproducing repeatedly when male, but only a few times as female. Decade-long data collection would be necessary to fully evaluate this hypothesis.

Whatever the proximate machinery for changing sex expression may be, the coming decades with variable climates will most certainly have an effect on this species. With increased warming and reduced polar ice modifying weather patterns, we expect increased frequency of severe weather events (Beniston and Stephenson 2004; Millar *et al.* 2007; Hultine *et al.* 2016; Whytlaw *et al.* 2017). These storms cause loss of branches from canopy trees, incidents that can dramatically damage the understory striped maples. I propose that the increased storm damage will likely result in an increased number of females. While this might initially lead to increased seed set and recruitment of juvenile trees, the result over prolonged periods of time remains to be seen. For population persistence over evolutionary time, female mortality must not outpace recruitment. At the community level, increased recruitment of striped maples does not bode well for recruitment of other species of hardwood seedlings, waging their own struggle against deer, drought, and a dearth of light flecks as they struggle to grow under a mini-canopy of broad striped maple leaves.

In conclusion, I have documented the patterns of sex expression in striped maples over four years, showing that conventional theory regarding environmental sex determination cannot be applied to all plant species without consideration of their biology. I have shown that while size has little importance in female expression, general

health and increased non-structural carbohydrates correlate with femaleness. Severe damage may cue full female sex expression and do so within three weeks of flowering. Males are over-represented in populations, due at least in part to high mortality. This work is important, not only for our understanding regarding environmental sex determination, plasticity, sex ratios, and life histories, but also for the maintenance of diversity in forest communities in the coming decades.

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## Appendices

### **I. Analysis of Accuracy of Sex Expression Methods**

To ascertain my accuracy in assessing the sex of large trees, I conducted a methods sensitivity analysis in 2015. At two populations (Jenny Jump and High Point Bathhouse) I selected 11 male, 7 female, and 9 monoecious trees (27 trees divided approximately equally between two sites). The objective was to compare accuracy of sex determination of inflorescences at a distance (as they grow on trees) versus inflorescences close-up.

I first conducted my normal visual sampling routine below (**Table 1**) which involves viewing inflorescences in high tree branches by eye or via binoculars. I then removed every flowering branch from the tree with pole pruners and determined the sex of flowers again. This allows me to determine the accuracy of my non-destructive visual sampling protocol by comparing it to a destructive method involving inflorescence removal.

# inflorescences on one individual	5	10	15	20
1. <i>Visually sampled from</i>	3 from bottom branches  2 from middle branches	+ 2 from middle branches,  3 from top branches	+ 2 from bottom,  3 from top	+ 5 from any un-sampled branches
2. <i>Spot check inflorescences with binoculars.</i>				
3. <i>Count all inflorescences.</i>				

**Table 1.** Routine sampling protocol for trees over 1.5 dbh.

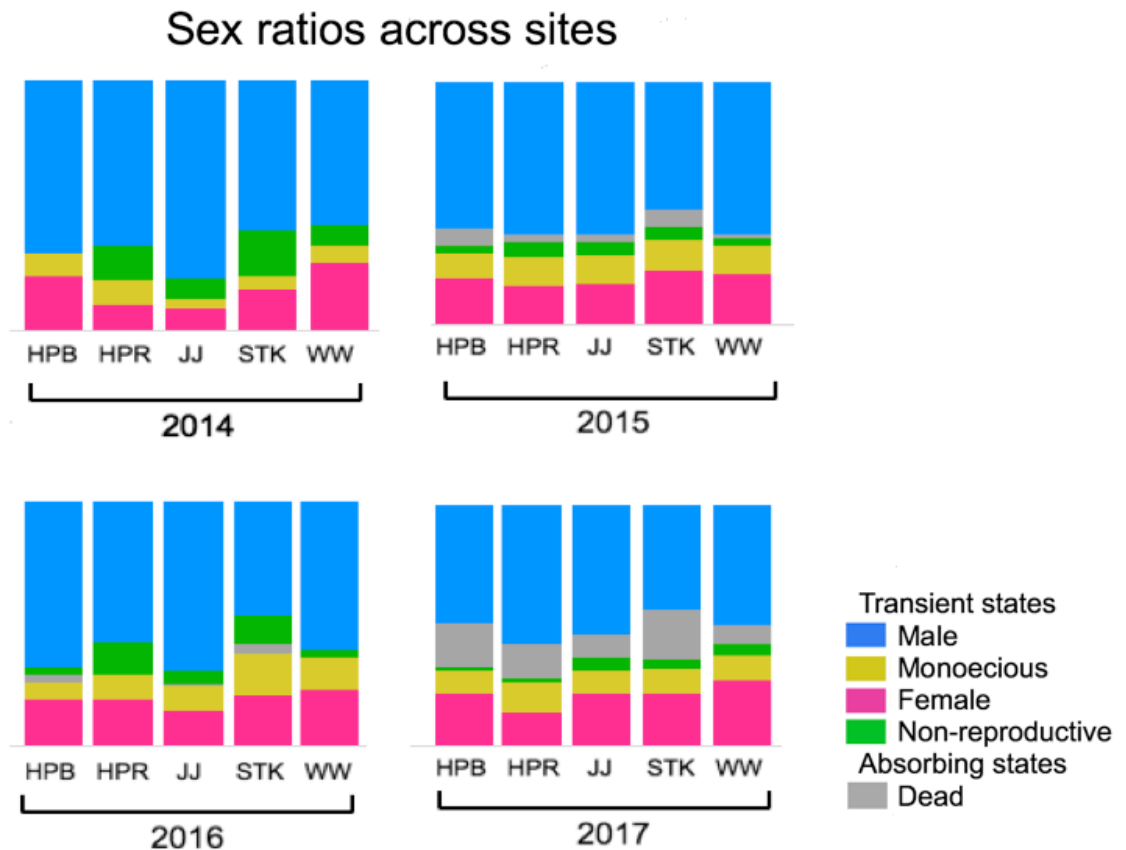
In 23 of 27 pruned trees, I could accurately assess the sex of a tree using step 1 of the above protocol and visually assessing only 5 inflorescences. Three trees needed a minimum of 10 and 15 inflorescences to correctly ascertain the sex. The remaining tree I accurately assessed the sex of by following the protocol through the end of step 2.



Following the full protocol leads to the most accurate sex expression, but an abbreviated protocol leads to 95% accuracy. Destructive sampling, therefore, is not needed to accurately assess the sex of tree. I used my full methodological protocol for all unmanipulated long-term, defoliated, and ringed study trees and abbreviated study protocol (steps 1-2) for trees in the forced flower and pruning investigation.

## II. Sex Ratios

Sex ratios across sites were relatively constant over time, despite increases in plot size between 2014 and 2015. (Figure 2.1 depicts sex ratios across sites over four years.) Over time, trees accumulate in the “dead” category because dead is an absorbing state.



**Figure 1.** Sex ratios across sites. Blue represents male trees, pink female trees, yellow monoecious trees, green non-reproductive trees (i.e – non-flowering), grey dead trees. Sites: HPB = High point state park site 1, HPR = High point state park site 2, JJ = Jenny Jump state forest, STK = Stokes state forest, WW = Wawayanda state park.

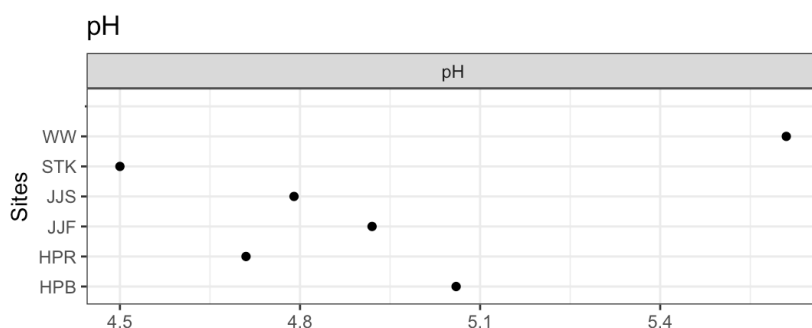
I compared sex ratios across sites and years using a multivariate analysis of variance. Neither site nor year had statistically significant impact on sex ratios (site:  $f\text{-value} = 0$ ,  $df = 4$ ,  $p = 1$ ; year:  $f\text{-value} = 5.738e-21$ ,  $df = 3$ ,  $p = 1$ ). Because of this, I consider the sex ratio among sites to be comparable. Thus, all site data are lumped in analyses unless otherwise noted.

### III. Soil Nutrient Analysis

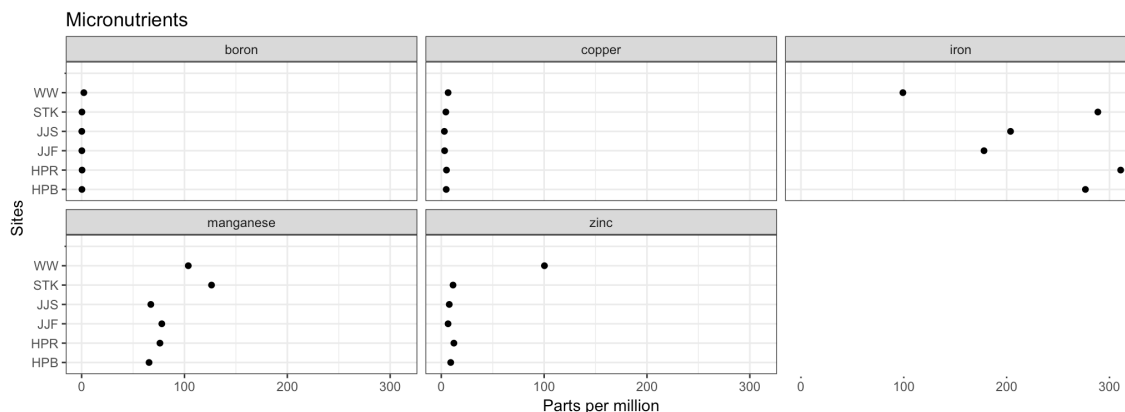
In summer of 2014 I collected soil samples from the field sites by taking 10 spadefulls of soil from different locations within a site and then mixing them together into one sample. In the largest site at Jenny Jump State Park I compared two samples.

Soils are predominantly clay with less organic matter. At one site, Wawayanda State Park, trees grow between boulders in a massive rock slide on the side of the mountain. The soil I collected there was low in clay content and high in organic matter.

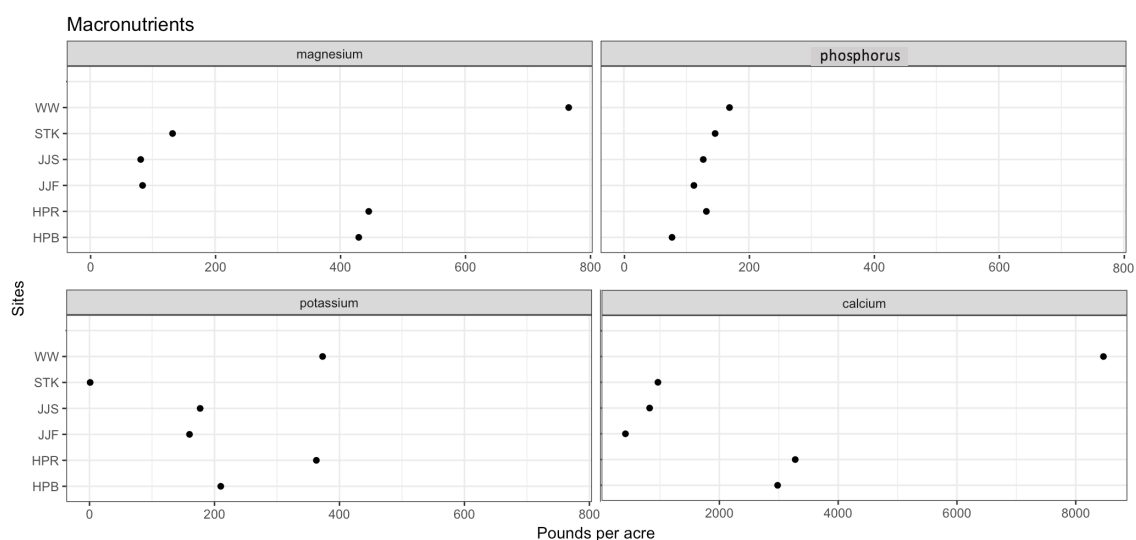
Samples were analyzed via Mehlich 3 extraction by the Soil Testing Laboratory at Rutgers. A summary of these findings are below.



**Figure 1.** Soils are acidic across sites. Sites: HPB = High point state park site 1, HPR = High point state park site 2, JJ = Jenny Jump state forest, STK = Stokes state forest, WW = Wawayanda state park.



**Figure 2.** Micronutrients (zinc, copper, manganese, iron, and boron) are measured in parts per million. Zinc, copper, manganese, and boron are similar across sites, while iron varies. Sites: HPB = High point state park site 1, HPR = High point state park site 2, JJ = Jenny Jump state forest, STK = Stokes state forest, WW = Wawayanda state park.



**Figure 3.** Macronutrients (potassium, phosphorus, magnesium, and calcium) are measured in pounds per acre. Note the different scales for calcium compared to others. Phosphorus is similar across sites, while magnesium, potassium and calcium, in particular, vary. Sites: HPB = High point state park site 1, HPR = High point state park

site 2, JJ = Jenny Jump state forest, STK = Stokes state forest, WW = Wawayanda state park.



















































Later analyses showed that site was not a significant predictor of sex expression (Chapter 2) or sex ratios (Appendix II). These data are consistent with the idea that large differences in some macronutrients (calcium) and moderate differences in other nutrients such as potassium, magnesium, or iron are insufficient to affect sex ratios at the population level.

#### **IV. Preliminary Forced Flowering Results From 2015**


In the late winter of 2015 I conducted a pilot experiment on striped maple phenology. Starting mid-March I collected branches at two-week intervals from 10 trees of unknown sex growing at High Point State Park. At each collection time, I also sampled two buds for fixation and eventual paraffin embedding and sectioning (which was unsuccessful).


These samples were kept in a cooler with dry ice and brought back to the greenhouse where the stems were recut underwater. They were individually put in aluminum-foil wrapped wide-mouthed glass mason jars with  $\frac{1}{2}$  liter of tap water mixed with 2.5 teaspoons commercially-produced “flower fresh” powder. As they were in the floriculture greenhouse, they were misted multiple times a day like their neighboring plants.


Of the 10 individuals, five exhibited sex expression in the greenhouse different from final expression in the field. Three individuals flowered in the green house as they did in the field. Two individuals produced only leaves in the greenhouse.


Date collected Sample	3.13.2015	3.27.2015	4.10.2015	4.25.2015	field expression
High Point 1					
High Point 2					
High Point 3					
High Point 4					
High Point 5					
High Point 6					
High Point 7					
High Point 8					
High Point 9					
High Point 10					

LEGEND

Female flowers 

Male flowers 

Both male and female flowers 

Leaves only 

**Figure 1.** Tree icons in pink indicate branches produced female flowers only, blue = male flowers only, yellow = male and female flowers in same or different inflorescences, green leaves indicate that branches did not flower. Sample trees 1, 2, and 8 flowered consistently the same sex in the cut branches in the greenhouse and on the tree in the field. Sample trees 3-7, highlighted in orange, flowered differently in the greenhouse and in the field. Some individuals (5 and 6) make a complete shift as late as three weeks before flowering in the field the week of May 15<sup>th</sup>, 2015.

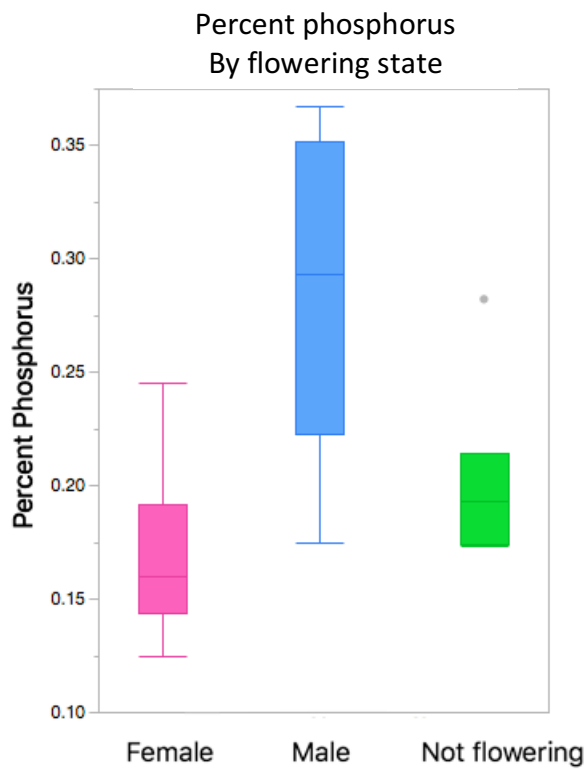


## **V. Initial phosphorus analyses**

I collected leaf tissue sample from trees blooming male, female, or not at all during my defoliation experiments. I was curious to see whether trees seem to be maintaining similar stoichiometric ratios across flowering sexes.

I dried the leaf tissue and ground it with mortar and pestle, washing mortar and pestle with phosphate-free soap between samples. Rachel Paseka performed the preliminary nutrient analysis using a spectrophotometer in the Department of Marine Sciences. Samples of approximately 4mg were combusted at 500° C, digested in acid, then analyzed using the molybdate method (Solorzano and Sharp 1980). Percent phosphorus is calculated using molar concentrations and describes the percentage dry mass of phosphorus in samples.

Of the initial 21 samples (seven males, seven females, and seven non-flowering trees), there was a significant difference in percent phosphorous among groups (ANOVA,  $n = 17$ ,  $df = 2$ ,  $F\text{-ratio} = 9.25$ ,  $p = .0017$ ). A student's t-test indicated that males are significantly different from female flowering and non-flowering trees.



**Figure 1.** Relative amounts of phosphorus found in leaf tissues of male, female, and non-flowering striped maple trees.

**Solorzano L, Sharp JH. 1980.** Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnology and Oceanography* **25**: 754–758.

## **VI. Initial Photosynthetic Rate Analyses**

In mid-September of 2017, I conducted preliminary analyses on photosynthetic rates on striped maples. In three sites (Jenny Jump, Stokes, High Point) I randomly selected trees that had flowered completely male or completely female in the spring. Because I wanted to assess photosynthetic rates on leaves while they were still attached to the tree, individuals with all branches higher than 1.5 m above the ground were necessarily excluded to enable the photosynthetic rate meter (LI6400) to reach the leaves.

Photosynthesis rates are often taken at saturating light levels of 1500 PAR (photosynthetically active radiation). In the understory this is often unrealistic: only momentary sunflecks approach this level of PAR. Because of this, I chose to use two levels of PAR – low (100) and high (1000) for each leaf. It should be noted that even 100 can be high for ambient light in the understory. Understory light levels were often in the 20-50 PAR range, occasionally as low as 2 PAR and once up to 280PAR.

I performed readings on a minimum of two leaves per tree, at two light levels. I used the standard LED setting on the fluorescence head. Ambient CO<sub>2</sub> levels were kept at 400 ppm, ambient humidity between 40% and 75% (averaging 60%), depending on the time of day. Readings were started at approximately 9 am and taken until 2:30pm. Weather at two of three field sites was partly cloudy; weather at Stokes was foggy and wet before clearing up mid-day.

After finding a tree I would select a branch and leaf that was neither in direct sun or deep shade. If the leaf was damp I would dry it with a cloth, set the PAR, and clamp the leaf into the chamber. I recorded readings when the photosynthetic, conductance, and humidity rates were stable, using the built-in stability indicator (indicated by 111\_ under

“PCHF”). On the same leaf in the same spot I would then increase the PAR and take another reading. Choosing a different leaf I would repeat the process.

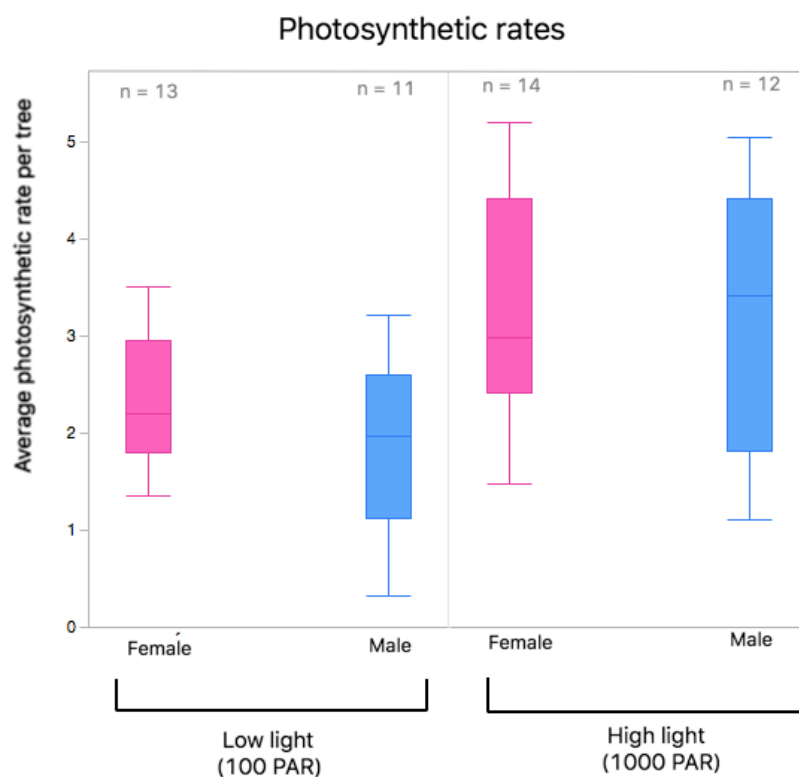


**Figure 1.** Setup for measuring photosynthetic rates using the LiCor 6400.

Although reaching stability could occur quickly (within two minutes), at least half of the time stability took 2-4 minutes to achieve. In situations when stability would not be reached after 10 minutes, I would open the chamber and re-clamp the leaf in the same approximate place and try again.

I noted that different readings from the same region of a leaf on a single plant were highly consistent, as hoped. On the other hand, different readings from different leaves on

a single plant could be very similar or very different. At 100PAR readings from different leaves could be as little as 8% different (e.g. 1.59 vs 1.57) or up to 150% different (e.g. 1.97 vs 0.27); at 1000 PAR 2% - 122% different. Although this inter-individual variability is rarely evaluated in studies of photosynthetic rates, it shows the necessity for multiple readings per individual and for further evaluation criteria (over and above “stability”) to evaluate the accuracy of collected data for the purpose of drawing conclusions about overall photosynthetic rates. Although I am trained on the instrument, these are my first data collected using the LI6400; user error cannot be ruled out for these preliminary analyses.



**Figure 2.** Photosynthetic rates measured for trees blooming male and female in 2017 at two light levels.

## VII. Boosted Classification Tree notes

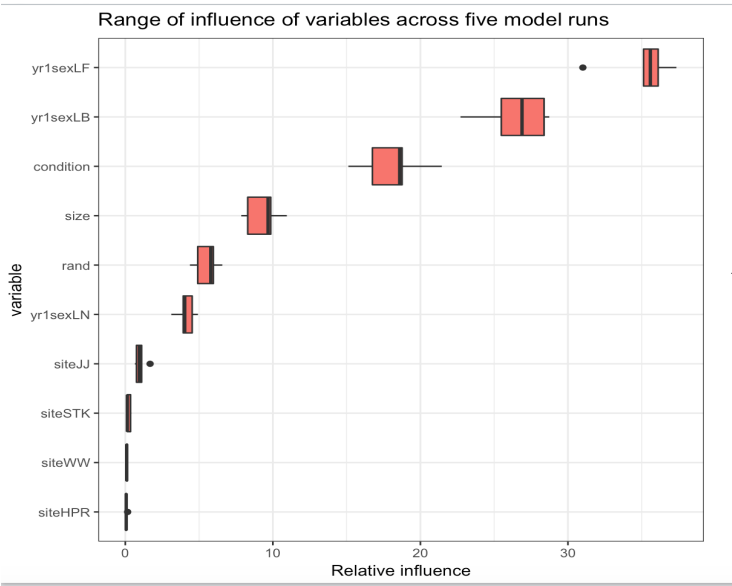
Boosted classification trees, also known as boosted regression trees, are not frequently used in ecology or evolution. Unlike classic hypothesis testing, BCTs use a machine learning approach.

There are many benefits to using machine learning, particularly when data do not conform to expectations. For example, with BCTs, there aren't many assumptions in what is the "baseline" level or whether data are independent. Misclassification errors are less influential. Categorical data is not a problem. The interpretability of main effects is not reduced in the presence of an interaction.

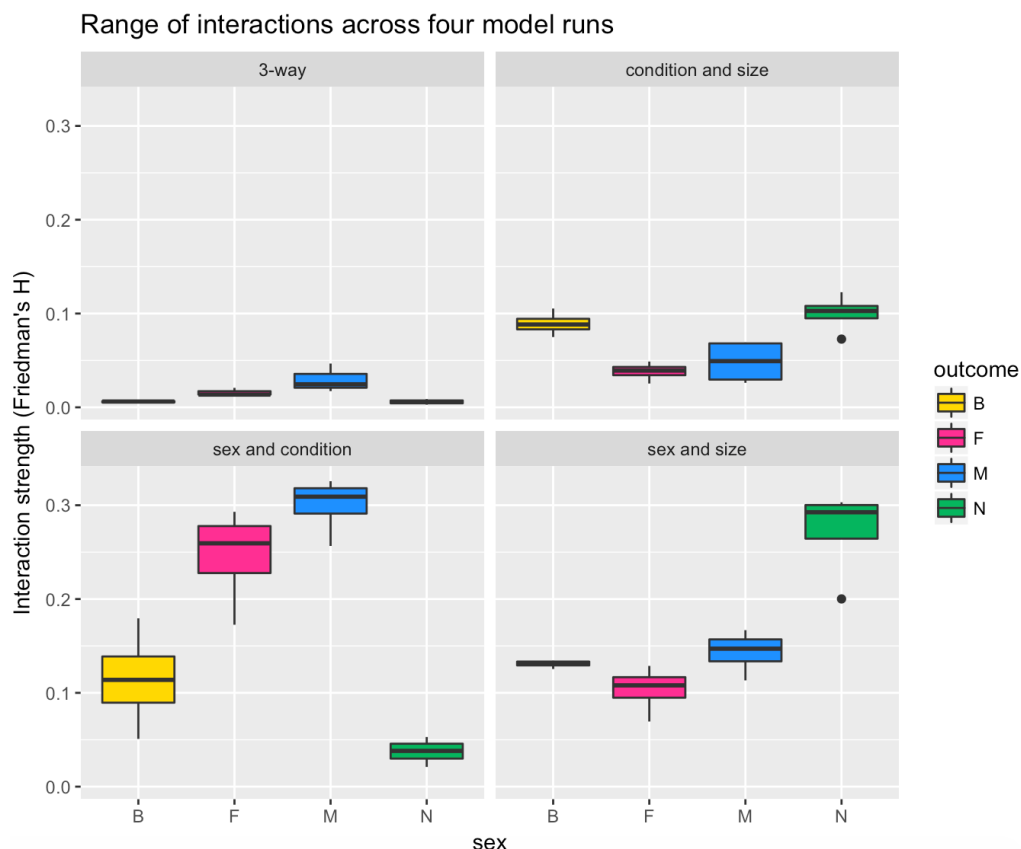
On the other hand, the black-box approach can be very uncomfortable as researchers may not understand exactly what is going on within the computer program. The natural stochasticity in machine learning will also lead to slightly different results if a random number "seed" is not set in the computer.

Although within the program, the model runs thousands of times and selects the best model given certain criteria, I was curious to see how initial data partitioning affected results. I ran the model five times using five different random separations of data into training and partitioning data sets. I found that the best fit model criteria varied: number of training trees varied between 1,000 and 6,000; interaction depth 2-3; and learning rate between .001-.005. Kappa fluctuated between .383 and .523. Relative influence also changed, but not that much. In all but one case, the variables worse than random stayed worse than random and those better than random remained better and in the same ranking (**Figure 1**). Differences in interaction strength also varied due to data

partitioning, but not much. Interactions vary from 0 (no interaction) to 1 (total correlation; **Figure 2**).



**Figure 1.** Variability in relative influence results, based on initial data partitioning.



**Figure 2.** Interactions varied across model runs but were larger for the interaction between sex and condition for flowering trees and between sex and size for non-flowering trees. Yellow = monoecious trees, pink = female trees, blue = male trees, green = non-flowering trees.

I then looked at the breakdown of data into the training (75% of data) and testing (25% of data) sets to ensure they were commensurate. The random seed I set (3) led a comparable data partitioning, with all deviations less than 1.5%, and most (14/16) less than 1%. Having partitioned data that is equivalent is important so that the model can reflect what we see in nature, which is often a skewed distributions of events.



Interestingly, I found that more disproportionate data partitions could lead to more accurate models. Although I am not a statistician, I would imagine that gain comes from inadvertently front-loading the training set with more “unusual” cases so that the computer could learn more rapidly. I did not follow this (potentially dangerous) path, but think it might warrant further investigation for those building predictive models.

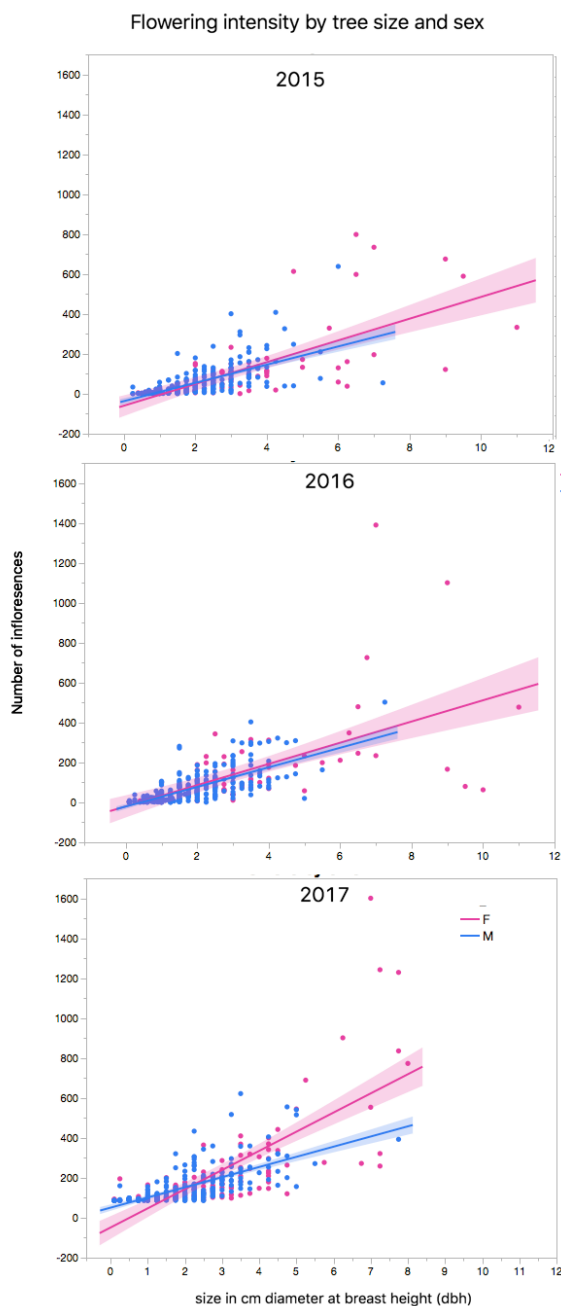
I think the results of the BCT model are useful and preferable to modeling with a generalized linear model such as logistic regression, when enough data are available. The lack of p-values, while slightly disturbing, also forces readers to think more deeply about the data. Because of the inherent stochasticity in machine learning, the results are less concrete. It is fair to say that, in my analysis, condition is 2-3 times more important than size (**figure 2.1**); however, I am cautious not to claim specific understanding at the finer scale of the “relative importance” values (Chapter 2, Supplementary information, table S5).

Generalized Linear Model with logit link	Boosted Classification Tree
<b>Answers question:</b> To what extent are the predictors correlated with the outcome?	<b>Answers question:</b> How important are the predictors, relative to each other and to a random predictor? How well can the created model actually predict the future?
<b>Findings:</b> Starting sex, size, condition are all significant predictors of ending sex. Increased size increases your odds of being fully or partially female compared to male, while increased size decreases your chances of being non-reproductive compared to being male (assuming you have flowered at least once previously). Decreases in overall health increase your odds of flowering partially of fully female, and most increase your odds of not-flowering.  The only significant interaction was between monocious trees and condition, therefore females, males, and non-reproductive trees all have similar shaped responses to changing condition.	<b>Findings:</b> If you flowered, the sex you are (Male or Female) has a large relative influence on your sex the next year, as well as your condition and size. Condition is more important than size.  The influence of where you are growing (site) not informative.  There is an interaction of condition with sex for Males, Females, and hermaphrodites and sex and size for non-reproductive trees.  We can predict well with this model.
<b>Benefits:</b> More common method P-values Directions inherent in odds ratios	<b>Benefits:</b> Predictive ability Good graphic representations Repeated measures allowed Lack of assumptions Miss-classification less of a problem
<b>Drawbacks:</b> Odd values non-intuitive Assumptions hard to assess	<b>Drawbacks:</b> Non-intuitive, black-boxy No p-values Small differences between simulations due to data partitioning

**Table 1.** Comparison of analysis methods between multiple multinomial logistic regression (left) and boosted classification tree (right).

### VIII. Flowering intensity by sex

For a given size, male and female trees produced similar numbers of inflorescences in 2015 and 2016. In 2017, larger female trees produced proportionally more inflorescences than did males (**figure 1**).



Amongst trees, mean number of flowers per inflorescence varies from 9 - 24. Females have slightly higher numbers of flowers per inflorescence (mean = 15.2 flowers / inflorescence, std dev = 2.68) than do males (mean = 13.9 flowers / inflorescence, std dev = 2.02). The biological difference is small. Females have, on average, 1 more flower per inflorescence than do males ( t-test for equal variances  $p = .011$ ,  $n = 86$ ,  $df = 78$ ).

**Figure 1.** The intensity of flowering per sex, measured as the number of inflorescences (y axis) for a tree of a certain size, measured as dbh in cm (x axis). Males are shown in blue, females in pink. Data from 2015 (top), 2016 (middle), and 2017 (bottom).

## IX. Public abstract

Forests are changing. As the overall global temperature increases, warming leads to changing climate patterns and more frequent severe weather events such as storms, droughts, and even pest-outbreaks. These major events can be stressful for forests, which are responsible for important processes like carbon processing and watershed health in the heavily-populated east coast of North America. In my research I've found that the physical damage often caused by severe weather events may affect trees in unusual ways. In the case of a native understory maple tree, striped maple, experiencing physical trauma can cause a tree to switch sex. In most plants and animals, sex is a consistent characteristic: a creature remains the same sex throughout its life. In some rare cases, though, a creature may switch sex during the course of its lifetime. For plants, sex is demonstrated via flowers. In striped maple, I demonstrated that the individual sex of a tree can change from year to year. Through long-term experiments in the woods I have found that in these trees, switches in sex correlate with decreasing health and that femaleness may be cued when the tree sustains extreme damage. In the lab, I found that the internal resource status of a tree (sugary sap) correlates with sex switching behavior. Females, and males changing to female, have higher concentrations of sugars than do males who stay male. In combined field and greenhouse studies I investigated the timing of flower development in striped maple flower buds. Unlike the long developmental period of buds in most tree species, striped maple may switch the sex expression of its flower buds within three weeks prior to flowering and does so in branches in response to being cut off from the tree. Taken together, these findings show that the increased levels of stress a forest experiences may shift striped maple populations from having an

overabundance of males to having more equal numbers of males and females. The increased numbers of females may change the amount of seed produced as well as the rates of mortality at a speed previously unheard of for trees. In addition to practical implications for forest and watershed management, this striped maple research extends our understanding of how individual plants deal with devoting limited energy to competing tasks like growth, reproduction, health maintenance, and survival.

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