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Interspecific variation in clonality in temperate lianas revealed by genetic analysis: Do clonal proliferation processes differ among lianas?

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Abstract

Lianas are important components of forest communities with a considerable impact on overall forest structure and function. Lianas are characterized by extensive clonal reproduction on the forest floor, which is important for their establishment and growth. Previous studies have suggested that clonal reproduction strategies vary substantially among liana species; however, few studies have quantitatively evaluated the clonality strategy in multiple co-occurring liana species. The primary objective of the present study was to evaluate the relative contribution of clonal reproduction and to understand the clonal proliferation processes in co-occurring liana species by assessing both small stems on the forest floor and mature stems that climbed trees. The clonal reproduction strategy in four common liana species (stem twiner: *Wisteria floribunda*; root climbers: *Schizophragma hydrangeoides*, *Euonymus fortunei* and *Rhus ambigua*) in a 6-ha plot and a belt transect within an old-growth temperate forest in central Japan was evaluated using genetic analysis. The contribution of clonal reproduction was smaller in root climbers than in *W. floribunda*. All *W. floribunda* genets with small ramets in the understory had genetically identical ramets that climbed trees, whereas few such ramets were found in root climbers. This indicates that *W. floribunda* mature ramets laterally produce small ramets via clonal proliferation, whereas seedlings of root climbers grow horizontally to climb trees. The results indicate that the clonal reproduction processes differ greatly among lianas and the clonal growth in lianas plays a contrasting role in their life-history strategy.

KEYWORDS

climbing mechanisms, clonal reproduction, Ogawa Forest reserve, temperate forest, vegetative growth

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1 | INTRODUCTION

A liana (woody vine) is a plant life-form that germinates in the soil and grows up to the forest canopy, although the exact mechanisms of liana growth are species dependent. A growing number of studies indicate that lianas can have a considerable impact on forest structure and function due to their influence on the regeneration and composition of the tree community (reviewed by Schnitzer & Bongers, 2002, 2011). Therefore, evaluation of the mechanisms controlling the abundance, establishment and growth of lianas in forests is necessary to understand and predict liana community dynamics and the impact of lianas on forest structure and function.

Lianas can establish in and colonize favorable environments not only via seed reproduction but also via vegetative growth (clonal reproduction; Penalosa, 1984; Schnitzer, Dalling, & Carson, 2000) on the forest floor and in multiple host trees; in contrast, most tree species typically rely solely on seed reproduction for establishment and colonization in favorable environments (Schnitzer & Bongers, 2011). Indeed, clonal reproduction in lianas is considered to be important for the formation of populations and determining their spatial distribution in forests (Ledo & Schnitzer, 2014; Mori et al., 2018). It is important to note that the processes of clonal growth in lianas vary greatly among species and climbing mechanisms (e.g., Gerwing, 2004; Kato, Kanematsu, Kawakubo, & Komiyama, 2012), such as in stem twiners and root

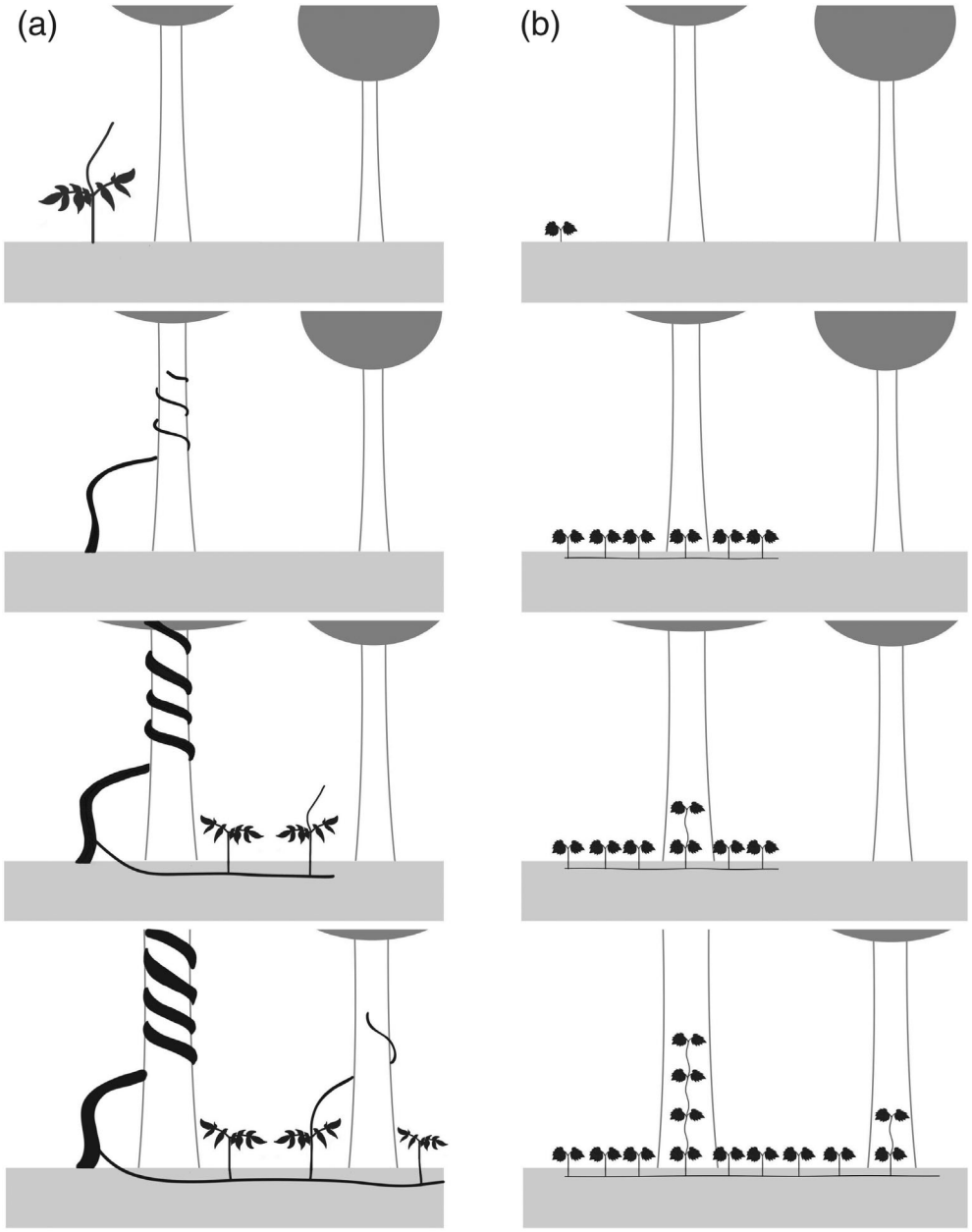


FIGURE 1 Illustration of the two different processes of clonal reproduction in lianas. (a) The climb-prioritizing strategy. After establishment via seed the shade-intolerant plants grow towards the forest canopy. Once the forest canopy is reached, adult plants produce stolons that grow towards the forest floor, resulting in the generation of small ramets. (b) The spread-prioritizing strategy. Small shade-tolerant plants (seedlings) derived from seed reproduction initially grow clonally on the forest floor and then start climbing trees once they encounter tree stems, eventually reaching the forest canopy

climbers (Putz & Holbrook, 1991). Root climbers are often suppressed under the host tree canopy, exhibiting less shoot production and slower growth than fast-growing and light-demanding stem twiners (Ichihashi & Tateno, 2015). Also, the role of clonal reproduction in the establishment and growth of lianas may differ considerably depending on the clonal reproduction strategy utilized to grow from the understory to the forest canopy. Therefore, assessing not only clonal structure but also clonal reproduction strategies across multiple liana species is essential for understanding the mechanisms of liana establishment and growth in forests.

Although few studies have reported clonal reproduction strategies in lianas so far, two different types of clonal reproduction strategy are generally recognized (illustrated in Figure 1): (a) a climb-prioritizing strategy, whereby after establishment via seed the shade-intolerant plants grow towards the forest canopy; once the forest canopy is reached adult plants produce stolons (or rhizomes) that grow towards the forest floor, resulting in the generation of small, clonally reproduced individuals (hereafter, "ramets"); and (b) a spread-prioritizing strategy, whereby small shade-tolerant plants (seedlings) derived from seed reproduction initially grow clonally on the forest floor and then start climbing trees once they encounter tree stems, eventually reaching the forest canopy. The climb-prioritizing strategy could be more beneficial than the spread-prioritizing strategy in terms of enabling rapid growth and establishment through long-distance clonal reproduction in the understory because mature lianas in the forest canopy are able to produce large amounts of stolons and ramets on the forest floor (e.g., Sakai, Nomiya, & Suzuki, 2002). On the other hand, the spread-prioritizing strategy could be more beneficial than the climb-prioritizing strategy in terms of allowing long-term persistence and growth in the dark understory, providing a longer window of opportunity for successful establishment on trees (Kato et al., 2012; Kato, Hosoi, Kawakubo, & Komiyama, 2011; Leicht & Silander, 2006). Therefore, examining both plants attached to host trees and small stems on the forest floor that have yet to begin climbing (hereafter termed "on-tree stage" and "on-floor stage", respectively) and clarifying the distinction between the two types of clonal reproduction are necessary for the evaluation of the significance of clonal reproduction in the processes of liana growth and reproduction.

Clonality in lianas can be evaluated by observing aboveground (liana inventory; Gerwing et al., 2006; Schnitzer, Rutishauser, & Aguilar, 2008; Schnitzer et al., 2012) and belowground connections (excavation; Putz, 1984; Sakai et al., 2002), and by determining stem genotypes using genetic tools (DNA markers; Mori et al., 2018). However, above- and belowground observations have limitations in terms of detecting clones when underground excavation is not practical or because physical connections have decayed over time. On the other hand, genetic tools such as microsatellite markers are able to precisely determine the size and distribution of clones (Arnaud-Haond, Duarte, Alberto, & Serrao, 2007) without relying on physical connections. However, studies on the clonality of liana species both at the on-tree and the on-floor stages using genetic tools are surprisingly limited.

Mori et al. (2018) previously evaluated the clonal structure of a liana species (*Wisteria floribunda* [Willd.] DC.) at the on-tree stage (i.e., only evaluating individuals that were growing on trees higher than 1.3 m above ground) in a temperate forest using 10 microsatellite markers (Mori et al. 2016b) and found evidence that clonally reproduced ramets comprised 71% of the total number of stems of this species. The authors also reported that they did not find stems with aboveground physical connections to other stems (apparent genets; Gerwing et al., 2006). This indicates the application of genetic tools is necessary to obtain a precise understanding of clonal reproduction in lianas in forests. However, the study was conducted on a single liana species at the on-tree stage, and whether differences in clonal strategy between co-occurring liana species, and between on-tree and on-floor stages, exist remains unknown.

This study aims to evaluate the clonal structure of four common liana species (*W. floribunda*, *Schizophragma hydrangeoides* Siebold & Zucc., *Euonymus fortunei* [Turcz.] Hand.-Mazz. and *Rhus ambigua* Lavallée ex Dippel) at the on-floor and the on-tree stages in an old-growth temperate forest in Japan. The clonal structure of *W. floribunda* at the on-tree stages was as reported at the same study site by Mori et al. (2018). Specifically, the following questions were addressed. (a) Does clonal reproduction of lianas at the on-tree stage contribute similarly to the number of stems in three liana species (*E. fortunei*, *S. hydrangeoides* and *R. ambigua*) as in *W. floribunda*? (b) Do small clonal ramets at the on-floor stage belong to the same genets of the surrounding ramets at the on-tree stage? (c) Which clonal reproduction strategy (i.e., climb- or spread-prioritizing) is more common?

2 | MATRIALS AND METHODS

2.1 | Study site

This study was conducted in a 6-ha plot in Ogawa Forest Reserve, which is located in northern Ibaraki Prefecture, Japan (36°56′ N, 140°35′ E, elevation 610–660 m) (Nakashizuka & Matsumoto, 2002). The mean monthly temperature and mean annual precipitation are 10.7°C and 1910 mm, respectively (Mizoguchi, Morisawa, &

Ohtani, 2002). Ogawa Forest Reserve is an old-growth forest that is dominated, in order of basal area, by *Quercus serrata*, *Fagus japonica* and *F. crenata* (Masaki et al., 1992). The present study site underwent large-scale human disturbance in the 1930s (probably due to fire; Masaki et al., 1992; Sakai, Nomiya, & Suzuki, 2002), and these disturbances occurred both at the edge of and outside the plot (Tanaka & Nakashizuka, 1997). Inventories of trees (diameter at breast height [DBH] ≥ 5 cm) in this plot have been conducted once every 2 or 4 years since 1987 (Nakashizuka et al., 1992). The species name, DBH, host tree and location of all lianas higher than 1.3 m above the ground on trees of DBH ≥ 5 cm were measured in 2013 (Mori, Kamijo, & Masaki, 2016a).

2.2 | Study species

Euonymus fortunei (Celastraceae), *S. hydrangeoides* (Hydrangeaceae) and *R. ambigua* (Anacardiaceae) are root climbers (Iwatsuki, 1999; Noshiro, 1999; Ohba, 2001) and are members of genera that are commonly found in other temperate forests (Mori, Kamijo, & Masaki, 2016a). In contrast, *W. floribunda* (Fabaceae) is a stem twiner (Ohashi, 2001) and is the most dominant liana species in this temperate forest (Mori, Kamijo, & Masaki, 2016a). Extensive clonal reproduction via stolons has been reported for *W. floribunda* (Sakai et al., 2002). These four liana species constitute 93 and 97% of the total abundance and basal area, respectively, of the liana community in the study forest (Mori, Kamijo, & Masaki, 2016a). *Euonymus fortunei* is an evergreen species, whereas the other three are deciduous species. The four studied liana species are all native to the region of the study site (Iwatsuki, 1999; Noshiro, 1999; Ohashi, 2001; Ohba, 2001). Moreover, mature individuals (DBH > 5 cm) of these four species have been recorded in the study plot for over 30 years (unpublished data) and specifically in 2013 (Mori, Kamijo, & Masaki, 2016a). The maximum DBHs of the study species in the 6-ha plot in 2013 were 16.6, 18.1, 14.7 and 40.4 cm for *S. hydrangeoides*, *E. fortunei*, *R. ambigua* and *W. floribunda*, respectively (Mori, Kamijo, & Masaki, 2016a).

2.3 | Sampling, DNA extraction and genotyping

For lianas at the on-tree stage, fresh leaves were collected from all stems of the study species (*E. fortunei*, *S. hydrangeoides* and *R. ambigua*) that were more than 1.3 m above the ground in 2016 based on the liana inventory conducted in 2013. For lianas at the on-floor stage, leaf samples were collected in a 10 m × 120 m belt transect established in the center of the study plot to include various landforms (Figure 2; Figure S1). This is because clonal growth on the forest floor via stolons and rhizomes is often influenced by microtopography and landforms (Mori et al., 2018; Parks & Werth, 1993; Suyama, Obayashi, & Hayashi, 2000). Samples of *W. floribunda* and the root climber species (*E. fortunei*, *S. hydrangeoides* and *R. ambigua*) were collected using a different sampling method because of differences in the morphology of stems at the on-floor stage. There are two types of *W. floribunda* stems found on the forest floor: upright stems with pairs of leaves, and vegetative shoots (searcher shoot; Ichihashi, Nagashima, & Tateno, 2008) that are often thin and creep along the forest floor. Because the present study aimed to evaluate upright stems rather than searcher shoots at the on-floor stage, stems that were more than 15 cm above the ground were collected to avoid sampling searcher shoots. On the other hand, stems of *E. fortunei*, *S. hydrangeoides* and *R. ambigua* at the on-floor stage formed dense carpet-like stolons. To collect samples across the transect we divided it into 1 m × 1 m cells (total 1,200 cells) and randomly collected one stem from each cell. To confirm whether small stems at the on-floor stage in the belt transect had genetically identical ramets at the on-tree stage outside the study plot, we also surveyed liana stems on trees (DBH ≥ 5 cm) in a 30 m × 30 m quadrat established outside the study plot. Thus, we investigated liana individuals that climbed trees outside the study area in 2017 (Figure S2), and we found no liana stems on trees in the 30 m × 30 m quadrat.

All samples were stored at −30°C prior to DNA extraction using a DNeasy kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction was performed using microsatellite markers designed for each liana species; 10 and nine microsatellite markers were used for *E. fortunei* and *S. hydrangeoides*, respectively (Mori et al., 2017), nine microsatellite markers were used for *R. ambigua* (Hsu, Shih, Kuo, Chiang, & Chiang, 2013) and 10 microsatellite markers were used for *W. floribunda* (Mori et al., 2016b). Genotyping data were checked and binned using Geneious R9.0 (Kearse et al., 2012) and the R package "Fragman" (Covarrubias-Pazaran, Diaz-Garcia, Schlautman, Salazar, & Zalapa, 2016).

2.4 | Data analysis

Clones were identified based on the methods proposed by Arnaud-Haond et al. (2007) as follows. The ability of the microsatellite markers to distinguish multi-locus genotypes (MLGs) was tested by calculating the number of MLGs for all combinations of a given locus, and the results were verified based on the plateaus of the genotype accumulation curves (Figure S3). To ascertain whether stems of the same MLG belonged to the same

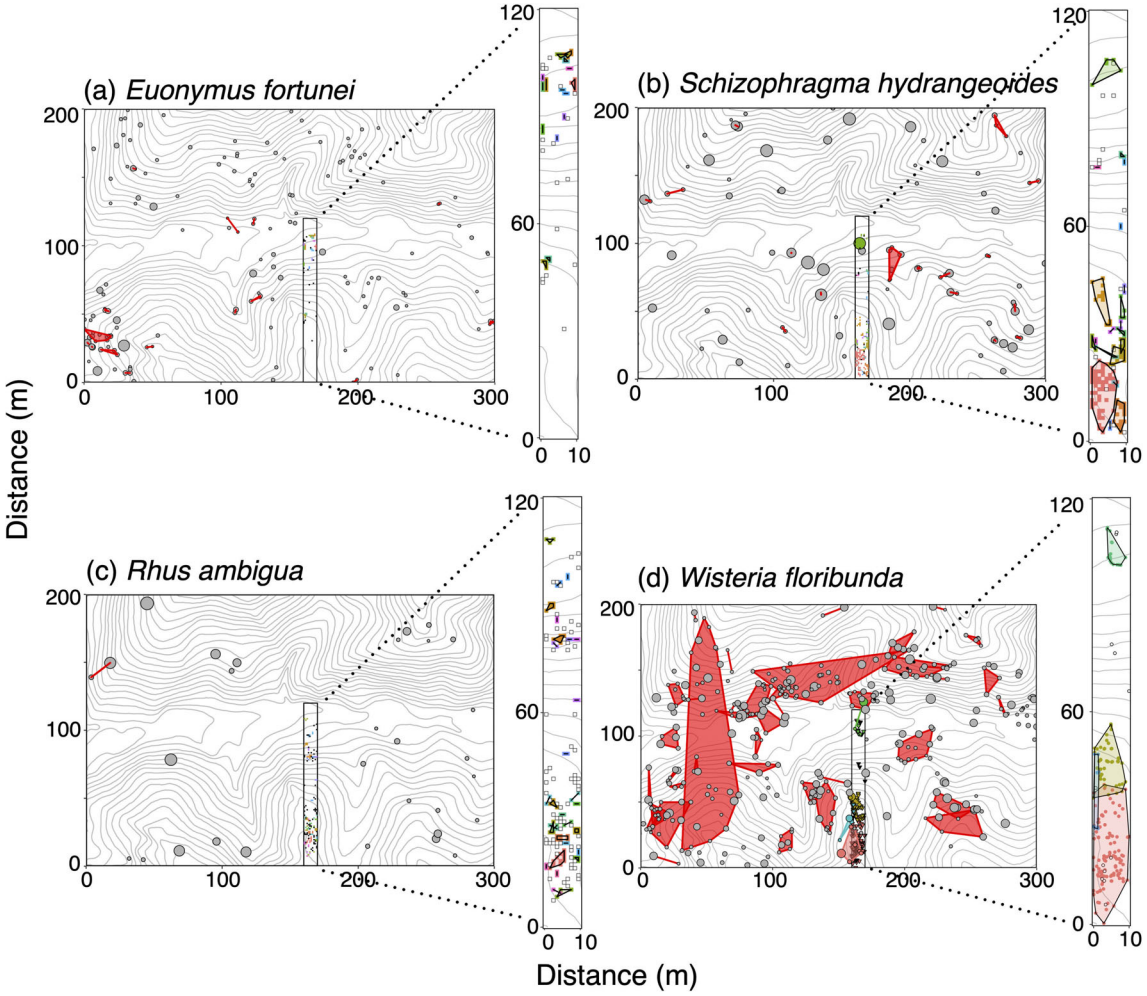


FIGURE 2 Distribution map of genets of liana species at the on-tree stage (in a 6-ha plot; left panel) and at the on-floor stage (in a belt transect; left and right panels). At the on-tree stage, gray circles represent sampled stems, with the size of the circles proportional to their diameter at breast height (DBH). The spatial distributions of multi-stem genets identified only at the on-tree stage are indicated by red polygons connecting each stem. The clonal structure of *W. floribunda* at the on-tree stage was obtained from Mori et al. (2018). At the on-floor stage, sampled individuals are represented by small squares, with empty squares representing single-stem genets and multi-stem genets indicated by colored polygons. Genets that occurred both at the on-tree stage and at the on-floor stage are shown in colors other than red. The interval between contour lines is 2 m

clone, the probability of a given MLG occurring in a population under Hardy–Weinberg equilibrium was calculated using the equation $P_{gen} = \sum_{i=1}^{l}(f_i)2^h$ (Parks & Werth, 1993), where f_i is the frequency of each allele at the i-th locus estimated with a round-robin method, and h is the number of heterozygous loci. Then, the probability of obtaining n repeated MLGs from a population more than once by chance in N samples (P_{sex}) was calculated using the equation $P_{sex} = \sum_{i=n}^{N} \frac{N!}{i!(N-i!)} \left[P_{gen}\right]^{i} \left[1-P_{gen}\right]^{N-i}$ (Parks & Werth, 1993). P_{gen} and P_{sex} were calculated for the study species, with the exception of P_{sex} of *R. ambigua* because this species had very few clones (see Results). To distinguish each distinct MLG that belonged to a distinct clone, multi-locus lineages (MLLs) were defined based on pairwise genetic distances. This procedure was necessary to prevent the false detection of clones due to slightly different MLGs resulting from somatic mutation or genotyping errors. The pairwise genetic distance threshold was determined as one by changing the threshold from zero to five following the recommendations of Meirmans and Van Tienderen (2004) (Tables S1 and S2; Text S1: Methods for Tables S1 and S2). MLLs are equivalent to genets in ecological studies, and we will use this term hereafter for MLLs. To evaluate the degree of aggregation versus intermingling (overlapping) among genets, the aggregation index was obtained using the equation $\left(P_{sg} - P_{sp}\right)/P_{sg}$, where P_{sg} is the average probability of clonal identity (being the same genet) of all stem pairs and P_{sp} is the average probability of clonal identity among pairwise

nearest neighbors. P_{sg} and P_{sp} for the given pair of stems are zero when they do not belong to the same clone. The aggregation index ranges from zero when the probability of the nearest neighbor does not differ from the overall average (all genets are intermingled) to one when all nearest neighbors share the same genet (all genets are aggregated or distinctly distributed). The statistical significance of the aggregation index was tested against the null hypothesis of spatially random distribution of the stems and the results of the significance test were obtained as *p* value. The aggregation index was not calculated for *R. ambigua* at the on-tree stage because there were very few clones of this species at this stage, leading to unreliable results (see Results). For calculation of P_{gen} and P_{sex}, R version 3.6.2 (R Core Team, 2019) was used with the R package "poppr" (Kamvar, Brooks, & Grünwald, 2015; Kamvar, Tabima, & Grünwald, 2014). Genets (i.e., MLLs) were determined with GenoDive version 2.0 (Meirmans & Van Tienderen, 2004). The aggregation index was calculated with the R package "RClone" (Bailleul, Stoeckel, Arnaud-Haond, & Poisot, 2016).

The clonal reproduction processes of the lianas were evaluated based on the results of genetic analysis. When most ramets of genets identified at the on-floor stage (in the belt transect) also had ramets at the on-tree stage (in the 6-ha plot), the ramets at the on-floor stage were assumed to have been produced from the (mature) ramets on the trees. This type of liana species was considered to exhibit the climb-prioritizing strategy. On the other hand, when most of the ramets of genets identified on the forest floor did not have ramets at the on-tree stage, the ramets at the on-floor stage were assumed to have been produced clonally from individuals derived from seed reproduction. This type of liana species was considered to exhibit the spread-prioritizing strategy.

3 | RESULTS

In total, leaf samples from 865 stems were collected in the present study (N = 275 for lianas at the on-tree stage, N = 590 for lianas at the on-floor stage), 98% of which (N = 852) were successfully genotyped (Table 1).

3.1 | Lianas at the on-tree stage

Distribution maps of genets of the three root climbers at the on-tree stage are shown in Figure 1. All samples of *E. fortunei* (N = 157), *S. hydrangeoides* (N = 88) and *R. ambigua* (N = 30) collected were successfully genotyped with the exception of *E. fortunei* (151 samples genotyped) (Table 2). The probability of the study species obtaining a given genotype ($p_{gen} < 0.001$) or obtaining repeated genotypes that originated from distinct sexual reproductive events by chance ($p_{sex} < 0.001$) were low; thus, it was assumed that errors in the identification of clones were unlikely. The number of genets of *E. fortunei*, *S. hydrangeoides* and *R. ambigua* at the on-tree stage was 127, 67 and 28, respectively (Table 2; Figure 2). Clonal reproduction contributed 16, 24 and 3% of the total number of stems of *E. fortunei*, *S. hydrangeoides* and *R. ambigua* at the on-tree stage, respectively. The aggregation index for *E. fortunei* and *S. hydrangeoides* was 0.17 ($p < 0.001$) and 0.32 ($p < 0.001$), respectively.

3.2 | Lianas at the on-floor stage

Distribution maps for genets of four liana species at the on-floor stage are also shown in Figure 1. Of all of the samples of *E. fortunei* (N = 69), *S. hydrangeoides* (N = 154), *R. ambigua* (N = 159) and *W. floribunda* (N = 208) collected, 65, 153, 159 and 207 samples, respectively, were successfully genotyped (Table 3). As in lianas at the on-tree stage, the probability of the four species at the on-floor stage obtaining a given genotype ($p_{gen} < 0.001$) or obtaining repeated genotypes that originated from distinct sexual reproductive events by chance ($p_{sex} < 0.001$) was low, and errors in the identification of clones were unlikely. The number of genets of *E. fortunei*, *S. hydrangeoides*, *R. ambigua* and *W. floribunda* was 34, 36, 100 and 18, respectively (Table 3;

TABLE 1 Liana species investigated in the present study and the number of stems collected from trees and from the forest floor. Climbing mechanism types are abbreviated as RC (root climber) and ST (stem twiner)

			Number of stems	
Family	**Species**	**Climbing type**	**On trees**	**On the forest floor**
Celastraceae	*Euonymus fortunei*	RC	157	69
Hydrangeaceae	*Schizophragma hydrangeoides*	RC	88	154
Anacardiaceae	*Rhus ambigua*	RC	30	159
Fabaceae	*Wisteria floribunda*	ST	391[a]	208

[a]Data from Mori et al. (2018).

TABLE 2 Summary of interspecific variation in clonality and clonal indices of four liana species on trees in a 6-ha plot. Some clonal indices were not calculated for *R. ambigua* as there were very few clones of this species on trees (see Methods)

	Euonymus fortunei	*Schizophragma hydrangeoides*	*Rhus ambigua*	*Wisteria floribunda*[a]
Number of stems	151	88	29	391
Number of genets (multiple-stem genets[b])	127 (16)	67 (16)	28 (1)	168 (53)
Maximum number of ramets within one genet	8	4	2	29
Percentage of clonal stems[c] (%)	26 (16)	42 (24)	7 (3)	71 (57)
Aggregation index[d] (A_c)	0.17***	0.32***	–	0.38***

[a]Reported in Mori et al. (2018).
[b]Number in parentheses represents the number of genets composed of two or more ramets.
[c]Ramets that constitute a multiple-stem genet. Number in parentheses represents the percentage of clonal stems when a ramet was excluded from each genet.
[d]Asterisks represent the level of significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

TABLE 3 Summary of interspecific variation in clonality and clonal indices of the four liana species on the forest floor in the belt transect

	Euonymus fortunei	*Schizophragma hydrangeoides*	*Rhus ambigua*	*Wisteria floribunda*
Number of stems	65	153	159	207
Number of genets (multiple-stem genets[a])	34 (15)	36 (21)	100 (31)	18 (4)
Maximum number of stems within one genet	5	50	11	119
Percentage of clonal stems[b] (%)	71 (48)	90 (76)	57 (37)	93 (91)
Percentage of multiple-stem genets with genetically identical ramets that climbed on trees (%)	0	5	0	100
Aggregation index[c] (A_c)	0.72***	0.76***	0.70***	0.27***

[a]Number in parentheses represents the number of genets composed of two or more ramets.
[b]Ramets that constitute multiple-stem genets. Number in parentheses represents the percentage of clonal ramets when a ramet was excluded from each genet.
[c]Asterisks represent the level of significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 2). Clonal reproduction contributed 48, 76, 37 and 91% of the total number of stems of *E. fortunei*, *S. hydrangeoides*, *R. ambigua* and *W. floribunda*, respectively. The maximum number of ramets within a genet was 5, 50, 11 and 119 for *E. fortunei*, *S. hydrangeoides*, *R. ambigua* and *W. floribunda*, respectively (Table 3). The aggregation index of *E. fortunei*, *S. hydrangeoides*, *R. ambigua* and *W. floribunda* was 0.72, 0.76, 0.70 and 0.27, respectively, and the significance level of these values was $p < 0.001$.

3.3 | Genets both at the on-tree and the on-floor stages

Genets (>1.3 m above the ground) observed both at the on-tree stage in a 6-ha plot and at the on-floor stage in a belt transect were found for all multiple-stem genets of *W. floribunda* (Figure 2; Table 3). In contrast, only one *S. hydrangeoides* genet and no *E. fortunei* or *R. ambigua* genets were found to have ramets belonging to the same genet both at the on-tree and the on-floor stages (Figure 2; Table 3).

4 | DISCUSSION

4.1 | Interspecific variation in clonal reproduction in lianas at the on-tree stage

There was considerable variation in clonality in lianas at the on-tree stage among the four liana species, and the contribution of clonal reproduction in *W. floribunda* was substantially larger than that in the root climbers; clonal reproduction contributed 57% (when a ramet was excluded from each genet) to the total abundance of *W. floribunda* (Mori et al., 2018), whereas the contribution of clonal reproduction in the root climbers ranged from 3 to 24%. This was also consistent with the maximum number of ramets within a genet (*W. floribunda*: 29; root climbers: 2–

8). These results indicate that stems of root climbers at the on-tree stage were mostly single-stem genets, which are unlikely to be derived from clonal reproduction, implying that root climbers maintain their populations mainly via seed reproduction rather than clonal reproduction. The relatively small contribution of clonal reproduction in root climbers could be due to their high shade tolerance (Valladares, Gianoli, & Saldana, 2011) allowing root climbers to reproduce via seeds under a closed canopy.

Differences in clonality between root climbers and stem twiners could also be due to the characteristics of their stolons. Root climbers are known to produce dense carpet-like stolons at the on-floor stage (e.g., Swearingen, Reshetiloff, Slattery, & Zwicker, 2010), whereas stolons of *W. floribunda* (a stem twiner) are known to have a web-like distribution (Sakai et al., 2002). This indicates that the density of ramets of root climbers tends to be higher, and that root climbers are less able to spread their stolons over large areas than stem twiners. Furthermore, although root climbers are not able to change hosts, switching (laddering) between host tree crowns is common in stem twiners (e.g., Putz, 1984). This is an important part of the clonal reproduction process because host switching enables stem twiners to expand their distribution horizontally via clonal reproduction not only in the understory through stolons but also in the forest canopy layer (Mori et al., 2018). As such, stem twiners may have a greater ability to produce more ramets that climb trees and expand their distribution of genets horizontally via clonal growth than root climbers, which may partly explain the differences in clonality found between lianas employing different climbing mechanisms.

In contrast to the above results, the aggregation index of lianas at the on-tree stage did not differ based on climbing mechanism, and the aggregation index of *E. fortunei* was smaller than that of *W. floribunda* and *S. hydrangeoides*. This indicates that the level of intermingling among genets of the study species is high in *E. fortunei*, probably due to the large number of single-stem genets distributed in the study plot.

4.2 | Interspecific variation in clonal reproduction in lianas at the on-floor stage

Among the study species, the contribution of clonal reproduction to stems at the on-floor stage was largest for *W. floribunda*. Only 18 genets were found among 207 *W. floribunda* stems, and four out of these 18 genets were multiple-stem genets. This indicates that *W. floribunda* genets produced a substantial number of ramets at the on-floor stage from a few genets, and suggests that clonal reproduction via stolons in *W. floribunda* at the on-floor stage greatly contributes to the expansion and persistence of ramets of this species in the understory. On the other hand, single-stem genets constituted only 7% in total number of stems (i.e., 14 out of 207). This indicates that the contribution of seed reproduction in *W. floribunda* to clonal reproduction is relatively small in terms of the number of stems at the on-floor stage. The large difference between climbing mechanisms was also confirmed by the aggregation indices; *W. floribunda* had a smaller aggregation index than the root climbers, indicating that *W. floribunda* genets at the on-floor stage are highly intermingled (overlapped) and the genets of root climbers are distinctly distributed.

It is important to note that clonality also varied greatly among the three root climbers, which indicates that the contribution of clonal reproduction also varies among species with the same climbing mechanism. Among the root climbers, the contribution of clonal reproduction was largest in *S. hydrangeoides* and relatively small in *E. fortunei* and *R. ambigua*. This indicates that a relatively large proportion of genets are established via seed reproduction in *E. fortunei* and *R. ambigua* compared to *S. hydrangeoides*, and that *E. fortunei* and *R. ambigua* might be able to establish from seeds and survive in the dark understory. On the other hand, it is possible that *S. hydrangeoides* may have a higher clonal proliferative ability in the understory than either *E. fortunei* or *R. ambigua*. Although this information on variation in clonality between climbing mechanisms is important for understanding the role of clonal reproduction in the establishment and growth of lianas at the species level, we currently do not have enough knowledge to explain the possible causes of these results. Further studies on liana species, focusing on not only clonal reproduction but also on other life history traits (e.g., seed reproduction), are necessary to evaluate the mechanisms underlying variation in clonality among species employing different climbing mechanisms.

Overall, the contribution of clonal reproduction was greater in stems at the on-floor stage than in stems at the on-tree stage, although it is important to note that direct comparison of clonality between lianas at the on-tree stage and lianas at the on-floor stage is difficult because the sampling design and area were different for the trees and the forest floor. One possible explanation for this result is that temperate lianas employ a "sit and wait" strategy that allows them to persist in the understory for long periods of time until the canopy opens (Greenberg, Smith, & Levey, 2001; Leicht & Silander, 2006; Ladwig & Meiners, 2015); this was first reported in the seedlings of a temperate liana *Celastrus orbiclatus* in North America, and is also reported in a study of current-year *W. floribunda* seedlings in the same study plot of the present study (Mori, Masaki, Tsunamoto, & Naoe, 2020). However, some studies on

temperate lianas have reported that the light environment has little or no significant effect on liana abundance (Gianoli, Saldana, & Jimenez-Castillo, 2012; Gianoli, Saldaña, Jiménez-Castillo, & Valladares, 2010). Further evaluation of clonality in multiple liana species affected by disturbances of different intensities is needed to develop a comprehensive understanding of the role of clonal reproduction in lianas at the on-floor stage.

4.3 | Implications of differences in clonal reproduction processes among lianas

The proportion of genets found both at the on-tree and the on-floor stages varied significantly among the liana species; all multiple-stem genets of *W. floribunda* were found both at the on-tree and the on-floor stages, whereas only one multiple-stem *S. hydrangeoides* genet and no multiple-stem *E. fortunei* or *R. ambigua* genets were found both at the on-tree and the on-floor stages. These results indicate that *W. floribunda* exhibits the climb-prioritizing strategy (Figure 1a), whereas the other three liana species exhibit the spread-prioritizing strategy (Figure 1b). Previous studies on several herbaceous clonal plant species have reported that nutrients are translocated from ramets in resource-rich locations to those in resource-poor locations (Alpert, 1996; Roiloa, Antelo, & Retuerto, 2014; Saitoh, Seiwa, & Nishiwaki, 2002, 2006). If nutrient sharing among ramets in patchy environments is common in clonal plant species, the climb-prioritizing strategy of *W. floribunda* may enable small ramets to survive in unfavorable environments (e.g., a dark understory), possibly via nutrient sharing by the mother ramet in the tree canopy, which may enable fast-growing and light-demanding stem twiners to employ the "sit and wait" strategy, also described above.

It is important to note that the spread-prioritizing strategy in root climbers could be related to the life-history strategy in understory shrubs (Abe et al., 2008; Kanno & Seiwa, 2004). These studies reported that small ramets of understory shrubs can persist under a closed canopy via vegetative growth until canopy gap formation. Although it is possible that root climbers exhibit the same life-history strategy with understory shrubs, root climbers are known to optimize light capture efficiency and thrive in closed canopy forests (Valladares et al., 2011). This indicates that the clonal growth strategies of root climbers and understory shrubs may differ in terms of light conditions, which necessitates further studies on clonal proliferation both in lianas and non-liana clonal plants across heterogeneous environments in forests.

5 | CONCLUSIONS

Using genetic tools, the clonal structures of four liana species at the on-tree stage and the on-floor stages in a temperate forest were evaluated in this study; a large amount of interspecific variation was found among these liana species. The findings imply that there are two different patterns of clonal reproduction in temperate lianas that are characterized by the presence or absence of genetically identical ramets shared between the on-tree and on-floor stages. These results indicate that the role of clonality in establishment and growth and the process of clonal reproduction in lianas may differ greatly depending on climbing mechanism and species, which further emphasizes the importance of understanding the life-history strategies (e.g., clonal reproduction, seed reproduction) of lianas in forests at the species level. Because this study was conducted in a single forest stand, further studies on clonality in lianas both at the on-tree and the on-floor stages in multiple forest stands using genetic tools are necessary to generalize the findings and to further investigate the hypotheses raised in the present study.

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