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Seedling Regeneration Process of *Sasa veitchii* var. *tyugokuensis* Over 12 Years Following Small-Scale Flowering

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ABSTRACT

Sasa species (Poaceae: Bambusoideae) typically regenerate following synchronous mass flowering and die-off. However, small-scale flowering is often observed and its contribution to seedling recruitment has been overlooked. To examine the ecological significance of small-scale flowering, we investigated the regeneration process of *Sasa veitchii* var. *tyugokuensis* from small-scale flowering to seedling establishment over 11 years, and to vegetation recovery in the 12th year. Seven genets flowered over an area of 2300 m^2. The flowering culms subsequently died, whereas the nonflowering culms remained in two of the flowered genets, indicating that they only partially flowered. Of the emerged seedlings, 83% died within the first 2 years and 5.8% (0.1 genets/m^2) survived for 11 years. Genets with greater initial growth and higher heterozygosity exhibited higher survival rates. However, seedling cover remained low even after 12 years, and nonflowered *Sasa* culms from the surrounding area had expanded into the seedling cover area. The pattern of initial high mortality was similar to the regeneration process after mass flowering, while seedling growth was slower after the small-scale flowering event. Seedling regeneration may only occur in areas free from the expansion of nonflowered *Sasa* culms. These findings suggest that small-scale flowering contributes to the regeneration of *Sasa* species and reduces the risk of regeneration failure associated with monocarpy and mass flowering.

1 | Introduction

Some plant species flower synchronously over wide areas only once in their lifetime, including bamboos (Janzen 1976), *Strobilanthes* (Kakishima et al. 2011), and *Isoglossa* (Tsvuura et al. 2011). After flowering, parent plants set seeds and die (i.e., monocarpy), and populations are regenerated by seedlings. These synchronous mass flowerings and subsequent die-offs are considered adaptive phenomena because they enhance pollination efficiency (Kelly et al. 2001), satiate flower and seed predators (Janzen 1976), and reduce competition between parents and offspring (Simmonds 1980). Although asynchronous small-scale flowering (referred to as "sporadic flowering" or "off-year flowering") also occurs, the offspring derived from such flowering are thought to be eliminated from the population by selection due to their lower fitness caused by inbreeding depression and intense competition with nonflowering individuals (Janzen 1976; Kakishima et al. 2011; Tsvuura et al. 2011; Tachiki et al. 2015).

Sasa species (Poaceae: Bambusoideae) are long-lived monocarpic plants that exhibit highly synchronous mass flowering similar to that of other bamboos (Janzen 1976; Makita 1998a; Kobayashi 2017). *Sasa* species are widely distributed in cool-temperate to subalpine deciduous forests and often form dense undergrowth in forests (Suzuki 1978; Matsuo et al. 2018). After

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a long period of clonal expansion via rhizomes (> 100 years), numerous individuals (genets: genetically identical units derived from a single seed) flower and die synchronously across a wide geographical area, ranging from several dozen hectares to over hundreds of kilometers (Makita 1992; Abe and Shibata 2012; Akashi 2024). This phenomenon has attracted the attention of many researchers, and its adaptive significance has been studied (Wu et al. 2024). Synchronous flowering of many genets promotes cross-pollination (Matsuo et al. 2014). Spatiotemporally isolated mass flowering increases seed survival by satiating florivores (Sakata et al. 2022), and successful seedling regeneration after mass flowering has been reported (e.g., Makita 1992; Makita et al. 1993; Tomimatsu et al. 2020).

In contrast, asynchronous small-scale flowering (Suyama et al. 2010) is commonly observed in *Sasa* spp. in years other than mass flowering events. In most cases of small-scale flowering, only one or several genets exhibit partial flowering (Mizuki et al. 2014). Therefore, the self-fertilization rate and seed predation rate are higher for these events than mass flowering events (Kitamura and Kawahara 2011; Mizuki et al. 2014; Matsuo et al. 2014; Sakata et al. 2022). Seedling regeneration after small-scale flowering has been assumed to be unsuccessful, as seedlings are rarely observed (Makita and Konno 1989; Yamazaki and Nakagoshi 2005). A few studies have reported seed production (Mizuki et al. 2014; Sato and Mochida 2019) and seedling occurrence (Koyama 2000; Kudo et al. 1994; Kitamura and Kawahara 2011). The ecological significance of these events has been overlooked. Thus, long-term seedling dynamics following small-scale flowering should be investigated.

In 2011, *Sasa veitchii* var. *tyugokuensis* (hereafter, *S. veitchii*) flowered and died off over an area of 2,300 m^2 in a deciduous forest of Akita City, Akita Prefecture, Japan. The area of this event was comparable to small-scale flowering events of the genus (e.g., 12–1790 m^2: Makita and Konno 1989; Mizuki et al. 2014). We considered this event "small-scale" because no mass flowering events were observed in this area in the same time period. After small-scale flowering and die-off, *Sasa* recovery exhibits two potential tracks: seedling regeneration and surrounding nonflowered *Sasa* culm expansion, which may inhibit seedling regeneration. Therefore, to determine whether small-scale flowering contributes to seedling regeneration, we investigated (1) the clonal structure and seed production of flowering genets, (2) seedling population dynamics over 11 years, (3) factors affecting seedling survival and growth, and (4) recovery of *Sasa* vegetation 12 years after flowering.

2 | Material and Methods

2.1 | Study Species and Site

S. veitchii (Carrière) Rehder var. *tyugokuensis* (Makino) Sad. Suzuki, which belongs to sect. *Sasa* (Kobayashi 2017), is a self-compatible (e.g., Matsuo et al. 2014; Mizuki et al. 2014) evergreen clonal plant that grows to a height of 1–2 m. In our study region, there are no historical records of mass flowering within the genus *Sasa*. However, we assume that this species has a mass flowering habit because mass flowering of the same species has been observed in other regions of Japan (Abe and Shibata 2012; Matsuo et al. 2014).

We conducted the study in the secondary deciduous forest adjacent to the Akita Botanical Garden in Akita City, Akita Prefecture (39°48′N, 140°13′E, 148 m a.s.l.). The forest is composed of *Quercus crispula*, *Castanea crenata*, and *Magnolia obovata*. The understory is dominated by the genus *Sasa*, including *S. veitchii*, *S. megalophylla* and the *S. kurilensis–S. senanensis* complex (Kobayashi 2017). These taxa do not exhibit significant ecological differences. Small-scale flowering of *S. veitchii* occurred in 2011, and although a few flowering culms were observed in 2012, no seeds were observed.

2.2 | Sampling and Seed Production Estimation During Flowering

From June to July 2011, we established a 100 m × 60 m study plot that included flowering and nonflowering areas to determine the genet structure of the population (Figure 1). If culms were observed within a 2 m radius of the 10 m grid points, then leaf or culm tissues were sampled for DNA analysis ($N = 66$).

We counted the number of flowering culms, inflorescences, and nonflowering culms within 2 m × 2 m quadrats at 10 m grid points in the flowering area and calculated their densities ($N = 31$). To investigate the seed set, inflorescences were randomly selected at 10 m grid points and covered with mesh bags after fertilization to prevent seed loss. After seed maturation, inflorescences were collected ($N = 38$), and the number of sterile florets and mature seeds was counted per inflorescence. Seed set was calculated by dividing the number of mature seeds by the number of florets per inflorescence at 10 m grid points. Then, the number of seeds produced per 1 m^2 was estimated by multiplying the number of mature seeds per inflorescence by the inflorescence density. The representative of seed set value for the study site was also calculated as the total number of mature seeds divided by the total number of florets across all collected inflorescences.

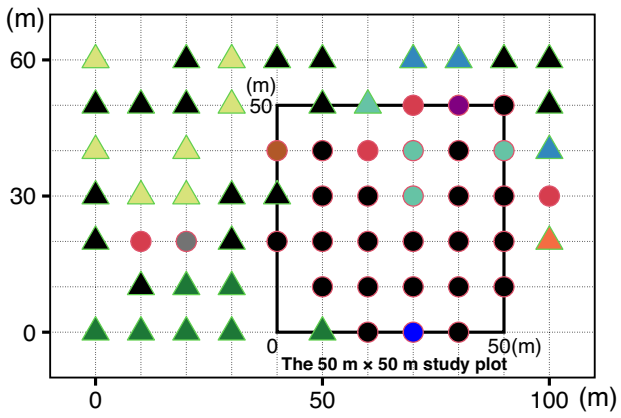


FIGURE 1 | Spatial distribution of genets in the flowering year (2011) within the 100 m × 60 m study plot. Circles and triangles represent flowering and nonflowering culms, respectively. Symbols of the same color represent culms belonging to the same genet. The 50 m × 50 m study plot (black square) was established to cover the main part of the flowering area for the seedling surveys.

2.3 | Seedling Demography

A 50 m × 50 m study plot was established in the flowering area (Figure 1). Within this plot, seedling quadrats of 2 m × 2 m ($N = 30$) and 5 m × 5 m ($N = 6$) were established at 10 m intervals to monitor the survival and growth of seedlings ($N = 36$, total 270 m^2; Figure S1). The 5 m × 5 m quadrats were established to track genet growth more accurately. In October 2012 and 2013, all genets were tagged and the height (maximum culm height) and number of culms (number of main stems) for each genet were measured (Figure S2). After 2013, we also tagged newly emerged culms when their connection to existing culms through rhizomes was unclear. Leaf tissue was sampled once from each tagged genet for DNA analysis (total leaf samples: $N = 1132$). The survey was conducted every fall from 2012 to 2022. Genet density, mortality, and growth were calculated for the seedlings that germinated in 2012, whereas culm density comprised all culms, including those from new genets recruited between 2013 and 2022.

To evaluate genet growth, we estimated the seedling biomass per genet (dry weight). In August 2022, we randomly selected 20 seedlings outside the quadrats. These were distinguished from non-flowered *Sasa* culms based on culm height and diameter: seedling culms were less than 60 cm in height and less than 4.0 mm in diameter, whereas nonflowered *Sasa* culms were typically more than 100 cm in height and more than 5.0 mm in diameter. We measured the height and number of culms of each seedling and then dug them up. The samples were oven-dried at 80°C for 48 h and then weighed. We then constructed a generalized linear model (GLM) based on the gamma distribution with a log-link to estimate the biomass. Biomass was set as the response variable, and the log-transformed height and number of culms were set as the explanatory variables. The best model was selected based on the Akaike information criterion (AIC: Burnham and Anderson 2002). We then obtained the following equation to calculate the genet biomass that survived in 2022: [Biomass of 11-year genet (g)] = exp. {−1.62 + 0.73 × log [number of culms] + 0.79 × log [height (cm)]} (Nagelkerke's $R^2 = 0.79$).

2.4 | Microsatellite Genotyping and Genet Identification

Fresh leaf samples were frozen at −20°C prior to DNA analysis. We extracted DNA from 2 mg of leaves using a modified cetyltrimethylammonium bromide (CTAB) protocol (Murray and Thompson 1980). Flowering and nonflowering culm samples were genotyped using seven polymorphic microsatellite (SSR) loci: Sasa223, Sasa500, Sasa718, and Sasa946 (Kitamura et al. 2009), BWSS-6 and BWSS-7 (Miyazaki, Ohnishi, Hirayama, and Nagata 2009), and ST57 (Sumiyoshi et al., unpublished; shown in Matsuo et al. 2018). For seedling genotyping, Sasa946 was excluded from the above primer set because of the low polymorphisms (only one allele was detected at this locus in most seedlings) and BWSS-3 (Miyazaki, Ohnishi, Hirayama, and Nagata 2009) was added instead. A pig-tail sequence (GTTTCTT) was attached to the 5′ end of the reverse primers to avoid scoring problems due to nontemplated nucleotide addition by Taq DNA polymerase (Brownstein et al. 1996). Multiplex polymerase chain reactions (PCR) were performed using a Multiplex PCR Kit (Qiagen, Hilden, Germany) in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, Foster City, California, USA). GeneScan 500 LIZ dye Size Standard (Applied Biosystems) was used. The lengths of the amplified PCR products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems), and GeneMapper version 5 (Applied Biosystems). Identical multilocus genotypes (MLGs) shared among samples were identified using GenAlEx 6.51 (Peakall and Smouse 2006). When MLGs differed by only two base pairs in one allele at one locus but shared the same alleles at all other loci, we considered that the variation between them was derived from somatic mutations and grouped them into a single genet (Matsuo et al. 2014, 2018). To distinguish between the seedlings originating from outside the quadrats and the nonflowered *Sasa* culms, new MLGs detected from 2014 to 2022 were compared with the MLGs of nonflowered *Sasa* culms. The detailed process of genet identification is described in the Supporting Information (Text S1). We also calculated the heterozygosity for each genet and the inbreeding coefficient (F_{IS}) for flowering genets and seedlings for each year using GenAlEx.

2.5 | Light Environment in the Forest Understory

To investigate the effect of light availability on seedlings, we quantified canopy openness. Hemispherical photographs were taken in 36 seedling quadrats at two time points: September 2013 and August 2022. In addition, to assess seasonal variations, we took photographs monthly from June 2022 to May 2023 at eight quadrats where seedlings had survived until 2021. All photographs were taken at the center of each seedling quadrat at 1 m above the ground using a digital camera (PowerShot G11, Canon Inc., Tokyo, Japan) and a fisheye lens (UWC-0195, Fit. Co. Ltd., Nagano, Japan). Photographs were analyzed using CanopOn 2.03 to calculate the canopy openness (Takenaka 2009).

2.6 | Vegetation Cover 12 Years After Flowering

To evaluate the recovery of *Sasa* vegetation in the flowered area, vegetation cover was measured in August 2023 (12 years after flowering). The 50 m × 50 m study plot was divided into 5 m × 5 m subplots, and the vegetation cover of plants less than 2 m in height was recorded using Londo's decimal scale (Londo 1976). Plant cover was categorized into four groups: *S. veitchii* seedlings, *Sasa* culms derived from genets that had nonflowering culms in 2011 (hereafter "nonflowered *Sasa*," which included *S. veitchii*, *S. megalophylla*, and the *S. kurilensis–S. senanensis* complex), woody plants, and herbaceous plants.

2.7 | Statistical Analysis of Seedling Survival and Growth

To examine the effects of initial growth, genetic traits, and environmental factors (light availability and nonflowered *Sasa* culm density) on the survival and growth of seedlings, we built four models, as described below. All explanatory variables were standardized. The seedling quadrats were set as the random effect in all models where applicable. We confirmed that the degree

of multicollinearity among the explanatory variables was low as all variance inflation factors (VIFs), an indicator of multicollinearity, were less than five (Montgomery et al. 2012). The significance of the explanatory variables was determined using the likelihood ratio test and compared with the chi-squared distribution. Statistical analyses were performed using the *lme4* package v.1.1.35 (Bates et al. 2015) and *car* package v.3.1.2 (Fox and Weisberg 2019) in R ver.4.3.2 (R Development Core Team 2023).

a. Survival in early life stage (2013–2014): We built a generalized linear mixed model (GLMM) based on the binomial distribution with a logit link to analyze the factors affecting survival from the second to the third year after seedling emergence. The survival of seedlings (survived or not) in 2014 was set as the response variable. Heterozygosity (genetic traits), height, number of culms per genet in 2013 (initial growth), and canopy openness in 2013 (light availability) were set as the explanatory variables.

b. Survival over a long-term period (2013–2022): We built a GLMM based on the binomial distribution with a logit link to analyze the factors affecting survival from the second to the 11th year after seedling emergence. The survival of seedlings in 2022 was set as the response variable. Heterozygosity, height, and number of culms in 2013 were set as the explanatory variables. Both models (a) and (b) were restricted to the period from the second year (2013) onward to use 2013 data (height and number of culms) as initial growth indicators.

c. Biomass in 2022: We built a GLM based on the gamma distribution with a log-link to analyze the factors affecting the estimated biomass in 2022, with the estimated biomass of seedlings as the response variable. Heterozygosity, canopy openness in 2022, and nonflowered *Sasa* culm density in 2022 were set as the explanatory variables. The random effect (seedling quadrats) was not included in this model because its inclusion resulted in a singular fit (i.e., the estimated variance of the random effect was zero), indicating that the random effect for seedling quadrats did not contribute to model performance.

d. Height from 2012 to 2022: We built a GLMM based on the gamma distribution with a log-link to analyze whether the changes in seedling height over the years were affected by heterozygosity (i.e., the interaction effect of year and heterozygosity). Height in each year from 2012 to 2022 was set as the response variable for seedlings surviving until 2022. Heterozygosity, year, and the interaction between these two variables were set as the explanatory variables. Genet identity was set as a random effect in addition to the seedling quadrats.

3 | Result

3.1 | Clonal Structure and Seed Production of Flowering Genets

Seven flowering genets were detected. One dominant genet accounted for 64.5% of the flowering grid points, while the others were small (Figure 1). Two of the flowering genets, including the dominant genet, also had nonflowering culms (Figure 1). This result indicates that these genets flowered only partially. Although seven genets flowered, allelic variation among them was low (Table S1).

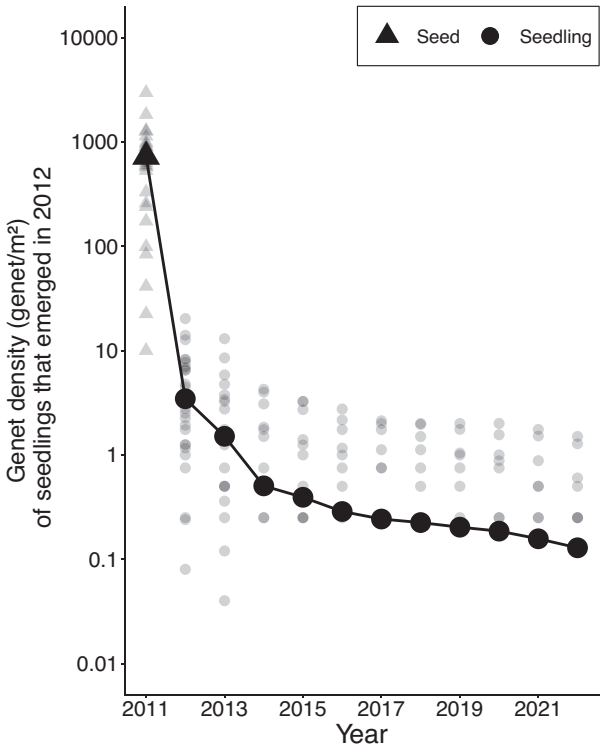


FIGURE 2 | Survivorship curve of *Sasa veitchii* seedlings that emerged in 2012, excluding those recruited between 2013 and 2022. Triangles and circles represent the estimated seed and seedling density, respectively. Small symbols represent the values for each quadrat, and large black symbols represent the mean values per year. Note that quadrats with zero values are not shown as the *y*-axis is on a logarithmic scale.

Flowering culm density was 11.6 ± 6.9 culms/m^2 (mean ± SD), and inflorescence density was 14.4 ± 8.4/m^2. Nonflowering culm density was 6.3 ± 8.4 culms/m^2. The mean flowering culm rate per quadrat was 65.8%. The representative of seed set value for the study site was 20.5%, and the values at 10 m grid points ranged from 1.5% to 41.4%. The estimated number of seeds produced was 722 ± 624 seeds/m^2 (10–2968 seeds/m^2; Figure 2).

3.2 | Seedling Population Dynamics Over 11 Years

The mortality of the seedling was particularly high during the first 2 years and declined gradually thereafter (Figure 2). In 2012, 1007 genets were observed across 25 quadrats, with a mean density of 3.5 ± 4.7 genets/m^2, varying widely among quadrats (0–20.3 genets/m^2; Figure 3a). Of the estimated seed production in 2011, 99.5% of seeds failed to emerge as seedlings (Figure 2). Of the genets that emerged in 2012, 422 genets (41.9%; 1.5 ± 2.7 genets/m^2) survived to 2013, and 176 genets (17.5%; 0.5 ± 1.1 genets/m^2) survived to 2014 (Figure 2). The seedling distribution area also decreased substantially by 2014

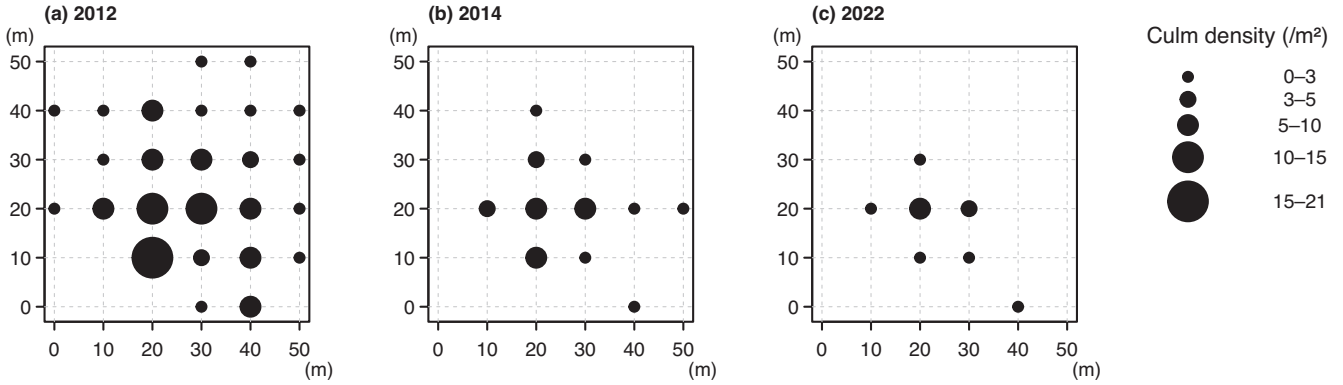


FIGURE 3 | Spatial distribution of seedling culm density in the quadrats in (a) 2012, (b) 2014, and (c) 2022. These values include seedling recruited between 2013 and 2022. Larger circles represent higher culm density.

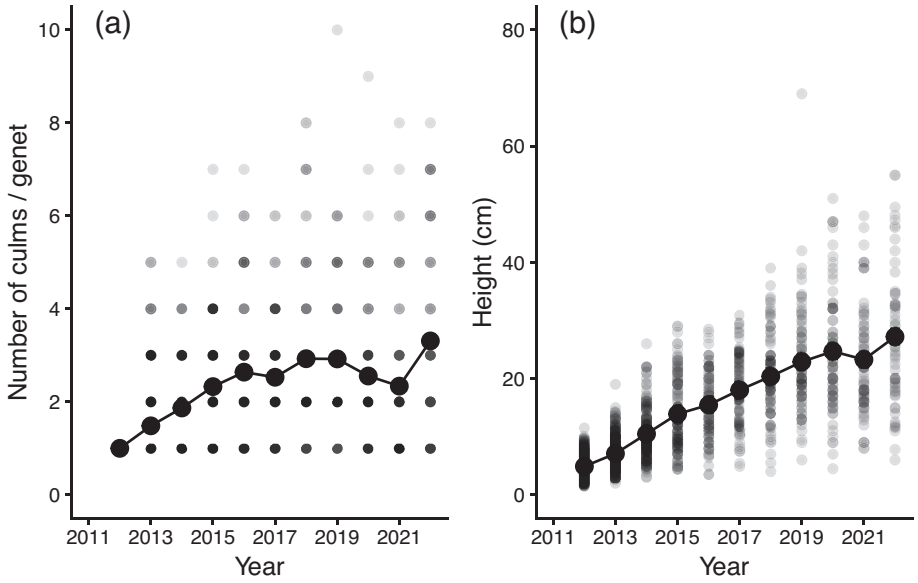


FIGURE 4 | Changes in the (a) number of culms per genet, and (b) height of seedlings, excluding seedlings recruited between 2013 and 2022. Small symbols represent the values for each quadrat, and large black symbols represent the mean values per year.

(Figure 3b). By 2022, only 58 of the original 1007 genets (5.8%) survived, resulting in a mean density of 0.1 ± 0.3 genets/m^2 (0–1.5 genets/m^2 across seven quadrats; Figure 2). Similarly, culm density declined substantially during the first 2 years following seedling emergence (Figure 3a,b). In 2022, culm density was 0.6 ± 1.7 culms/m^2 (0–8.75 culms/m^2; Figure 3c). In addition, a total of 98 new genets of seedlings were detected between 2013 and 2022. Based on size and rhizome connections, we identified that these seedlings either germinated within the quadrats after the initial survey ($N = 38$) or colonized the quadrats via rhizome extension from outside after establishing elsewhere ($N = 60$).

All seedlings that germinated in 2012 initially possessed a single culm, which increased to a mean of 3.3 culms by 2022 (range: 1–8 culms; Figure 4a). During the same period, their mean height increased from 4.9 cm (1.5–11.5 cm) to 27.2 cm (6–55 cm; Figure 4b). The growth of a few genets could not be accurately assessed, as they appeared to have spread beyond the quadrats.

3.3 | Factors Affecting Survival and Growth of Seedlings

The canopy above the seedlings was generally closed. Canopy openness in summer 2022 was $2.3\% \pm 3.5\%$, with little variation among the seedling quadrats. Across the eight quadrats where seedlings survived, openness remained low throughout the summer and fall but increased in November after the canopy trees had shed their leaves (Figure S3). Quadrats near the edge of the study plot (quadrats 20_10 and 40_0) had to be darker than the interior quadrats (Figure S3). The allelic variation of seedlings was low (Table S1). The F_{IS} value among surviving seedlings decreased from 2012 to 2022 (Figure S4).

Seedling survival in the early life stage (2013–2014) was positively affected by height and the number of culms per genet in 2013 (Table 1a). Long-term survival (2013–2022) was positively affected by heterozygosity and the number of culms in 2013 (Table 1b). Biomass in 2022 was not significantly affected

TABLE 1 | Results of GLMM and GLM analyses examining the effects of initial growth, genetic traits, and environmental factors on survival and growth of seedling: (a) survival in the early life stage (2013–2014), (b) survival over a long-term period (2013–2022), (c) biomass in 2022, and (d) height (2012–2022).

Parameter	Estimate	χ^2	p
(a) Survival from 2013 to 2014 ($N = 377$)			
Heterozygosity	0.21	2.91	0.09
Height in 2013 (cm)	0.45	11.78	< 0.001
Number of culms in 2013	0.91	37.30	< 0.001
Canopy openness in 2013 (%)	−0.10	0.42	0.52
(b) Survival from 2013 to 2022 ($N = 377$)			
Heterozygosity	0.40	5.78	0.02
Height in 2013 (cm)	0.22	1.74	0.19
Number of culms in 2013	0.30	4.09	0.04
(c) Biomass in 2022 ($N = 77$)			
Heterozygosity	0.02	0.09	0.77
Canopy openness in 2022 (%)	−0.18	1.22	0.27
Nonflowered *Sasa* culm density in 2022 (/m^2)	−0.05	0.11	0.74
(d) Height from 2012 to 2022 ($N = 583$)			
Heterozygosity	−0.01	0.02	0.88
Year	0.49	889.39	< 0.001
Heterozygosity × year	0.02	0.92	0.34

Note: Estimated values represent the model results. χ^2 and p values are from the likelihood ratio tests. All explanatory variables were standardized. Seedling quadrat was set as the random effect in models (a) and (b), but was excluded in model (c). Both genet identity and quadrat were set as the random effects in model (d).

by heterozygosity, canopy openness, or the non-flowered *Sasa* culm density (Table 1c). Height increased significantly over the years. However, this growth was not affected by heterozygosity, as the interaction term with heterozygosity and year was not significant (Table 1d).

3.4 | Recovery of *Sasa* Vegetation 12 Years After Flowering

In 2023, *S. veitchii* seedling cover was only 0.7%, which was much lower than that of other plant categories (Figure 5). In contrast, nonflowered *Sasa* occupied 45% of the study plot (Figure 5). The non-flowered *Sasa* cover was not uniformly high across the study plot but tended to be higher at the edges, while seedlings were mostly distributed in areas where nonflowered *Sasa* cover was relatively low (Figure 6).

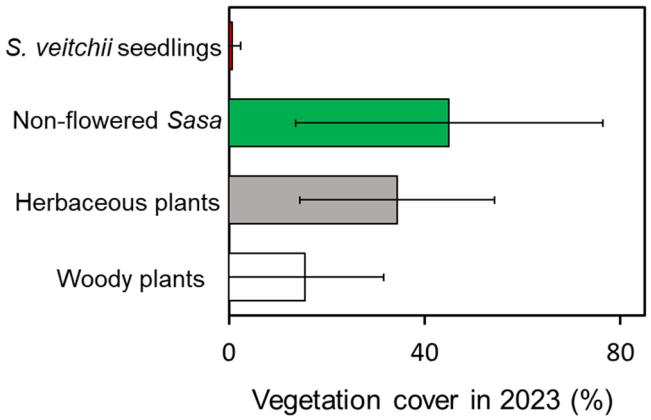


FIGURE 5 | Vegetation cover (mean ± SD) of *Sasa veitchii* seedlings, nonflowered *Sasa*, herbaceous plants, and woody plants in 2023 (12 years after small-scale flowering).

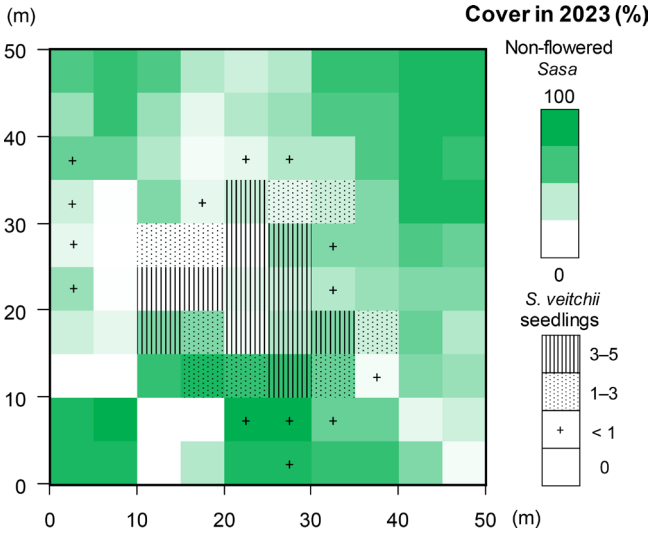


FIGURE 6 | Spatial distribution of vegetation cover for *Sasa veitchii* seedlings and non-flowered *Sasa* across the 50 m × 50 m study plot in 2023. The differences in color and pattern represent the cover of non-flowered *Sasa* and *S. veitchii* seedlings, respectively.

4 | Discussion

4.1 | Clonal Structure and Seed Production of Flowering Genets

The substantial seed production and seedling germination observed in this study suggest that small-scale flowering can contribute to seedling regeneration, despite the conventional assumption that successful recruitment in *Sasa* only occurs following mass flowering. Previous studies have suggested that the low seed set in small-scale flowering is due to limited flowering areas and high predation rates (Sakata et al. 2022; Mizuki et al. 2014), and low pollen density (Nishiwaki and Konno 1990). Flowering restricted to a few genets is likely to increase self-fertilization rates (Mizuki et al. 2014) and subsequent inbreeding depression (Kitamura and Kawahara 2011; Matsuo et al. 2014). The flowering event observed in this study involved seven genets across 2300 m^2, with one genet dominating, and

two genets exhibiting partial flowering. This pattern was distinct from monocarpic mass flowering; however, the scale was larger than the typical small-scale flowering involving only one to four genets (Makita and Konno 1989; Kitamura and Kawahara 2011; Mizuki et al. 2014). Furthermore, nonflowering culm density (6.3 culms/m^2) was lower than that reported in studies of small-scale flowering (9–34.8 culms/m^2; Makita and Konno 1989; Yamazaki and Nakagoshi 2005). Both seed production (722 seeds/m^2) and seedling emergence (3.4 genets/m^2) were higher than those reported in previous studies (403–609 seeds/m^2: Makita and Konno 1989; 0.11–0.35 genets/m^2: Kudo et al. 1994; Koyama 2000).

However, seed and seedling densities were lower than those reported after mass flowering (480–13 810 seeds/m^2; 8.7–978.1 genets/m^2: Makita et al. 1993; Abe and Shibata 2014a; Nishiwaki and Makita 1998). Although seven genets flowered, they were not uniformly intermingled. The spatial dominance of a single genet suggests that a substantial proportion of seeds were likely produced through inbreeding.

4.2 | Seedling Population Dynamics Over 11 Years and Factors Affecting Them

Our study provides the first evidence that seedlings derived from small-scale flowering in *Sasa* can persist for over 10 years. Makita (1992) divided the early regeneration process after mass flowering of *S. kurilensis* into three phases: "establishment phase," with high initial mortality; "density-stable phase," with low mortality for several years; and "thinning phase," with decreasing culm density. The population dynamics in this study corresponded to the first two phases. The initial period (2012–2014) corresponded to the "establishment phase," characterized by extremely low emergence (only 0.48% of the seeds emerged by 2014) and high subsequent mortality (83% of the emerged genets died by 2014; Figure 2). This trend is consistent with previously reported patterns in other *Sasa* spp. after mass flowering (Makita et al. 1993; Abe and Shibata 2014b; Suzuki and Kajimura 2025). Subsequently, the period from 2014 onward corresponded to the "density-stable phase," which exhibited low mortality (Figure 2).

In the establishment phase, initial growth significantly affected survival. Genets with a larger number of culms and greater height in 2013 exhibited higher survival rates in 2014 (Table 1a). During the establishment and density-stable phases, initial growth and genetic factors significantly affected survival. Genets with a larger number of culms in 2013 and higher heterozygosity exhibited higher survival rates in 2022 (Table 1b). Although most of the seedlings exhibited limited allelic variation (Table S1), the decrease in F_{IS} among surviving seedlings from 2012 to 2022 suggested inbreeding depression (Figure S4).

Seedling growth in this study was substantially slower than that reported after mass flowering of *Sasa* spp. For example, the culm density at this study site after 11 years (0.6 culms/m^2; Figure 3c) was lower than the value reported 5 years after a mass flowering event of the same species (26.6 culms/m^2; Abe and Shibata 2014a). Similarly, the number of culms per genet (3.3 culms; Figure 4a) and height (27.2 cm; Figure 4b) after 11 years were lower than the values reported for *Sasa tsuboiana*, which shares the same rhizome type as *S. veitchii* (Makita 1998b), after mass flowering (6.9 culms in 5 years, and height reached approximately 90 cm within 10 years; Makita et al. 1993). The third "thinning phase," which follows the "density-stable phase," occurs after seedlings have grown and the population has reached full density (Makita 1992, 1998a). In this study, however, both the culm density and seedling cover remained low (Figures 5 and 6) because of slow seedling growth. As a result, the population had not yet reached full density, and the "thinning phase" was not observed.

Two factors may explain this slow seedling growth. First, low light availability may limit seedling growth. As mass flowering occurs on a large scale, seedlings can grow under various light conditions. Matsuo et al. (2018) examined the regeneration process of *S. kurilensis* after mass flowering and showed that faster biomass recovery occurred in seedlings under canopy gaps than in those under closed canopies. These fast-growing genets colonized areas under closed canopies, thereby contributing to overall regeneration (Tomimatsu et al. 2020). However, the canopy at the study site was almost completely closed. Uniformly low light availability may have contributed to slow seedling growth. Second, their slower growth may be attributed to inbreeding depression. Although seven genets flowered in this event, the allelic variation of the flowering genets and seedling was lower than that observed after mass flowering, in which many genets synchronously flower (Table S1; Matsuo et al. 2014). Most seedlings in this study may be selfed offspring of the single dominant flowering genet. However, heterozygosity did not significantly affect seedling growth (Table 1c,d), which may be due to the short observation period or small genetic differences among genets.

4.3 | Recovery of *Sasa* Vegetation 12 Years After Flowering

Twelve years after small-scale flowering, nonflowered *Sasa* had expanded into the flowered area from the surrounding area (Figure 6) and exhibited greater cover than the seedlings (Figure 5). Sato and Mochida (2019) showed that small-scale flowering areas were gradually dominated by nonflowering *S. senanensis* after 16 years. Well-developed nonflowering *Sasa* spp. possess large resource reserves, allowing them to spread extensively across heterogeneous forest environments (Tomimatsu et al. 2020) and translocate resources via their rhizomes (Saitoh et al. 2002). These advantages enable them to rapidly produce larger culms than recently established seedlings. Within the study plot, edge quadrats with high nonflowered *Sasa* cover (90% in quadrat 20_10 and 65% in quadrat 40_0; Figure 6) had lower light availability than the interior quadrats from late autumn to spring (Figure S3). This is because evergreen *Sasa* culms remained dominant in the edge quadrats even after the canopy leaves had fallen. Although no significant effects of the nonflowered *Sasa* culm density and light availability on seedling growth were detected (Table 1c), nonflowered *Sasa* will likely continue to spread into the flowered areas and shade seedlings out. Therefore, seedling regeneration may occur only where the expansion of nonflowered *Sasa* is slow or restricted.

4.4 | Ecological Significance of Small-Scale Flowering

The monocarpic strategy, which is typical for *Sasa* spp., is considered inherently risky because individuals invest all their resources and rely on a single reproductive event that is affected by variable environmental conditions (Read et al. 2008). Damage from weather or insects during mass flowering may cause the extinction of the entire population (e.g., Kobayashi 2017). In contrast, small-scale flowering genets often retain both flowering and nonflowering culms (Kitamura and Kawahara 2009; Mizuki et al. 2014). The successful establishment of seedlings in our study suggests that *Sasa* spp. are potentially polycarpic (Miyazaki, Ohnishi, Takafumi, and Hiura 2009; Kitamura and Kawahara 2009; Mizuki et al. 2014). Therefore, the seedling regeneration derived from partial flowering within individuals may reduce the regeneration failure risk associated with a strictly monocarpic and mass flowering life history (Suyama et al. 2010). To clarify whether *Sasa* spp. exhibit a polycarpic reproductive system, future studies should determine whether the remaining non-flowered *Sasa* culms of the genets that previously flowered will flower again. Our study may help illuminate the ecological role of asynchronous small-scale flowering in species that exhibit synchronous mass flowering.

Author Contributions

A.M., C.O., I.M., and R.O. conceived the study and designed the methodology and all authors contributed to data collection. R.O. and Y.S. analyzed the data and wrote the draft of the manuscript. All authors contributed to writing and editing the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Distribution of the seedling quadrats within the 50 m × 50 m study plot. A total of 36 seedling quadrats (total 270 m^2), consisting of 2 m × 2 m ($N = 30$) and 5 m × 5 m ($N = 6$) quadrats were established at 10 m intervals. Smaller black squares represent the 2 m × 2 m quadrats, and larger black squares represent the 5 m × 5 m quadrats. White squares represent quadrats where seedlings survived. The coordinates of the horizontal (x) and vertical (y) axes are used consistently in Figures 1, 3, 6. **Figure S2:** Eleven-year-old seedling derived from small-scale flowering. In this study, "height" was defined as maximum culm height and "number of culms" was defined as number of main stems with leaves. **Table S1:** Characteristics of microsatellite loci used in this study, including the number of alleles per locus (N_A), effective number of alleles per locus (N_E), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficient (F_{IS}), for both the flowering genets and seedling of *S. veitchii*. We also tested for significant deviations of F_{IS} from Hardy–Weinberg equilibrium (HWE) using a chi-squared test in GenAlEx. Seven flowering genets were detected from 39 flowering culms. A total of 1104 genets were detected from 1236 seedling samples from 2012 to 2022. Of these, 104 genets were identified by field survey alone, and 1000 genets were identified through DNA analysis of 1132 seedling leaf samples. **Figure S3:** Seasonal change in canopy openness in eight quadrats where seedlings survived until 2021. Different colors represent different quadrats. Plot numbers (e.g., 40_0) correspond to the horizontal (x) and vertical (y) axis coordinates in the 50 m × 50 m study plot (Figure 6 and Figure S1). Measurements were not performed between December and March because the seedlings were covered with snow. **Figure S4:** Change in F_{IS} (mean ± SD) of *S. veitchii* flowering and seedling populations from 2011 to 2022. The 2011 value represents the flowering population, whereas the values from 2012 to 2022 represent the seedling population. F_{IS} typically ranges from 0 to 1, however, since it is calculated as $F_{IS} = 1 - H_O/H_E$, it can result in a negative value when $H_O > H_E$.