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Cryptic Japanese maple species exhibit different drought tolerance, suggesting reproductive isolation

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Abstract

Acer mono is thought to comprise seven varieties, but its classification and nomenclature are controversial. A recent molecular phylogenetic study suggested that *A. mono* varieties *glabrum* (G) and *mayrii* (M) do not interbreed, despite occurring syntopically (same location) and having a common genetic origin. However, if these are separate, reproductively isolated species, the basis for that isolation remains unclear. Here, we tested whether G and M can be considered separate species that differ in multiple ecological characteristics. We compared G and M in terms of distribution, habitats, drought tolerance of leaves, and flowering phenology of trees, and we verified their reproductive isolation (RI) in Japan. G showed higher drought and salt tolerance than M, which is consistent with the distribution of G in salt-stressed coastal areas with relatively low precipitation. In contrast, M occurs in snowy, mesic, and mountain habitats. Even in neighboring M and G trees, the onset of flowering in M is earlier than that of G. Similarly, flower drop in M is completed sooner than in G. Although the presence of post-zygotic reproductive barriers was not tested, the high calculated RI index probably contributes to RI. Two varieties of *A. mono* with a common genetic origin but different drought tolerance should be considered separate species. They likely shifted their climatic niches, involving local adaptation to different climatic conditions. This is roughly supported by an ENM-based niche analysis and significant differences in flowering time. These findings help to understand the physiological diversification of *A. mono*.

KEYWORDS

Acer mono var. *glabrum* and var. *mayrii*, drought and salt stress tolerance, ecological niche modeling (ENM), phenology of flowering and leafing, reproductive isolation

Shigeta Mori and Yoko Kurosawa should be considered joint first authors.

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1 | INTRODUCTION

The genus *Acer* is a major component of deciduous, broad-leaved forests in the northern hemisphere and comprises 129–156 diverse species adapted to a wide range of environments (Dawson & Ehleringer, 1993; Gelderen, 1994; Ogata, 1965, 1967; Schumann et al., 2019; Tanaka et al., 2008; Tissier et al., 2004). The study of species diversity in the genus *Acer*, with a focus on molecular phylogenetics, has been one of the most active research areas in recent forest resource management (Areces-Berazain et al., 2021; Gao et al., 2020; Grossman, 2021; Li et al., 2019; Liu et al., 2014; Qiu et al., 2011). However, among species of *Acer*, classification and nomenclature of forms of *Acer mono* Maxim. have been highly controversial (Liu et al., 2017; Ogata, 1965; Ohashi, 1993; van Rijckevorsel, 2008; Wijnands, 1990). One reason for this confusion is the high physiological and morphological diversity among varieties that have been shaped by global environmental changes (Alberto et al., 2013; Areces-Berazain et al., 2021; Baack et al., 2015; Ferrio et al., 2003; Kakishima et al., 2015; Nosil et al., 2005; Qian & Ricklefs, 2000). Disentangling this complex situation requires both molecular phylogenetic and ecophysiological investigations (Lowry et al., 2008; Mayr, 1947).

Acer mono is a representative species in East Asia that is distributed across Russia, China, North Korea, South Korea, and Japan. It is generally thought to comprise seven varieties or subspecies (Ogata, 1965; Ohashi, 1993). Among these, *A. mono* varieties *mayrii* (M) and var. *glabrum* (G) are the major forms in northern Japan. Ogata (1965) reported distinct geographical and morphological differences between varieties M and G and suggested that they may be distinct species. However, this possibility has not been seriously considered so far because they are believed to interbreed as intraspecific varieties with partial range overlap (Liu et al., 2017; Ogata, 1965). Furthermore, M and G are difficult to distinguish because smaller trees are less likely to flower and they exhibit plasticity in leaf morphology, especially in young trees (Kikuchi et al., 2009; Ogata, 1965). Therefore, the classification and nomenclature of *A. mono* has remained controversial, causing confusion in studies of diversity within this genus (Liu et al., 2017; Ogata, 1965; Ohashi, 1993; van Rijckevorsel, 2008; Wijnands, 1990). Notably, in a molecular phylogenetic study, Liu et al. (2017) showed that M and G do not interbreed, suggesting that they may be genetically differentiated, even in the same forest. For this reason, Liu et al. (2017) proposed that M and G have diverged as separate species over time, despite a common origin, because nuclear simple sequence repeat analysis showed clusters with 98% support that share a chloroplast DNA haplotype. Recently, Yahara et al. (2024) proposed that G and M are probably separate species based on genetic analysis using multiplexed inter-simple sequence repeat genotyping by sequencing (Suyama & Matsuki, 2015). However, the mechanism of reproductive isolation (RI) of G and M has not been confirmed, and if they are separate species, the ecological basis for this separation has not been identified.

Ogata (1965) reported that G is distributed near the coast of the Sea of Japan, where salt stress is expected, and in inland Japan, the Kuril Islands, and the Sakhalin Oblast of Russia, where rainfall is relatively low. In contrast, M is distributed mainly in snowy and humid mountain areas and is endemic to Japan (Ogata, 1965, 1967). Therefore, we hypothesized that G and M differ in drought and salt tolerance. However, the drought and salt tolerance of G and M have not yet been assessed in ecophysiological studies. In this study, we postulated that differences in the drought tolerance of G and M reflect differences in the environments from which they originated. We sought to clarify the ecophysiological reasons why these two varieties should be considered different species with RI. Ecophysiological characterization of G and M will facilitate resource management under a changing climate.

2 | MATERIALS AND METHODS

2.1 | Study species

We focused on *A. mono* varieties G and M, and compared their distributions, flower and leaf phenology, and drought tolerance. We examined the taxonomy and nomenclature for G and M, as well as two other varieties of *A. mono* distributed in southwest Japan, adjacent to the distributions of M and G (Table 1) (Liu et al., 2017; Ogata, 1965; Ohashi, 1993). Ohashi (1993) modified the taxonomy and nomenclature by recognizing *A. pictum* instead of *A. mono* and by designating subspecies instead of varieties. However, in previous studies of molecular phylogenetics (Guo et al., 2014; Kikuchi et al., 2009; Liu et al., 2017) and ecology (Seiwa, 1998, 1999; Zhang et al., 2019), *A. mono* and its varieties have been more frequently adopted than *A. pictum* and its subspecies (Liu et al., 2017). Additionally, van Rijckevorsel (2008) suggested that "Rejecting the name *A. pictum*, a long-persistent source of error and confusion, will simplify and stabilise botanical nomenclature." Therefore, we use *A. mono*, following previous studies (Liu et al., 2017; Ogata, 1965; van Rijckevorsel, 2008; Wijnands, 1990).

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TABLE 1 Compilation of varieties by Liu et al. (2017).

Abbreviations in this paper	Ogata (1965)	Ohashi (1993)	Japanese name
	Acer mono Maxim.	*Acer pictum* Thunb.	Itayakaede (sensu lato)
G	var. *glabrum* (Lév. et Vnt.) Hara f. *glabrum*	subsp. *mono* (Maxim.) H. Ohashi	Ezo-itaya
M	var. *mayrii* (Schwerin) Sugimoto	subsp. *mayrii* (Schwer.) H. Ohashi	Aka-itaya
MA	var. *marmoratum* (Nichols.) Hara f. *marmoratum*	subsp. *dissectum* (Wesm.) H. Ohashi f. *dissectum*	Itayakaede (Enkokaede)[a]
CO	var. *marmoratum* (Nichols.) Hara f. *connivens* (Nichols.) Rehder	subsp. *dissectum* (Wesm.) H. Ohashi f. *connivens* (G. Nicholson) H. Ohashi	Urage-Enkokaede

[a]Liu et al. (2017) and Ogata (1965) tentatively identified Enkokaede (MA) as Itayakaede. However, the name Itayakaede is still commonly used for all varieties of *Acer mono*, leading to confusion in identifying the different varieties in Japan.

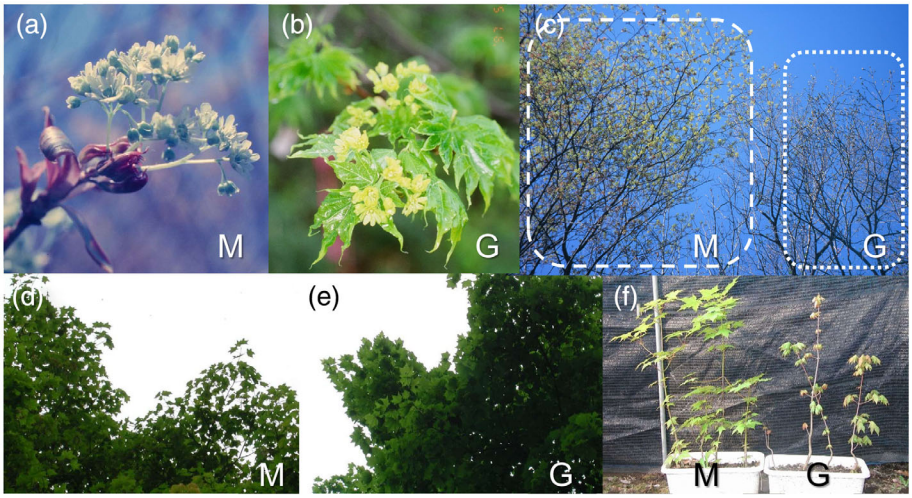

FIGURE 1 Images of *Acer mono* var. *mayrii* (M) and *Acer mono* var. *glabrum* (G). (a) Flowering of M precedes leafing. After reaching full bloom, leaves begin to open (Sapporo on May 6, 1997, Figure 5a). (b) Flowering and leafing of G proceed concurrently (Sapporo on May 16, 1997, Figure 5a). (c) Flowering of M was earlier than that of adjacent G (Sapporo on May 13, 1997, Figure 5a). (d) Relatively sparse canopy of M. (e) Relatively dense canopy of G. (f) In juvenile trees, M opened leaves earlier than G (Morioka on May 6, 2000).

2.2 | Distribution survey and niche differentiation analysis

We surveyed the distributions of G and M in northern Japan every year from 1995 to 2023. We distinguished M from G by observing flowers during the flowering season (Figure 1a–c). Identification of M and G outside the flowering stage was conducted by investigating the leaf morphology and canopy structure of adult trees (Figure 1d,e), since it is somewhat difficult to distinguish between G and M during the juvenile stage (Ogata, 1965, Figure 1f). We added confirmed distribution sites to the distribution map in the area spanning southern to northern Japan as reported by Ogata (1965) and created a new distribution map of M and G. Environments of these distributions in Japan and elsewhere in East Asia were examined based on mean annual precipitation and maximum snow depth from 1991 to 2020 (Japan Meteorological Agency). These distributional data were then used to test the hypothesis for niche differentiation between these varieties. First, PCA was performed to compare climatic variables at various locations using the R package ade4 (Dray & Dufour, 2007). Climatic variables included 19 bioclimatic data layers (BIO1-19) at 30-arcsec resolution, available from the WorldClim 1.4 database (Hijmans et al., 2005), as well as annual maximum snow depth averaged from 1991 to 2020, provided by the National Spatial Planning and Regional Policy Bureau (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-G02.html) of the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) of Japan. All climatic data were projected to the same projection system, resampled to the same cell size (30 s), and covered the same geographical range (therefore only including the Japanese Archipelago), using QGIS software version 3.24.3 (QGIS.org, 2022).

Moreover, the niche differentiation hypothesis was also tested using an ecological niche modeling (ENM) approach: We built ENMs for each variety using maximum entropy modeling implemented in MAXENT v3.3.3 (Phillips et al., 2006). We then performed niche

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identity and background similarity tests with ENMTools v.1.4 (Warren et al., 2010). In the niche identity test, we compared Schoener's *D* index of niche overlap between the varieties with simulated values generated by 100 replicates under the null hypothesis that the niches of the varieties are identical. To determine whether niche differences arise from niche divergence or simply from different background availability, we also performed a background test with 100 pseudoreplicates in reciprocal directions, comparing the niche of var. *glabrum* to the background of var. *mayrii*, and vice versa. We used the minimum training presence threshold calculated by MAXENT to define the background areas of each variety.

2.3 | Phenology of flowering, leafing, and RI

Flowering and leafing phenology were investigated in secondary, deciduous, broad-leaved forests in Sapporo, Hokkaido (42.99°N, 141.39°E), and Morioka, Iwate (39.75°N, 141.14°E), Japan. Mean annual precipitation, mean annual temperature, and mean maximum snow depth for 1991–2020 were 1146.1 mm, 9.2°C, and 97 cm in Sapporo, and 1279.9 mm, 10.6°C, and 36 cm in Morioka (ClimatView, Japan Meteorological Agency). At both the Sapporo and Morioka study sites, G and M trees were intermixed. For phenological observations, we selected 10 healthy trees each of both M and G that were at the reproductive stage. Flowering periods were synchronized separately in M and G, even within a few kilometers of the target trees, which should be sufficient to observe differences in phenology despite the small number of trees. Heights of selected trees were approximately the same, ranging from ~10 to 15 m. Ten shoots were selected from each tree in the outer part of the canopy that was exposed to sufficient light, and the numbers of flowers and open leaves per shoot were counted from the beginning to the completion of flowering and leafing. The number of fallen flowers was also counted on the same shoots. This observation was conducted every day in Sapporo from April 24 to June 1, 1997, and in Morioka from April 24 to May 17, 2000. Varieties of *A. mono* have a form of sex expression, or heterodichogamy, in which protandrous (male first) and protogynous (female first) individuals coexist, with a system that avoids selfing and promotes disassortative mating (Gao et al., 2015; Kikuchi et al., 2009; Shang et al., 2012; Shibata et al., 2009). However, we investigated phenology without distinguishing between the two kinds of individuals because the distinction between male and female periods was unclear (Kikuchi et al., 2009).

RI between the varieties can be evaluated as the ratio of conspecific mating frequency to total mating (Martin & Willis, 2007). With the flowering intensity at each time denoted as *Ft*, the relative reproductive contribution of flowers at time *t* to the total mating can be described as:

$$Ft/\sum_t Ft.$$

We assigned the rate of flowering shoots within the population (Table S1) to *Ft*, as we evaluated flowering phenology at the population level based on the same number of trees from each variety, without considering individual-level phenological variation. Moreover, we simply assumed that flowers of this species behave as if they were hermaphroditic, and that each flowering tree in full bloom makes an equal contribution to mating. Here, we assumed that conspecific and heterospecific mating opportunities within a population of each variety at a given period *t* depend only on the relative abundance of conspecific flowers (equal to *Ft*) and hetero-specific flowers (described as *Ht*) in the population at that time. Then, the RI for each variety can be calculated as:

$$\mathrm{RI} = \sum_t Ft\left(\frac{Ft}{Ft+Ht}\right)/\sum_t Ft.$$

The value of RI defined here approaches 1 if a variety represents exclusively conspecific mating (i.e., isolation is complete), and to 0 if only heterospecific mating occurs.

2.4 | Drought tolerance evaluated by pressure–volume (P–V) curves

To compare drought tolerance of G and M leaves, leaf P–V curves (Tyree & Hammel, 1972) were generated. We selected four adjacent G and M trees in Sapporo (Figure 1c) and collected their shoots every day from June 22 to 25, 1998. Shoots for these measurements were collected from the surface of the canopy in full sunlight. Collected shoots were enclosed in plastic bags and immediately brought to the laboratory near the study site, cut under water, and rehydrated for 2 h. After reaching full turgor, leaves were dehydrated using a pressure chamber Model 3000 (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). During dehydration, we repeatedly measured fresh mass and leaf water potential. Leaves were then oven-dried at 70°C for 3 days, and their dry mass was measured. From fresh and dry masses, relative water content RWC and free water content FWC (relative

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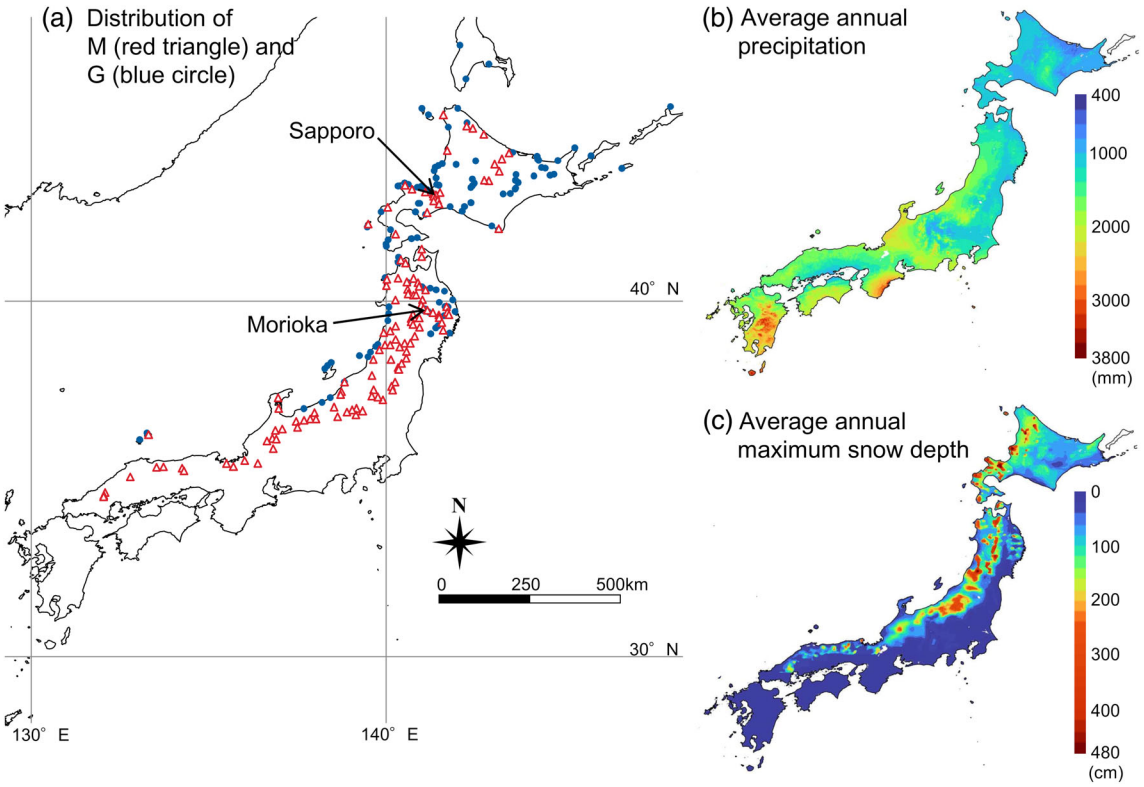

FIGURE 2 (a) Distribution of M (red open triangles, *Acer mono* var. *mayrii*), and G (blue filled circles, *Acer mono* var. *glabrum*), (b) Average annual precipitation data from 1960 to 1990 (BIO12) obtained from WorldClim 1.4. (c) Annual maximum snow depth averaged from 1991 to 2020 from the National Spatial Planning and Regional Policy Bureau (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-G02.html) of the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) of Japan. This figure (a) was prepared by adding observed distribution sites to the figure by Ogata (1965).

values of symplastic water content) at each leaf water potential were calculated. From P–V curves, we evaluated the osmotic potential at full turgor (φ_{osat}, MPa), the water potential at the leaf turgor loss point (φ_{tlp}, MPa), the relative water content at turgor loss (RWC_{tlp}), the maximum bulk module of elasticity (ε_{max}, max. slope of turgor potential to FWC; MPa), and leaf water content per unit leaf area V_t/LA (mL/m^2) at full turgor. We also calculated dry matter content (DMC, leaf dry mass/fresh mass) and specific leaf area (SLA, cm^2/g).

3 | RESULTS

3.1 | Distribution area and niche differentiation

The habitat of variety G includes windy slopes facing the Sea of Japan, where plants are likely to be exposed to salt stress (Ogata, 1965, Figure 2a). Variety G also tends to occur in inland areas in Northern Japan, characterized by relatively low precipitation (Figure 2a,b). Furthermore, based on specimens preserved in Japanese museums, variety G has been confirmed elsewhere in eastern Asia, in the Sakhalin Oblast and the Kuril Islands of Russia, where annual precipitation is relatively low (883.7 mm, Yuzhno-Sakhalinsk, Russia) (Ogata, 1965). Its distribution has also been confirmed on small islands in the Sea of Japan (Ogata, 1965, Figure 2a).

Variety M, an endemic Japanese variety, is not distributed on windy slopes facing the Sea of Japan where G grows, but occurs mainly inland in snowy and humid montane regions, with annual precipitation over 2000 mm (Figure 2a–c) (Ogata, 1965, 1967). It does not occur in the Sakhalin Oblast or the Kuril Islands (Figure 2a). In areas with intermediate annual precipitation, such as in Morioka City, Iwate Prefecture (annual precipitation, 1279.9 mm) and Sapporo City, Hokkaido Prefecture (1146.1 mm), we observed that varieties G and M coexist (Figure 2a,b).

PCA was conducted for bioclimatic data extracted from 125 and 103 points in M and G varieties, respectively. The first (PCA1) and second components (PCA2) accounted for 47.9% and 18.0% of the total variation, respectively. These varieties have overlapping but differentiated climate clouds, with a between-group inertia

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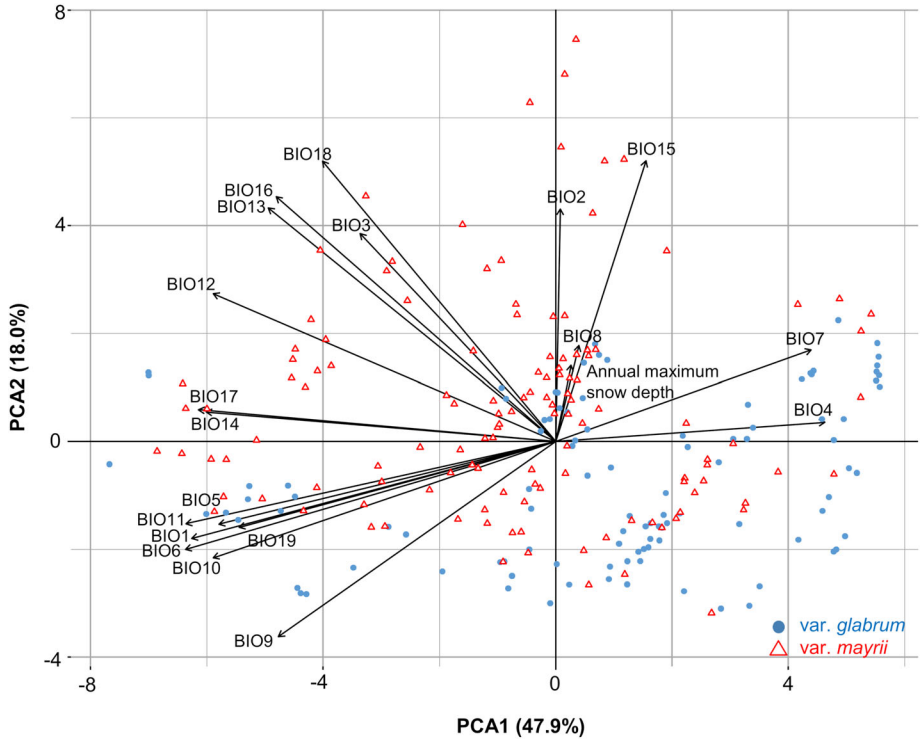

FIGURE 3 Principal component analysis based on 20 climatic variables at locations of M (red open triangles, *Acer mono* var. *mayrii*) and G (blue filled circles, *Acer mono* var. *glabrum*). BIO1, annual mean temperature; BIO2, mean diurnal range (mean of monthly (max temp − min temp)); BIO3, isothermality (BIO2/BIO7) (×100); BIO4, temperature seasonality (standard deviation × 100); BIO5, max temperature of warmest month; BIO6, min temperature of coldest month; BIO7, temperature annual range (BIO5 − BIO6); BIO8, mean temperature of wettest quarter; BIO9, mean temperature of driest quarter; BIO10, mean temperature of warmest quarter; BIO11, mean temperature of coldest quarter; BIO12, annual precipitation; BIO13, precipitation of wettest month; BIO14, precipitation of driest month; BIO15, precipitation seasonality (coefficient of variation); BIO16, precipitation of wettest quarter; BIO17, precipitation of driest quarter; BIO18, precipitation of warmest quarter; BIO19, precipitation of coldest quarter; annual maximum snow depth = average value from 1991 to 2020 from the National Spatial Planning and Regional Policy Bureau (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-G02.html) of the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) of Japan.

value of 0.069 ($p = 0.001$; Figure 3). Niche differentiation was related to bioclimatic factors BIO 2, 3, 8, 12, 13, 15, 16, 18, and annual maximum snow depth, indicating that higher precipitation in summer and heavier snowfall in winter are favorable to variety M. ENMs were constructed for each variety, which received high AUC scores (>0.99), indicating good predictive model performance (Figure 4). The most important contributing factor for both varieties (65.2% for M and 32.8% for G), was the annual maximum snow depth, although the response curve generated by MAXENT revealed that M prefers deep-snow conditions, whereas G avoids them (Figure S1). The second important factor was precipitation of the driest month for M (16.7%) and precipitation of the warmest quarter for G (20.6%). Schoener's *D* (Schoener, 1968) between the two varieties was 0.481, which was significantly lower ($p < 0.05$) than expected from the null hypotheses ($D = 0.838 \pm$ SE 0.973) of niche identity, and also lower than the null distribution in background similarity tests (G vs. the background area of M:D = 0.532 ± SE 0.00203; M vs. the background area of G:D = 0.607 ± SE 0.00253).

3.2 | Phenology of flowering, leafing, and RI index

At study sites in both Sapporo and Morioka, flowering and leafing occurred simultaneously for G (Figures 1b and 5a,b, Table S1), whereas flowering precedes leafing for M (Figures 1a and 5a,b). The onset of flowering of M preceded that of G by 13 days in Sapporo and by 7 days in Morioka (Figure 5a,b). Similarly, the falling of flowers for M was completed 12 days earlier than for G in Sapporo, and 6 days earlier in Morioka. The duration of blossoming (100% of flowering rate) for M was 9 days in Sapporo and 5 days in Morioka, and for G, it was 8 days in Sapporo and 3 days in Morioka. Blooming of G commenced 4 days later than that of M in Sapporo, and by 2 days in Morioka. Thus, the flowering peaks for M and

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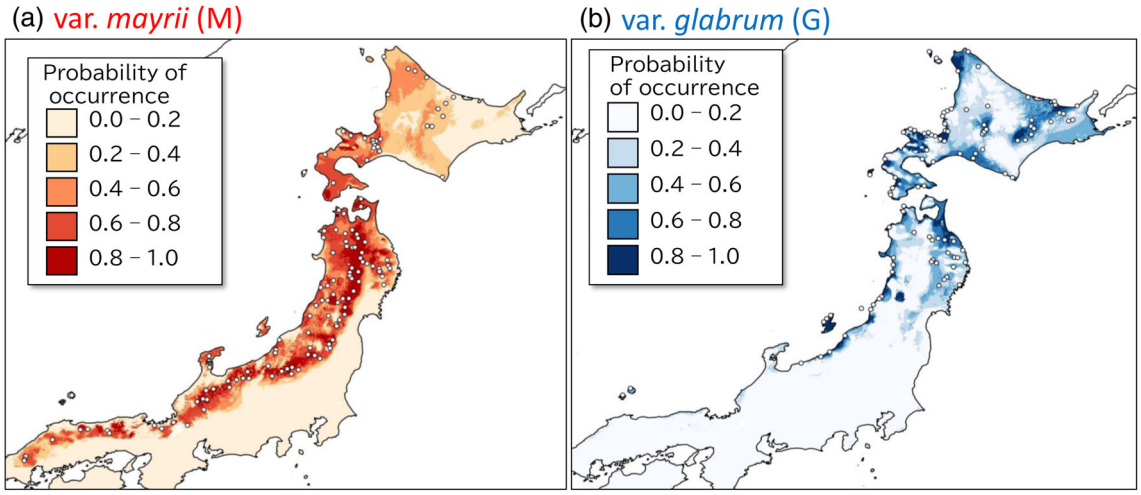

FIGURE 4 Ecological niche models (ENMs) showing potential distributions of the two varieties of *Acer mono*, var. *mayrii* (a) and var. *glabrum* (b). Open circles indicate the locations of samples where climatic data were used for ecological niche modeling.

G have limited overlap, suggesting that M and G are reproductively isolated. Calculated values of RI for M (conspecific mating in M) were 0.86 and 0.83 in Sapporo and in Morioka, respectively, whereas values of RI for G were 0.84 and 0.75 in Sapporo and in Morioka, respectively.

3.3 | Leaf P–V characteristics

Leaf physiological characteristics derived from P–V curves are related to drought and salt tolerance (Hsiao et al., 1976). We examined differences in physiological characteristics between varieties G ($n = 4$) and M ($n = 4$) using Student's t test (Table 2, Table S2). There were no significant differences between G and M in leaf thickness (LT) (Figure 6a) or SLA (Figure 6b). M showed significantly lower DMC (Figure 6c) and higher V_t/LA (Figure 6d) than G. However, despite the higher moisture content of M, there was no significant difference between the two varieties in osmotic potential at full turgor (φ_{osat}) (Figure 6e). Compared to M, in leaf wilting, G had significantly lower leaf water potential (φ_{tlp}) and lower relative water content at the turgor loss point (RWC_{tlp}) (Figure 6f,g). The maximum bulk module of elasticity (ε_{max}) of M tended to be higher (i.e., less elastic cell walls) than that of G, although the difference was only marginally significant (Figure 6h). PCA was conducted for these physiological characteristics in M and G. The first (PCA1) and second components (PCA2) accounted for 57.7% and 16.5% of the total variation, respectively (Figure 7). Thus, physiological differentiation was related to DMC, V_t/LA, φ_{tlp}, RWC_{tlp}, and ε_{max}, indicating that G was more drought tolerant than M.

4 | DISCUSSION

We tested the hypothesis that M and G with a probable common origin (Liu et al., 2017), have developed divergent ecophysiological and flowering habits under different environmental conditions. Accumulation of such ecological barriers may lead to ecological speciation, resulting in isolated species that are unable to exchange genes, even when syntopic (co-occurring). However, further studies, including artificial crosses, reciprocal transplants, and population genetic analyses, will be needed to determine whether complete isolation exists in these syntopic species (Baack et al., 2015; Colautti & Lau, 2015). Furthermore, RI may increase if differences in adaptation to contrasting environments reduce the likelihood of survival or fitness of hybrids (Nosil et al., 2005). Therefore, differences in drought tolerance should also be considered part of the reproductive barrier (Sakaguchi et al., 2013).

Lower leaf wilting points (significantly lower φ_{tlp}) and elastic cell walls (marginally lower ε_{max}) in G leaves compared to M leaves (Figure 6f,h) indicate that G leaves are more drought and salinity tolerant than M leaves. Despite the higher water content of M leaves than of G (Figure 6d), even from adjacent trees, there was no significant difference in osmotic potential at full turgor (φ_{osat}, Figure 6e). These facts indicate that M leaves have a lower water potential due to a higher proportion of apoplastic than symplastic water than G leaves. M leaves increase leaf osmoles by decreasing apoplastic water, rather than by osmotic adjustment (Bartlett et al., 2012; Harayama et al., 2006; Ishida et al., 1992). The higher apoplastic water content of M may require relatively greater water supply to leaves than in G (Plett

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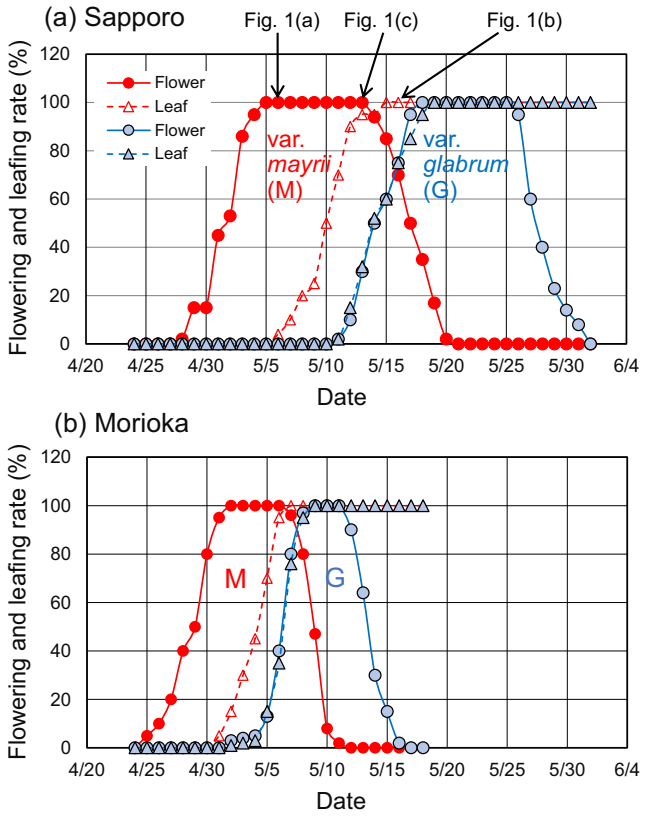

FIGURE 5 Flowering and leafing of M (red symbols, *Acer mono* var. *mayrii*) and G (blue symbols, *Acer mono* var. *glabrum*) in Sapporo (a) and Morioka (b). In panel (a), three arrows indicate the dates when photos in Figure 1a–c were taken.

et al., 2020; Scharwies & Dinneny, 2019). Additionally, cell walls of M leaves tend to be less elastic, indicating that even a slight decrease in leaf water content can easily lower the leaf water potential and increase leaf water absorption (Ishida et al., 1992; Lo Gullo & Salleo, 1988). Under water stress, the more elastic cell walls of G would experience a greater water loss (confirmed by the lower RWC_{tlp}). This, together with a lower proportion of apoplastic water, suggests more conservative water use in G, achieving lower water potentials without turgor loss, which in turn induces stomatal closure (Novick et al., 2016). The ability to maintain turgor pressure despite changes in cell volume has been described as an adaptive response to moderate water stress (Corcuera et al., 2002; Dawson & Bliss, 1989). The results of ENM in this study indicated these leaf characteristics are associated with climatic conditions where they grow. M is well adapted to snowy and humid (mesic) habitats, whereas G occurs in drier conditions. Ecophysiologically detailed comparisons of cryptic G and M will be the subject of a future study, considering the coordinated response of stem and leaves to drought/salinity stress

TABLE 2 Statistics (t), degrees of freedom (df), and p values (p) comparing drought tolerance of leaves of M and G in Figure 6 using Student's t test.

	t	df	p
LT (mm)	0.32	6	0.759
SLA (cm^2/g)	1.09	6	0.316
DMC	−3.29	6	0.017
V_t/LA (mL/m^2)	2.90	6	0.027
φ_{osat} (MPa)	1.85	6	0.114
φ_{tlp} (MPa)	3.91	6	0.008
RWC_{tlp}	3.44	6	0.014
ε_{max} (MPa)	2.41	6	0.052

Note: LT (mm), leaf thickness; SLA (cm^2/g), specific leaf area; DMC (leaf dry mass/fresh mass), dry matter content; V_t/LA (mL/m^2), water content per leaf area at full turgor; φ_{osat} (MPa), osmotic potential at full turgor; φ_{tlp} (MPa), water potential at turgor loss point; RWC_{tlp}, relative water content at turgor loss point; ε_{max} (MPa), the maximum bulk module of elasticity.

(Alonso-Forn et al., 2021), as well as individual responses (Kurosawa et al., 2021, 2023; Mori et al., 2010).

Given G's distribution and higher drought and salt tolerance (Bartels & Sunkar, 2007), it is probable that G expanded its distribution from environments with relatively lower precipitation and/or higher salt stress (Figures 2a–c and 4). Niche differentiation between the varieties shown in this study supports the habitat preference for precipitation and moisture from melting snow (Figure 3). Although the presence of post-zygotic reproductive barriers was not specifically tested in this study, the high RI index suggests that the time lag in flowering between G and M likely contributes to their RI (Baack et al., 2015). They are clearly different, even in leafing phenology during flowering (Figure 5a,b), highlighting the need to treat them as separate species. This difference in leaf phenology was also observed in potted juvenile trees grown in a common garden from seeds collected in the forest (Figure 1f), and was therefore assumed to be a genetic trait. The factors behind the differences in flowering and leaf phenology in M and G are not clear at present but need to be investigated in the future, including the effects of global warming (Buonaiuto & Wolkovich, 2021; Ettinger et al., 2018). Considering the phenological characteristics and lower drought tolerance in M, it may have expanded its distribution from southeastern Japan via snowy, humid mountains to northern Hokkaido. However, the ecophysiological characteristics of M may have prevented it from expanding to the Sakhalin Oblast and the Kuril Islands, where drought and salt stress are expected (Figure 2a). M and G have differentiated in drought tolerance and flowering phenology, and they have ultimately become syntopic, but they remain

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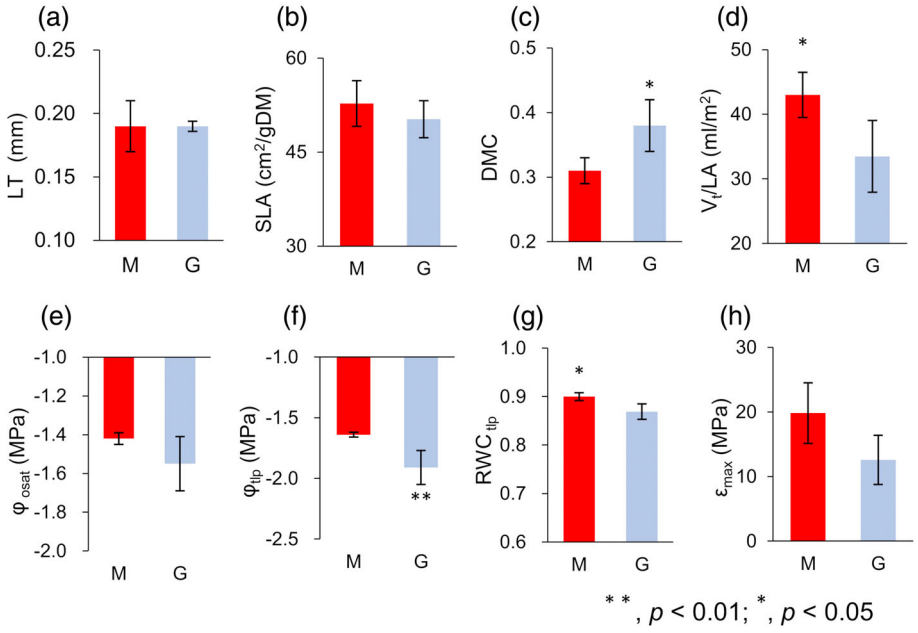

FIGURE 6 Leaf characteristics of M (*Acer mono* var. *mayrii*) and G (*Acer mono* var. *glabrum*) as indicators of drought and salt tolerance. (a) LT (mm), leaf thickness; (b) SLA (cm^2/g), specific leaf area; (c) DMC (leaf dry mass/fresh mass), dry matter content; (d) V_t/LA (mL/m^2), water content per leaf area at full turgor; (e) φ_{osat} (MPa), osmotic potential at full turgor; (f) φ_{tlp} (MPa), water potential at turgor loss point; (g) RWC_{tlp}, relative water content at turgor loss point; and (h) ε_{max} (MPa), the maximum bulk module of elasticity.

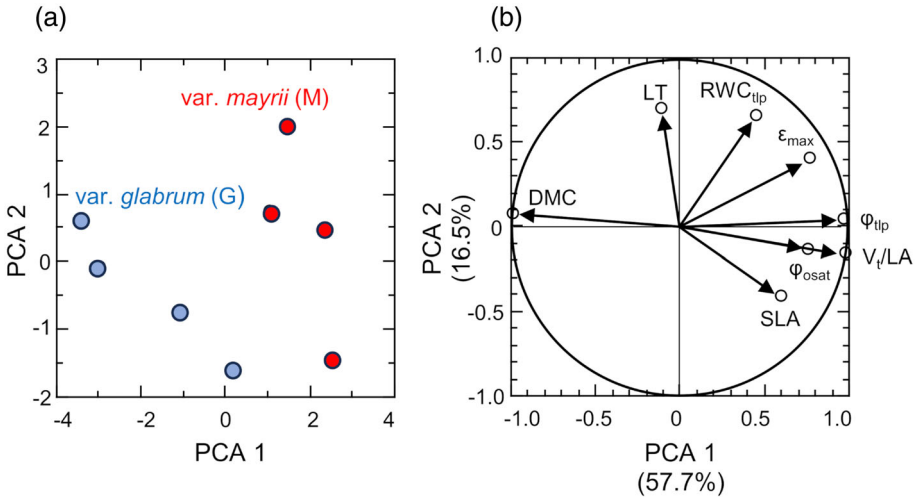

FIGURE 7 (a) Biplot of principal component analysis based on eight leaf characteristics of M (red circles, *Acer mono* var. *mayrii*) and G (blue circles, *Acer mono* var. *glabrum*). (b) Loading plot of eight leaf characteristics. Abbreviations of eight leaf characteristics are given in Figure 6.

reproductively isolated species. Our study suggests that M and G should be recognized as legitimate species, as proposed by Yahara et al. (2024), thereby explaining some of the physiological diversity in *A. mono*. This speciation process may be unique to the Japanese archipelago, locally associated with heavy snowfall, which is composed of highly diverse plant species from continental North and South Asia. This will need to be confirmed with higher-resolution genetic markers in subsequent studies (Kikuchi & Shibata, 2008; Ye et al., 2015).

Acer mono var. *marmoratum* (Nichols.) Hara f. *connivens* (Nichols.) Rehder (CO) and *Acer mono* var. *marmoratum* (Nichols.) Hara f. *marmoratum* (MA) (Table 1) are distributed in southwest Japan, adjacent to the distributions of M and G (Liu et al., 2017; Ogata, 1965; Qiu et al., 2011). In the future, the genetic phylogenetic relationship among M, G, MO, and CO will allow us to investigate the origin of M and G to clarify unresolved issues of *A. mono* varieties in Japan. Additionally, Ye et al. (2015) stated, "We can still conclude that Japanese [*Acer mono*] populations are genetically closer to populations in the subtropical region than the temperate region." Considering results of recent molecular phylogenetic studies (Liu et al., 2017; Newton et al., 1999; Qiu et al., 2011; Ye et al., 2015), we suggest that M expanded its distribution from southwestern Japan northward into northern Hokkaido, but not into the Sakhalin Oblast and the Kuril Islands. Among varieties of *A. mono*, G is the only one distributed widely in the Amur River basin, Sakhalin Oblast, and the Kuril Islands

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(Ogata, 1965). Although not fully demonstrated phylogeographically, distributions of G and M appear to support this hypothesis (Figure 2a).

Ogata (1965) also mentioned the possibility that the seven varieties of *A. mono* are separate species because of different distributions. Furthermore, considering various recent genetic analyses (Guo et al., 2014; Liu et al., 2014, 2017; Wang et al., 2020; Yahara et al., 2024; Ye et al., 2015), *A. mono* may be a complex containing several clades or species with different physiological characteristics. Future collaborative analyses of molecular phylogenetics covering *A. mono* in Russia, North Korea, South Korea, China, and Japan (Liu et al., 2014; Pfosser et al., 2002) need to be combined with an ecophysiological perspective to inform forest management under a changing climate.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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