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 change in individuals.

**PREMISE:** Plant sex is usually fixed, but in rare cases, sex expression is flexible and may be influenced by environmental factors. Theory links female sex expression to better health, but manipulative work involving the experimental change of health via injury is limited, particularly in sexually plastic species. A better understanding of mechanisms influencing shifts in sex is essential to our understanding of life history theory regarding trade-offs in sex allocation and differential mortality.

**METHODS:** We investigated the relationship between physiological stress and sex expression in sexually plastic striped maple trees (*Acer pensylvanicum*) by inflicting damage of various intensities (crown pruning, defoliation, and hydraulic restriction). We then monitored the sex expression of injured and control individuals for 2 years to assess the extent to which injury may cue changes in sex expression.

**RESULTS:** We found that severe damage such as full defoliation or severe pruning increased odds of changing sex to female and decreased odds of changing to male. In fact, no pruned male trees flowered male 2 years later, while all males in the control group flowered partially or fully male. After full defoliation, trees had 4.5 times higher odds of flowering female. Not all injury is equal; less-severe physical trauma did not affect the frequency of sex change to femaleness.

**CONCLUSIONS:** This work demonstrates that physical trauma in striped maple appears to exhibit a threshold effect in which only the most stressful of physiological cues instigate changes in sex expression, a phenomenon previously unknown, and that damage stress is strongly correlated with switching to femaleness. These findings have implications for population sex ratios and sustainability within an increasing stressful climate regime.

**KEY WORDS** *Acer pensylvanicum*; defoliation; dioecy; environmental sex determination; injury; mortality; pruning; Sapindaceae; sex expression; sex change; sexual plasticity.
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 a change in sex 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 explanation for ESD in plants, but it does not apply in all species. For example, in Acer pensylvanicum, little support has been found linking sex expression and body size (Hibbs and Fischer, 1979; Blake-Mahmud and Struwe, 2019).

The second model hypothesizes an alternate explanation for ESD—patchy environments. In this model, sex expression results from environmental variables that are unevenly distributed in time or space. This model is often interpreted as an increased availability of water, light, or nutrients, which correlate with increased female sex expression (see Heslop-Harrison, 1957; Freeman et al., 1980; Korpelainen, 1998; Bachtrog et al., 2014 for reviews). This aspect of the patchy environment model is most clearly demonstrated in the spatial segregation of the sexes (Bierzychudek and Eckhart, 1988).

The evidence of size-based spatial distribution varies by species, with some dioecious species exhibiting spatial segregation, such as Acer grandidentatum (Barker et al., 1982), while other studies, such as with A. rubrum (Sakai, 1990), found a lack of sex-based spatial structure. The influence of increased abiotic resources is also mixed, with males and females sometimes responding differently to resources (e.g., Krischik and Denno, 1990), and other times responding in a similar manner (e.g., Gehring and Linhart, 1993).

While often viewed from a landscape level, environments exhibit patchiness at smaller scales, so that even individuals rooted next to each other might experience very different phenomena (Bell and Lechowicz, 1994). This stochasticity is perhaps most salient in the experience of physical trauma. One plant may be crushed by a falling tree while a neighbor is unaffected, or one individual may sustain complete defoliation by caterpillars with an adjacent plant of the same species left undisturbed. These hyper-local environments are potentially crucial to understanding ESD in species with little spatial variation in sex expression and are understudied as proxies in individuals (Blake-Mahmud and Struwe, 2019) and that branch excision from male trees leads to female-flowering branches (Blake-Mahmud and Struwe, 2018). The influence of size on sex expression is minimal (Blake-Mahmud and Struwe, 2019). However, there are no published causative links between physical trauma and sex expression at the individual level in striped maple or in other Acer species.

Through a series of damage manipulations, we sought to address the following questions: (1) What are the effects of differing levels and kinds of physiological stress (caused by severe crown pruning, complete or partial defoliation, or hydraulic disruption via partial xylem removal) on sex expression in individuals? (2) Does physiological stress caused by defoliation or pruning have similar effects on trees of different sexes?

**MATERIALS AND METHODS**

**Experimental manipulations**

Our 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 to 2016, we conducted four experimental manipulations on separate individuals along a gradient of intensity to elucidate the effects of physical damage 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 removal in March 2016 and August 2016, and (4) partial defoliation in August 2016. We monitored manipulated and control trees in the flowering seasons of 2015, 2016, and 2017 for sex expression, flowering, and mortality (using methods described by Blake-Mahmud and Struwe, 2019).

**Pruning**—In April and May of 2015, we selected 26 striped maple trees of 1.5–6.0 cm DBH, that were flowering male (10 trees), female (7 trees), or monoeciously (9 trees). These trees were confined to two 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. We used pole-pruners to remove every forest along the Appalachian Mountain range in eastern North America. It prefers rocky soils and is well adapted to shade (Hibbs et al., 1980). Individuals frequently incur damage to branches and foliage from herbivores (including insects, caterpillars, and deer), and structural damage to trunks and branches (from falling canopy trees or branches, bark splits due to infection or rapid warming, and deer via antler rubbing). 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). Experimental work has indicated that flower sex may be determined within 3 weeks of flowering (Blake-Mahmud and Struwe, 2018). Males outnumber females by more that three to one (Hibbs and Fischer, 1979), possibly as a consequence of high female mortality (Blake-Mahmud and Struwe, 2019). Previous studies have shown that in striped maple, deteriorating health correlates with increased female sex expression in individuals (Blake-Mahmud and Struwe, 2019) and that branch excision from male trees leads to female-flowering branches (Blake-Mahmud and Struwe, 2018). The influence of size on sex expression is minimal (Blake-Mahmud and Struwe, 2019). However, there are no published causative links between physical trauma and sex expression at the individual level in striped maple or in other Acer species.
inflorrescence and its associated pair of leaves. This damage to every major and minor branch resulted in extreme damage to the tree’s crowns and left trees with less than 5% of their original leaf cover. These were compared to 26 control trees of similar size (in DBH) and starting sex from the same sites. Starting sex was calculated as the percentage female sex expression by dividing the number of female inflorrescences by the total number of inflorrescences on the tree, which yielded sex as a continuous predictor, with females as 1, males as 0, and monoecious flowering trees taking values between 0 and 1. We chose to compute percentage female expression 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.

We monitored the sex expression and fate of these trees throughout the growing season of 2015, 2016, and 2017. In the spring, we classified the trees as belonging to one of five mutually exclusive states: male, female, monoecious, nonflowering, or dead. 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 the intervening 5 months. Trees were considered dead when their normally green, photosynthetic bark turned purple-black, and they either failed to put out any leaves or had all current leaves shrivel and die before leaf drop in the fall. Dead trees were reassessed each following year to ensure the initial mortality assessment was accurate. We assessed the health, sex expression, and mortality of individuals in the springs of 2016 and 2017.

**Full defoliation**—In the summer of 2015, we 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). We selected only healthy trees that were either nonflowering (did not reproduce) or flowered male in 2015. We excluded females from this manipulation because females this small are generally in poor condition, resulting in insufficient numbers of small, healthy females for a balanced study at the time. Study trees were compared to a control group of 44 trees of similar DBH and starting sex from the same sites.

Using scissors, we removed every leaf from each tree at the petiole. No branches were cut, and all buds and stem tissue were left intact. The fully defoliated trees were then monitored for the rest of the summer and fall. Of the 44 individuals, only 14 developed new leaves between July and leaf drop in the fall. In the spring of 2016 and 2017, we recorded the health and sex expression of the study trees, by classifying the trees as belonging to one of five states: male, female, monoecious, nonflowering, or dead.

**Partial defoliation**—Following the heavy mortality of trees fully defoliated in July of 2016, we performed a less drastic defoliation later in the 2016 growing season. In August, we selected a new set of 22 small single-sexed trees (12 female, 10 male), with DBH between 0.5 and 2.0 cm, divided amongst two sites (High Point State Park and Jenny Jump State Forest). In striped maple, leaves are opposite, i.e., in pairs, along the branch. We removed one leaf from each pair across the entire tree for approximately 50% defoliation. If one leaf in a pair was noticeably less healthy (due to tissue necrosis or herbivory), that leaf was removed. In cases where only a single leaf remained at a branch node, that leaf was left intact. No branches were cut, and all buds and stem tissue remained. In the spring of 2017 and 2018, we assessed the sex and health of individuals by classifying the trees as belonging to one of five states: male, female, monoecious, nonflowering, or dead.

**Hydraulic restriction**—In March of 2016, we selected male trees in one site (Jenny Jump State Forest) to examine the effects of experimentally reducing vascular connection via controlled ringbarking on flowering (Pepper, 2008). We selected 68 trees to compare the effects of experimentally reducing vascular connection via 50% phloem removal. On 34 trees, we excised two strips of bark approximately 3 mm wide from 50% of the branch 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 reduce the risk of infection. The removal of this outer ring restricts nutrient and water flow above and below the ringed area. Thirty-four trees of similar size and starting sex were selected as controls. In August of 2016, we refreshed the wounds and recovered with masking tape. We assessed flowering sex in the spring of 2016, 2017, and 2018 for this study by classifying the trees as belonging to one of five mutually exclusive states: male, female, monoecious, nonflowering, or dead.

**Statistical analyses**

For the pruning and defoliation analyses, we analyzed the data using multivariate multinomial logistic regression models. These models generalize binomial logistic regression to allow for multiple categorical outcomes and multiple categorical or continuous predictors. 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 mosaic diagrams were created in JMP Pro 13 (SAS Institute, Cary, NC, USA) and sankey diagrams using SankeyMATIC (Bogart, 2016).

To examine the effects of experimental treatments on sex expression in 2 years, we conducted multivariate multinomial logistic regressions. In each case, initial models included the following potential predictors: the treatment, starting sex, site, size, and the interaction of sex and treatment. The outcome variable may belong to one of five mutually exclusive states: male, female, monoecious, nonflowering, or dead. Data were collected for these states at one time each year (that is, trees were not assessed first for mortality, then for flowering, then for sex, with manipulations between assessments, an experimental design that would lead to hierarchical models). The experimental structure supports using a single multinomial approach. For the full and partial defoliations, pruning analyses, and hydraulic restriction via phloem removal, we looked at the effect of site, size, treatment, starting sex (the first year of the study), and the interaction of sex and treatment on expressed sex (in the final year of the study).

We conducted post hoc analyses examining the correlation of treatment with mortality and with incidence of flowering. More specifically, we performed a one-tailed Barnard’s test to examine whether the treatment correlated with (1) increased mortality compared to control; (2) decreased flowering compared to control. Barnard’s test is similar to a χ² or Fisher’s exact test for a 2 × 2 matrix of categorical outcomes. Barnard’s test is more powerful for small sample sizes and does not assume that row and column totals are fixed, as Fisher’s exact test does, making it a more appropriate choice for post hoc analyses (Berger, 1994; Mehta and Senchaudhuri, 2003). Effect sizes were analyzed using Cramer’s V, measured on a scale of 0 to 1, where
larger numbers represent larger effect sizes. For $2 \times 2$ tables, small effect sizes are generally considered to be from 0.1 to 0.3, medium effect sizes from 0.3 to 0.5, and large effect sizes $>0.5$ (Hatcher, 2018).

RESULTS

Pruning (most severe treatment)

We investigated whether the pruning treatment, starting sex (measured as percentage female in 2015), site, size, or the interaction of sex and treatment were significant predictors for survival or sex in 2017. We used forward-selection model building procedures, selecting the best model based on the Akaike information criterion (AIC). Our final multinomial multiple logistic regression model includes the pruning treatment and the starting sex in 2015 (measured as percentage female) as predictors for sex expression in 2017 (Table 1, $n = 52, df = 12$). The effect of the pruning treatment completely separated the data along the nonflowering and male outcomes. All of the nonflowering trees in 2017 belonged to the treatment group, while all of the male trees in 2017 belonged to the control group. The strength of the treatment as a predictor results in quasi-complete separation and yields extreme odds ratios. Of all the trees in the study, none of the pruned trees flowered completely male 2 years later (seven were female, two monoecious), and all the nonflowering trees in 2017 were from the pruned group (three nonflowering: Fig. 1A).

The pruning treatment had a significant impact on flowering sex in 2017 of females, males, and nonflowering trees (Fig. 1). Compared to monoecious trees, pruning significantly increased the odds of flowering female 2 years in the future (odds ratio = 3.94e4, $P < 0.001$). Pruned trees also had much higher odds of not flowering at all than of flowering monoeciously (odds ratio = 1.78e8, $P < 0.001$). Conversely, 2 years after a pruning treatment, trees had miniscule odds of flowering male (odds ratio = 2.81e-3, $P < 0.001$). Because of the strength of the treatment as a predictor, the odds ratios were either very large or very small; importantly, the confidence intervals for the significant coefficients did not overlap with zero. Starting sex was also a significant predictor for trees that did not flower in 2017. Effectively, any tree flowering even 1% female in 2015 had zero odds of being nonflowering in 2017. The only trees that did not reproduce in 2017 were pruned males.

Mortality was high in the study group. Of the 52 trees, nine died between the spring of 2015 and spring of 2016, an additional five died during 2016–2017. Compared to control trees, pruned trees had significantly higher odds of dying than expressing both sexes (odds ratio = 4.09e5, $P < 0.001$). Furthermore, for an increase of a single point in female sex expression, an individual had odds 1.05 times higher of dying within 2 years. Effectively, a 25% increase in female flowering increased the odds of dying by 3.5. A tree flowering 50% male, 50% female in 2015 had 12 times higher odds of being dead in 2017 than of flowering monoeciously again. Fully flowering females had odds 153 times higher of dying in 2 years than of flowering monoeciously (Table 1; Appendix S1). Barnard’s test indicated that pruning treatment was significantly correlated with mortality (n = 52, Wald’s $W = 3.25$, $P < 0.001$; Fig 1B) with a moderate effect size ($V = 0.451$). Pruning treatment was significantly correlated with flowering (n = 35, $W = 2.51$, $P = 0.035$; Fig. 1C, Table 3), with a moderate effect size ($V = 0.424$).

Full defoliation (severe treatment)

We investigated whether the defoliation treatment, starting sex in 2015, site, size, and the interaction of sex and treatment were significant predictors for sex in 2017. With forward-selection model building procedures, size, site, and the interaction of sex and treatment were shown to be nonsignificant. The final model with lowest AIC contained only starting sex and treatment as predictors (Table 2). Compared to control trees 2 years after defoliation, treated trees of any starting sex had odds 9.6 times higher of dying than of being male. Fully defoliated trees had

| TABLE 1. The effect of pruning and (previous) sex on survival and sex expression. Coefficients and their confidence intervals, and odds ratios for model parameters examining the effect of pruning on tree state. Model parameters include the pruning treatment and starting sex of individual Acer pensylvanicum trees, measured as a continuous variable from 0% female (that is, male) to 100% female. Untreated control trees are the category to which pruned trees are compared. For the outcome (sex in 2017), monoecy is the comparison category. Trees with significant odds ratios greater than one indicate outcomes with odds more likely than flowering monoeciously; odds ratios less than one indicate the outcome is less likely than flowering monoeciously. |
|-----------------|----------|-----------------|-----------------|-----------------|
| 2017 sex | Coefficient | Odds ratio | Lower Coef CI (2.5%) | Upper Coef CI (97.5%) | P-value |
| Intercept | | | | |
| Male | 0.47 | 1.59 | -0.86 | 1.80 | 0.492 |
| Female | -0.33 | 0.72 | -1.86 | 1.20 | 0.671 |
| Nonflowering | -9.23 | 9.76e-5 | -10.12 | -8.35 | 0.001*** |
| Dead | -3.50 | 3.02e-2 | -6.04 | -0.96 | 0.01*** |
| Pruning treatment | | | | |
| Male | -5.88 | 2.81e-3 | 5.88 | 6th decimal | 0.001*** |
| Female | 0.58 | 3.94e4 | 9.74 | 11.42 | 0.001*** |
| Nonflowering | 19.00 | 1.78e8 | 18.11 | 19.89 | 0.001*** |
| Dead | 12.92 | 4.09e5 | 11.79 | 14.05 | 0.001*** |
| Sex in 2015 (continuous; 0-1 scale) | | | | |
| Male | -7.38e-3 | 0.99 | -4.46e-2 | 2.99e-2 | 0.698 |
| Female | 2.67e-2 | 1.03 | -3.52e-3 | 5.70e-2 | 0.083 |
| Nonflowering | -7.57 | 5.1e-4 | -7.57 | -7.57 | 0.001*** |
| Dead | 5.03e-2 | 1.05 | 1.51e-2 | 8.54e-2 | 0.01*** |
**FIGURE 1.** (A) Sankey diagram showing sex expression and mortality over 3 years for *Acer pensylvanicum* trees. Control trees are shown on the left and pruned trees on the right, with sex expression in 2015 indicated with the darker bars on the far left of each top panel; sex in 2017 is indicated with the darker bars on far right in each panel. Males are in blue, females in pink, monoecious in yellow, nonflowering in green, and dead in gray. The paths representing individual trees take the color representing their sex in 2017. Treatment significantly affected sex expression in 2017. \( N = 52 \), 1B and C. Mosaic plots showing comparisons between groups for (B) mortality and (C) flowering after pruning. Control trees are on left; treated trees are on the right. Starting sex is on the bottom, with trees color-coded by status in 2017. For mortality (B), live trees are in light gray, trees that died are in dark gray. For flowering (C), trees that flowered are in dark purple; trees that did not flower are in light purple. Treatment significantly affected survival and flowering.
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4.5 times higher odds of being female than being male, regardless of their starting sex. Compared to male trees, trees that were nonflowering have much higher odds (39 times higher) of remaining nonreproductive than changing to male (Fig. 2). Twice as many defoliated trees expressed the female sex 2 years later, compared to control trees. Eleven of the 32 (34.4%) starting defoliated males died, compared to two (6.3%) control males (Appendix S2). Barnard’s test indicated that full defoliation significantly correlated with mortality ($n = 88$, $W = 2.76$, $P = 0.012$; Fig 2A) with a small effect size ($V = 0.295$) but did not correlate with flowering ($n = 72$, $W = 1.16$, $P = 0.148$; Fig 2B, Table 3).

**Partial defoliation (less severe treatment)**

Of the models examining the effect of partial defoliation on sex expression, 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 and nonsignificant $P$-values for the effect of treatment. Females had much higher odds of remaining female (odds ratio = 31 times, $P = 0.004$) or being nonflowering (odds ratio = 28 times, $P = 0.008$) than being male (Fig. 3; Appendix S3). Barnard’s test confirmed that partial defoliation did not significantly affect mortality or incidence of flowering ($n = 44$, $W = 0.374$, $P = 0.528$, Fig 3B; $n = 35$, $W = 0.077$, $P = 0.0577$, Fig. 3C, Table 3).

**Hydraulic disruption (less severe treatment)**

There were no statistically significant effects of phloem removal on sex expression (multinomial logistic regression, $n = 68$; on males: $P = 0.0768$, on females: $P = 0.0876$, on nonflowering: $P = 0.943$, on dying: $P = 0.314$). The simplest multinomial logistic regression model with lowest AIC had the hydraulic restriction treatment as the sole predictor of sex expression. This result indicated a trend for trees to flower either male or female, rather than monoeciously, following hydraulic disruption (Appendices S4, S5). The trend is not statistically significant.

**DISCUSSION**

This is the first experimental study of the effects of vegetative trauma on sex expression in a woody angiosperm. This series of multiple
FIGURE 2. (A) Sankey diagram showing sex expression over 3 years for defoliated *Acer pensylvanicum* trees (right) and control trees (left). Males are in blue, females in pink, monoecious in yellow, nonflowering in green, and dead in gray. Treatment significantly affected sex expression in 2017. *N* = 88, 1B and C. Mosaic plots comparing (B) mortality and (C) flowering after full defoliation. Control trees are on left; treated trees are on the right. Starting sex is on the bottom, with trees color-coded by status in 2017. For mortality (B), live trees are in light gray; trees that died are in dark gray. For flowering (C), trees that flowered are in dark purple; trees that did not flower are in light purple. Treatment significantly affected survival but not flowering.
FIGURE 3. (A) Sankey diagram showing sex expression over 3 years for partially defoliated *Acer pensylvanicum* trees (right) and control trees (left). Males are in blue, females in pink, monoecious in yellow, nonflowering in green, and dead in grey. Treatment did not significantly affect sex expression in 2017. \( N = 44 \), 1b and c. Mosaic plots depict no significant differences in (B) mortality and (C) flowering after partial defoliation. Control trees are on left; treated trees are on the right. Starting sex is on the bottom, with trees color-coded by status in 2017. For mortality (B), live trees are in light gray; trees that died are in dark gray. For flowering (C), trees that flowered are in dark purple; trees that did not flower are in light purple. Control and treatment groups did not significantly differ for rates of survival and flowering.
damage manipulations along a gradient of intensity, from moderate (hydraulic disruption and partial defoliation in late summer) through extreme damage (crown removal and full defoliation in mid-summer), showed clear patterns of intensity-dependent reactions in these striped maple trees. Only the most severe damage manipulations impacted sex expression in subsequent years, as seen in full defoliation and crown pruning experiments, where damage resulted in an increased prevalence of female sex expression and increased mortality.

Given the high mortality that resulted from the extreme damage manipulations, we expected to see some response to moderate levels of trauma. It was surprising that the less-severe treatments induced little response in individuals. While the high mortality among pruned and defoliated trees was significant (Table 3), it is not directly responsible for the increased prevalence of female flowering. That is, it is not simply that all the pruned male trees died and left females overrepresented among study trees. Rather, pruned trees of every starting sex died, and half of pruned males became fully or partially female; none remained male (Fig. 1; Appendix S1). Fully defoliated male trees had approximately equal probabilities of staying male (31%, 10/32), dying (34%, 11/32), or changing to fully or partially female (31%, 10/32).

There is no demonstrable correlation between treatment and state change in either the less severe partial defoliation or hydraulic restriction treatments (Fig. 3; Appendices S3, S5). These data point to the possibility of a threshold level of stress, below which the tree does not respond. Although reducing phloem connection in a branch by half had no effect on the flowering sex of that branch, 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 within 3 weeks (Blake-Mahmud and Struwe, 2018), these new data show that the legacy of damage persists and continues to affect sex expression in trees for 2 or more years.

The increased odds of female expression and nonflowering in trees that underwent severe crown pruning suggest that this kind of severe injury constitutes an important cue for sex change. The two trees classified as monoecious in 2017 provide interesting anecdotes that support this hypothesis (Fig. 1A). 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 2 years flowered male. Looking at 2 years of sex expression data for pruned trees seems to indicate that severe damage pushes monoecious trees toward complete female sex expression. Injury does not prohibit trees from basal sprouting from latent buds on mature trunks and adding male flowers in the future, but it does suggest one cause of sexual transitions between monoecy and female sex expression (Appendix S1).

Examples of the effects of trauma on sex expression in other species show conflicting results. In some taxa, trauma causes shifts toward male expression. For instance, chronic herbivory in monoecious Pinus edulis leads to increased male sex expression (Cobb et al., 2002). Cannabis sativa (Cannabaceae), a dioecious species with heteromorphic sex chromosomes (Berhin et al., 2014), is known to be sensitive to environmental cues including photoperiod (Schaffner, 1931; Freeman et al., 1980). Work done by Pritchard in 1916 showed that the removal of leaves and flowers could cue sex reversals in either sex, and flower removal cued a change in sex expression in two-thirds of 45 individuals. Specifically, removal of flowers in females led to future monoecy (i.e., increased male flowering) in all 27 females, while three of 14 males became monoecious after flower removal. In sexually plastic Arisaema triphyllum (Araceae), a reduction of the underground storage corm (Maekawa, 1924) or of leaf tissue (Bierzychudek, 1984) caused increased numbers of male plants.

Mutilation has the opposite effect in other species. For example, removal of flower heads increased female flowering in monoecious Cleome spinosa (Capparaceae; reviewed by Heslop-Harrison, 1957). In Zea mays (Poaceae), trauma encourages female flowering, and in Carica papaya (Caricaceae), a dioecious species with sex chromosomes and without documented ESD, severe pruning of males can trigger female flowering (Heslop-Harrison, 1957). In Mercurialis annua (Euphorbiaceae), a subdioecious annual herb with sex chromosomes (Pannell, 2017), pruning of males leads to temporary femaleness before returning to male sex expression (reviewed by Heslop-Harrison, 1957).

Most of the aforementioned results derive from work done in species without demonstrated ESD and therefore show the potential importance of trauma in modifying sex allocation within hermaphrodites (Lloyd and Bawa, 1984). Even when sex is known to be the result of sex chromosomes, as for C. papaya, M. annua, and Cannabis sativa, environment can play a modulating role and is perhaps responsible for the “leakiness” present in some plant sexual systems (J. Pannell, Université de Lausanne, personal communication). The effects of damage in large-scale changes between sexual phases (that is, in known species with ESD) has been limited to studies in A. triphyllum until now. In contrast to our findings, those studies indicated that trauma cued changes from female to male sex expression (Maekawa, 1924; Bierzychudek, 1984, reviewed by Korpelainen, 1998).

In Acer pensylvanicum populations, males outnumber females by more than three to one (Hibbs and Fischer, 1979; Blake-Mahmud and Struwe, 2019). 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 as a female would have higher predicted relative parentage of the next generation than if the same tree flowered male. Femaleness, however, is often considered more costly than male sex expression, due to the resources needed to provision fruits (Obeso, 2002; Case and Ashman, 2005). 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. Earlier work has shown that female mortality is disproportionately high in this species (Hibbs and Fischer, 1979; Blake-Mahmud and Struwe, 2019). 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 nonreproduction before switching to the higher-mortality sex. While under frequent investigation in animal systems, the influence of mortality on sex expression in plants has received little attention (Iwasa, 1991; de Jong and Klinkhamer, 2005; but see Nanami et al., 2004).

Defoliation and pruning presumably have multiple physiological impacts. These likely involve the production of a cue signaling damage and the reduction in concentrations of nonstructural carbohydrates, among other outcomes. Because of the effects of physical trauma in vascular 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, or flower removal, the reduction in nonstructural carbohydrates (NSCs) cannot be separated from the wounding process. In the full defoliation experiment, we confirmed that NSC concentration decreased for trees following leaf removal (J. Blake-Mahmud, unpublished data). However, separate studies indicated that females maintain higher concentrations of NSCs (Blake-Mahmud and Struwe, 2020, in this issue), lending support to the hypothesis that it is the damage cue, and not the NSC reduction, that is an important trigger for increased 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 (Metzger, 1995), so it is difficult to speculate on which hormones might be responsible without testing hormones. Many hormones are associated with responses to wounding, including ethylene, jasmonic acid, or salicylic acid (Bari and Jones, 2009). Gibberellins are associated with both stem elongation and maleness and produced in developing shoots (Davies, 2010); this hormone would have likely been decreased in severe pruning manipulations. Alternatively, there could be a bud hormone building up in the branch tips and cueing female flower development through active promotion (or lack of inhibition), such as auxins (Metzger, 1995; Davies, 2010). It seems less likely that a root hormone, such as cytokinin, is involved. While cytokinins seem to affect flower sex (Meilan, 1997) and are associated with femaleness in some plants such as Cannabis sativa (Metzger, 1995), it would make little sense that more female sex expression is observed when this female-enhancing hormone is prevented from fully reaching the developing buds. Direct exploration of the roles of hormones would be needed to understand the mechanistic links with sex expression.

Correlative and experimental studies support the findings that females in striped maple are in poorer overall health (Blake-Mahmud and Struwe, 2019) and that severe damage can cue female sex expression. Our study shows that Acer pensylvanicum is a novel and excellent model species for investigations into long-lived woody plants with ESD. Several unanswered questions remain regarding sex expression in this species that will be investigated in future studies. What are the hormonal, physiological, or genetic mechanisms underpinning these changes in sex? What is the role of monoeciously flowering individuals in striped maple populations? What are the demographic consequences of shifting sex ratios in this species? In addition to these questions, future work will model the evolution of threshold effects for injury and the conditions under which such a sex-determining mechanism would be maintained.

CONCLUSIONS

In conclusion, we have shown here that the hyper-local environment of an individual can inform understanding of sex expression in ways previously unexplored in species with ESD. We also show that severe damage may be an important sex-determining factor in sexually plastic species such as 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 increase the incidence of extreme weather events and disease outbreaks (Dale et al., 2001; Rosenzweig et al., 2001; Beniston and Innes, 2006; Findell et al., 2017; Moran et al., 2017), which may increase the incidence of insect herbivory and physical damage in understory tree populations like Acer pensylvanicum. In the short term, such damage may increase female expression in the population. However, given the high female mortality, it is questionable whether striped maple populations will remain demographically stable over extended periods in an increasingly stressful environment. In the long term, environmental cues affecting sex change might affect the survivability of this species in northeastern deciduous forests in North America.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Sex expression in pruned trees.

APPENDIX S2. Sex expression in fully defoliated trees.

APPENDIX S3. Sex expression in partially defoliated trees.

APPENDIX S4. Effect of hydraulic disruption on sex expression.

APPENDIX S5. Sex expression in trees with hydraulic restriction.

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