Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Fine-scale clonal structure of the lingonberry Vaccinium vitis-idaea under the nurse plant Pinus pumila vegetation in alpine region, Mt. Norikura.

Kensuke Sugimoto
Nihon University - Sakurajosui Campus: Nihon Daigaku - Sakurajosui Campus
Rio Kawai
Nihon University - Sakurajosui Campus: Nihon Daigaku - Sakurajosui Campus
Takahiko Koizumi
Tokyo University of Agriculture and Technology: Tokyo Noko Daigaku
Ayumi Matsuo
Tohoku University: Tohoku Daigaku
Yoshihisa Suyama
Tohoku University: Tohoku Daigaku
INOUE MIZUKI
inoue.mizuki@nihon-u.ac.jp

Nihon University - Sakurajosui Campus: Nihon Daigaku - Sakurajosui Campus https://orcid.org/0000-0001-7390-3655

Research Article

Keywords: clonal diversity, MIG-seq, nursery, SNPs, stolon

Posted Date: May 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2950446/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Journal of Plant Research on April 3rd, 2024. See the published version at https://doi.org/10.1007/s10265-024-01537-0.

Abstract

Nursery effect is a positive interaction wherein a nurse plant improves the abiotic environment for another species (beneficiary plant) and facilitates its establishment. The evergreen shrub *Vaccinium vitis-idaea* (beneficiary plant) grows mainly under the dwarf shrub *Pinus pumila* (nurse plant) in the alpine regions of central Japan. However, whether *V. vitis-idaea* shrubs under various *P. pumila* shrubs spread through clonal growth and/or seeds remains unclear. Here, we aimed to investigate the clonal structure of *V. vitis-idaea* under the nurse plant, *P. pumila*, in Japanese alpine regions. MIG-seq analysis was conducted to compare isolated and patchy *P. pumila* plots on the ridge (PATs) and plots covered by dense *P. pumila* on the slope (MAT) on Mt. Norikura, Japan. We detected 15 and 17 multi-locus genotypes in 209 ramets across 11 PATs and 130 ramets in one MAT, respectively. Approximately half of the PATs consisted of a single genet. These results suggest that the seeds of *V. vitis-idaea* were dispersed under small *P. pumila* patchy shrubs and spread by clonal growth in small PATs. The genet abundance curve of MAT was more moderate than that of PATs, and the clonal diversity of MAT was lower than that of PATs on a small scale. Therefore, spatial spread of the nurse plant *P. pumila* facilitates germination via the seeds of *V. vitis-idaea.* Our results show that the nursery effect on the genetic diversity of beneficiary plant increased positively with the spatial size of nurse plants in alpine regions, leading the sustainability of the beneficiary populations.

Introduction

Interactions between organisms, such as competition, symbiosis, parasitism, and predation, are noteworthy ecological topics. A positive interaction, known as the nursery effect, has been observed in stressful environments such as salt marshes, Arctic regions, and deserts (Arroyo et al. 2003; Haase et al. 1996; López et al. 2007). Canopies of adult shrubs or trees (nurse plants) improve the abiotic environment of nurse plants and facilitate the establishment of other species (beneficiary plants). For example, in the Sonoran Desert, shading of the nurse plant bunchgrass, *Hilaria rigida*, reduces maximal soil surface temperatures, which are lethal to most desert succulents, and provides shelter microhabitats for succulent seedlings, *Agave deserti* (Franco and Nobel 1988). Furthermore, it is known that the spatial size of nurse plants plays a crucial role in plant facilitation (Peláez et al. 2019; Tewksbury and Lloyd 2001); the larger the nurse plant, the greater the nursery effect.

Evergreen shrubs, lingonberry, *Vaccinium vitis-idaea* (Ericaceae), which can expand clonally by stolons, are common in the Arctic; they are also distributed in Japanese alpine regions (Ikeda et al. 2015). In the alpine regions in central Japan, *V. vits-idaea* grows mainly under the dwarf shrub *Pinus pumila* (Pinaceae), whose trunk lies on the ground surface and forms a dense monodominant dwarf forest above the tree line. Shrubs of *P. pumila* mitigate the strong ultraviolet light and wind peculiar to alpine regions, harboring various alpine plants such as *V. vitis-idaea*. Therefore, *P. pumila* is known as a nurse plant for *V. vitis-idaea* (Nakano 1996).

On the ridge of Mt. Norikura in Japan, many shrubs of *P. pumila* spread in dots on rocky bare ground, while on the slopes, *P. pumila* is densely and widely distributed. As nurse plant spatial size is related to the nurse plant facilitation effect (Peláez et al. 2019), differences in the spatial spreading patterns of *P. pumila* would also alter the strength of nursery effect. If the nursery effect is sufficient, seedlings via the seeds of the beneficiary plant species under nurse plants germinate and establish themselves. However, it remains unclear whether *V. vitis-idaea* shrubs under various *P. pumila* shrubs spread through clonal growth and/or seeds. From the viewpoint of the sustainability of *V. vitis-idaea* populations, it is important to clarify the number of genets consisting of *V. vitis-idaea* shrubs according to *P. pumila* shrub spatial size.

In this study, we used MIG-seq analysis (Suyama and Matsuki 2015), to investigate the clonal structure of *V. vitis-idaea* under the nurse plant, *P. pumila*, in Japanese alpine regions. We considered the effect of the spatial size of *P. pumila* shrubs on the clonal composition of *V. vitis-idaea*. We compared isolated and patchy *P. pumila* plots on the ridge (patchy plots) and plots covered by dense *P. pumila* on a slope (mat plot) at Mt. Norikura in Japan. The results of this study can aid in a better understanding of the process of colonization and expansion of *V. vitis-idaea* under *P. pumila* vegetation.

Material and Methods

Ethics Statement

Permission to survey *V. vitis-idaea* was obtained from the Ministry of the Environment and Forestry Agency of Japan.

Study species

V. vitis-idaea ranges across the Northern Hemisphere. It is an evergreen dwarf shrub with creeping stems (Ritchie 1955) and stolons (Persson and Gustavsson 2001). It is pollinated by bees (Jacquemart 1993; Persson and Gustavsson 2001) and dispersed by birds, such as *Lagopus mutus* (Kobayashi and Nakamura 2011).

Study sites and samples

The study sites are located at Mt. Norikura (36º74 N, 137º33 E, 2,770–2,790 m a.s.l.) in central Japan (Fig. 1a). Mean annual temperature and rainfall were − 1.2 ºC and 2,738 mm, respectively. In 2019, the lowest and highest temperatures were − 14.2 ºC and 12.0 ºC, respectively. Snow generally covers the area until late June.

On the ridge, there were several different-sized isolated shrubs of *P. pumila*, whose heights were less than 0.4 m. *V. vitis-idaea* was mainly distributed under the shrubs of *P. pumila*. In contrast, the plot on the slope was covered with dense *P. pumila*, with a height of approximately 2.0 m.

We established 11 plots on the ridge and 1 plot on the eastern slope of the mountain (Fig. 1b). At the 11 plots of patchy *P. pumila* shrubs (patchy plots; PATs), we estimated patch sizes based on the areas of ellipses by measuring the lengths of the major and minor axes of a PAT. We collected the leaves of *V. vitis-idaea* at every 150-cm grid point in each PAT in August 2020. When we could not collect more than 16 leaves at every 150 cm grid point, we changed the grid size from 20 to 150 cm, depending on the size of the PATs. On the slope, we set a 45 m × 6 m plot of *P. pumila* vegetation (a mat plot; a MAT). We collected leaves of *V. vitis-idaea* at every 150 cm grid point in the MAT in August 2021. When there were no leaves of *V. vitis-idaea* near the grid points, we only recorded them. All leaves were stored with silica gel and at − 30°C until DNA extraction for MIG-seq analysis.

MIG-seq analysis

Total genomic DNA was extracted from 50 mg of leaves using the cetyltrimethylammonium bromide (CTAB) method as described by Murray and Thompson (1980). We performed multiplex inter-simple sequence repeat (ISSR) genotyping by sequence analysis, known as MIG-seq, which is a useful method for detecting genome-wide single-nucleotide polymorphisms (SNPs) (Suyama and Matsuki 2015). Standard experimental conditions have been described by Suyama (2022). First, ISSRs were amplified from 50 ng of DNA using eight pairs of multiplex ISSR primers. The first PCR product from each sample was purified/equalized, and short fragments (ca. <250 bp) were removed using AMPure XP (Beckman Coulter, Brea, CA, USA) and used as a template for the second PCR. The second PCR was performed to add complementary sequences for the oligonucleotides that coat the Illumina sequencing flow cell, annealing sites of the DNA sequencing primers, and indices for the first PCR products. The concentration of each PCR product (library) was measured using a Microchip Electrophoresis System (MultiNA, Shimadzu, Tokyo, Japan) with a DNA-2500 Reagent Kit (Shimadzu). Libraries from each sample, each with a different index, were pooled at equimolar concentrations. Fragments in the size range of 350–800 bp in the purified library were isolated using the magnetic bead method (AM Pure XP). The final concentration was measured using an SYBR Green quantitative PCR assay (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) with primers specific to the Illumina constructs. Libraries (final concentration of 10 pM) were sequenced on an Illumina MiSeq System (Illumina) using the MiSeq Reagent Kit v3 (150 cycles, Illumina).

Low-quality reads and primer sequences were eliminated from the raw data using trimmomatic 0.39 (Bolger et al. 2014). The quality-filtered sequence data were demultiplexed and filtered using Stacks v2.41 software (Catchen et al. 2011; Catchen et al. 2013; Rochette et al. 2019). We used “ustacks” with the following option settings: minimum depth of coverage required to create a stack (m) = 3; maximum distance allowed between stacks (M) = 2; and maximum distance allowed to align secondary reads to primary stacks (N) = 2. We used “cstacks” with the option settings for the number of mismatches allowed between sample loci when building the catalog (n) = 2, followed by “sstacks.” We used “populations” with the following option settings: minimum proportion of individuals required to process a locus across all data (r) = 0.8; restricting data analysis to a single SNP per locus (write_single_snp); the minimum number of populations in a locus (p) = 1; the minimum minor allele frequency required to process a nucleotide site

at a locus (min_maf) = 0.01; and the maximum observed heterozygosity required to process a nucleotide site at a locus (max_obs_het) = 0.95.

Genet identification and clonal diversity

We obtained 18,803,564 and 4,964,890 raw reads for the PAT and MAT samples, respectively, including duplications, by MIG-seq. After quality control, a total of 95, 948 PAT and 20,783 MAT reads were used for further analyses. The mean number of highly common reads per sample was 459.0 and 163.6 for PAT and MAT, respectively, suggesting that the data were reliable. Therefore, we estimated the similarity between the samples. We considered ramets belonging to identical genets if they had over 90% of similarity to 779 SNPs (Fig. 2).

After identifying the genets, we estimated the genotypic richness and three measures of clonal diversity. Genotypic richness was estimated according to Dorken and Eckert (2001) as $R = (G-1) / (N-1)$, where G and N are the numbers of genets and ramets, respectively. Clonal diversity was calculated using:

1. Simpson's index of diversity corrected for sample size (Pielou 1966), D, which represents the probability that two ramets selected at random from N ramets are from different genets: $D = 1 - \{[\Sigma\, n_i (n_i - 1)] / [N (N - 1)]\}$, where n_i is the number of ramets of each genet.
2. H values of Shannon–Wiener (Shannon 1949), $H = -\Sigma (n_i / N) * \ln (n_i / N)$.

To consider the effect of the survey area of plots on Simpson's D, we estimated the values at R1.5 area size (radius = 1.5 m, area size = 7.1 m^2) using each grid point excluding the grid points at all edges (n = 87) at MAT. In addition, we estimated the values at R3.0 (radius = 3.0 m, area size = 19.6 m^2) using central 27 grid points (n = 27).

Statistical Analysis

To investigate the relationship between Simpson's D and survey area, we used a binomial generalized linear model (GLM). We used a linear model to investigate the relationship between the maximum distance within the same genotype and the number of ramets within the same genotype. The best model was selected based on the AIC. Statistical analyses were performed using R4.0.3 (R Development Core Team 2019).

Results

Based on MIG-seq analysis, we detected 15 and 17 multi-locus genotypes in 209 ramets across 11 PATs and 130 ramets in the MAT, respectively. At each PAT, we found between one and five genets (Table 1). Approximately half of the PATs (5 of 11 sites) consisted of a single genet. Some of the PATs shared the same genets (Fig. 3a). In two large PATs and the MAT, some genets were clumped and some were scattered (Fig. 3b).

Table 1

Plot name, (Plot NO.), size of plots (Area, m^2), numbers of ramets we sampled (*N*), number of genets we identified (*G*), genotypic richness (R), and two measures of diversity (Simpson's *D* and Shannon–Wiener's *H*).

Plot NO.	Area (m^2)	*N*	*G*	*R*	Simpson's *D*	Shannon-Wiener's *H'*
PAT10	80.94	30	5	0.14	0.68	1.32
PAT11	54.30	33	3	0.06	0.39	0.69
PAT13	2.98	14	1	0	0	0
PAT23	22.36	6	1	0	0	0
PAT27	19.16	15	3	0.14	0.64	1.06
PAT28	21.18	20	3	0.11	0.41	0.73
PAT32	11.72	16	1	0	0	0
PAT33	11.12	16	3	0.13	0.23	0.46
PAT36	6.87	16	3	0.13	0.40	0.70
PAT37	5.51	13	1	0	0	0
PAT38	5.73	13	1	0	0	0
MAT	270.00	130	17	0.12	0.86	2.33

Genotypic richness was 0–0.14 (mean = 0.06) and 0.13 in 11 PAT and 1 MAT, respectively (Table 1). Clonal diversity in MAT (Simpson's $D = 0.87$, Shannon–Wiener's $H = 2.33$) was higher than that in PATs (mean Simpson's $D = 0.25$, mean Shannon–Wiener's $H = 0.45$). Simpson's *D* was positively correlated with the survey area at both sites (Fig. 4a). Simpson's *D* in the MAT was higher than that in the PATs on a small scale.

The relative abundance curves of the PATs were steeper than those of the MAT (Fig. 4b). The spatial extent of a genet was positively related to the number of ramets within the same genets (adjusted $R^2 = 0.38$, $p = 0.009$, AIC = 115.5; Fig. 4c).

Discussion

The results of our study showed that nursery effect on the genetic diversity of beneficiary plants increased with the spatial size of nurse plants. It was facilitated by germination and establishment via the seeds of beneficiary plant.

MIG-seq is a useful tool for investigating the clonal structure of *V. vitis-idaea* populations. We identified 32 genets in the PAT and MAT plots. Clonal diversity in the MAT (Simpson's $D = 0.87$) is similar to that of a previous study on *V. vitis-idaea* conducted in Sweden (Simpson's $D = 0.84$ (Persson and Gustavsson

2001). Furthermore, the clonal diversity of large populations of *V. vitis-idaea* is generally comparable to the values obtained in a meta-analysis of clonal plant species (mean weighted Simpson's $D = 0.85$ for 55 species; Honnay and Jacquemyn 2008).

The clonal diversity in PATs was extremely low (mean Simpson's $D = 0.25$, mean Shannon–Wiener's $H = 0.45$). Furthermore, several PATs belong to a single genet. On a small scale, Simpson's D values in the PATs were smaller than those in the MAT. These results suggest that the seeds of *V. vitis-idaea* were dispersed under small *P. pumila* patchy shrubs, and one of them germinated by chance and spread by clonal growth in small PATs, that is, young nurse plant sites. The genets of *V. vitis-idaea* spread linearly up to 30 m (Garkava-Gustavsson et al. 2005). In our study, three genets expanded to over 30 m in the MAT. The contribution of the clonal growth of *V. vitis-idaea* would also be high, even in the MAT.

The number of genets and clonal diversity were positively related with the spatial size of the PATs. This finding has two possible explanations. First, small PATs would expand by clonal growth, neighboring each other. Otherwise, further seed dispersal and germination would occur in the larger PATs after the establishment of the *V. vitis-idaea* genet. The clonal spatial structures of PAT10 and PAT11 might support the former possibility, because there were some genets with a large number of ramets. However, the steeper curves of PATs supported the latter possibility, because most PATs consisted of a few major genets and some minor genets.

Seedlings via seeds would contribute to the vegetation of MAT because the genet abundance curve of MAT would be more moderate than that of PATs, and the clonal diversity of MAT would be higher than that of PATs on a small scale. This suggests that on the local scale of MAT, many genets intermingle with each other by further seed dispersal and germination. Thus, the nurse plant *P. pumila* facilitated the germination and establishment of seeds of *V. vitis-idaea*, as shown by Turner et al. (1966).

Our results showed that the nursery effect increased with the spatial size of the nurse plant, *P. pumila*, which supports the findings of previous studies (Peláez et al. 2019; Pugnaire et al. 1996; Tewksbury and Lloyd 2001). Nurse plant spatial size played a crucial role in the genetic diversity of beneficiary plants. Although one of the nursery systems (a beneficiary plant, *Euphorbia nicaeensis*, whose nurse plant is *Juniperus sabina* in a Mediterranean mountain environment) has exhibited the effects of the gene pool in beneficiary plant populations on nurse plants in harsh environments (Castellanos et al. 2014), our study is the first to reveal that the positive effects on genetic diversity of beneficiary plants increased with the size of the nurse plant.

In conclusion, the spatial size of nurse plants plays a crucial role in plant facilitation; the larger the spatial spread of the nurse plant, the higher the chances to be germinated and established by seeds of beneficiary plant, leading to the larger nursery effect on the genetic diversity of beneficiary plant. We also showed the importance of spread by clonal growth of beneficiary plant in small nurse plant shrubs to maintain the genets of beneficiary plant. The nurse plant, *P. pumila*, and the plant community under the species in the alpine ecosystem, Mt. Norikura, would be an excellent system to examine the scale effects of nursery effects, similar to those in the desert (Tewksbury and Lloyd 2001), because of the various sizes

of *P. pumila* shrubs. In future studies, we would investigate the relationship between *P. pumila* and other plant species, not only on genetic diversity but also on the growth, recruitment, abundance, and richness of plant communities to clarify the sustainability of alpine plant populations vulnerable to global warming.

Declarations

Competing interests

The authors declare no conflict of interest.

Acknowledgments

The authors thank Shiori Narizuka and Yuki Ohkawa for their help with the fieldwork. We would like to thank Editage (www.editage.com) for English language editing. I.M received support from JSPS KAKENHI Grant Number JP19K06129 and JP22K05754.

References

1. Arroyo MTK, Cavieres LA, Peñaloza A, Arroyo-Kalin MA (2003) Positive associations between the cushion plant Azorella monantha (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. Plant Ecol 169:121–129. 10.1023/A:1026281405115
2. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. 10.1093/bioinformatics/btu170
3. Castellanos MC, Donat-Caerols S, González-Martínez SC, Verdú M (2014) Can facilitation influence the spatial genetics of the beneficiary plant population? J Ecol 102:1214–1221. 10.1111/1365-2745.12278
4. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Mol Ecol 22:3124–3140. 10.1111/mec.12354
5. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH (2011) Stacks: building and genotyping loci de novo from short-read sequences. G3 (Bethesda) 1:171–182. 10.1534/g3.111.000240
6. Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, Decodon verticillatus (Lythraceae). J Ecol 89:339–350. 10.1046/j.1365-2745.2001.00558.x
7. Fager EW (1972) Diversity: a sampling study. Am Nat 106:293–310. 10.1086/282772
8. Franco AC, Nobel PS (1988) Interactions between seedlings of Agave deserti and the nurse plant Hilaria rigida. Ecology 69:1731–1740. 10.2307/1941151

9. Garkava-Gustavsson L, Persson HA, Nybom H, Rumpunen K, Gustavsson BA, Bartish IV (2005) RAPD-based analysis of genetic diversity and selection of lingonberry (Vaccinium vitis-idaea L.) material for ex situ conservation. Genet Resour Crop Evol 52:723–735. 10.1007/s10722-003-6123-4
10. Haase P, Pugnaire FI, Clark SC, Incoll LD (1996) Spatial patterns in a two-tiered semi-arid shrubland in southeastern Spain. J Veg Sci 7:527–534. 10.2307/3236301
11. Honnay O, Jacquemyn H (2008) A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. Evol Ecol 22:299–312. 10.1007/s10682-007-9202-8
12. Ikeda H, Yoneta Y, Higashi H, Eidesen PB, Barkalov V, Yakubov V, Brochmann C, Setoguchi H (2015) Persistent history of the bird-dispersed arctic-alpine plant Vaccinium vitis-idaea L. (Ericaceae) in Japan. J Plant Res 128:437–444. 10.1007/s10265-015-0709-8
13. Jacquemart AL (1993) Floral visitors of Vaccinium species in the high Ardennes, Belgium. Flora 188:263–273. 10.1016/S0367-2530(17)32276-4
14. Kobayashi A, Nakamura H (2011) Seasonal change of food items of the Japanese Rock Ptarmigan (in Japanese with English summary). Jpn J Ornithol 60:200–215. 10.3838/jjo.60.200
15. López RP, Valdivia S, Sanjinés N, de la Quintana D (2007) The role of nurse plants in the establishment of shrub seedlings in the semi-arid subtropical Andes. Oecologia 152:779–790. 10.1007/s00442-007-0698-y
16. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325. 10.1093/nar/8.19.4321
17. Nakano T (1996) Ecology of Vaccinium vitis-idaea in a wind exposed area of alpine region of Mt. Norikura. Ph.D. thesis. In: Tokyo Metropolitan University
18. Peláez M, Dirzo R, Fernandes GW, Perea R (2019) Nurse plant size and biotic stress determine quantity and quality of plant facilitation in oak savannas. For Ecol Manag 437:435–442. 10.1016/j.foreco.2019.02.010
19. Persson HA, Gustavsson BA (2001) The extent of clonality and genetic diversity in lingonberry (Vaccinium vitis-idaea L.) revealed by RAPDs and leaf-shape analysis. Mol Ecol 10:1385–1397. 10.1046/j.1365-294x.2001.01280.x
20. Pielou EC (1966) The Measurement of diversity in different types of biological collections. J Theor Biol 13:131–144. 10.1016/0022-5193(66)90013-0
21. Pugnaire FI, Haase P, Puigdefábregas J, Cueto M, Clark SC, Incoll LD (1996) Facilitation and succession under the canopy of a leguminous shrub, Retama sphaerocarpa, in a semi-arid environment in south-east Spain. Oikos 76:455–464. 10.2307/3546339
22. Ritchie JC (1955) Vaccinium vitis-idaea L. J Ecol 43:701–708. 10.2307/2257030
23. Rochette NC, Rivera-Colón AG, Catchen JM (2019) Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol Ecol 28:4737–4754. 10.1111/mec.15253

24. R Development Core Team (2019) R version 3.6.1: A language and environment for statistical computing. https://www.R-project.org. R Foundation for Statistical Computing, Vienna, (p 21/06/2021)
25. Shannon CE (1949) The mathematical theory of communication. University of Illinois Press
26. Suyama Y, Matsuki Y (2015) MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. Sci Rep 5:16963. 10.1038/srep16963
27. Suyama Y, Hirota SK, Matsuo A, Tsunamoto Y, Mitsuyuki C, Shimura A, Okano K (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. Ecol Res 37:171–181. 10.1111/1440-1703.12270
28. Tewksbury JJ, Lloyd JD (2001) Positive interactions under nurse-plants: spatial scale, stress gradients and benefactor size. Oecologia 127:425–434. 10.1007/s004420000614
29. Turner RM, Alcorn SM, Olin G, Booth JA (1966) The influence of shade, soil, and water on saguaro seedling establishment. Bot Gaz 127:95–102. 10.1086/336348

Figures

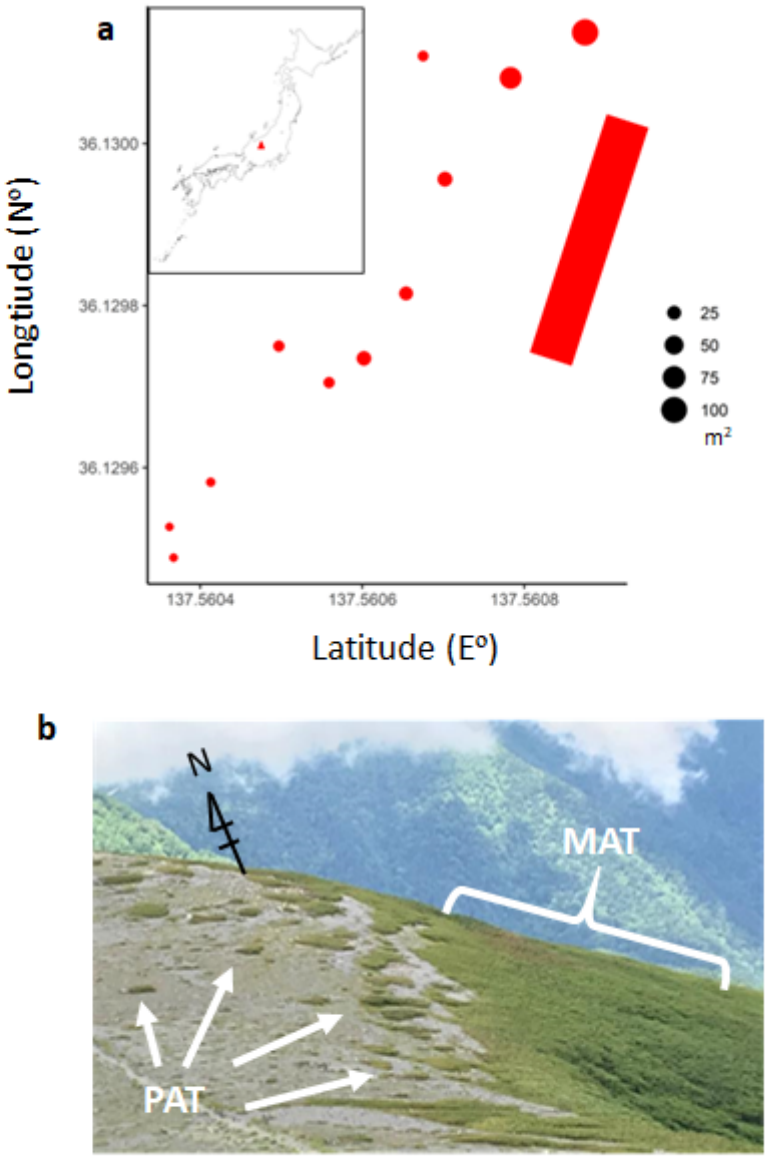

Fig.1

Figure 1

Location of study sites (a). Circles: patchy plots (PAT) of *V. vitis-idaea* under the *P. pumila* shrub. Rectangle: 45 m × 6 m plot (MAT) of *V. vitis-idaea* under *P. pumila* dense vegetation. A photo of the study sites' landscape (b)

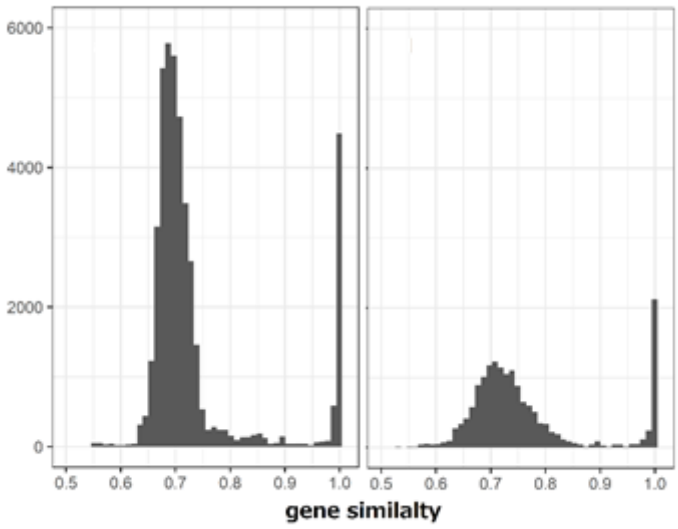

Figure 2

Histograms of similarity among 209 ramets across 11 PAT (a) and those among 130 ramets of the MAT (b) based on 779 SNPs

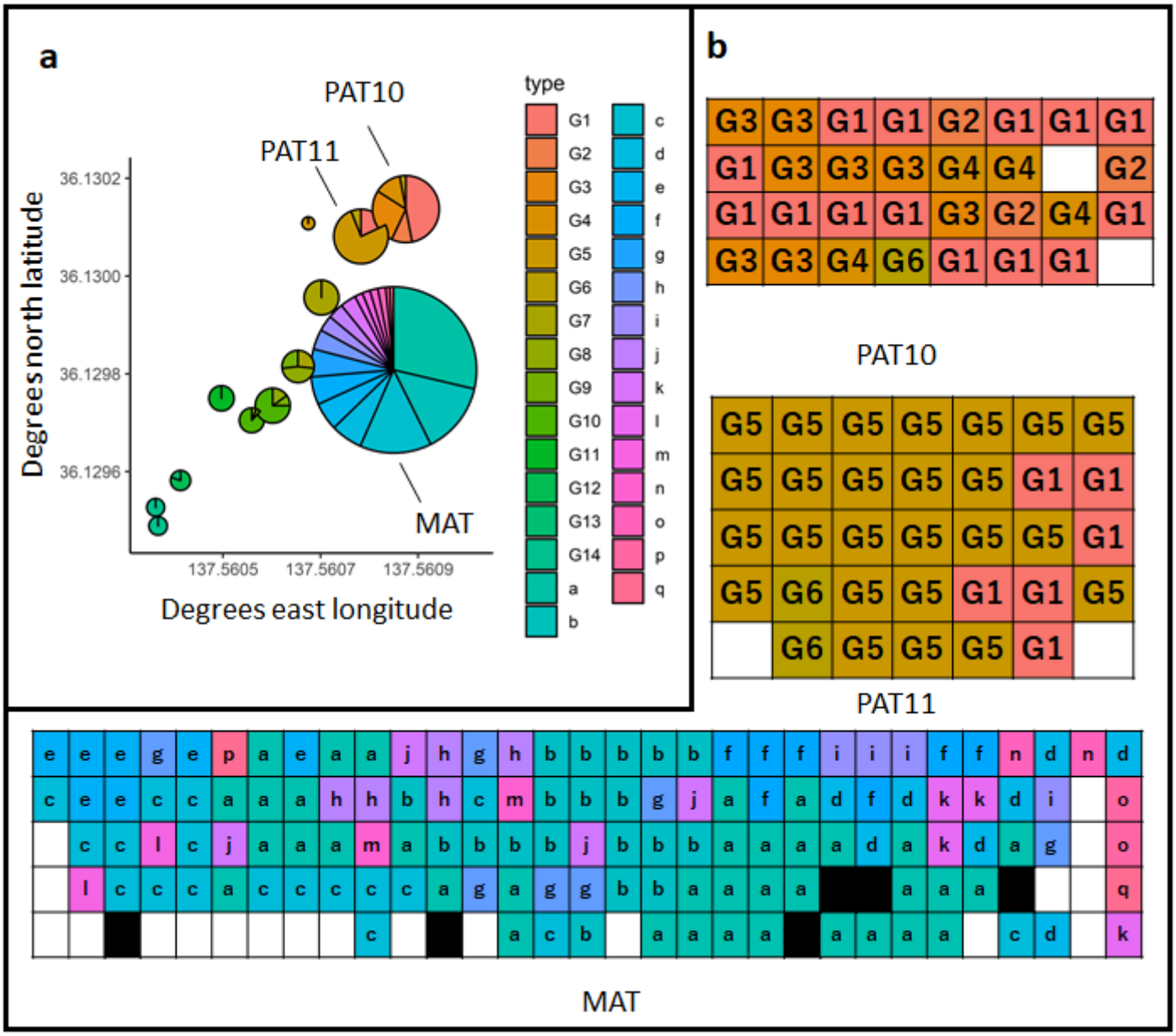

Figure 3

Clonal composition of 11 PAT and the MAT (a) and clonal spatial structure of PAT10, PAT11, and MAT (b). Each genet is labeled using a different color and alphabet. Samples in which we could not find any leaves and those in which we missed the analysis are shown as white and black, respectively

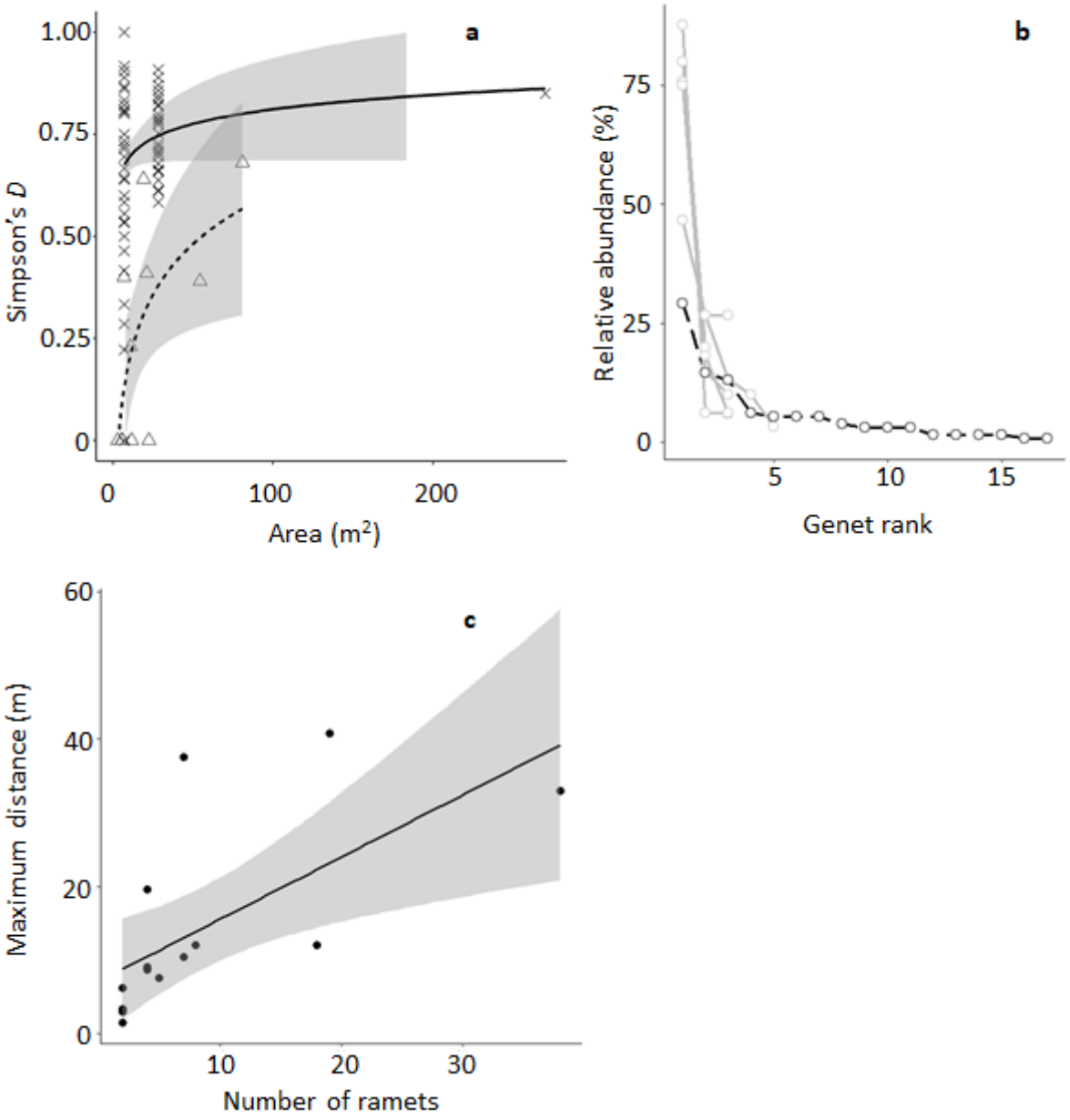

Figure 4

Relationship between Simpson's *D* and the survey area (a). Triangles show each value of PAT. Circles show each value calculated by different size (7.1 m^2, 28.3 m^2, and whole plot) of MAT. The solid and dashed lines indicate the logarithmic curves of PAT and MAT, respectively. The 95% confidence interval is denoted by light gray. Genet rank abundance curve of MAT (b). The black dashed and grey solid lines indicate the values of each plot of PAT and MAT, respectively. The points were excluded when the plots consisted of only one genet. Relationship between the number of ramets within genets and the spatial extent of the genet of MAT (c). The line indicates the simple regression analysis of MAT. The 95 % confidence interval is denoted by light gray