# Intercontinental dispersal and whole-genome duplication contribute to loss of self-incompatibility in a polyploid complex

Brittany L. Sutherland[1,3], Brandie M. Quarles[1,2], and Laura F. Galloway[1]

**PREMISE OF THE STUDY**: Angiosperm species often shift from self-incompatibility to self-compatibility following population bottlenecks. Across the range of a species, population bottlenecks may result from multiple factors, each of which may affect the geographic distribution and magnitude of mating-system shifts. We describe how intercontinental dispersal and genome duplication facilitate loss of self-incompatibility.

**METHODS**: Self and outcross pollinations were performed on plants from 24 populations of the *Campanula rotundifolia* polyploid complex. Populations spanned the geographic distribution and three dominant cytotypes of the species (diploid, tetraploid, hexaploid).

**KEY RESULTS**: Loss of self-incompatibility was associated with both intercontinental dispersal and genome duplication. European plants were largely self-incompatible, whereas North American plants were intermediately to fully self-compatible. Within both European and North American populations, loss of self-incompatibility increased as ploidy increased. Ploidy change and intercontinental dispersal both contributed to loss of self-incompatibility in North America, but range expansion did not affect self-incompatibility within Europe or North America.

**CONCLUSIONS**: When species are subject to population bottlenecks arising through multiple factors, each factor can contribute to self-incompatibility loss. In a widespread polyploid complex, the loss of self-incompatibility can be predicted by the cumulative effects of whole-genome duplication and intercontinental dispersal.

**KEY WORDS**   *Campanula rotundifolia*; Campanulaceae; gametophytic self-incompatibility; hexaploid; long-distance dispersal; mating system; polyploidy; tetraploid.

In many angiosperms, the cost of inbreeding depression is so high (Lande and Schemske, 1985) that numerous mechanisms have evolved to prevent plants from self-fertilizing (Charlesworth and Charlesworth, 1987). One such mechanism, self-incompatibility (SI), is found in many plant families and allows individuals to recognize and prevent fertilization by related pollen (East, 1926; Bateman, 1952; de Nettancourt, 1977). Although SI is common (Allen and Hiscock, 2008), numerous losses have been documented when selection favors the capacity to self-fertilize (Barrett, 1988; Mable et al., 2005; Busch and Schoen, 2008; Igic et al., 2008). Loss of SI frequently occurs following population bottlenecks, when the cost of inbreeding depression declines and reproductive assurance becomes critical to the persistence of small, isolated populations (Baker, 1955; Busch and Schoen, 2008; Igic and Busch, 2013; Pannell et al., 2015). Population bottlenecks often arise during range expansion or following long-distance, particularly intercontinental, dispersal.

In plants, they can also occur after whole-genome duplication. As drivers of population bottlenecks, each of these processes—range expansion, intercontinental dispersal, and whole-genome duplication—differ in duration, severity, and underlying genetic effects on mating system (see below). In polyploid complexes with expansive or disjunct ranges, one or more of these processes may contribute to overall loss of SI. Therefore, to understand mating-system evolution in widespread polyploid complexes, it is necessary to assess the individual and cumulative effects of range expansion, intercontinental dispersal, and whole-genome duplication across the cytotypic and geographic range of the complex.

Both range expansion and intercontinental dispersal are known to cause population bottlenecks at range edges (Lande and Schemske, 1985; Porcher and Lande, 2005; Busch et al., 2011; Griffin and Willi, 2014), but expectations for their effects on effective population size differ. Intercontinental dispersal is a relatively rare event that often

involves only one or a few founder individuals that experience little or no gene flow with the source population. Population bottlenecks during intercontinental dispersal are often profound; they create a demographic sieve in which individuals that can self are more likely to persist, thereby enriching the frequency of self-compatibility (SC) among colonists. This phenomenon has been described as "Baker's law" (Baker, 1955; Pannell et al., 2015). However, during range expansion, population bottlenecks are typically less severe and edge populations may experience gene flow with more central populations (Wilson et al., 2009). Here, as leading-edge populations become progressively smaller and more genetically homogeneous, the cost of inbreeding is reduced and the need for reproductive assurance increases. This change in selective pressures can favor self-compatible individuals over obligate outcrossers (Hargreaves and Eckert, 2014). Because this process is gradual, it may take more time for loss of SI following intracontinental range expansion than following intercontinental dispersal (Pannell, 2015).

Severe population bottlenecks can also occur following whole-genome duplication (Husband et al., 2008). When tetraploids arise in an otherwise diploid population via genome duplication, any reproduction between cytotypes is likely to result in hybrid triploid embryos, which are subject to severe fitness deficits due to low viability and fertility (Miller and Venable, 2000; Mable et al., 2004; Husband et al., 2008). This creates substantial reproductive isolation between diploids and polyploids. Under such conditions, polyploids experience strong selection against SI (Sabara et al., 2013). Indeed, most emergent polyploids within diploid populations are transient individuals that fall victim to minority cytotype exclusion because most mating attempts result in unfit hybrids, and only those individuals that are self-compatible tend to persist (Husband, 2000; Pannell et al., 2004). This selective pressure for loss of SI is prevalent enough that shifts in mating system have been correlated with changes in ploidy (Entani et al., 1999). In addition to strong selection for SC, polyploids may also be preadapted for SI loss (Hauck, 2006; Tao and Iezzoni, 2010). Genome duplication has been shown to reduce the efficiency of SI systems, particularly gametophytic self-incompatibility (GSI) systems in which self-recognition is mediated by the haploid pollen genotype (Hauck et al., 2006; Miller et al., 2008).

In polyploid systems that have undergone range expansion or long-distance dispersal, successive bottlenecks may result in stepwise loss of SI. Earlier bottlenecks may actually facilitate later ones. For example, following genome-duplication, a population of polyploids may be more self-compatible than diploids, and thus more likely to establish after intracontinental range expansion or intercontinental dispersal than diploids (Linder and Barker, 2014). Following such dispersal, potential colonists may experience a severe population bottleneck and be subject to further loss of SI. The facilitation of subsequent dispersal by more self-compatible polyploids is consistent with the overrepresentation of polyploids following intercontinental (Crawford et al., 2009) or island colonizations (te Beest et al., 2012).

The *Campanula rotundifolia* polyploid complex is a tractable system in which to compare the relative effects of range expansion, intercontinental dispersal, and whole-genome duplication on loss of SI. The complex has a broad circumboreal range encompassing the northern latitudes of Europe and North America and comprises three dominant cytotypes (Shetler, 1982; Stevens et al., 2012). It is thought to have originated in southeastern Europe ~2.3 mya. It has a history of relatively gradual range expansion throughout Europe,

transatlantic dispersal to North America ~220,000 years BP, and subsequent range expansion throughout northern North America (Sutherland, 2017). Tetraploids originated from diploids and hexaploids from tetraploids at least four times each via whole-genome duplication (Sutherland, 2017). The complex is considered self-incompatible (Bielawska, 1973; Nyman, 1992). However, anecdotal accounts of selfing (Shetler, 1982; Giblin, 2005) suggest that the mating system may be variable.

Here, we seek to understand the patterns of SI loss throughout the geographic distribution of the *C. rotundifolia* polyploid complex. We assess SI in multiple European and North American populations that represent all dominant cytotypes to address four questions. Is the *C. rotundifolia* polyploid complex self-incompatible throughout its geographic and cytotypic range? Is range expansion or intercontinental dispersal associated with any loss of SI? Is whole-genome duplication associated with any loss of SI? Is loss of SI predictable based on the cumulative effects of range expansion, intercontinental dispersal, and genome duplication? By determining the extent to which bottlenecks due to multiple factors contribute to loss of SI, we can better understand patterns of variation in SI across widespread polyploid complexes.

## MATERIALS AND METHODS

### Study species

*Campanula rotundifolia* L. is a short-lived perennial wildflower with a circumboreal distribution covering much of the northern latitudes of Europe and North America (Shetler, 1982; Stevens et al., 2012). It exists as an autopolyploid complex comprising diploids in Central and Northern Europe, tetraploids throughout the distribution, and hexaploids in the British Isles and North America (Shetler, 1982; Stevens et al., 2012; Sutherland, 2017). *Campanula rotundifolia* is protandrous; prior to anthesis, anthers deposit pollen onto stylar hairs, which results in secondary pollen presentation. The stigmatic lobes remain closed for the first 24–48 hr post-anthesis. Pollinator visitation stimulates stylar hairs to retract and stigmatic lobes to open and begin curling under (Shetler, 1982), placing receptive stigmas closer to their own pollen. The mechanism of SI in *C. rotundifolia* is not known. However, the congener *C. rapunculoides* exhibits a GSI system in which self-recognition is mediated by coat protein alleles in the haploid pollen grain (Good-Avila et al., 2008), and it is likely that *C. rotundifolia* shares this mechanism.

We chose 24 populations of *C. rotundifolia* that spanned the geographic range and ploidy variation present within the complex (Fig. 1) and comprised 11 European and 13 North American populations (Appendix S1; see Supplemental Data with this article). To assess the effect of ploidy variation on SI, we included all dominant cytotypes. In Europe, these samples comprised three diploid, seven tetraploid, and one hexaploid population, and in North America accessions comprised eight tetraploid and five hexaploid populations. Although diploids have been reported in North America (Löve and Löve, 1966), multiple attempts to resample a diploid population in the northeastern United States were unsuccessful, and the population may be extirpated. Populations were sampled across the three main clades identified in a chloroplast phylogeny (Sutherland, 2017) that roughly correspond to three locations: Central and Southern Europe, Western and Northern Europe, and North America (Fig. 1). This sampling design permitted evaluating the effects of both
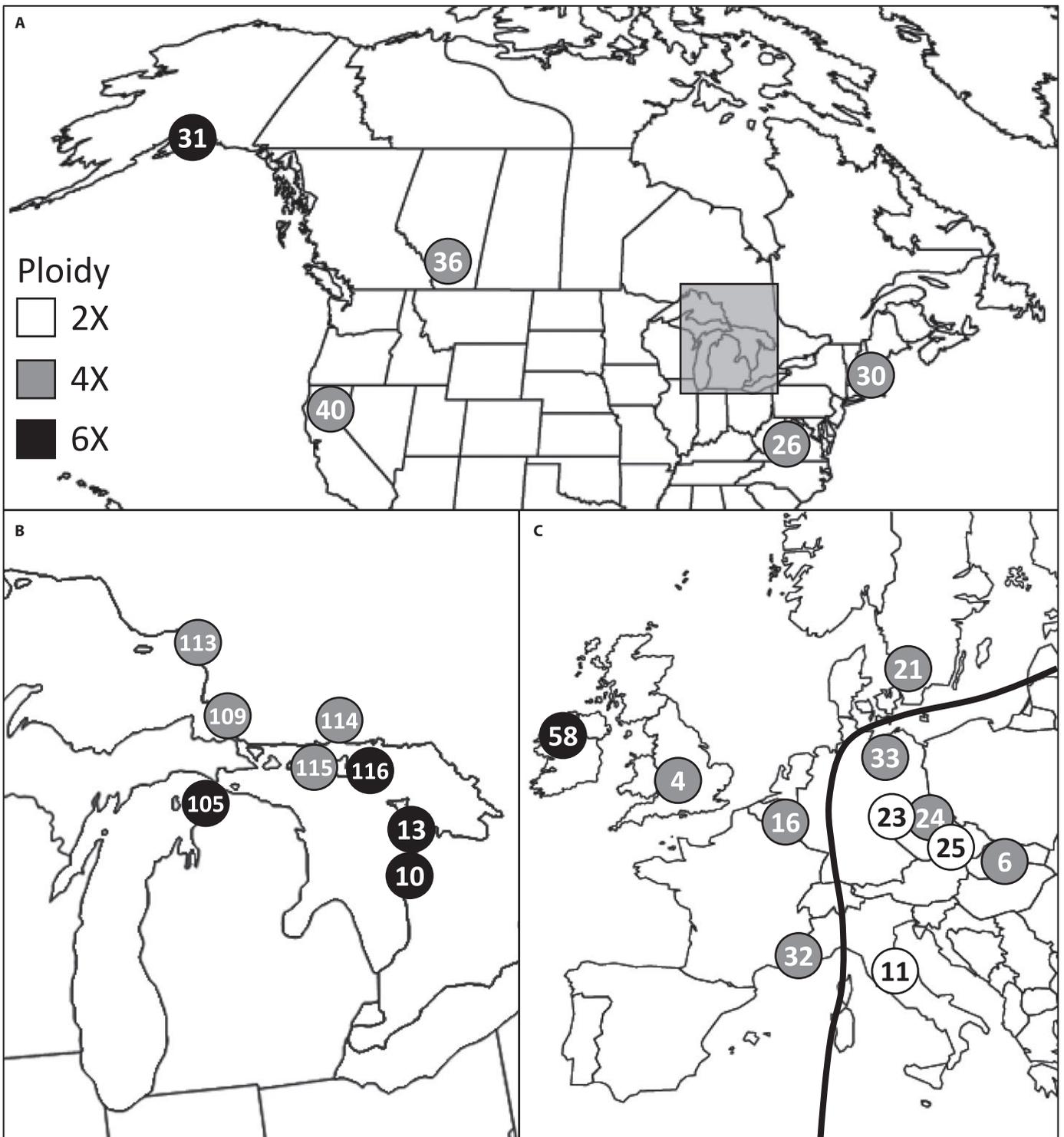
**FIGURE 1.** Maps of sampled diploid, tetraploid, and hexaploid *Campanula rotundifolia* populations. (A) North American distribution of sampled populations, with inset gray square showing location of (B) distribution of populations centered around the Great Lakes. (C) European distribution of sampled populations. Numbers denote populations listed in Appendix S1, and black line denotes partition between populations assigned to Central and Western European locations.

gradual range expansion (Central/Southern to Western/Northern Europe and within North America) and intercontinental dispersal (Western/Northern Europe to North America). Additional North American populations were chosen as well to assess SI in a narrowly defined contact zone between tetraploids and hexaploids along the Great Lakes (Fig. 1B). Ploidy was assessed for greenhouse-grown

plants of all populations using a modified Otto 2-step protocol (Otto, 1990; Sutherland and Galloway, 2017).

## Crossing method

Crosses were performed on greenhouse plants grown from wild-collected seed. We performed one outcross and one self-cross per plant for an average (± SD) of 10.7 ± 4.2 plants for each population (minimum = 6; $N$ = 248 pairs of self-crosses and outcrosses). Pollinated flowers were surgically emasculated in the bud prior to anther dehiscence to prevent potential within-flower selfing. Preliminary crosses showed no difference in seed set between intact maternal flowers and those for which anthers had been removed. Once stigmas opened, pollen-bearing styles of paternal flowers were rubbed on stigmatic lobes of maternal flowers, depositing an excess of pollen. For outcrosses, paternal flowers were chosen from different plants within the same population; for self crosses, paternal flowers were chosen from a different flowering stem on the same plant. Mature fruits were collected prior to dehiscence, and fully developed brown, inflated seeds were counted. Flowers that failed to set fruit were recorded as seed set of zero. If outcrosses set <10 seeds, indicating poor pollination success (6.6% of crosses), they were replaced along with their matched self-cross by an additional pair of crosses. No outcrosses set <10 seeds twice consecutively.

## Statistical analysis

Self-incompatibility was scored by calculating the index of self-incompatibility (ISI; Lloyd, 1965), or 1 minus the ratio of seeds produced by self-fertilization in relation to outcrossed seed produced; ISI can range from 0 to 1. Although fruit set is more commonly used to calculate ISI (Raduski et al., 2012), we used seed set because polyploids that display GSI often have multiple pollen-coat genotypes in one paternal flower, some of which may successfully self-fertilize ovules (Tao and Iezzoni, 2010). This variability leads to high fruit formation but reduced seed formation. We calculated ISI for each plant. If selfed seed exceeded outcrossed seed for an individual, the individual was deemed fully self-compatible, and ISI was set to zero. Individuals have historically been classified as self-incompatible, intermediately self-incompatible, or self-compatible on the basis of threshold values (Lloyd, 1965); self-incompatible individuals have ISI > 0.8, and self-compatible individuals have ISI < 0.2.

To evaluate the effect of ploidy, range expansion, and intercontinental dispersal on SI, we used a generalized mixed model in PROC GLIMMIX (SAS 9.3; SAS Institute, Cary, North Carolina, USA). A logit transformation of seed set ratio (selfed/outcrossed seed per plant) was performed to normalize the data prior to calculating ISI. The model included ploidy and location as fixed effects, and population nested within ploidy and location as a random effect. Location was treated as a categorical variable with three levels (Central/Southern Europe, Western/Northern Europe, and North America). Ploidy is nonrandomly distributed throughout the range, with most hexaploids in North America and all diploids in Central Europe (Fig. 1). Because of this distribution, the model was unavoidably unbalanced, and as such, an interaction term for these factors was not included in the model.

To address this imbalance and to test the effect of range expansion apart from intercontinental dispersal, analyses were also performed on subsets of the data. First, the effect of ploidy was tested within each continent. All three cytotypes were compared in Europe, and North America was limited to tetraploids and hexaploids. Populations were compared using the same mixed model described above, but omitting location as a main effect. Second, tetraploid populations occur throughout the range and are fairly evenly distributed, permitting a test of the relationship between dispersal distance and SI. Specifically, linear regressions were performed to test the effect of distance (as a continuous variable) from the hypothesized center of origin on ISI, along hypothesized routes of migration (Sutherland, 2017). To minimize the effect of potential leverage on these regressions from having clusters of populations in North America and Europe, the distance between nearest European and North American populations was set equal to the longest distance between any other two adjacent populations (~1600 km). Three regressions were performed on population mean ISI: all tetraploid populations, only European tetraploids, and only North American tetraploids.

## RESULTS

Mean population ISI ranged from self-incompatible to self-compatible, with values from 0.915 to 0.131 (Fig. 2A), and location was strongly associated with ISI ($F_{2,19}$ = 10.17, $P$ < 0.001). Central European populations were self-incompatible, with an average ISI of 0.847 (Fig. 2B), while Western European populations had a slightly lower average ISI of 0.747, but this difference was not significant (Tukey-Kramer, $t$ = −0.97, $P$ = 0.60). North American populations had a significantly lower average ISI of 0.418 (Tukey-Kramer, $t$ = −4.50, $P$ < 0.001), indicating partial SC. One North American population, Michigan 105, was fully self-compatible, with an ISI of 0.140 (Fig. 2A). When analysis was limited to tetraploid populations, distance from the hypothesized center of origin was associated with decreasing SI ($t$ = −4.97, $P$ < 0.001, $\beta$ = −0.799, $R^2$ = 0.638; Fig. 3A). However, when samples were limited to either Europe or North America, distance was not significantly associated with loss of SI (Europe: $t$ = −1.08, $P$ = 0.323, $\beta$ = −0.402, $R^2$ = 0.162; North America: $t$ = 0.28, $P$ = 0.788, $\beta$ = 0.114, $R^2$ = 0.013; Fig. 3B,C).

Increasing ploidy was associated with decreasing SI (Fig. 2). Diploids were self-incompatible, with ISI values between 0.883 and 0.866 (mean ISI = 0.880). Self-incompatibility in tetraploids was highly variable, with ISI values ranging from 0.915 to 0.301 (mean ISI = 0.709). Hexaploids were partially to fully self-compatible, with ISI values between 0.529 and 0.131 (mean ISI = 0.361). This negative association between SI and ploidy was near significant for the whole dataset ($F_{2,19}$ = 3.38, $P$ = 0.056), with tetraploids not significantly different from either diploids or hexaploids, but with hexaploids having significantly lower SI than diploids (Tukey-Kramer, $t$ = −2.52, $P$ = 0.033). When ploidy was tested within each continent, increasing ploidy was significantly associated with decreasing SI in both Europe and North America (Europe: $F_{2,11}$ = 3.82, $P$ = 0.032; North America: $F_{1,12}$ = 5.56, $P$ = 0.020; Fig. 2).

## DISCUSSION

The *C. rotundifolia* polyploid complex is not uniformly self-incompatible, and loss of SI is consistent with a history of both whole-genome duplication and intercontinental dispersal, but not intracontinental range expansion. Some populations are strongly self-incompatible; however, most have experienced some loss of SI,
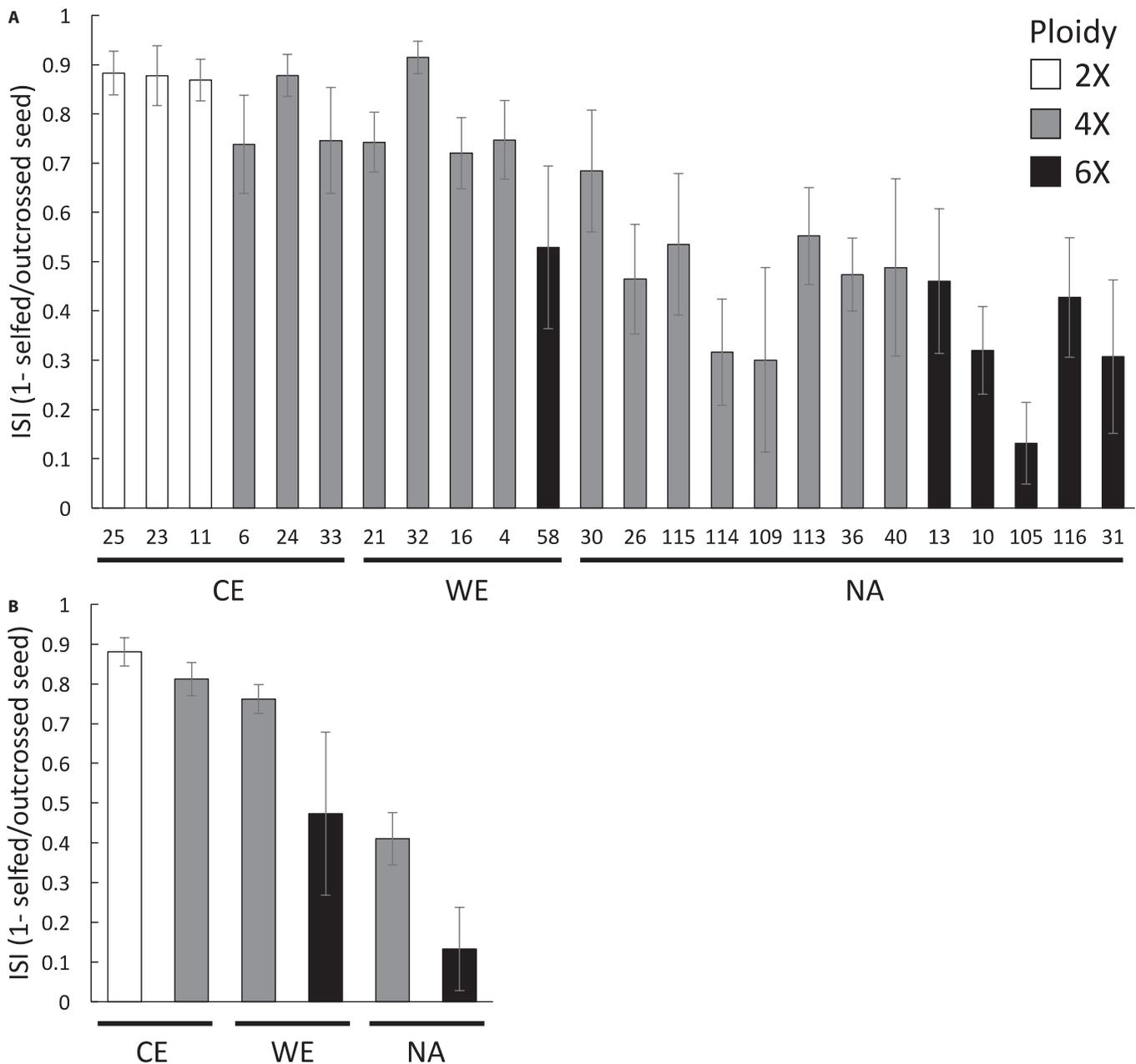
**FIGURE 2.** Index of self-incompatibility (ISI) in *Campanula rotundifolia* by (A) population and (B) location and ploidy, averaged across all maternal plants in a population. Error bars denote standard error. Location categories (CE = Central Europe, WE = Western Europe, NA = North America) are arranged east to west, and populations are arranged east to west within each location and ploidy. See Fig. 1 for population locations.

which contrasts with historical accounts of *C. rotundifolia*'s mating system (Bielawska, 1973; Nyman, 1992). Loss of SI was most pronounced in North American populations and in hexaploids, with almost complete SI loss in some North American hexaploids, which reflects the cumulative effects of bottlenecks caused by intercontinental dispersal and whole-genome duplication.

Loss of SI in *C. rotundifolia* has been influenced by a history of intercontinental dispersal, but not gradual range expansion. Based on nuclear (Sutherland, 2017) and chloroplast (Mansion et al., 2012, Sutherland, 2017) phylogenies, *C. rotundifolia* is hypothesized to have originated in south-central Europe. From that center

of origin, the complex has undergone gradual range expansion in Europe, intercontinental dispersal to North America, then gradual range expansion in North America. However, SI loss was only significantly associated with intercontinental dispersal between Europe and North America. While Western European populations had slightly lower ISI than Central European ones, this loss was modest and not significant. By contrast, ISI was 46% lower in North America than in Europe. Although the hypothesized single dispersal event from Europe to North America (Sutherland, 2017) makes it difficult to definitively ascribe loss of SI to intercontinental dispersal, loss of SI following long-distance dispersal
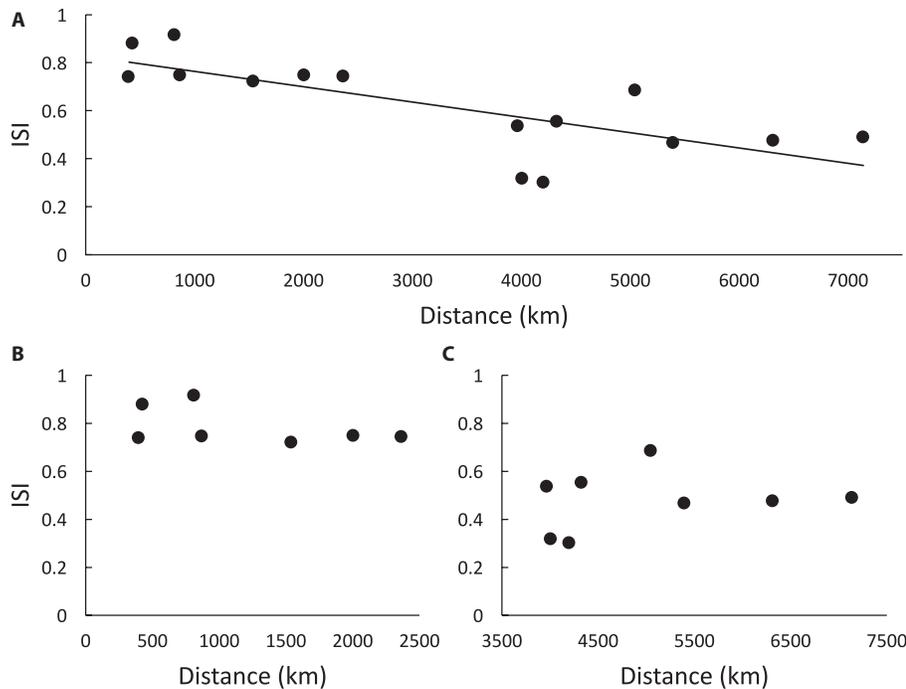
**FIGURE 3.** Effect of distance from hypothesized center of origin on index of self-incompatibility (ISI) for tetraploid *Campanula rotundifolia* populations. Linear regression of population means by distance for (A) all tetraploid populations, (B) populations in Europe only, and (C) populations in North America only.

each successive genome duplication (for example, from tetraploidy to hexaploidy) presents a new potential bottleneck event that can select for loss of SI. The SI loss seen in tetraploid and hexaploid *C. rotundifolia* is consistent with selection for reproductive assurance during population bottlenecks (e.g., Guo et al., 2009) caused by successive rounds of genome duplication (Mable, 2004).

Alternatively, loss of SI following genome duplication may be due to breakdown of GSI, which relies on linked pollen-coat proteins and stylar RNases to prevent growth of pollen tubes from related pollen (Tao and Iezzoni, 2010). In diploid plants, haploid pollen expresses a single GSI coat protein, and if that protein is recognized by stylar RNases, pollen-tube arrest is complete or near complete (Luu et al., 2000). However, recognition efficiency decreases when multiple coat proteins are present, which is possible in heterozygous pollen from tetraploids and hexaploids (Hauck et al., 2006). Because heterozygous autotetraploids will produce a mixture of heteroallelic and homoallelic pollen, self-fertilization will be reduced but not eliminated. In hexaploids, a higher proportion of heteroallelic pollen is possible, resulting in a further decrease in pollen tube recognition and arrest. As a result, SI may be lost cumulatively through subsequent genome duplication events. This mechanism is consistent with loss of SI observed in *C. rotundifolia*, with progressively lower ISI values as ploidy increases.

Breakdown of GSI may also explain the unexpectedly high frequency of intermediate ISI values observed in *C. rotundifolia*. Although mixed mating systems are well documented, most angiosperms tend to be either self-incompatible or self-compatible (Raduski et al., 2012), and intermediate SI has been considered a transient state (Schemske and Lande, 1985). By contrast, only *C. rotundifolia* diploids are universally self-incompatible; most tetraploid and hexaploid populations exhibit intermediate ISI values. The high proportion of intermediate ISI throughout the distribution suggests that the capacity for outcrossing has been maintained in this system, and that polyploids may not fit the broader pattern of bimodality observed for SI among diploids. Intermediate values of ISI may reflect GSI breakdown associated with ploidy change. As such, changes in SI are not associated with selection on mating system that may favor outcrossing or selfing, and therefore may be more likely to be stable.

Patterns of SI loss in *C. rotundifolia* are consistent with a stepwise process in which loss of SI accumulates over successive population bottleneck events, with some bottlenecks facilitated by those that preceded them. Slight loss of SI with whole-genome duplication in European tetraploids and more pronounced loss in European hexaploids may have increased their likelihood of successful establishment following long-distance dispersal when populations are small and mates are limited. As Baker's law suggests (Baker, 1955; Pannell et al., 2015), because individuals that are more self-compatible have

events—including intracontinental dispersal (Šingliarová et al., 2008), intercontinental dispersal (Costa et al., 2017), and island colonization (Grossenbacher et al., 2017)—has been observed across disparate taxa, in both diploid and polyploid systems (e.g., Barrett, 2015). These findings support the conclusion that intercontinental dispersal has led to a reduction in SI in *C. rotundifolia*.

The lack of significant loss of SI within European tetraploids or North American tetraploids is consistent with less severe bottlenecks associated with range expansion rather than intercontinental dispersal. European taxa expanded after glacial maximum from refugia both north and south of the Alps (Sutherland, 2017) and likely experienced mild population contractions at the leading edge while maintaining some gene flow with the core distribution (e.g., Wilson et al., 2009). By contrast, dispersal from Europe to North America required long-distance transoceanic migration, effectively cutting off gene flow from source populations (e.g., Wilson et al., 2009). This abrupt lack of gene flow, coupled with small population sizes among colonists, would have more strongly selected for reproductive assurance than in leading-edge populations of range expansion, and is a likely explanation for the considerable loss of SI observed in North American taxa.

Whole-genome duplication was also associated with SI loss. Diploid populations were strongly self-incompatible, whereas tetraploid populations had ISI values 22% lower. Hexaploids were weakly self-incompatible to self-compatible, with ISI values 42% lower than those of tetraploids and 60% lower than those of diploids. Given that newly established polyploids appear to undergo extreme population bottlenecks (Husband et al., 2008; Sabara et al., 2013), strong selection for the capacity to self is expected in order to provide reproductive assurance (Barringer, 2007). Furthermore,

a higher likelihood of establishment, colonist populations can have reduced average SI compared to the home range. In keeping with this pattern, North American tetraploids and hexaploids, which already had some SC due to whole-genome duplication, experienced further loss of SI following intercontinental dispersal. Polyploid complexes can have broad ranges and are often known to span continents (Bleeker et al., 2002; Al-Shehbaz et al., 2006; Brochmann and Brysting, 2008). As such, successive bottlenecks that facilitate predictable stepwise loss of SI may be common as these lineages undergo genome duplication and long-distance dispersal events.

The high variation in SI in *C. rotundifolia* and the pattern of SI loss with ploidy increase and intercontinental dispersal suggest two things. First, loss of SI following genome duplication may be partial, and populations may persist with intermediate SI. Second, while gradual range expansion appears to play little or no role in SI loss in *C. rotundifolia*, both intercontinental dispersal and whole-genome duplication appear to have contributed, independently and successively, to SI loss. The repeated effects of whole-genome duplication inducing SI loss in a putatively GSI system, followed by enrichment for self-compatible individuals in colonist populations, appear to have mediated wide variation in mating system across the range of *C. rotundifolia*. The stepwise changes in SI observed in this system are not consistent with either whole-genome duplication or intercontinental dispersal alone but can be predicted as the result of both processes acting consecutively. To fully understand mating-system evolution in widespread polyploid complexes, it is critical to determine how changes in both distribution and cytotype influence each other in the maintenance or loss of self-incompatibility.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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