

What causes female bias in the secondary sex ratios of the dioecious woody shrub *Salix sitchensis* colonizing a primary successional landscape?¹

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PREMISE OF THE STUDY: Females often outnumber males in *Salix* populations, although the mechanisms behind female bias are not well understood and could be caused by both genetic and ecological factors. We investigated several ecological factors that could bias secondary sex ratios of *Salix sitchensis* colonizing Mount St. Helens after the 1980 eruption.

METHODS: We determined whether *S. sitchensis* secondary sex ratios varied across disturbance zones created by the eruption and across mesic and hydric habitats within each zone. For one population, we tracked adult mortality, whole-plant reproductive allocation, the number of stems, and plant size for 2 years. In a field experiment, we created artificial streams to test whether vegetative reproduction via stem fragments was sex-biased.

KEY RESULTS: We found a consistent 2:1 female bias in *S. sitchensis* secondary sex ratios across all disturbance zones and habitats. Despite female plants sometimes allocating more resources (in terms of carbon, nitrogen, and phosphorus) to reproduction than males, we found no evidence of sex-biased mortality. The establishment rate of *S. sitchensis* experimental stems did not differ between the sexes, indicating that vegetative reproduction was not distorting secondary sex ratios.

CONCLUSIONS: We hypothesize that *S. sitchensis* secondary sex ratios depend on either early-acting genetic factors affecting the seed sex ratio or sex-specific germination or survival rates before maturity, as opposed to factors associated with reproduction in adult plants.

KEY WORDS dioecy; fragmentation; primary succession; reproductive allocation; Salicaceae; *Salix*; sex ratio; survivorship; vegetative reproduction; willow

Dioecy (separate male and female individuals) occurs in a small, but widely distributed, number of angiosperm taxa, evolving independently multiple times from an ancestral cosexual state (Charlesworth, 1985; Renner and Ricklefs, 1995). Dioecious plant populations often exhibit some form of biased sex ratio, typically measured as a sex-based difference in the frequency of flowering ramets (Delph, 1999; Sinclair et al., 2012; Field et al., 2013a). Of the cases where sex ratios are biased, the norm appears to be for male bias, with female bias occurring half as often (Sinclair et al., 2012; Field et al., 2013a). What has remained unclear are which factors

(genetic or environmental) are responsible for the biased sex ratios observed in mature plant populations, and when during a plant's life cycle are biases introduced?

Diverse early-acting genetic factors have been advanced to explain deviations from the 1:1 primary or seed sex ratio (the sex ratio at fertilization) predicted under the null model of negative frequency-dependent selection proposed by Düsing (Edwards, 2000) and Fisher (1930). These include prezygotic factors, such as Y-chromosome degradation (Vyskot and Hobza, 2004; Stehlik et al., 2007) leading to gametic viability selection (Stehlik and Barrett, 2005; Stehlik et al., 2008), as well as sexual distortion via sex-linked meiotic drive (Taylor and Ingvarsson, 2003) or sexual conflict (Taylor, 1994). In addition, ecological factors affecting reproduction and dispersal can result in biased evolutionarily stable seed sex ratios. For example, a high degree of sib mating may cause mild female-bias, while local resource competition can male-bias seed sex ratios, the more pollen dispersal distance exceeds that of seed (Maynard Smith, 1978; Bulmer and Taylor, 1980; de Jong et al., 2002).

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Post-germination ecological factors, which affect the secondary sex ratio (the post-germination sex ratio, hereafter referred to as sex ratio), are often rooted in the difference in reproductive allocation between the sexes. These differences give rise to sexual dimorphism in traits known to affect the frequency and distribution of each sex in a population at multiple spatial scales. For example, females typically allocate greater amounts of carbon or total biomass to reproduction than do males, which can in turn lead to higher reproductive costs, such as delayed age or size at first flowering, less frequent flowering, higher mortality following reproductive events or in response to herbivory, and lower rates of clonal growth (see reviews by Delph, 1999; Obeso, 2002; Cornelissen and Stiling, 2005; Sinclair et al., 2012; Field et al., 2013a). These first three reproductive costs can cause male-bias in the sex ratios of flowering genets (Barrett et al., 2010; Field et al., 2013a).

Females can display differing sensitivity to environmental stress than males (Popp and Reinartz, 1988; Verdú and García-Fayos, 1998; Espírito-Santo et al., 2003; Li et al., 2007; Randriamanana et al., 2015), causing broad-scale geographic variation in sex ratios when one sex is found less frequently in harsh sites (Grant and Mitton, 1979; Marques et al., 2002; Ortiz et al., 2002; Li et al., 2007). Male-bias in less favorable environments is also seen at finer spatial scales, when sexes segregate by habitat, based on resource availability (typically water) (Bierzychudek and Eckhart, 1988). This spatial segregation of the sexes could be due to differential mortality between the sexes as a direct reproductive cost (Bierzychudek and Eckhart, 1988), habitat specialization as an adaptive response to overcome high reproductive costs (Dawson and Ehleringer, 1993), or niche partitioning as a means to reduce intersexual competition (Freeman et al., 1976; Cox, 1981; Ågren, 1988; Mercer and Eppley, 2010).

Several difficulties arise in determining the cause of biased sex ratios in dioecious plant populations. Ecological and genetic factors may simultaneously or sequentially reinforce or attenuate one another, causing a population's sex ratio to vary with respect to age, life stage, or location, and making it problematic to isolate the effects of individual factors. For example, the female bias in *Rumex nivialis* seed sex ratios due to certation becomes more pronounced in the sex ratios of later life stages from higher male mortality associated with Y-chromosome degradation (Stehlik and Barrett, 2005, 2006). Furthermore, while there are numerous explanations for male-biased sex ratios or female-biased seed sex ratios in plant families known to possess heteromorphic sex chromosomes, causes of strongly female-biased sex ratios in families with homomorphic sex chromosomes remain less understood.

Strongly female-biased sex ratios are restricted mainly to clonal shrubs with abiotic pollen dispersal that colonize disturbed habitat (Sinclair et al., 2012; Field et al., 2013a). For these plants, male allocation to reproduction may exceed female allocation for certain resource currencies (such as nitrogen or phosphorus), raising the possibility that the resources that limit plant growth are sex-specific (Chapin, 1989; Antos and Allen, 1990; Ashman and Baker, 1992). Such differences could result in higher male reproductive costs in species where males allocate large amounts of nitrogen to pollen production (Harris and Pannell, 2008) and could be a contributing factor to female bias (Field et al., 2013a). Such a scenario might especially pertain to long-lived woody plants colonizing disturbed areas (such as early successional systems where nutrient limitation is common) but remains unexplored.

While sex bias in woody plant clonality is well documented (Obeso, 2002), no studies have investigated sex bias in the establishment

of asexual propagules such as stem fragments. Sexual dimorphism in vegetative reproduction via fragmentation should distort sex ratios and would most strongly affect dioecious populations that rely on vegetative reproduction following disturbance events as a means of expansion (Walker and del Moral, 2003).

In this study, we examined the adult plant (genet) sex ratios of three populations of *Salix sitchensis* (Sitka willow), a dioecious, pioneering woody shrub currently colonizing Mount St. Helens 29–31 yr after the 1980 eruption. Sex ratios in *Salix* are typically female-biased (Myers-Smith and Hik, 2012, and references therein), and we investigated sexual dimorphism in several life history traits well known to cause sex ratio bias. Common garden experiments with *Salix repens* and *Salix viminalis* suggest that sex determination in *Salix* is under genetic, not environmental control (Alström-Rapaport et al., 1997; de Jong and Meijden, 2004). However, the sex-determining genetic mechanism is not well understood, and whether *Salix* species have a common sex determination (SD) locus remains in dispute (Alström-Rapaport et al., 1998; Gunter et al., 2003; Semerikov et al., 2003; Temmel et al., 2007). Recently, Pucholt et al. (2015) reported that sex determination in *S. viminalis* is female heterogametic, with the SD locus found on chromosome XV. Hou et al. (2015) confirmed female heterogamety in *Salix suchowensis* as well, with the SD locus also on chromosome XV. Pucholt et al. (2015) and Galdes et al. (2015) posited that the lack of heteromorphic sex chromosomes observed in Salicaceae is due to turnover (the process by which a new SD locus replaces an older one on nascent sex chromosomes), which has slowed the evolution of heteromorphic sex chromosomes in this family (see Perrin, 2009; Bachtrog, 2013). While sex determination in *S. sitchensis* is unknown, none of the *S. sitchensis* plants monitored in this study demonstrated di- or produced hermaphroditic flowers, consistent with genetic and not environmental sex determination. In addition, we did not observe any instances where flowers on experimentally planted ramets (either in this study or in other common garden plots on Mount St. Helens) differed from the sex of the parent plant from which they were harvested.

We asked five questions: (1) Are populations of *S. sitchensis* colonizing three disturbance zones on Mount St. Helens sex-biased? (2) Do *S. sitchensis* populations in these three disturbance zones exhibit spatial segregation of the sexes between upland (mesic) and riparian (hydric) habitats? Is sexual dimorphism in (3) adult survivorship or (4) vegetative reproduction through stem fragments responsible for any observed sex bias seen in riparian habitat, and (5) does nutrient and carbon reproductive allocation differ by sex and habitat?

MATERIALS AND METHODS

Study system—The 1980 eruption of Mount St. Helens involved a complex set of geophysical forces that transformed a 600 km² area supporting forest, riparian, and meadow habitats into a complex mosaic of disturbance zones. These zones differed dramatically in the types and abundance of biological legacies remaining in the post-eruption environment (Zobel and Antos, 1997; Crisafulli et al., 2005b; Swanson and Major, 2005). At one end of the disturbance gradient are primary successional areas (the debris avalanche and pyroclastic flow) where all vestiges of life were removed or deeply buried by sterile volcanic rock (Fig. 1). A region of intermediate disturbance (the blowdown zone), included leveled forests

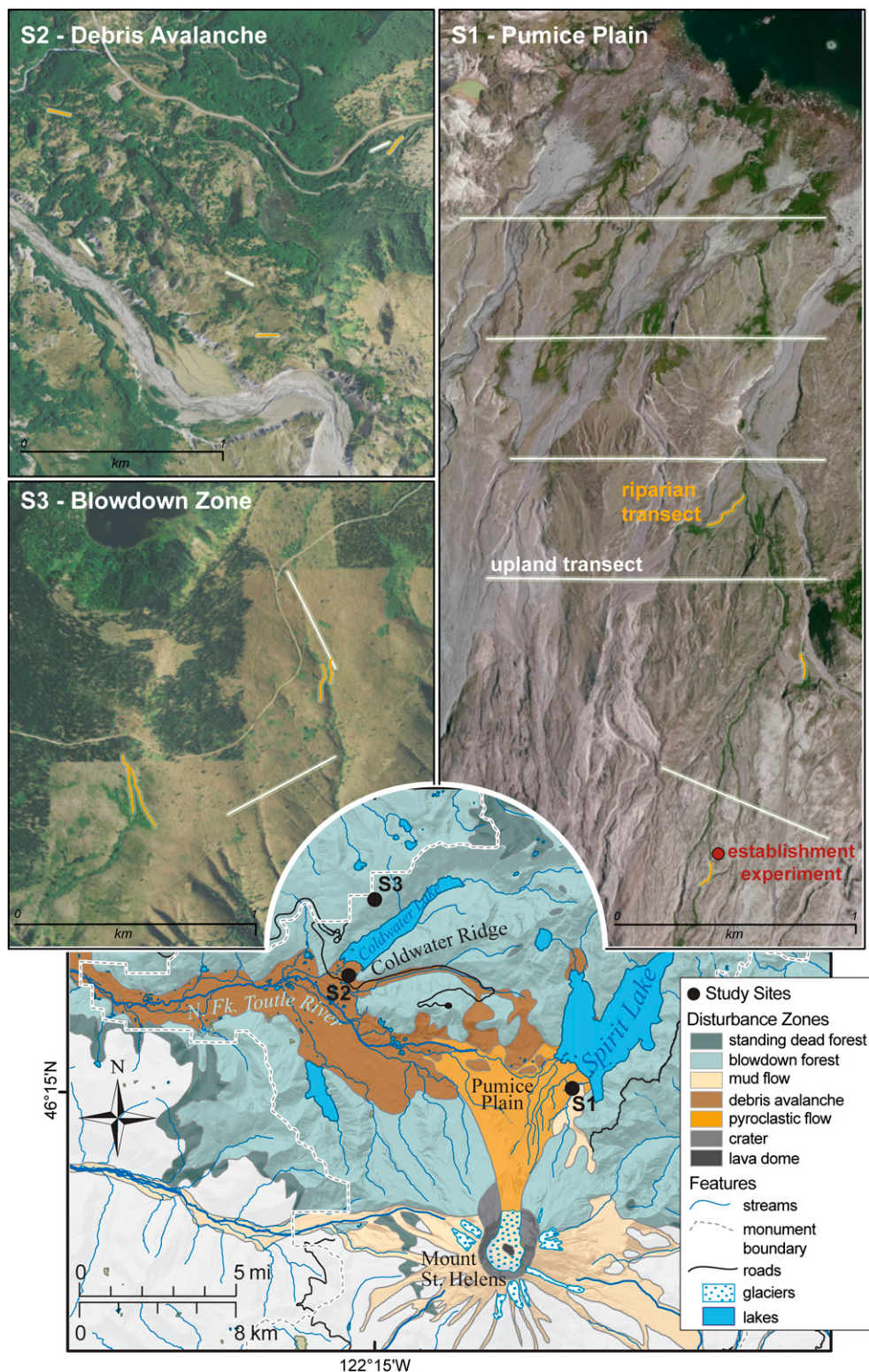


FIGURE 1 The distribution of primary volcanic deposits from the 1980 Mount St. Helens eruptions (adapted from Swanson and Major, 2005). We denoted upland (white lines) and riparian (orange lines) transects along which we conducted sex surveys of *Salix sitchensis* plants in 2011 and in the case of the Pumice Plain, where we tagged and monitored *S. sitchensis* plants from 2009 to 2011 and collected *S. sitchensis* catkins in 2011 and 2012. We denoted the location of the ramet establishment experiment with a red dot.

with scattered and often isolated refugia that collectively contained most species assumed to be present in the pre-eruption landscape, but at vastly reduced abundances. We focused on *S. sitchensis* populations colonizing upland and riparian habitats in the (1) blowdown zone, (2) debris avalanche, and (3) a 15 km² area of mainly pyroclastic flow known as the Pumice Plain, areas that represent a gradient of disturbance intensities, biological legacies, and other environmental conditions.

Pockets of *S. sitchensis* in the blowdown zone that survived the 1980 eruption likely served as the donor population for the initial colonization of the debris avalanche and Pumice Plain via long-distance seed dispersal. Subsequently, colonization has become increasingly locally controlled as *S. sitchensis* spread along riparian corridors, where it grows densely, and in adjacent upland areas, where it is sparsely distributed (Wood and del Moral, 1988, 2000). During our study, upland *S. sitchensis* plants flowered on the Pumice Plain for several weeks in late May to early June, just before leaf emergence, while in nearby riparian zones flowering was delayed by 1–2 wk.

Salix is dual pollinated by wind and insects, although which mechanism predominates depends on the species and abiotic conditions during flowering (Argus, 1974; Sacchi and Price, 1988; Tamura and Kudo, 2000). For *S. sitchensis* colonizing Mount St. Helens, we do not know whether wind or insect pollination is more responsible for successful fertilization, but we have observed bees and unknown dipterans visiting flowers on Pumice Plain plants. Seed set occurs in early July on the Pumice Plain, and seeds are dispersed by wind and running water (Johnson, 2000; Karrenberg et al., 2002). Seeds are short-lived, nondormant, and depend critically on favorable microsites for germination (Densmore and Zasada, 1983). *Salix* spreads vegetatively through root suckering or layering, (a process where a still-attached branch is buried and develops roots and new aerial shoots), and the establishment of stem fragments (Krasny et al., 1988; Karrenberg et al., 2002; Moggridge and Gurnell, 2009). On Mount St. Helens,

we have observed both the clonal spread of *S. sitchensis* via layering and the establishment of large numbers of stems fragmented from their parent plants following seasonal hydrological disturbance (Fig. 2).

Salix herbivory is common on Mount St. Helens; larvae of the poplar-willow weevil (*Cryptorynchus lapathi*) routinely attack stems, elk (*Cervus elaphus*) browse new shoots, and *Microtus* and *Peromyscus* rodents denude riparian plants of their bark (Crisafulli et al., 2005a; Che-Castaldo, 2014). Weevil larvae are the primary herbivore on *Salix* plants in this system, causing high levels of stem mortality. Although stem reproduction increases larval attack, weevil herbivory is not sex-biased (Che-Castaldo, 2014). Localized herbivory by beavers (*Castor canadensis*) also contributes to *Salix* spread via fragmentation.

Plant survivorship, reproduction, and size—We tagged 261 *S. sitchensis* genets in 2009 on the Pumice Plain and monitored their demography for three growing seasons (2009–2011). In upland habitat, we selected plants at 100-m intervals along 1-km subsets of four 2-km and one 1-km permanent transects. These transects form a grid overlaid on the Pumice Plain, with each transect separated by 500 m (Fig. 1).

In 2009, we determined the sex (if possible) and marked the five *S. sitchensis* plants nearest to each transect point that had at least

one ramet that originated from the ground or caudex (defined as a 1st order stem) ≥ 15 mm in basal diameter. The minimum stem size requirement was to ensure that we only tagged well-established willow plants (hereafter referred to as adult). We tracked some plants that were initially nonreproductive, a subset of which eventually flowered allowing us to assign their sex. Given the high frequency of flowering observed for plants of a known sex, we categorized plants that did not flower during our study as “nonreproductive” and all other plants as “reproductively capable”. Individual upland plants could usually be distinguished visually, but when plants were located very close to one another, or when we were unable to see an obvious caudex due to burial of stems by sediment, we traced shallow roots from each plant to group ramets by genet. In riparian habitat, we established paired transects 100–140 m in length along both sides of three permanent streams dominated by dense thickets of *Salix* and *Alnus viridis* (Fig. 1). Visual separation of ramets by genets was not possible due to the high density of *Salix* in riparian zones. To ensure that marked plants represented different individuals, we located plants at 5-m intervals and sexed and marked only plants that had at least one ramet ≥ 15 mm in basal diameter and whose ramets could be clearly traced back to its caudex.

We visited these plants twice each season for up to 3 years. In June of 2010 and 2011, we recorded whether the plant was flowering, and, if so, the number of catkins produced. In August of each



FIGURE 2 (A) Large numbers of buried *Salix sitchensis* plants and stem fragments establishing on the Pumice Plain near Spirit Lake in 2014. (B) A partially buried *S. sitchensis* stem fragment on the Pumice Plain in 2014 that is producing shoots and roots from dormant buds.

year, we recorded plant survivorship and the basal stem area of all 1st order stems and any branches directly attached to these stems (defined as 2nd order stems) ≥ 12 mm in basal diameter on each tagged plant. We used the sum of these basal stem areas (hereafter referred to as total basal area) as a proxy for aboveground plant size. Plants were scored as alive if they possessed any ramets with living foliage and dead otherwise.

Plant flowering sex ratios—We conducted sex surveys of flowering *S. sitchensis* genets in 2011 on the Pumice Plain along the same transects used for tagging and monitoring adult plants. We conducted similar surveys in riparian and upland habitat on the debris avalanche and blowdown zone (Fig. 1). For all surveys, we scored plants that had at least one stem ≥ 15 mm in basal diameter as male, female, or unknown (nonreproductive) based on observations of sex-specific catkin morphology. To ensure that we surveyed individual upland genets, we employed the methodology described in the previous section for tagging upland plants. On the Pumice Plain, we scored all adult plants within 25 m of each surveyed upland transect point. In the debris avalanche and blowdown zones, we established two (blowdown zone) or three (debris avalanche) belt transects (100 $\times$ 10 m) in upland areas and scored all plants meeting the minimum size requirement within these belts. For riparian plants, we surveyed plants at two (blowdown zone) or three (Pumice Plain and debris avalanche) riparian sites in each disturbance zone using the method described previously (Fig. 1). Riparian transects ranged from 100 to 320 m long, depending on the stream.

Catkin biomass and nutrient content—In addition to counting all catkins produced on tagged plants, we collected catkins from untagged *S. sitchensis* plants on the Pumice Plain to measure their size and nutrient content. We selected only male catkins whose flowers had reached reproductive maturity but had not yet lost their pollen to wind or pollinators and female catkins whose seeds had matured but had not yet dispersed. Due to the shortness of these phenophases and the spatial and temporal heterogeneity in *S. sitchensis* flowering observed on and between individual plants on Mount St. Helens, collection was done opportunistically along a subset of the transects used for the genet sex surveys. For upland *S. sitchensis*, we harvested 1–5 catkins per plant from 25 male plants (June 2011) and from 62 female plants (July 2011) located within a belt 50 m wide centered on the transect points whose length matched the start and end points used in the genet sex surveys. For riparian *S. sitchensis*, we harvested a similar number of catkins from 20 male and 48 female plants (July 2012) along two of the Pumice Plain riparian transects.

We dried all catkins within 24–48 h of collecting at 60°C for 3–5 d in a drying oven prior to weighing. We ground catkins using a ball mill (Retsch, Newtown, Pennsylvania, USA), and then pooled these for each plant. We determined catkin %P by mass by placing a known mass (~ 2 mg) in a muffle furnace at 550°C for 2 h (Miller, 1998), followed by colorimetric analysis using the ammonium molybdate method (Clesceri et al., 1998). We determined catkin %N and %C by combusting samples for carbon and nitrogen elemental analysis with an ECS 4010 elemental analyzer (Costech Analytical, Valencia, California, USA). We separated N_2 and CO_2 gases with a 3.0 m GC column (40°C) and analyzed for total area with a DELTA^{PLUS} XP continuous flow isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) or the thermal conductivity detector on the elemental analyzer (Brenna et al., 1997).

Ramet establishment experiment—We created artificial streams to experimentally mimic conditions in which *S. sitchensis* spreads vegetatively through the establishment of severed stems after a hydrological disturbance. In the spring of 2007, we harvested *S. sitchensis* ramets from plants of known sex in three locations on the Pumice Plain that had high densities of flowering willow plants. We selected a single living ramet per genet whose basal diameter ranged from 1.5 to 3.5 cm and was free of insect attack and signs of cankers or injuries. We removed all side branches, trimmed cuttings to a length of 60–100 cm, and stored them submerged in cold water for several days before planting in an experimental plot on the Pumice Plain just south of Forsyth Creek's initial branch point on the northeast flank of Mount St. Helens (46.230881°N, 122.164084°W, elevation 1282 m a.s.l.). This plot contained two dried, willow-free rill beds 10 m apart, each roughly 1 m wide, 65 m long, and several centimeters deep (Fig. 3).

We used a gravity-fed irrigation system to continually deliver water from nearby Forsyth Creek to each rill at 1135–2270 L/h during the dry summer months of July to early September from 2007 to 2009. The slope of the experimental plot and topography of the rills channeled irrigated water in such a way as to create two artificial streams when these rills were watered. Using a completely randomized design, we planted 60 male and 60 female ramets along both streams randomized by sex and source location, where each ramet was separated by 50 cm from its neighbors. We inserted ramets to a depth of at least 50 cm and sprayed them twice annually with the broad-spectrum pyrethroid bifenthrin (Onyx insecticide; FMC Agricultural Solutions, Philadelphia, Pennsylvania, USA) to prevent stem attack by the poplar-willow weevil. We constructed an electric fence around both rills to discourage elk browse. We allowed ramets to establish over two growing seasons and in August 2009 scored each ramet as alive or dead based on the presence of living shoots and foliage. We did not observe elk, rodent, or weevil herbivory on the experimentally planted ramets.

Data analysis—We used binomial generalized linear models to estimate *S. sitchensis* flowering sex ratios, reproductive and flowering frequencies, and ramet and adult survivorship. To account for overdispersion, we used a hierarchical zero truncated Poisson model to estimate the number of stems per plant. We used linear regression models to estimate catkin nutrient content and log normal regression models to estimate catkin production, aboveground plant size, and catkin mass. Specifically we estimated:

- The adult flowering plant sex ratios as $F_{ij} \sim \text{binomial}(T_{1,ij}, p_{1,ij})$, where $T_{1,ij}$ is the number of flowering plants surveyed in 2011 in the i_{th} habitat and j_{th} disturbance zone, F_{ij} is the number of these plants that were female, and $p_{1,ij} = P(F|R)$ is the probability of being female given that a reproductively capable adult plant is flowering.
- The Pumice Plain sex-specific flowering frequencies as $R_{ik} \sim \text{binomial}(S_{1,ik}, p_{2,ik})$, where $S_{1,ik}$ is the number of tagged plants in 2011 in the i_{th} habitat and of the k_{th} sex, R_{ik} is the number of these plants that flowered in 2011, $p_{2,i1} = P(R|F)$ is the probability of flowering given that a reproductively capable adult plant is female, and $p_{2,i2} = P(R|M)$ is the probability of flowering given that a reproductively capable adult plant is male.
- The Pumice Plain overall flowering frequency as $N_i \sim \text{binomial}(X_i, p_{3,i})$, where $X_i = S_{1,i1} + S_{1,i2}$ is the number of tagged male and female plants in 2011 in the i_{th} habitat, $N_i = R_{i1} + R_{i2}$ is the

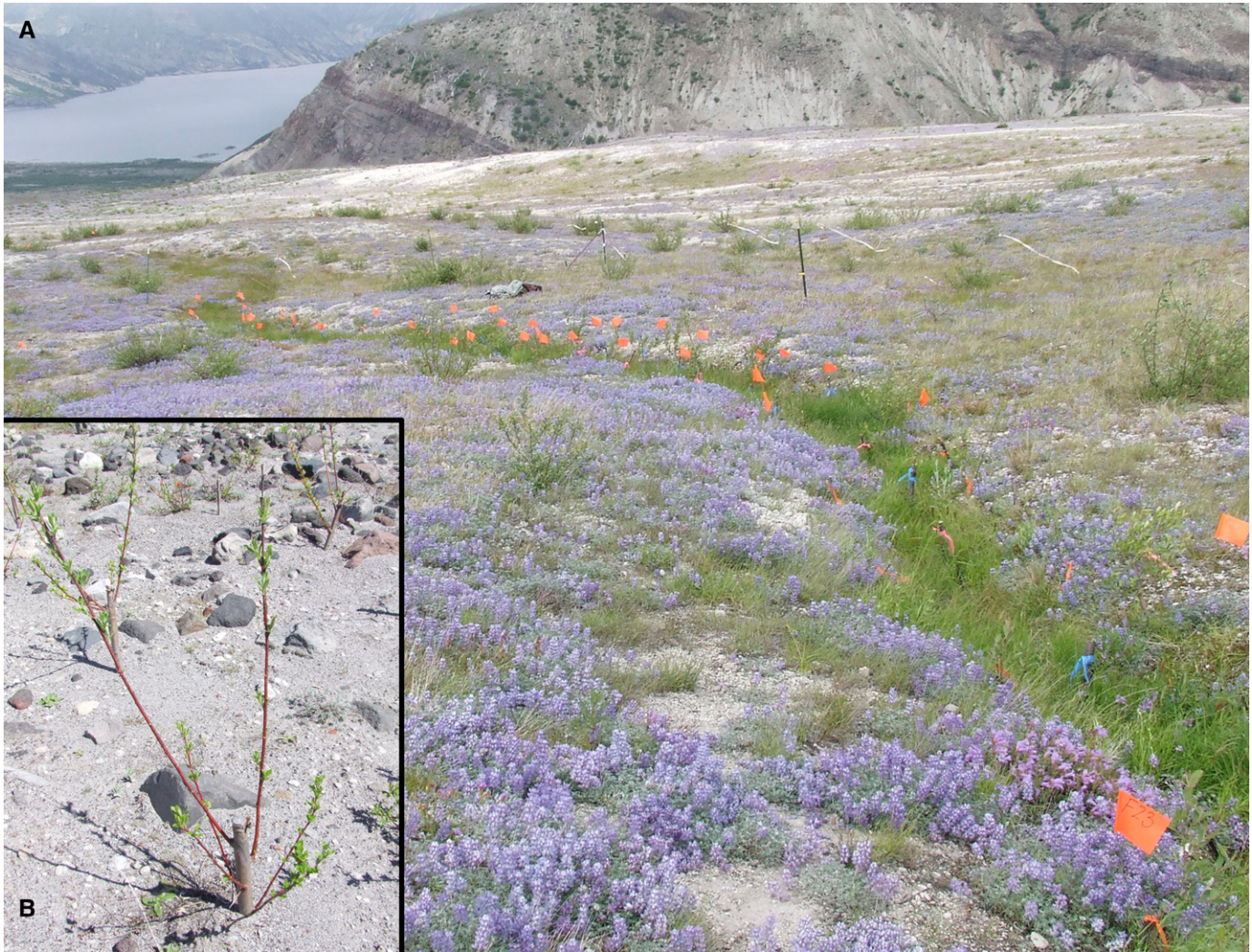


FIGURE 3 (A) Layout of the *Salix sitchensis* ramet establishment experiment on the Pumice Plain in summer 2009, just after measuring ramet survivorship. The colored flags mark surviving ramets in the eastern rill. (B) An experimentally planted ramet producing shoots and roots from dormant buds.

number of these plants that flowered in 2011, and $p_{3,i} = P(R)$ is the probability of flowering for a reproductively capable adult plant.

- Adult plant survivorship as $S_{1,ik} \sim \text{binomial}(T_{2,ik}, \phi_{1,ik})$, where $T_{2,ik}$ is the number of plants tagged in 2009 in the i_{th} habitat and of the k_{th} sex, $S_{1,ik}$ is the number of these plants alive in 2011, and $\sqrt{\phi_{1,ik}}$ is the annual survival probability for adult reproductively capable plants.
- Ramet survivorship as $S_{2,k} \sim \text{binomial}(T_{3,k}, \phi_{2,k})$, where $T_{3,k}$ is the number of ramets experimentally planted in 2007 of the k_{th} sex, $S_{2,k}$ is the number of these ramets alive in 2009, and $\sqrt{\phi_{2,k}}$ is the annual survival probability for establishing ramets.
- Number of stems per plant as $ST_{ikpr} \sim \text{ZTP}(\lambda_{ikpr}) \times \text{gamma}(\lambda_{ikpr} | a_{ikr}, b_{ikr})$, where ZTP is the zero truncated Poisson distribution, ST_{ikpr} is the number of stems on the p_{th} tagged plant in the r_{th} year in the i_{th} habitat and of the k_{th} sex, and the mean number of stems per plant is

$$\eta_{ikr} = \frac{a_{ikr}}{b_{ikr}} \left(1 - e^{-\frac{a_{ikr}}{b_{ikr}}} \right)^{-1}.$$

- Aboveground plant size as $TBA_{ikpr} \sim \text{lognormal}(\mu_{1,ikr}, \sigma_{1,r})$, where TBA_{ikpr} is the total basal stem area (cm^2) of the p_{th} tagged plant, in the r_{th} year in the i_{th} habitat and of the k_{th} sex, and $e^{\mu_{1,ikr}}$ is the median total basal stem area per plant.
- Plant catkin production as $C_{ikpr}/TBA_{ikpr} \sim \text{lognormal}(\mu_{2,ikr}, \sigma_{2,r})$, where C_{ikpr} is the number of catkins on the p_{th} plant in the r_{th} year in the i_{th} habitat and of the k_{th} sex, and $e^{\mu_{2,ikr}}$ is the median whole plant catkin production per unit stem area (cm^2).
- Catkin mass as $M_{ikp} \sim \text{lognormal}(\mu_{3,ikr}, \sigma_{3,k})$, where M_{ikp} is the average mass of a catkin on the p_{th} plant in the i_{th} habitat and of the k_{th} sex and $e^{\mu_{3,ikr}}$ is the median catkin mass.
- Catkin nutrient content as $E_{iknp} \sim \text{normal}(\mu_{4,ikn}, \sigma_{4,n})$, where E_{iknp} is the average nutrient content of a catkin for the n_{th} nutrient on the p_{th} plant in the i_{th} habitat and of the k_{th} sex.

We fit all models using Bayesian methods and estimated the posterior distributions for all parameters using Markov chain Monte Carlo (MCMC) methods implemented in the program JAGS 3.4.0 (Plummer, 2013a) with the rjags package (Plummer, 2013b) in the R computing environment (R Core Team, 2013). We

chose vague uniform or gamma priors and computed three chains for each parameter, each with a different initial value. After a burn-in period of 10 000 iterations, we accumulated 5000 samples from each chain.

We evaluated convergence by visually inspecting trace plots to assure stationarity and homogeneous mixing and by using the diagnostics of Gelman (Brooks and Gelman, 1998). We assessed model fits with posterior predictive checks (Gelman et al., 2013). We used residual plots to confirm that variances between groups were homogeneous, where appropriate. We computed derived quantities for 2010 and 2011 whole-plant allocation of N, P, and C to reproduction, standardized for aboveground plant size, as $RA_{iknr} = e^{\mu_{2,jkr}} \times e^{\mu_{3,jkr}} \times \mu_{4,iknr}$, where RA_{iknr} is reproductive allocation in terms of the n_{th} nutrient, for plants in the r_{th} year, in the i_{th} habitat, and of the k_{th} sex.

A substantial number of monitored *S. sitchensis* plants remained nonreproductive during the study, which could cause the true population sex ratio to differ from the flowering sex ratio, if the nonreproductive class was disproportionately one sex. Sex-specific differences in the frequency of flowering could also distort the flowering sex ratio from the true population sex ratio (Field et al., 2013a). We addressed these two difficulties in the following way. First, in the absence of additional information, we were cautious to restrict our inferences regarding sex ratios to a subset of the population, specifically adult flowering *S. sitchensis* plants. Second, we investigated whether sexual dimorphism in flowering frequencies could cause the observed flowering sex ratio on the Pumice Plain to differ from the unobserved sex ratio of all reproductively capable willows of similar size. We employed Bayes' theorem to compute the habitat-specific probabilities that a reproductively capable Pumice Plain *S. sitchensis* plant was female as

$$P(F) = \frac{P(F|R)P(R)}{P(R|F)} = \frac{p_{1,i3}p_{2,i1}}{p_{3,i}}.$$

Alternatively, we could have estimated $P(R)$ from the flowering plant sex surveys instead of from the tagged plants. However, the degree to which the sex ratio of reproductively capable plants departs from the flowering sex ratio is determined by the ratio of $P(R)$ to $P(R|F)$. Temporal heterogeneity in *S. sitchensis* flowering on Mount St. Helens could affect this ratio, given that the Pumice Plain flowering plant sex surveys were done later in the growing season than the reproductive survey of tagged plants.

Given that all models were means-parameterized, we computed simple effects as the difference between two parameters' posterior distributions or the difference between functions of several parameters' posterior distributions. We considered the effect of sex, habitat, or disturbance zone to be meaningful if the Bayesian 95% credible interval of the derived quantity's posterior distribution did not overlap zero, and otherwise unimportant. Likewise, we considered the *S. sitchensis* flowering sex ratio in the i_{th} habitat and k_{th} disturbance zone to be sex biased if the Bayesian 95% credible interval for $p_{1,ik}$ did not overlap 0.5. Our decision to use Bayesian methods in this study was driven largely by the ability to calculate derived quantities, such as reproductive allocation and the sex ratio of reproductively capable plants, as functions of multiple parameters (often estimated from different data sets), while still fully accounting for the uncertainty in each of the parameter estimates.

RESULTS

In all disturbance zones, flowering *S. sitchensis* plant sex ratios were female-biased approximately 2:1 in both riparian and upland habitats (Fig. 4). Within each habitat, there was no evidence that flowering plant sex ratios differed across disturbance zones (Fig. 4). Within each disturbance zone, flowering *S. sitchensis* did not exhibit spatial segregation of the sexes by habitats (Fig. 4). The flowering frequencies of male and female plants did not differ from one another in 2011 (Fig. 5), and consequently, the sex ratios of flowering and reproductively capable plants on the Pumice Plain did not differ (Fig. 6).

Survivorship of adult *S. sitchensis* plants on the Pumice Plain was not sex-biased in either riparian or upland habitat, although survivorship was higher for female riparian plants compared with female upland plants (Fig. 7). Similarly, roughly equal fractions of experimentally planted male and female ramets established in the artificial streams (Fig. 7).

The number of stems and the total basal stem area per plant did not differ by sex in either habitat (Figs. 8, 9). In 2010, upland plants of both sexes had more 1st order stems than riparian plants did, while in 2011 all plants had the same number of 1st order stems (Fig. 8). Riparian plants had a much greater total stem area than did upland plants for both sexes in each year, indicating that riparian plants were typically "branchier", possessing more higher-order stems than did upland plants (Figs. 8, 9).

Whole plant catkin production did not differ by sex in either habitat for 2010 and 2011, although in 2011 male upland plants produced more catkins per unit stem area than did male riparian plants (Table 1). In both habitats, male catkins had higher concentrations of N and P than did female catkins, but female catkins were substantially larger (Table 2). In addition, catkins produced in upland

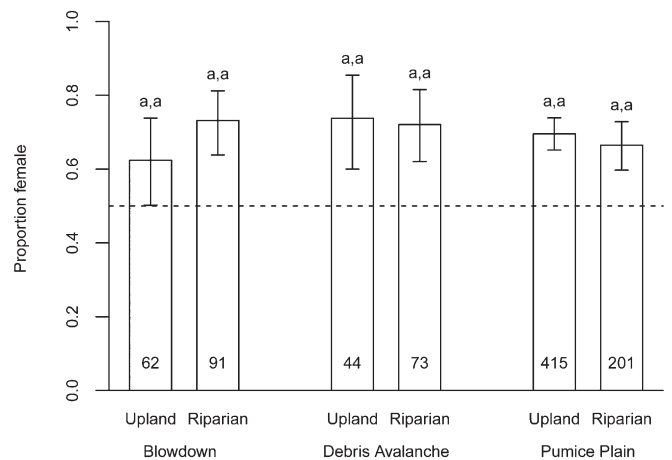


FIGURE 4 Mean sex ratios of flowering *Salix sitchensis* plants in upland and riparian areas surveyed in 2011 in three disturbance zones ($p_{1,ij}$). Error bars represent Bayesian 95% credible intervals of the posterior distributions of the means. The first letter in each pair is for comparisons between riparian and upland sex ratios within each disturbance zone. The second letter in each pair is for comparisons between disturbance zones within each habitat. The same letters for a comparison indicate that the 95% credible interval of the posterior distribution of the difference between the two means being compared overlaps zero. The sample sizes are listed at the base of the bars.

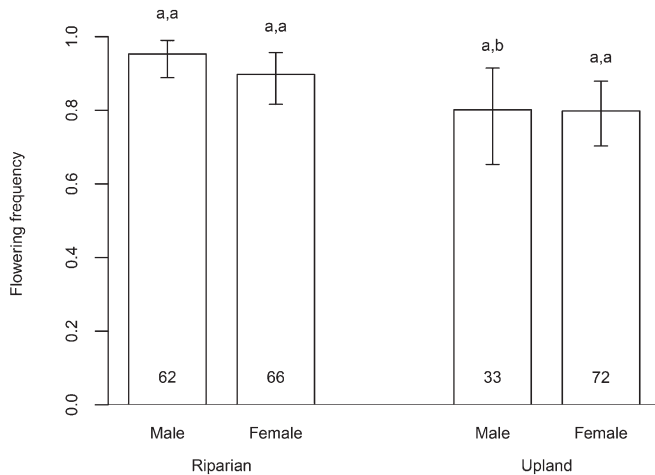


FIGURE 5 Mean flowering frequencies for tagged *Salix sitchensis* plants on the Pumice Plain in 2011 ($p_{2,ij}$). Error bars represent Bayesian 95% credible intervals of the posterior distributions of the means. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between adult riparian and upland plants within each sex. When present, different letters indicate that the 95% credible interval of the posterior distribution of the difference between the two means being compared does not overlap zero. The sample sizes are listed at the base of the bars.

areas were smaller than those produced in riparian zones on plants of the same sex (Table 2). At the whole plant level, female plants allocated the same amount of resources or more to flowering and seed set than male plants did to flowering and pollen production across all resource currencies measured (C, N, and P) (Fig. 10). Upland male and female plants allocated the same amount of N and P to reproduction (with the exception of 2011), while female riparian

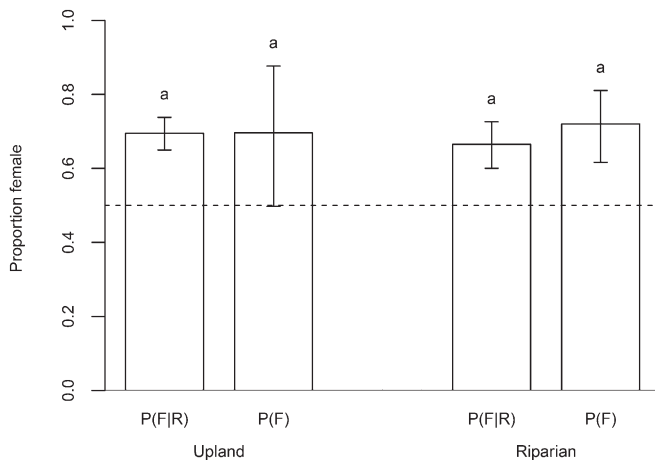


FIGURE 6 Mean sex ratios of flowering, $P(F|R)$, and all reproductively capable, $P(F)$, upland and riparian *Salix sitchensis* plants on the Pumice Plain in 2011. Error bars represent Bayesian 95% credible intervals of posterior distributions of the means. The letter is for comparisons between flowering and all reproductively capable plants in either upland or riparian habitat. The same letters for a comparison indicate that the 95% credible interval of the posterior distribution of the difference between the two means being compared overlaps zero.

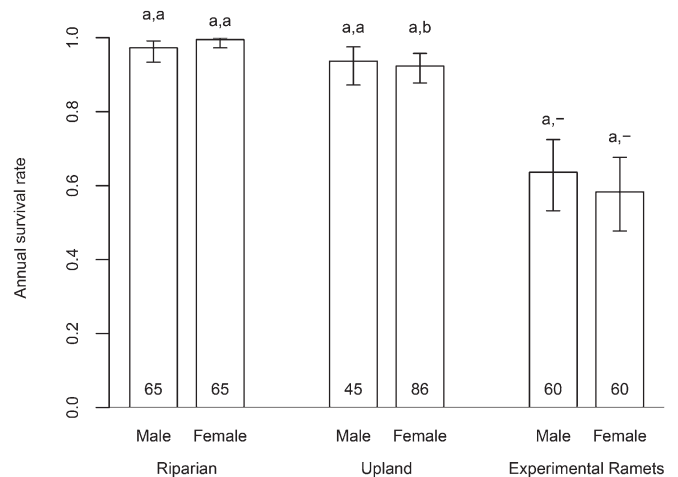


FIGURE 7 Median annual survival rate for tagged *Salix sitchensis* plants on the Pumice Plain from 2009 to 2011 ($\sqrt{\phi_{1,ik}}$) and for experimentally planted *S. sitchensis* ramets from 2007 to 2009 ($\sqrt{\phi_{2,ik}}$). Error bars represent Bayesian 95% credible intervals of the posterior distributions of the medians. The first letter in each pair is for comparisons between male and female plants or ramets. The second letter in each pair is for comparisons between adult riparian and upland plants within each sex (this is not applicable for ramets and is designated as a dash). When present, different letters indicate that the 95% credible interval of the posterior distribution of the difference between the two medians being compared does not overlap zero. The sample sizes are listed at the base of the bars.

plants always allocated more N and P to reproduction than did male riparian plants (Fig. 10). Female plants allocated more carbon to reproduction than did male plants in both habitats and years (Fig. 10).

DISCUSSION

We quantified the flowering plant sex ratios of three *S. sitchensis* populations colonizing Mount St. Helens. Two of these populations (debris avalanche and Pumice Plain) occupy primary successional landscapes, while the third relictual population (blowdown zone) likely served as part of their initial seed source. In all three disturbance zones, we observed 2:1 female-biased sex ratios, consistent with most other studies of sex ratio bias in *Salix* (Myers-Smith and Hik, 2012, and references therein). On the Pumice Plain, we showed that the sex ratio of all reproductively capable adult plants was female-biased by confirming that flowering frequencies did not vary by sex (Fig. 5). Our results are inconsistent with the generally observed trend (outside of *Salix*) that male plants are more common than female plants on highly disturbed or stressful sites (Grant and Mitton, 1979; Marques et al., 2002; Ortiz et al., 2002; Li et al., 2007). While we recognize that our efforts do not provide an explanation for the persistent female-bias in adult flowering *S. sitchensis* plants on Mount St. Helens, we are able to identify several factors that are not strong contributors.

Reproductive allocation and adult mortality—Sex-biased mortality (assumed to be a consequence of differing reproductive allocation between the sexes) is often implicated as the mechanism underlying biased sex ratios in dioecious plants (Lloyd and Webb,

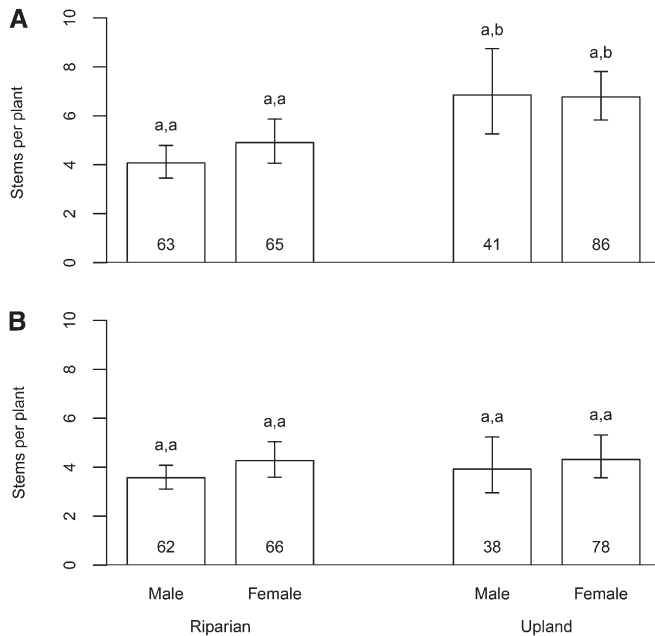


FIGURE 8 Mean number of stems per plant for tagged Pumice Plain *Salix sitchensis* plants in (A) 2010 and (B) 2011. Error bars represent Bayesian 95% credible intervals of the posterior distributions of the means. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between riparian and upland plants within each sex. When present, different letters indicate that the 95% credible interval of the posterior distribution of the difference between the two means being compared does not overlap zero. The sample sizes are listed at the base of the bars.

1977; Bell, 1980; Delph, 1999). Female-biased mortality, especially in the case of long-lived plants, can cause male-biased sex ratios (Escarré and Houssard, 1991; Allen and Antos, 1993; Cipollini et al., 1994). Conversely, Field et al. (2013a) hypothesized that in dioecious species with wind-dispersed pollen, male allocation to reproduction could possibly exceed female allocation when other resource currencies are considered (Harris and Pannell, 2008), resulting in female-biased sex ratios due to male-biased mortality. Reproductive costs are expected to increase when plants experience environmental stress (Delph, 1999), suggesting that primary successional landscapes, which are typically characterized by stressful edaphic conditions (Walker and del Moral, 2003), are likely to exacerbate sex-biased mortality for long-lived dioecious colonists that risk resource exhaustion through repeated bouts of reproduction (Åhman, 1997). Pumice Plain soils are immature, with poor water-holding capacity and low fertility, and non-N-fixing species growing here experience strong N limitation (del Moral and Bliss, 1993; Halvorson et al., 2005; Gill et al., 2006; Bishop et al., 2010; Schoenfelder et al., 2010; Marleau et al., 2011).

Whole plant reproductive allocation for C, N, and P by adult *S. sitchensis* on the Pumice Plain either did not differ by sex or was female-biased (mainly in 2011, a year of heavy flowering) (Fig. 10). Despite this female bias for the reproductive allocation of nutrients that are broadly limiting in this system, we did not observe sex-biased survivorship for adult Pumice Plain plants in either habitat (Fig. 7). Higher reproductive allocation by females, in terms of biomass or nutrients, has also been observed in other *Salix* popula-

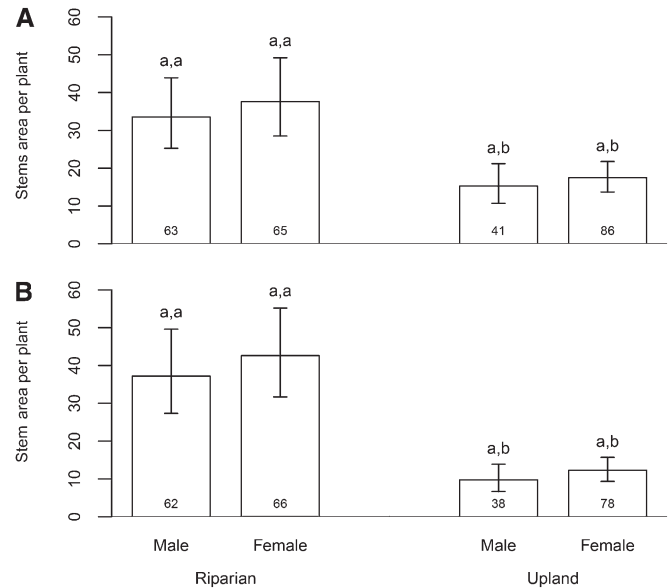


FIGURE 9 Median total basal stem area (cm²) per plant for tagged *Salix sitchensis* plants on the Pumice Plain in (A) 2010 and (B) 2011 ($e^{U_{1,ikr}}$). Error bars represent Bayesian 95% credible intervals of the posterior distributions of the medians. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between riparian and upland plants within each sex. When present, different letters indicate that the 95% credible interval of the posterior distribution of the difference between the two medians being compared does not overlap zero. The sample sizes are listed at the base of the bars.

tions with female-biased sex ratios (Turcotte and Houle, 2001; Ueno and Seiwa, 2003; Dudley, 2006; Ueno et al., 2006, 2007; Tozawa et al., 2009). Hence, adult mortality driven by reproductive allocation is unlikely to explain female-biased sex ratios in most *Salix* species.

Survivorship rates reported here are consistent with those from Ueno et al. (2007) for older *Salix sachalinensis* plants in northern Japan. These two studies provide the only information on sex bias in adult *Salix* mortality and run counter to the prevailing trend in dioecious woody plants of male-biased survivorship (Obeso, 2002). This disparity may be due to factors related to *Salix* ecology, such as compensatory mechanisms that mitigate reproductive costs. For example, female *Salix integra* and *S. sachalinensis* compensated for relatively higher reproductive costs compared with males by increasing the resource gathering capacity of vegetative shoots through a variety of physiological mechanisms (Ueno and Seiwa, 2003; Ueno et al., 2006; Tozawa et al., 2009). Also, it is possible that plants only experience reproductive costs when reproductive allocation is above a certain threshold relative to resource inputs (Tuomi et al., 1983). On the Pumice Plain, stem mortality due to weevil herbivory has resulted in upland plants that have extensively developed root systems relative to their aboveground biomass and reproductive effort may be below such a threshold. Lower reproductive effort could be an adaptive bet-hedging strategy for *Salix*, who experience high levels of seedling, relative to adult, mortality (Stearns, 1976; Obeso, 2002). This hypothesis warrants further study.

TABLE 1. Whole plant catkin production.

Year	Habitat	Male			Female		
		N	Median (cm ⁻²)	95% CI	N	Median (cm ⁻²)	95% CI
2010	Riparian	52	1.24	(1.02–1.63) ^{a,a}	54	1.23	(1.01–1.62) ^{a,a}
	Upland	22	2.00	(1.20–3.24) ^{a,a}	47	1.27	(1.02–1.68) ^{a,a}
2011	Riparian	59	1.73	(1.35–2.23) ^{a,a}	60	2.28	(1.74–2.92) ^{a,a}
	Upland	28	2.91	(1.94–4.14) ^{a,b}	57	2.23	(1.71–2.85) ^{a,a}

Notes: Median whole plant number of catkins produced per square centimeter of stem basal area in 2010 and 2011 for tagged *Salix sitchensis* plants on the Pumice Plain ($e^{12.3/k}$). Bayesian 95% credible intervals were computed from the posterior distributions of the medians. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between riparian and upland plants within each sex. When present, different letters for a comparison indicate that the 95% credible interval of the posterior distribution of the difference between the two medians being compared does not overlap zero.

Vegetative reproduction—Pioneering plants invading primary successional environments depend heavily on vegetative reproduction as a means of expansion across these landscapes (Walker and del Moral, 2003). For example, the establishment of stem fragments was the main contributor to *Salix* spread along an Alaskan river floodplain subject to frequent flooding during primary succession (Krasny et al., 1988). On Mount St. Helens, episodic debris flows that bury and abrade riparian vegetation are an important source of post-eruption disturbance, particularly for areas that were denuded by the 1980 volcanic blast (Major, 2004). Debris flows were particularly acute during the February 1996 floods, resulting in *Salix* regeneration from thousands of stem fragments, covering expansive swaths across multiple drainages. On the Pumice Plain, disturbance events are more chronic than episodic. In high-gradient upper stream reaches, *Salix* plants are uprooted, fragmented, and redistributed downstream as fragments or whole plants, where many are deposited in depositional reaches near Spirit Lake (Figs. 1, 2). Annual shifting of channels and sediment transport of fine textured material also bury established *Salix* plants in these depositional reaches. Buried plants and stem fragments initiate root growth and send up aerial shoots, resulting in hydrologically mediated cycles of nudation and regeneration (Fig. 2).

Because stem fragments retain the sex of the parent plant, the sex ratio of these fragments is directly determined by the ramet sex ratio in the parent population. Consequently, sexual dimorphism in the

number or size of stem fragments produced during disturbance events, or in their subsequent ability to establish, could bias a population's sex ratio. For instance, modeling has shown that sexual dimorphism in the production of asexual propagules by the dioecious liverwort *Marchantia inflexa* may attenuate the strong female-bias observed in liverwort metapopulations by helping maintain the presence of competitively disadvantaged males (Crowley et al., 2005; García-Ramos et al., 2007). Shafroth et al. (1994) proposed that vegetative reproduction via stem fragmentation is responsible for the strong female-biased *Salix x rubens* sex ratios observed along several Colorado streams. While this hypothesis is indirectly supported by evidence from a greenhouse experiment showing that female *Salix myrsinifolia-phylicifolia* cuttings have higher shoot growth rates than male cuttings (Hughes et al., 2010), no previous studies have investigated sexual dimorphism in *Salix* stem fragment establishment under field conditions. We found no evidence for sex-based differences in the number or size of 1st order stems on *S. sitchensis* plants in either riparian or upland areas (Figs. 8, 9). In addition, we did not detect a sex-based difference in the establishment rate of stem fragments in the artificial streams (Fig. 7). These results suggest that episodic or chronic disturbances are expected to produce equal numbers of male and female *S. sitchensis* stem fragments per plant, but more female stem fragments overall. Fragments of either sex are equally likely to successfully establish in riparian zones, maintaining the female genet sex ratio bias in these locations.

TABLE 2. Plant level catkin nutrient content and mass.

Attribute	Habitat	Male			Female		
		N	Mean (mg)	95% CI	N	Mean (mg)	95% CI
%C	Riparian	20	44.6	(44.3–45.0) ^{a,a}	48	45.1	(44.9–45.3) ^{b,a}
	Upland	25	45.7	(45.4–46.0) ^{a,b}	62	44.6	(44.4–44.8) ^{b,b}
%N	Riparian	20	3.58	(3.41–3.74) ^{a,a}	48	2.69	(2.59–2.79) ^{b,a}
	Upland	25	3.60	(3.46–3.75) ^{a,a}	62	1.89	(1.80–1.98) ^{b,b}
%P	Riparian	20	0.63	(0.58–0.67) ^{a,a}	32	0.48	(0.44–0.51) ^{b,a}
	Upland	25	0.67	(0.63–0.71) ^{a,a}	54	0.49	(0.47–0.52) ^{b,a}
		N	Median (mg)	95% CI	N	Median (mg)	95% CI
Mass	Riparian	20	37.3	(31.3–44.0) ^{a,a}	48	175.8	(142.4–213.7) ^{b,a}
	Upland	45	26.0	(22.4–30.2) ^{a,b}	81	101.5	(84.9–120.9) ^{b,b}

Notes: Mean plant level catkin nutrient content ($\mu_{4,ik}$) and median mass ($e^{13.3/k}$) of catkins from *Salix sitchensis* plants on the Pumice Plain. Bayesian 95% credible intervals are computed from the posterior distributions of the means or medians. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between riparian and upland plants within each sex. When present, different letters for a comparison indicate that the 95% credible interval of the posterior distribution of the difference between the two means or medians being compared does not overlap zero.

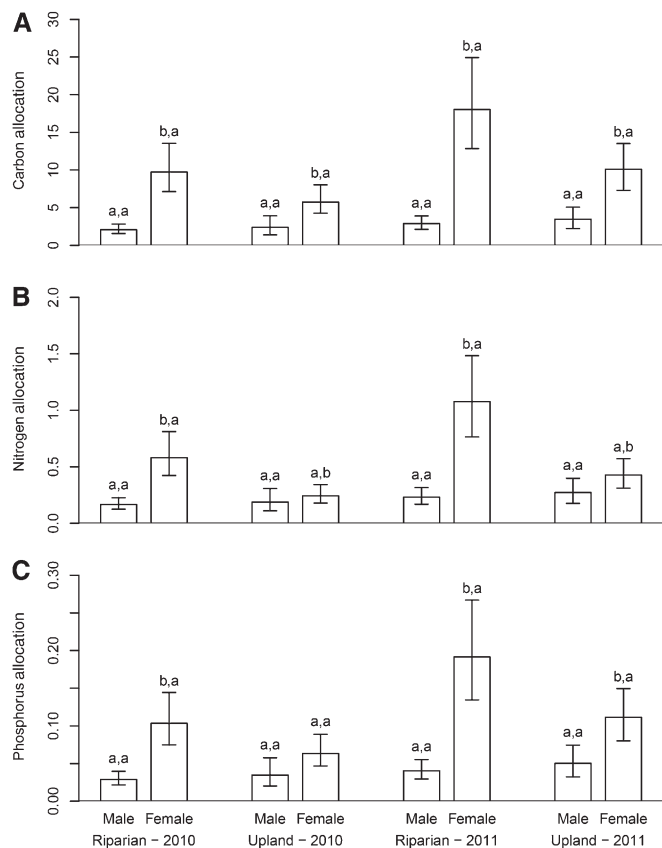


FIGURE 10 Whole plant reproductive allocation expressed in grams of (A) carbon, (B) nitrogen, and (C) phosphorus per unit stem area (cm^2) for tagged *Salix sitchensis* plants on the Pumice Plain in 2010 and 2011 (RA_{ikm}). Error bars represent Bayesian 95% credible intervals of the posterior distributions. The first letter in each pair is for comparisons between male and female plants within each habitat. The second letter in each pair is for comparisons between riparian and upland plants within each sex. When present, different letters indicate that the 95% credible interval of the posterior distribution of the difference between the two groups being compared does not overlap zero.

Spatial segregation of sexes—Although there is some evidence that male *Salix* plants are more drought-tolerant and more abundant in drier upland sites compared with females (Dawson and Bliss, 1989; Dudley, 2006), we did not find moisture-related spatial segregation of the sexes, because sex ratios did not differ by habitat in any of the three disturbance zones (Fig. 4). These results are largely consistent with other studies that find no evidence or weak support for spatial segregation of the sexes in *Salix* along gradients perpendicular to riparian corridors (Alliende and Harper, 1989; Ueno and Seiwa, 2003; Ueno et al., 2007; Hughes et al., 2010). It is possible that upland invasion by *S. sitchensis* is so heavily dependent on microsite availability and seasonal weather conditions (Shafroth et al., 1994; Walker and del Moral, 2003) that sex-biased mortality is minimized during establishment. If *S. sitchensis* must establish in years when conditions are less stressful, plant sex may have a weak effect on seedling survival in upland areas, and sex ratios of adult plants these areas would mimic those in adjacent riparian zones. This colonization process is consistent with the occasional, large establishment events of *S. sitchensis* observed in some years (del Moral and Wood, 2012).

Historical contingency—Colonization of new areas by dioecious plant species, either at the edge of species' ranges or when habitat becomes available through disturbance, can potentially alter seed and secondary sex ratios in colonizing, relative to donor, populations (Barrett et al., 2008; Field et al., 2013b). Founder effects may be persistent and especially pronounced in primary successional seres, if early colonists remain nonreproductive for long periods of time and colonization events are very rare (Walker and del Moral, 2003). The sex ratios of *S. sitchensis* populations on the Pumice Plain and the debris avalanche were likely governed initially by the seed sex ratio of blowdown zone *S. sitchensis* plants that survived the 1980 eruption. On the Pumice Plain, *S. sitchensis* first established shortly after the eruption (1985–1986) in a single riparian zone (Wood and del Moral, 1988, 2000). Persistent seed rain and vegetative reproduction led to recruitment into upland areas, adjacent riparian zones, and along riparian corridors (del Moral and Wood, 2012). The fact that *S. sitchensis* sex ratios in all three disturbance zones were virtually identical and matched those reported in the majority of other studies on *Salix* sex ratios (Myers-Smith and Hik, 2012, and references therein) suggests that factors other than historical contingency are responsible for the consistently observed female biases. *Salix* species are highly vagile, and repeated annual recruitment from seed since the mid-1980s has likely erased any potential founder effects on the sex ratios of early Pumice Plain and debris avalanche colonies of *S. sitchensis*.

Early-acting factors implicated—Sex ratios in *S. sitchensis* colonizing Mount St. Helens more likely depends on either early-acting genetic factors affecting the seed sex ratio, or sex-bias in germination or seedling mortality, rather than late-acting ecological factors associated with reproduction. Lack of well-developed heteromorphic sex chromosomes in *Salix* may preclude a variety of early-acting mechanisms known to female-bias seed sex ratios, such as Y-chromosome degradation (Vyskot and Hobza, 2004; Stehlik et al., 2007), gametic viability selection (Stehlik and Barrett, 2005; Stehlik et al., 2008), sex-linked meiotic drive (Taylor and Ingvarsson, 2003), or sexual conflict (Taylor, 1994). While *Salix* seeds may disperse long distances, successful establishment hinges on seeds landing in favorable microsites (Shafroth et al., 1994; Walker and del Moral, 2003) and could result in moderate levels of sib mating from clumping if resources are distributed heterogeneously due to topography, moisture, or nutrient availability. Also, pollen dispersal distances are likely to be substantially reduced by the wet weather conditions on Mount St. Helens that regularly coincide with pollen availability (Che-Castaldo, 2014). These factors are consistent with evolutionarily stable female-biased seed sex ratios proposed from theoretical models (Maynard Smith, 1978; Bulmer and Taylor, 1980; de Jong et al., 2002), and of the two studies on *Salix* seed sex ratios, both report strong female-bias (Alström-Rapaport et al., 1997; de Jong and Meijden, 2004). Currently, the lack of a molecular sex marker for sex determination in *Salix* makes measuring sexual dimorphism in germination, or seedling and juvenile plant survivorship impossible.

Small mammal herbivory—Male-biased seedling and shoot herbivory by small mammals has been hypothesized to cause female-biased *Salix* sex ratios, leading to the prediction that *Salix* populations should be more female-biased when herbivory is high (Elmqvist et al., 1988; Danell et al., 1991). Mount St. Helens provides an excellent natural system to test this prediction, as sharp

differences in small mammal herbivory pressure on *S. sitchensis* exist between riparian zones and adjacent upland areas and more broadly among the disturbance zones. Crisafulli et al. (2005a) found much higher richness and abundances of herbivorous small mammal species in Mount St. Helens riparian zones as compared with adjacent upland areas. They also observed a strong relationship between increased snowpack depth and duration, and small mammal herbivory on *Salix*; in years with little snowpack, herbivory is nearly nonexistent, whereas in heavy snow years *Salix* is often subjected to intense herbivory. The Pumice Plain is a largely barren, wind-driven system where snow is scoured from the upland environment and redeposited in the topographically low riparian zones to considerable depth (1–2 m). Small mammal herbivory on *S. sitchensis* is rarely observed in the snow-free uplands, but in the riparian habitats (as well as the blowdown zone) winter-active rodents exert substantial herbivory pressure. Despite the strongly contrasting small mammal community structures and herbivory pressure on *S. sitchensis* between habitats and disturbance zones, sex ratios were consistently female-biased on Mount St. Helens.

CONCLUSIONS

Adult *S. sitchensis* plants at Mount St. Helens had consistently female-biased flowering sex ratios across all habitats and disturbance zones. Determining the causes of sex ratio biases involves an investigation of multiple factors. We found no evidence this female bias is caused by historical contingency associated with the colonization of these areas, broad spatial differences in the distribution of the sexes in response to water or stress gradients, a survey artifact induced by sex-bias in flowering frequency or plant size, sexual dimorphism in vegetative reproduction from stem fragments, or sex-biased herbivory. Consistent with other studies in *Salix*, we found that females plants allocated equal or more resources to reproduction than males, yet populations of adult plants remained female-biased and adult mortality did not differ by sex. These results suggest that reproductive costs were either successfully mitigated or that a life history trade-off between survivorship and reproduction was being masked phenotypically by other confounding factors.

Our conclusions support a growing consensus that early-acting factors are likely the cause of female-biased sex ratios in *Salix* (Ueno et al., 2007; Myers-Smith and Hik, 2012). We hypothesize that the strong female sex ratio biases observed in all habitats and disturbance zones on Mount St. Helens is most likely caused by female-bias in the seed sex ratio, but also possibly by sex-specific germination or survival rates prior to reproductive maturity. We propose that, absent a molecular marker for sex determination, studies in *Salix* should focus on determining seed sex ratios from germinated seeds raised to sexual maturity under optimal conditions, especially in populations where the adult sex ratio is already known.

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