[Dear Professor Bohumil Mandak,

Associate Editor of *Flora*,

Revised parts based on the reviewer #1 are shaded in blue and the reviewer #2 in green]

Polyploidy in *Lilium lancifolium*: evidence of autotriploidy and no niche divergence between diploid and triploid cytotypes in their native ranges

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Highlights

► • Allozymes clearly indicate an autopolyploid origin for the triploid cytotype of *Lilium lancifolium*. ► • Ecological niche modeling suggests no niche divergence between diploid and triploids.

- The triploid cytotype of *L. lancifolium* has a broader niche breadth.
- Ecological differentiation is not a pre-requisite for new polyploid lineage establishment.

Abstract

Lilium lancifolium, the tiger lily, constitutes a polyploid complex with both diploids (reproduced by seeds and bulbils) and triploids (propagated exclusively via bulbils). An autopolyploid origin for the triploid forms has been previously suggested based on classical cytogenetics, chromosome mapping techniques, ecological data, and geographic distribution in its native range (Korea and the Japanese Tsushima Island). Using 13 allozyme loci, we comparatively assessed clonal structure and levels of genetic diversity in four diploid and 11 triploid populations in South Korea to test the autopolyploid origin of the triploid cytotype and to infer which seedling recruitment strategy is operating within the diploid populations. We also employed ecological niche modeling and multivariate analysis to determine whether triploids of *L. lancifolium* occupy different and broader niches to those of diploids in Korea and Tsushima Island. The diploids harbored higher levels of within-population genetic diversity than triploids, and allele profiles found in triploids were exactly subsets of those in diploids. Repeated seedling recruitment was inferred for the diploids, whereas all the studied triploid populations are monoclonal since there is no seedling (sexual) recruitment. Although we found no niche divergence between cytotypes

of *L. lancifolium*, the triploids have a broader niche breadth. Genetic data further confirm the autotriploid origin of *L. lancifolium*, and the lack of a clear, strong evidence for niche divergence between cytotypes of *L. lancifolium* supports the view that ecological differentiation is not a pre-requisite for the establishment of new polyploid lineages.

Keywords:

Clonal diversity

Conservation

Diploids

Ecological niche modeling (ENM)

Genetic diversity

Lilium lancifolium

MaxEnt

Origin

Triploids

Introduction

The genus *Lilium* consists of over 100 species that are widely distributed throughout boreal and temperate regions of the Northern Hemisphere, especially in East Asia (Liang and Tamura, 2000). All the species within the genus are diploid with the only exception of the tiger lily *Lilium lancifolium* Thunb. (= *L. tigrinum* Ker Gawl.). This taxon constitutes a polyploid complex including both diploid ($2n = 2x = 24$) and triploid ($2n = 3x = 36$) cytotypes (Noda, 1978, 1986; Kim et al., 2006a). The diploid cytotype, locally common, can be found in western and southern coastal areas of the Korean Peninsula (including their

small islands and islets), in Jeju Island, and in the Japanese islands of Tsushima and Iki (Fig. 1). Compared to the relatively limited distribution of diploid *L. lancifolium*, triploid forms have a much broader distribution: they occur in the inland areas of the Korean Peninsula (and very rarely in coastal areas) and in Jeju and Tsushima islands, but also in large portions of China, in the main Japanese islands (Hokkaido, Honshu, Kyushu, and Shikoku), and in the south-eastern tip of Russian Far East. In China, in Japan (except Tsushima and adjacent islets) and in Russia, however, the triploid cytotype is likely an archaeophyte (i.e., those non-native plants introduced in the wild before 1500 AD), given its complete sterility (see below) and the fact that it has been widely cultivated (in China and in Japan) during the last one or perhaps two millennia (Stout, 1926; Maekawa, 1943; Everett, 1981; but see Haw, 1986) for its edible bulbs and medicinal uses (Bailey et al., 1976; Noda, 1986; Liang and Tamura, 2000). In contrast, *L. lancifolium* has been rarely cultivated in Korea and never in Tsushima Island (Noda, 1986; J. M. Chung, Korea Forest Service, pers. comm.), where it should be regarded as a native plant. In Europe and North America, the triploids have been widely planted as ornamentals since the 19th century (i.e., they should be regarded as neophytes) and are common garden escapes, with several areas where the herb has naturalized (Skinner, 2002; NOBANIS, 2014).

The diploid cytotype of *L. lancifolium* is considered to be self-incompatible because artificial self-pollination results on the production of incomplete capsules at very low frequencies; artificial cross-pollination experiments, in contrast, produce viable capsules (Noda, 1986). The triploids are completely sterile because flowers do not produce capsules (Noda, 1986); in addition, pollen fertility of triploids is also low (Niikezi, 1961; Kim et al., 2006b; Liu and Jia, 2013). Both diploid and triploid forms of *L. lancifolium* produce large numbers of bulbils on the leaf axils along the stem. In diploids, bulbils are primarily

formed at the low-middle part of the stem (from late May to mid June), although secondary formation of bulbils occurs on the upper part of the stem and in the inflorescences (in late June). In triploids, in addition to this mode of bulbil formation, bulbils can be formed along the whole stem in early July but develop more slowly. Detached bulbils from mother plants bloom in their second or third year (Noda, 1986; M. Y Chung and M. G. Chung, pers. obs.). Therefore, the diploid forms are capable of reproducing by sexual (via seeds) and by asexual means (via bulbils), whereas completely sterile triploids propagate exclusively by asexual means (Noda, 1986).

For plants with sexual and asexual propagation, seedling recruitment strategy through sexual reproduction is closely linked with clonal structure and with levels of within-population genetic diversity (Eriksson, 1989, 1993). For example, if no recruitment occurs after the establishment of the initial cohort, this could result in a decrease of genetic diversity over time, and ultimately populations would be composed of a small number of large, old, and even-aged clones [the “initial seedling recruitment” (ISR) strategy; Eriksson, 1989]. By contrast, if there is steady recruitment of genets, populations will contain clones of variable age and size, largely maintaining local genetic variability [the “repeated seedling recruitment” (RSR) strategy; Eriksson, 1989]. As the diploid cytotype of *L. lancifolium* can reproduce by both seeds and bulbils, and plants are locally common in western and southern Korea, it is reasonable to expect that RSR strategy may operate within their populations. High levels of genetic diversity can also be expected for diploid populations of *L. lancifolium*, as lilies are usually insect-pollinated and have a high seed dispersal potential (traits that are associated with high within-population genetic variation; Hamrick and Godt, 1990). In contrast, we may expect a single clone (i.e., “monoclonal”) or a few clones in each triploid population, because triploid forms exclusively propagate via

bulbils [there is no seedling (sexual) recruitment within populations].

The origin of the triploid cytotype of *L. lancifolium* has been a matter of debate during the past half century. Noda (1966, 1986) hypothesized that the triploids may either be autotriploids directly originated from the diploid cytotype through the production of unreduced gametes or allotriploids produced by natural hybridization between the diploid cytotype and the closely related *L. maximowiczii*. Later, based on a study on the geographic distribution and the observed habitat preferences of diploid and triploid *L. lancifolium* in South Korea, Kim et al. (2006a) argued that an allotriploid origin is unlikely, because *L. maximowiczii* does not occur sympatrically or parapatrically with the diploids in the southern Korean Peninsula. More recently, Sultana et al. (2010) conducted genomic in situ hybridization and detected strong hybridization of genomes between diploid and triploid cytotypes of *L. lancifolium*, indicating that triploids are derived from diploid forms and not from *L. maximowiczii*. This hypothesis is also supported by the high frequency of trivalent formation in the meiosis as reported in recent studies (Hwang et al., 2012; Liu and Jia, 2013). Codominant genetic markers (such as allozymes and microsatellites) have been proved very useful to infer the mode of polyploidization in plants, mainly through the observation of banding patterns (López-Pujol et al., 2004; Landergott et al., 2006; Shinohara et al., 2010; Palop-Esteban et al., 2011). If the triploid cytotype of *L. lancifolium* is an autopolyploid, we expect triploids to have a subset of the alleles present in diploid populations, and no evidence of fixed heterozygosity. Alternatively, if the triploid cytotype is an allopolyploid, we expect it to have novel alleles (and fixed heterozygosity) indicating its hybrid origin. Fixed heterozygosity is a consequence of the combination of two divergent genomes (as usually occurs in allopolyploids); if these genomes are fixed for different alleles at a given loci, then all individuals will invariably show a heterozygous

banding pattern.

Ecological niche modeling (ENM) is increasingly used as a quantitative tool for investigating the possible role of ecological (niche) divergence in the establishment and persistence of polyploids (either in auto- or allopolyploids) and for comparing ecological breadth among ploidy levels of plant species (Glennon et al., 2012; Godsoe et al., 2013; Laport et al., 2013; Harbert et al., 2014; Thompson et al., 2014). This approach has the advantages of its relative simplicity and the availability of databases of occurrence data and environmental variables that can be freely accessed (Hijmans et al., 2005; Telenius, 2011). The spatial separation of cytotypes via niche divergence can prevent “minority cytotype exclusion” (Levin, 1975), and is generally considered as one of the primary mechanisms involved in polyploidy success (Fowler and Levin 1984; Thompson and Lumaret, 1992; Petit et al., 1999; Baack, 2005). However, several studies have failed to reject the null hypothesis of no climatic niche divergence—niche conservatism (Sampoux and Huyghe, 2009; Godsoe et al., 2013; Glennon et al., 2014; Soltis et al., 2014). In addition, polyploids are expected to show increased niche breadth (ecological tolerance) due to a better ability to tolerate stressful conditions such as low nutrient levels, cold temperatures, and drought (Levin, 2002). However, this hypothesis is not always empirically confirmed (Godsoe et al., 2013; Laport et al., 2013; Theodoridis et al., 2013; Glennon et al., 2014).

Based on the observations of Kim et al. (2006a), the diploid (reproduced via seeds and bulbils) and the triploid (propagated exclusively via bulbils) cytotypes of *L. lancifolium* rarely coexist on the Korean Peninsula; triploids seems to be adapted to inland habitats (along roadsides, streams, river banks or sites subject to human disturbance), whereas diploids are almost exclusively confined to natural coastal habitats (cliffs or beaches). These field observations suggest that some degree of habitat differentiation between the

two cytotypes would have occurred. This observed habitat differentiation, however, seems to be less marked or even absent in the Jeju and Tsushima islands, where in some locations (especially in Tsushima Island) diploid and triploid forms grow together (Noda, 1986). In addition, the fact that triploid forms have a much broader geographic distribution than diploids in the Korean Peninsula might indicate that the former has a broader niche than the latter. A rigorous test for niche divergence vs. niche conservatism between diploids and triploids of *L. lancifolium* is needed, because it would provide insights into the establishment of triploids and the reasons why no tetraploids or higher ploidy levels exist in this complex (Levin, 1975; Rodriguez, 1996; Baack, 2005).

In this study we use allozyme markers to (1) assess the clonal structure of both diploid and triploid cytotypes of *L. lancifolium* in Korea [and infer which seedling (sexual) recruitment strategy is operating within the diploid populations], (2) evaluate genetic diversity and population structure of both cytotypes in Korea, and (3) test the autopolyploid origin of the triploid cytotype of *L. lancifolium*. Here, we also build niche models for the two cytotypes of *L. lancifolium* in their truly native range (Korea plus Tsushima Island) to test: (4) whether the differential geographic distribution of diploid and triploids observed in Korea—as noted by Kim et al. (2006a)—is attributable to niche differentiation (i.e., whether the triploids have a different niche than from diploids) and (5) whether the triploids have a broader niche than diploids.

Materials and Methods

Study species

Lilium lancifolium is a short-lived herbaceous perennial, 0.8–1.5 (up to 2) m tall, with minute white woolly hairs on stem. Leaves are sessile and scattered along the stem, with bulbils on the axils of distal leaves. Three to six flowers, horizontal or nodding, are arranged in racemes. Flowers comprising six vermillion tepals, each 7–10 cm long, with dark purple spots, are open from July to August. Fruits (capsules in diploids) are narrowly ovate-oblong, 3–4 cm long. Butterflies visit flowers of *L. lancifolium* during day time (M. Y. Chung and M. G. Chung, pers. obser.). *Lilium lancifolium* produces many thin, flat seeds with a wider winged margin, having thus a high potential for seed dispersal by wind (McRae, 1998).

Population sampling

Kim et al. (2006a) provided a detailed distribution of diploid and triploid cytotypes of *L. lancifolium* throughout South Korea [by mapping 367 populations of 66 different localities (including 23 islands)]. According to their report (based on chromosome counts), diploids are concentrated on the western and southern coasts (including their minute associate islands as well as Jeju Island), but are absent from inland areas. By contrast, triploids are found in inland areas and also in Jeju Island. With this information, we collected 141 leaf samples from four populations within the diploid range (LLD-1 to LLD-4; Table 1 and Figure 1) and 459 samples from 11 populations within the region of triploids occurrence (LLT-1 to LLT-11; Table 2 and Fig. 1). We did not include samples from North Korea due to obvious political reasons. To verify the ploidy level of the sampled populations, we counted the number of chromosomes from one plant per population, with the root tip squash method using aceto-orcin as described in Jones and Luchsinger (1986:

pp. 181–182); ploidy level was consistent with our population assignment.

Enzyme electrophoresis

Enzymes were extracted from each sample by crushing the leaf in a buffer (Mitton et al., 1979) using a chilled mortar and pestle and absorbing the extract onto paper wicks (Whatman 3MM chromatography paper). We conducted electrophoresis on 13% starch gels, with two buffer systems. We used a modification (Haufler, 1985) of the system 6 of Soltis et al. (1983) to resolve alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*), fluorescent esterase (*Fe*), malic enzyme (*Me*), phosphoglucoisomerase (*Pgi-1*, *Pgi-2*), phosphoglucomutase (*Pgm*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*). We also used the morpholine-citrate buffer system (pH 6.1) of Clayton and Tretiak (1972) to resolve isocitrate dehydrogenase (*Idh*) and 6-phosphogluconate dehydrogenase (*6Pgd-1*, *6Pgd-2*). We followed stain recipes from Soltis et al. (1983) except for diaphorase (Cheliak and Pitel, 1984). We designated putative loci sequentially, with the most anodally migrating isozyme designated as *1*, the next 2, and so on. We also designated different alleles within each locus sequentially by alphabetical order. For diploids, the observed enzyme banding patterns were consistent with their typical subunit structure and subcellular compartmentalization in diploid plants (Weeden and Wendel, 1989). For triploids, putative genotypes of heterozygotes were inferred based on the “allele dosage” effect (the intensity of band staining) in isozyme patterns (e.g., Darnaedi et al., 1990; Suzuki and Iwatsuki, 1990; Naujoks et al., 1995; King et al., 1996). When it was difficult to identify genotypes because of the co-migration of “homodimeric” with “heterodimeric” bands (for example for the *Me* locus; Table 2), phenotypes were recorded as “a/b”.

Identification of clones and measures of clonal diversity

We considered a locus to be polymorphic when two or more alleles were observed, regardless of their frequencies. The triploids propagate exclusively by bulbils, and we can anticipate that all samples collected per population will be monoclonal (i.e., consisting of a single clone) unless triploids are formed repeatedly from diploids or triploids are dispersed from other populations. For diploids, plants can be formed via seeds and by bulbils, and multiple ramets (N_T , the number of shoots sampled that include ramets produced through clonal reproduction) representing allozyme-based identical multilocus genotypes (MLG) could result either from clonal propagation or distinct sexual reproduction events. Thus, it is important to discriminate these cases to correctly identify clonal ramets (Chung and Epperson, 1999). Using the program GenClone 2.0 (Arnaud-Haond and Belkhir, 2007), we calculated $P_{\text{gen}} F_{\text{IS}}$, the probability of the MLG occurring by chance due to sexual reproduction by taking into account departures from Hardy–Weinberg (H–W) equilibrium (Parks and Werth, 1993; Arnaud-Haond et al., 2007). We averaged $P_{\text{gen}} F_{\text{IS}}$ estimates generated from one such value for each MLG in each population and used a probability $P_{\text{gen}} < 0.05$ cut off for the discrimination of ramets versus genets (Chung et al., 2014). Under this criterion, we prepared a second data set excluding all but one clonal ramet per genet, resulting in each distinct MLG only represented once per population (N_G , the number of individuals excluding clonal ramets).

As Arnaud-Haond et al. (2007) recommended, we used four parameters to describe clonal diversity and distribution: genotypic richness [$R = (N_G - 1)/(N_T - 1)$; Dorken and Eckert, 2001], the Simpson diversity index (Pielou, 1969) of clonal heterogeneity [D , the probability of encountering distinct MLG when randomly taking two units (ramets) in a

population] and its equitability (ED , Simpson evenness; Hurlbert 1971), and the Pareto index β (Table 1; Arnaud-Haond et al., 2007). To characterize the genet size (X , the number of ramets belonging to each genet), we fitted a cumulative function of the Pareto distribution to the data following the method of Arnaud-Haond et al. (2007). This function takes the following form: $N_{\geq X} = a X^{-\beta}$, where $N_{\geq X}$ is the number of genets containing X or more ramets and a is a constant. The definitions and calculations for β associated parameters [regression slope (b_p) of $\log_{10}$ (reverse cumulative frequency of $N_{\geq X}$) vs. $\log_{10}$ (X), and its 95 % confidence intervals (CIs), and its associated coefficient of determination (R^2)] are well described in the footnote of Table 1 and Chung et al. (2014). For all these calculations, we also used GenClone 2.0 (Arnaud-Haond and Belkhir, 2007). Finally, a contingency χ^2 -test was conducted to determine whether distribution of clone sizes was significantly different between diploid populations.

Measures of genetic diversity and structure

For diploid populations, we estimated the following genetic diversity parameters using the N_G , with the aid of the programs POPGENE (Yeh et al., 1999) and FSTAT (Goudet, 1995): percent polymorphic loci ($\%P$), mean number of alleles per locus (A), allelic richness (AR) using a rarefaction method that compensates uneven population sample sizes (Hurlbert, 1971; El Mousadik and Petit, 1996), observed heterozygosity (H_o), and Nei's (1978) unbiased gene diversity or Hardy–Weinberg (H–W) expected heterozygosity (H_e).

For individual loci, we evaluated the difference between the H–W H_e and the equilibrium heterozygosity (H_{eq}) expected assuming mutation-drift equilibrium to test for

recent decreases in effective population size (bottlenecks). These differences were evaluated using a sign test and a Wilcoxon sign-rank test conducted across loci under an infinite allele model using the program BOTTLENECK (Cornuet and Luikart, 1996). Since allelic diversity is generally lost more rapidly than H_e (Nei et al., 1975), recently bottlenecked populations are expected to exhibit an excess of H–W equilibrium H_e relative to H_{eq} (Cornuet and Luikart, 1996; Luikart et al., 1998).

Using the program SPAGeDi (Hardy and Vekemans, 2002), we estimated population-level F_{IS} (inbreeding) and calculated its significance probability (P values) by gene permutation tests (999 replicates) under the null hypothesis ($F_{IS} = 0$). We also calculated Wright's (1965) F_{IS} and F_{ST} over loci following Weir and Cockerham (1984). These fixation indices measure the average deviation from H–W equilibrium of individuals relative to their local populations (F_{IS} , a measure of local inbreeding) and local populations relative to the total population (F_{ST} , also a measure of differentiation between local populations). The significance of multi-population F_{IS} and F_{ST} estimates was determined by permutation tests (999 randomizations of alleles between individuals within samples and 999 randomizations of genotypes between populations, respectively). These calculations were performed using FSTAT (Goudet, 1995).

Ecological niche modeling (ENM)

ENM was performed to evaluate the potential distribution of both diploid and triploid cytotypes of *L. lancifolium*. We employed the maximum entropy algorithm, as implemented in MaxEnt v. 3.3 (Phillips et al., 2006). The current distribution information for both cytotypes in their native areas was obtained from presence records included in the

Global Biodiversity Information Facility (www.gbif.org), from personal communications, from relevant literature (e.g., Kim et al., 2006a), and from the sampling sites of this study. In total, after removing duplicate records within each pixel (2.5 arc-min, ca. 5 km), we obtained 45 and 51 presence records for diploid and triploid *L. lancifolium*, respectively. A set of 19 bioclimatic variables at 2.5 arc-min resolution covering the native distribution range (and neighboring areas) for both cytotypes under current conditions (1950–2000) were downloaded from the WorldClim website (www.worldclim.org; Hijmans et al., 2005). After a correlation analysis in a random sample of 1,000 points, we selected a smaller set of seven (relatively) uncorrelated variables ($r < 0.9$): mean diurnal temperature range (bio2), isothermality (bio3), maximum temperature of the warmest month (bio5), minimum temperature of the coldest month (bio6), annual precipitation (bio12), precipitation of the wettest month (bio13), and precipitation seasonality (bio15).

Replicate runs (10) of MaxEnt (using the ‘subsampling’ method) were performed to ensure reliable results. Model performance was assessed using the area under the curve (AUC) of the receiver operating characteristic plot, with 20% of the localities randomly selected to test the model. AUC scores may range between 0.5 (randomness) and 1 (exact match), with those above 0.9 indicating a good performance of the model (Swets, 1988). A jackknife analysis was used to evaluate the relative importance of the seven bioclimatic variables employed, based on their gain values when used in isolation. All ENM predictions were visualized in ArcGIS v. 9.3 (ESRI, Redlands, CA, USA), with the aid of Hawth’s Analysis Tools (Beyer, 2004). Finally, we used the range overlap test implemented in the software ENMTools v. 1.4.3 (Warren et al., 2008, 2010) to estimate the amount of overlap between potential distributions of the two cytotypes (using the 10 percentile training presence logistic threshold as the presence/absence threshold).

Niche comparisons

To assess whether the triploid cytotype of *L. lancifolium* shows evidence of niche divergence from the diploid one, we computed two niche overlap indices, Hellinger-derived *I* and Schoener's *D*; these metrics are implemented in the software ENMTools v. 1.4.3 and measure similarity among ENMs. *I* and *D* values range from 0 (when the two species—in the present case, cytotypes—show completely discordant ENMs) to 1 (complete niche overlap), and are suitable for the geographic (G) space. We further used two quantitative tests of niche similarity that are also implemented in ENMTools, the “niche identity test” and the “background test”. The former was used to know whether ENMs obtained for the two cytotypes of *L. lancifolium* are identical. To do it, a one-tailed test was employed to compare the empirical *I* and *D* values to those generated from a number of pseudoreplicated datasets (100 for the present case) that were obtained by pooling all the occurrences of the two cytotypes and randomly splitting them into two new groups.

When two mainly allopatric entities (such as the two cytotypes of *L. lancifolium*) are compared, some niche differentiation may be due simply to the environmental differences that are expected to occur between two distinct geographic regions, which would make the identity test inappropriate (Warren et al., 2010). Thus, we further used the background test, which determines whether ENMs are more similar (or less similar) than expected by chance. A null distribution of similarities was generated by comparing the actual occurrence records of the diploid cytotypes with a set of randomly simulated occurrences within the range of the triploids. The same procedure was performed in the inverse direction (i.e., triploid cytotype occurrences vs. background ones within the diploid range). Finally, a two-tailed test was used to compare the empirical *I* and *D* values to those

obtained after running 100 pseudoreplicates of each cytotype-pair tested. The background point selection was done by creating a buffer zone (of 20 km) around the occurrence points of each cytotype, with the aid of the specific tools included in ArcGIS.

To test whether triploids have a broader niche than diploids, the niche breadth of each cytotype was measured. Values of niche breadth were obtained by calculating the inverse concentration statistic of Levins (1968), as implemented in ENMTools. Values can range from 0 (only one pixel in G-space shows suitability greater than zero) to 1 (all pixels equally suitable).

Niches of both cytotypes of *L. lancifolium* were also compared by means of direct observations in the multivariate environmental (E) space. It is highly advisable to combine these analyses with ENM because of the duality of environmental and geographic spaces (e.g., Soberón and Nakamura, 2009). We used the method proposed by McCormack et al. (2010) in which mean observed niche divergence (d_n) between the two cytotypes was tested against a null model of background environmental differences (background divergence, d_b) in the principal components analysis (PCA) axes. Values for the seven bioclimatic variables were sampled from all the occurrence points and from 1,000 random background points within the range of each cytotype (using the method of background point selection described above). The seven (relatively) uncorrelated variables (bio2, bio3, bio5, bio6, bio12, bio13, and bio15) were reduced with the PCA with varimax rotation; only the principal components (PCs) with an eigenvalue ≥ 1 were selected. Divergence on each niche axis (PC) was evaluated by comparing the differences between the mean scores of the occurrence points of the two compared cytotypes (d_n) to the differences between those of the 1,000 background points (d_b) of each cytotype, with the null hypothesis being $d_n = d_b$. Significance of d_n itself was assessed by means of *t*-tests. Distributions of d_n and d_b were

generated with 1,000 bootstrap resamplings, and d_n was compared to the 95% confidence intervals (CIs) of d_b . Niche divergence is supported if d_n is significantly greater than d_b (and d_n is significant), whereas niche conservatism is supported if d_n is significantly smaller than d_b . A measure of niche breadth for the two cytotypes of *L. lancifolium* in E-space was obtained by visualizing the scores of the occurrence points for each PCA axis (by using boxplots). All the multivariate analyses were conducted using SPSS v. 22 (SPSS Statistics, Chicago, IL, USA).

Results

Identification of clones and clonal diversity

For diploids, 10 (*Adh*, *Dia-1*, *Dia-2*, *Fe*, *Me*, *6Pgd-1*, *6Pgd-2*, *Pgi-2*, *Pgm*, and *Tpi-1*) of the 13 putative loci were polymorphic across four populations (Table 2). Thus, the power to discriminate clonal genotypes from sexually produced genotypes was high (with an average of 0.977; Table 1). At the population level, we identified a total of 85 unique genotypes (N_G) out of 141 N_T (Table 1). For triploids, five (*Dia-1*, *Me*, *6Pgd-1*, *6Pgd-2*, and *Pgi-2*) of the 13 putative loci were polymorphic across 11 populations (Table 2). At the population level, all 11 populations were monoclonal (i.e., consisting of a single MLG; Table 2). Only six different MLG out of 459 N_T were detected across 11 populations (Table 2).

For the four diploid populations, mean estimates of genotypic richness (R), Simpson diversity index (D), and Simpson evenness index (ED) were 0.611, 0.950, and 0.797, respectively (Table 1). In all populations, the $\log_{10}$ of the cumulative distribution of ramets

among genets was linearly related to the $\log_{10}$ of the genet size (X) or the number of ramets belonging to each genet (significant regress slope of b_P ; $R^2 = 0.735$ to 0.995 , $P < 0.05$; Table 1). Except for LLD-3, the values of the Pareto index β ($-1 \times b_P$) were moderate (Table 1). The b_P of LLD-4 was significantly steeper than that of LLD-3, as 95% CIs of b_P did not overlap (Table 1), whereas the differences were not significant for any other pairwise comparisons (Table 1). In accordance with this, we found no significant differences in the distribution of clone sizes among the four populations (contingency χ^2 -test, $\chi^2 = 25.6$, $df = 18$, $P = 0.110$; supplementary Appendix 1).

Genetic diversity and structure

The four diploid populations of *L. lancifolium* maintained moderate levels of allozyme variation ($\%P = 55.8$, $AR = 1.59$, $A = 1.59$, and $H_e = 0.164$; Table 3). Slightly higher levels of genetic variation were obtained in pooled samples ($\%P = 76.9$, $A = 1.92$, and $H_e = 0.171$; Table 3). We found no significant evidence of recent bottlenecks (supplementary Appendix 2). In contrast, the 11 triploid populations harbored very low levels of genetic variation ($\%P = 16.1$ and $A = 1.16$; Table 3). When samples were pooled, we found again slightly higher levels of genetic variation ($\%P = 38.5$ and $A = 1.38$; Table 3). Whereas up to seven alleles were specific to the diploid cytotype, the triploid populations did not show any exclusive allele. Moreover, the most common alleles within populations were identical between diploids and triploids (supplementary Appendix 3). Banding patterns exhibited no evidence of fixed heterozygosity at any of the analyzed loci in the triploid cytotype (Table 2).

All population-level F_{IS} estimates for all diploid populations were significantly

positive at the 0.05 level of significance (Table 3). This result, as well as the significant multi-population-level F_{IS} ($F_{IS} = 0.279$, $P = 0.001$; Table 3) indicates a substantial deficit of heterozygotes within populations. Deviation from H–W expectations due to allele frequency differences between diploid populations were significantly different from zero but very low ($F_{ST} = 0.053$, $P = 0.001$).

Ecological niche modeling (ENM)

The AUC scores averaged across 10 runs were high for both the diploid and the triploid cytotypes of *L. lancifolium* (mean $\pm$ SD, 0.978 ± 0.006 and 0.806 ± 0.022 , respectively), which supported the predictive power of the model. According to the jackknife tests, mean diurnal temperature range (bio2), minimum temperature of the coldest month (bio6), and maximum temperature of the warmest month (bio5), were the most informative for predicting the niche of the diploid cytotype; the former two (bio2 and bio6) were also important for the triploids' niche model, together with annual precipitation (bio12) (supplementary Appendix 4). On the basis of the 10 percentile training presence logistic threshold, the predicted distribution of the triploid cytotype of *L. lancifolium* was much larger (over five-fold) than that of the diploid one (being the latter limited to the coastal areas of Korea, and Jeju and Tsushima islands; Fig. 2). ENMs were largely congruent with the known occurrences of both cytotypes; the only significant exception is a ca. 150-km coastal stretch of predicted suitability for the diploid cytotype in the Pacific side of the Peninsula, but where it is not known to occur (Fig. 2). The potential distribution of the diploid mostly overlapped with the predicted suitable range for the triploids (81.5% of overlapping; Fig. 2).

Niche comparisons

Regarding the niche similarity analyses in G-space, the niche identity test revealed that both I and D values of the null distribution were significantly greater ($P < 0.01$) than the observed ones ($I_{\text{obs}} = 0.704$; $D_{\text{obs}} = 0.382$; Fig. 3A, B); thus, the null hypothesis of identical niches for both cytotypes can be rejected. The background test, in contrast, indicated that niches for both cytotypes are not divergent. When we compared occurrence records of diploids to the background points within the range of the triploids, the observed niche overlap was significantly larger than the null distribution of niche overlap ($P < 0.01$ for both I and D ; Fig. 3C, D). When the test was performed in the inverse direction (i.e., triploid occurrences vs. diploid range), the observed value of overlap was not significantly different from the null distribution ($P > 0.05$ for I and D ; Fig. 3E, F). These results unambiguously indicate that ENMs of diploids and triploids of *L. lancifolium* are not different than expected by chance; the differences observed with the niche identity test should be simply attributable to habitat differences in the geographic regions where diploids and triploids do not overlap. Finally, the niche breadth estimate based on ENMs was about 4 times smaller for the diploid cytotype compared to the triploid one (0.121 and 0.491, respectively).

PCA of the seven bioclimatic variables identified three PCs that explained collectively 85.5% of the overall variance in *L. lancifolium* (Table 4 and supplementary Appendix 5). The second axis was associated with rainfall, whereas the third axis was correlated with temperature; the first axis, in contrast, was associated with both temperature and rainfall variables (Table 4 and supplementary Appendix 5). None of the three axes did not show conclusive results regarding niche conservatism/divergence (because d_n

overlapped with the 95% CIs of d_b ; Table 4). However, the distribution of niche breadth values along the three axes suggested that the triploid cytotype has a somewhat wider climate niche than the diploid one (Fig. 4).

Discussion

Repeated seedling recruitments of the diploid L. lancifolium

The highly skewed distribution of genet size (75% of the genets comprised single ramets; supplementary Appendix 1) suggests that repeated recruitments of seedlings (i.e., RSR strategy) have taken place within the diploid populations of *L. lancifolium*, as predicted. Inference of the RSR model is further supported by levels of genotypic diversity. Levels of clonal (genotypic) diversity (R , D , ED , and the Pareto index β) found in the diploid populations of *L. lancifolium* are high compared to published data for other plant species. The Pareto index β is well suited for summarizing clonal diversity and for making comparisons among different studies (Arnaud-Haond et al., 2007; Ohsako, 2010). The mean value of β (1.127) for the diploid *L. lancifolium* populations is higher than the average value ($\beta = 0.930$) from 15 populations belonging to 11 terrestrial and marine plant species compiled by Ohsako (2010). In agreement with this distribution, genotypic richness (R) values found in the four diploid populations of *L. lancifolium* (mean $R = 0.611$) are higher than the average (0.294) reported by Ohsako (2010) for 11 clonal plant species. In contrast and as anticipated, all the studied triploid populations are monoclonal since there is no seedling (sexual) recruitment.

Moderate levels of genetic diversity and low genetic differentiation in the diploid *Lilium lancifolium*

Despite its small range, the diploid cytotype of *L. lancifolium* maintain moderate levels of within-population genetic variation, comparable to those of its northeastern Asia congeners and higher than those averaged for plants with a regional distribution, species with mixed-animal pollination, and species with wind-dispersed seeds (supplementary Appendix 6). Within the Korean Peninsula, the moderate to high levels of genetic diversity observed in three species of *Lilium* (*L. cernuum*, *L. distichum*, and *L. tsingtauense*) have been attributed to their occurrence along the Baekdudaegan; these mountains (which run north to south along the Korean Peninsula) harbored multiple, continuous refugia during the Last Glacial Maximum, which would have enabled plant species to maintain relatively large population sizes and high rates of recurrent gene flow (Chung and Chung, 2014). In the diploid *L. lancifolium*, although it occurs far away from these suggested refugia, large effective population sizes (there is no significant evidence of recent bottlenecks), repeated seedling recruitment (RSR) strategy, and clonal reproduction (which maintains genetic variation—once produced—within populations) might be involved in the high levels of within-population genetic variation.

According to an equilibrium equation between F_{IS} and t (outcrossing rates) [$F_{IS} = (1 - t)/(1 + t)$; Hedrick and Cockerham, 1986], we obtain a t of 0.564 ($F_{IS} = 0.279$) for the diploid *L. lancifolium*, suggesting a mixed-mating system for the species. As the diploid *L. lancifolium* is self-incompatible, the substantial deficit of heterozygotes found within populations must be attributed to biparental inbreeding (mating with relatives) and/or Wahlund effect (population subdivision).

The genetic differentiation between the four diploid populations of *L. lancifolium* ($F_{ST} = 0.053$) is low, suggesting high historical/contemporary gene flow between the populations. This value of genetic differentiation is substantially lower than those expected for short-lived herbaceous perennials and monocots ($G_{ST} = 0.233$ and 0.231 , respectively; Hamrick and Godt, 1990). However, as most of the studied *Lilium* species show low values of F_{ST} (Table 1), this might be regarded as a “typical” trait within the genus. The only species that shows a high value is *L. longiflorum* ($F_{ST} = 0.348$; Hiramatsu et al., 2001), although it should be noted that this species occurs on isolated islands across the Ryukyu Archipelago in southern Japan, stretching ca. 1300 km.

Autotriploid origin of the triploid cytotypes

We found that allele profiles detected in the triploids were exactly subsets of those in the diploids, as no alleles unique to triploids have been detected (but, instead, there are several alleles unique to diploids), with no evidence of fixed heterozygosity. In addition, we found that the most common alleles within populations were identical between diploids and triploids (supplementary Appendix 6). These results unambiguously indicate an autotriploid origin for the triploid cytotype of *L. lancifolium*, in agreement with biogeographic (Kim et al., 2006a) and cytogenetic studies (Sultana et al., 2010; Hwang et al., 2012; Liu and Jia, 2013). None of the allozyme phenotypes obtained for the 459 samples from the 11 populations of putative triploids showed a pattern consistent with tetraploidy, which indicates that the triploid cytotype of *L. lancifolium* has resulted from the union of reduced (n) and unreduced ($2n$) gametes of diploid individuals. A similar result has been reported in the western North American aspen (*Populus tremuloides*); based on allele frequency

comparisons between pools of diploid and triploid plants within populations of *P. tremuloides*, Mock et al. (2012) demonstrated an autotriploid origin of the triploid cytotype resulting from unreduced spore formation. On the contrary, an allotriploid origin was reported for the triploid, endemic to Korea *Lycoris flavescens* (Amaryllidaceae), which showed fixed heterozygosity for most of the studied loci as a consequence of the combination of the two parental genomes (a diploid and a tetraploid one; Lee et al., 2001).

The origin of the triploid cytotype of *L. lancifolium* from the diploid one seems to be analogous to the model of “progenitor–derivative (P–D)” species pairs. First, the spectrum of alleles observed in triploid *L. lancifolium* (the D) is a subset of those found in the diploid forms (the P), with few, if any, unique alleles. Second, levels of within-population genetic variation observed in triploids are lower than in diploids (Gottlieb, 1973; Crawford, 1983). In fact, two cases of a P–D species pair in *Lilium* are known so far; *L. distichium*/*L. tsingtauense* (Chung et al., 2015) and *L. longiflorum*/*L. formosanum* (Hiramatsu et al., 2001). These two cases, coupled with the polyploid complex studied here, can be regarded as examples of ongoing speciation processes within the genus, for which East Asia is the main center of diversification (Gao et al., 2013). Apart from these pairs, in which most alleles are shared and unique alleles are rare, East Asian *Lilium* species usually do not share many alleles: for example, Nei’s (1978) unbiased genetic identity between the diploid form of *L. lancifolium* and other five species native to Korea ranges from 0.440 (the diploid *L. lancifolium* vs. *L. amabile*) to 0.647 (the diploid *L. lancifolium* vs. *L. cernuum*; M. Y. Chung et al., unpublished manuscript). These results also support an autotriploid origin for the triploid *L. lancifolium*; if the triploid was produced by natural hybridization between the diploid *L. lancifolium* and *L. maximowiczii*, at least a few alleles from the latter would be found in the triploid cytotype.

It should be noted that, despite the low levels of genetic variation, the six different MLG found in the triploid forms of *L. lancifolium* suggest their multiple origins in South Korea. Two of these MLG (A and B; Table 2), in addition, occur in geographically clustered populations (Fig. 1). Based on 10 allozyme loci (nine polymorphic), Ozaki et al. (2002) reported 12 MLG from 27 triploid populations of *L. lancifolium* on Tsushima Island (see Fig. 1) in Japan. This and our findings strongly suggest that formation of triploid *L. lancifolium* has occurred recurrently, probably both in space and time. In fact, multiple origins in polyploidy seems to be the rule and not the exception, and usually increase the genetic variation of a polyploid species, which might facilitate its persistence (Ellstrand and Roose, 1987; Segraves et al., 1999; Soltis and Soltis, 1999, 2000).

No niche divergence but broader niche breadth for triploids

Our results clearly support that the diploid and the triploid cytotypes of *L. lancifolium* show niche conservatism in G-space. First, the potential distribution of the diploid cytotype, although much smaller, was almost entirely included within the predicted range of the triploid cytotype (81.5% of the diploid potential range overlapped with the triploid's one). Second, the niche similarity analyses (background test) indicate that ENMs of diploid and triploid cytotypes of *L. lancifolium* are not different than expected by chance. If we move to E-space, no niche divergence was detected between diploids and triploids, although niche conservatism cannot be assumed either (for PC1, the niches of diploids and triploids are different, but this may simply reflect divergence in their background environments; for PC2 and PC3, the niches of diploids and triploids appear to be similar, but this might simply reflect similarity in their background environments).

The lack of a clear, strong niche divergence between cytotypes of *L. lancifolium* supports the view that ecological differentiation is not a pre-requisite for the establishment of new polyploid lineages (e.g., Glennon et al., 2014). However, this issue remains controversial, as there are studies often reporting contrasting results. For example, Sampoux and Huyghe (2009) found that differences in ploidy levels barely influenced niche differentiation in *Festuca* subg. *Festuca*. More recently, Godsoe et al. (2013) also reported niche conservatism between diploid and autotetraploid cytotypes of *Heuchera cylindrica*. McIntyre (2012) found niche differentiation between diploid and polyploid cytotypes of *Claytonia* but not when tetraploids were compared to hexaploids. Similarly, Glennon et al. (2012) reported niche divergence between cytotypes of *Houstonia longifolia* but not for *H. purpurea*. In a last example, ecological differentiation was detected at different ploidy levels for the four species of *Primula* sect. *Aleuritia* (Theodoridis et al., 2013). Nevertheless, in a recent study (Glennon et al., 2014), the authors tested climate shifts between cytotypes in 20 plant species from Europe and North America (using the ordination method proposed by Broennimann et al., 2012) and did not find any evidence for niche shift between diploids and polyploids. According to Glennon et al. (2014), polyploid persistence is better explained by dispersal capabilities or other life-history traits, rather than niche divergence.

The lack of niche differentiation between diploid and triploid cytotypes of *L. lancifolium* may seem surprising given the rather contrasting differences in the current distribution of both forms in South Korea (Kim et al., 2006a). It should be noted, however, that diploids and triploids coexist in several places in Tsushima Island, in one locality in Jeju island, and also in one in mainland Korea (Incheon, near Seoul) (Noda, 1986; Kim et al., 2006a). Moreover, 23% of the triploid populations in Korea occur in coastal cliffs (Kim

et al., 2006a), the typical habitat of the diploid cytotype. These data suggest that the low rates of cytotype coexistence between cytotypes of *L. lancifolium* may be simply due to full occupancy of the suitable habitat by one of the cytotypes. In other words, most of the coastal niches for *L. lancifolium* might have already been occupied by diploid individuals at the time of triploid formation (i.e., competitive exclusion). Competitive exclusion might be favoured by *a priori* higher reproductive performance of diploids compared to triploids (as diploids can reproduce by means of sexual and asexual means); however, such reproductive advantage of the diploid cytotype of *L. lancifolium* remains to be tested.

The broader ecological tolerance of the triploids compared to diploids (detected both in G- and E- space), in addition, would have allowed the colonization of vacant and/or newly formed habitats (roadsides, arable lands, and other habitats subjected to frequent disturbance such as river banks). A very close situation has been already reported in other polyploid complexes. For example, in north-eastern Spain the diploid cytotype of *Dactylis glomerata* is confined to low-density woodlands of ancient origin, whereas the tetraploid cytotype is more widespread and tends to occur in open areas with some level of anthropogenic disturbance (Lumaret et al., 1987). Similarly, the establishment of the tetraploid cytotype of *Heuchera cylindrica* has been directly linked to its expansion into previously unoccupied habitats (due to the glacial retreat and/or scouring floods; Godsoe et al., 2013).

Although the presumed broader niche breadth for polyploids remains as a highly controversial issue (Martin and Husband, 2009; te Beest et al., 2012; Glennon et al., 2014), there are numerous examples in the literature of polyploid complexes for which the polyploids show wider ecological tolerances and larger geographic ranges (e.g., Lowry and Lester, 2006; Treier et al., 2009; McIntyre, 2012). The superior colonization ability showed

by many polyploids is usually attributed to their increased genetic and biochemical diversity (Levin, 1983; Otto and Whitton, 2000; Lowry and Lester, 2006), but it can also be linked to other changes in the morphology and physiology that seem to be inherent to polyploids. Polyploids are reported to possess longer life spans and to show more vigorous growth, often having much taller plant height and larger flowers and seeds than diploids (reviewed in te Beest et al. 2012). Some of these advantages may apply to the triploid cytotype of *L. lancifolium*, whose individuals have been observed to be taller than diploids (M. Y. Chung and M. G. Chung, personal observations), although more detailed studies are needed.

The competitive ability and the establishment of polyploids are also promoted by vegetative (asexual) multiplication and perennial habit (Stebbins, 1950; Otto and Whitton, 2000; Soltis and Soltis, 2000; te Beest et al., 2012). Given its low levels of within-population genetic diversity, the vigorous vegetative reproduction showed by the triploid cytotype of *L. lancifolium* is undoubtedly behind its successful establishment and spread within the diploid's native range (having colonized most of the Korean Peninsula). The great success in clonal reproduction of triploid forms may have also facilitated its spread throughout most of China (it is only absent from its north-western region), Japan, and the south-eastern tip of Russian Far East by escaping from cultivation (the plant is widely cultivated for its medicinal and edible properties in China and Japan). Axillary bulbils detachment has also allowed *L. lancifolium* to be a common garden escape in Europe and North America. Horticulture has become a major pathway of introduction of alien plants (Reichard and White, 2001), and there is at least one reported instance of diploid/triploid pairs in which the triploids have become more widespread than the diploid because of its economic uses (*Butomus umbellatus*; Lui et al., 2005).

Acknowledgements

The authors thank Dong Yuk Yeo, Beom Jin Shim and Myeong Soon Park for laboratory assistances. Dr. Yukio Ozaki of Kyushu University in Japan kindly provided allozyme-based abstract related to this study. This research was supported by the Basic Science Program through the National Research Foundation of Korea (NRF-2013R1A1A2063524) funded by the Ministry of Education, Science and Technology, the Republic of Korea to M.G.C.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.flora.201x.xx.xxx>.

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Table 1

Summary of clonal diversity measures examined in four diploid populations of *Lilium lancifolium*. The following parameters are shown: N_T number of shoots sampled (including clonal ramets), N_G number of individuals excluding clonal ramets, $P_{\text{gen}} F_{\text{IS}}$ probability of the identical multilocus genotypes (MLG) occurring by chance due to sexual reproduction by taking into account departures from Hardy–Weinberg (H–W) equilibrium, R genotypic richness, D Simpson diversity index of clonal heterogeneity, ED Simpson evenness index, β the Pareto index describing the Pareto distribution [$-1 \times$ regression slope, b_P , of the double logarithmic linear regression of reverse cumulative frequency of the number of genets containing X or more ramets ($N_{\geq X}$) on the number of sampled ramets belonging to a genet (X)], CIs confidence intervals, R^2 coefficient of determination (square of correlation coefficient of the double logarithmic linear regression).

Population	N_T	N_G	$P_{\text{gen}} F_{\text{IS}}$	R	D	ED	β (95% CIs for b_P^a)	R^2
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LLD-1	36	25	0.009	0.686	0.967	0.774	1.128 (−1.521, −0.735)	0.965
LLD-2	30	22	0.027	0.724	0.970	0.753	1.322 (−2.458, −0.187)	0.926
LLD-3	41	12	0.043	0.275	0.884	0.882	0.538 (−0.909, −0.167)	0.735
LLD-4	34	26	0.012	0.758	0.979	0.780	1.521 (−1.842, −1.201)	0.995
Average	35	21	0.023	0.611	0.950	0.797	1.127	0.905

^a All log-log regressions indicated significance with $P < 0.05$.

Table 2

Alleles found in the four diploid populations of *Lilium lancifolium* based on 13 loci and six multilocus genotypes (MLG) found in 11 triploid populations. Heterozygous genotypes found in triploids are indicated by boxes.

Population	MLG	<i>Adh</i>	<i>Dia-1</i>	<i>Dia-2</i>	<i>Fe</i>	<i>Idh</i>	<i>Me</i>	<i>6Pgd-1</i>	<i>6Pgd-2</i>	<i>Pgi-1</i>	<i>Pgi-2</i>	<i>Pgm</i>	<i>Tpi-1</i>	<i>Tpi-2</i>
Diploid														
LLD-1		<i>a,b</i>	<i>a,c</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a,b,c</i>	<i>a</i>
LLD-2		<i>a,b</i>	<i>a,c</i>	<i>a,b</i>	<i>b</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a,b,c</i>	<i>a</i>
LLD-3		<i>a</i>	<i>a,b</i>	<i>b</i>	<i>a,b</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>
LLD-4		<i>a</i>	<i>a,b</i>	<i>b</i>	<i>a,b</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>	<i>a</i>	<i>a,b</i>	<i>a,b</i>	<i>a,b</i>	<i>a</i>
Triploid														
LLT-1 (30) ^a	A	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b ^b	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-2 (30)	A	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-3 (40)	A	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-4 (20)	A	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-5 (48)	A	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-6 (33)	B	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	abb	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-7 (35)	B	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	abb	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-8 (22)	C	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>	<i>aaa</i>	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-9 (61)	D	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	abb	<i>aaa</i>	<i>aaa</i>	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-10 (81)	E	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	abb	<i>aaa</i>	<i>aaa</i>	aab	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>
LLT-11 (59)	F	<i>aaa</i>	aab	<i>bbb</i>	<i>bbb</i>	<i>aaa</i>	a/b	abb	aab	<i>aaa</i>	<i>aaa</i>	<i>aaa</i>	<i>bbb</i>	<i>aaa</i>

^a sample size for allozyme analysis.

^b When it was difficult to infer genotypes (because of deviation of expected band intensity), phenotypes were recorded as “a/b”.

Table 3

Levels of genetic diversity in four diploid populations and 11 triploid populations of *Lilium lancifolium* in Korea. The following parameters are shown: N_G the number of individuals excluding clonal ramets, % P percentage of polymorphic loci, AR mean allelic richness (adjusted for a sample size of 12 individuals), A mean number of alleles per locus, H_o observed heterozygosity, H_e H-W expected heterozygosity or genetic diversity, SE standard error, F_{IS} fixation index within populations.

Population	N_G	% P	AR	A	H_o (SE)	H_e (SE)	F_{IS}
Diploid							
LLD-1	25	61.5	1.69	1.69	0.108 (0.030)	0.163 (0.050)	0.338 ^a
LLD-2	22	53.9	1.61	1.58	0.119 (0.039)	0.151 (0.052)	0.213 ^a
LLD-3	12	53.9	1.51	1.54	0.135 (0.047)	0.167 (0.056)	0.192 ^a
LLD-4	26	53.9	1.54	1.53	0.121 (0.040)	0.176 (0.056)	0.313 ^a
Average	21	55.8	1.59	1.59	0.121 (0.006)	0.164 (0.005)	0.279 ^b
Pooled samples	129	76.9		1.92		0.171 (0.046)	
Triploid							
LLT-1	1	15.4		1.15			
LLT-2	1	15.4		1.15			
LLT-3	1	15.4		1.15			
LLT-4	1	15.4		1.15			
LLT-5	1	15.4		1.15			
LLT-6	1	15.4		1.15			
LLT-7	1	15.4		1.15			
LLT-8	1	15.4		1.00			
LLT-9	1	15.4		1.15			
LLT-10	1	15.4		1.23			
LLT-11	1	15.4		1.31			
Average	1	16.1		1.16			
Pooled samples	11	38.5		1.38			

^a Significance ($P < 0.05$) based on permutation (999 replicates) under the null hypothesis of $F_{IS} = 0$.

^b Significant (at the 0.05 level) Weir and Cockerham (1984) estimate of F_{IS} over populations.

Table 4

Test of niche divergence on each principal component (PC) between diploid and triploid cytotypes of *Lilium lancifolium*. The following parameters are shown: d_n mean of observed niche divergence, d_b mean of background divergence (CIs, confidence intervals), NC ‘not conclusive of niche divergence or niche conservatism’ (d_n is not significantly greater than d_b , i.e., $d_n = d_b$ or d_n is significantly greater than d_b , but d_n is not significant; Wooten and Gibbs, 2012). Note that for ‘niche conservatism’ d_n should be significantly smaller than d_b , whereas for ‘niche divergence’ d_n should be both significant and significantly greater than d_b (McCormack et al., 2010).

	PC1	PC2	PC3
d_n	0.960 [*]	0.092	0.389
d_b (95% CIs)	0.904 (0.806, 1.003)	0.041 (-0.082, 0.153)	0.428 (0.320, 0.530)
Niche divergence pattern	NC	NC	NC
Percentage of variance explained	50.1	20.9	14.5
Most important variables ^a	bio2, bio3, bio6, bio15	bio12, bio13	bio5
Biological interpretation	Temperature / Rainfall	Rainfall	Temperature

^{*} Denotes significance ($P < 0.05$).

^a See Supplemental data with the online version of this article, supplementary Appendix 5 for details.

Figure legends

Fig. 1. Locations of sampled diploid (LLD-1 to LLD-4) and triploid populations (LLT-1 to LLT-11) of *Lilium lancifolium* in South Korea.

Fig. 2. Potential distributions as probability of occurrence for the two cytotypes of *Lilium lancifolium* in their native ranges using MaxEnt (Phillips et al., 2006), at the present time (A, diploid cytotype; B, triploid cytotype). Black circles represent extant occurrence points of the cytotypes. Predicted distribution probabilities (in logistic values) are shown in each 2.5 arc-min pixel.

Fig. 3. Results of the two tests of niche similarity in *Lilium lancifolium* as obtained using ENMTools. (A) and (B), niche identity tests between diploids and triploids based on *I* and *D*, respectively; (C) and (D), background tests between diploid occurrences and triploid background points based on *I* and *D*, respectively; (E) and (F), background tests between triploid occurrences and diploid background points based on *I* and *D*, respectively. The arrow represents the observed niche overlap between ENMs (i.e., the empirical values of *I* and *D*), whereas the histograms are those expected under the null hypotheses.

Fig. 4. Niche breadth of the diploid (D) and triploid (T) cytotypes of *Lilium lancifolium* along the four PCA axes. In each boxplot, the bold line represents the median, box limits are the first and third quartiles, whiskers indicate 1.5 times the interquartile range, and black circles are outliers.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4