

**COMPETITION BETWEEN CYTOTYPES CHANGES ACROSS
A LONGITUDINAL GRADIENT IN *CENTAUREA STOEBE*
(*ASTERACEAE*)¹**

ALEXANDRA R. COLLINS^{2,4}, RUHOLLAH NADERI^{2,3}, AND HEINZ MUELLER-SCHAERER²

²University of Fribourg, Ecology and Evolution Department, Chemin du musée 10, Fribourg 1700, Switzerland; and ³Shiraz University, Department of Crop Production and Plant Breeding, P.O. Box 1585, Shiraz 71345, Iran

- *Premise of the Study:* Polyploidy resulting from whole genome duplication has contributed to the adaptive evolution of many plant species. However, the conditions necessary for successful polyploid evolution and subsequent establishment and persistence in sympatry with diploid progenitors are often quite limited. One condition thought to be necessary for establishment is a substantial competitive superiority of the polyploid.
- *Methods:* We conducted a pairwise competition experiment using diploid and tetraploid cytotypes of *Centaurea stoebe* L. to determine whether (1) tetraploids have greater competitive ability than diploids, (2) cytotypes from mixed-cytotype populations have more balanced competitive abilities than single-cytotype populations, and (3) competitive abilities change along a longitudinal gradient.
- *Key Results:* Across sampling localities, tetraploids did not produce greater aboveground biomass than diploids but suffered from greater intracytotypic competition. Tetraploids allocated greater biomass belowground than diploids, regardless of competition treatment, and had greater performance for traits associated with long-term persistence (bolted more frequently and produced more accessory rosettes). Competitive ability of tetraploids did not differ between single- and mixed-cytotype populations but varied along a longitudinal gradient. Tetraploids were stronger intercytotypic competitors in Western Europe (Switzerland and Germany) than in Eastern Europe (Hungary and Slovakia), which indicates that cytotype coexistence may be more likely in Eastern Europe, the proposed origin of tetraploids, than in Western Europe.
- *Conclusions:* Our study addresses the importance of examining competitive interactions between cytotypes across their distributional range, as competitive interactions were not consistent across sampling localities.

Key words: intracytotypic competition; intercytotypic competition; minority cytotype; mixed ploidy; polyploidy; sympatry.

Polyploidy resulting from whole genome duplications has contributed to the adaptive evolution of many plant species (Levin, 1983; Ramsey and Schemske, 1998; Otto, 2007). Yet the success of polyploids is not always certain: evolutionary models find that the conditions necessary for successful polyploid evolution in sympatry with diploids are often quite limited (Fowler and Levin, 1984; Felber, 1991; Rodriguez, 1996). One explanation for this is that rare polyploids within a mixed-ploidy population will be subject to frequency-dependent selection, which Levin (1975) referred to as the “minority cytotype disadvantage.” To overcome this disadvantage, two conditions seem to be particularly important for successful establishment of polyploids: (1) there must be partial niche separation between compatible cytotypes and/or (2) there must be a substantial competitive superiority of the polyploid (Maceira et al., 1993).

In natural populations in which two or more cytotypes co-occur, few studies have focused on competitive differences between cytotypes; instead, most experimental work has focused on niche separation, such as when coexistence of cytotypes occurs through habitat differentiation (Felber, 1991; Meerts, 1992; Felber-Girard et al., 1996; Johnson et al., 2003; Baack, 2004; Schönswetter et al., 2007) and/or reproductive isolation via differences in flowering time (Vandijk and Bijlsma, 1994; Bretagnolle and Thompson, 1996; Husband and Schemske, 2000) and/or ploidy barriers that lead to seed abortion (Vinkenoog et al., 2003). A better understanding of the coexistence of mixed-ploidy populations will require the study of the small-scale competitive interactions between cytotypes, because this is also relevant for (small-scale) niche separation (Levin, 1975).

When examining competition between two species (and/or genotypes), coexistence is often explained as a balance between intra- and interspecific (genotypic) competition, whereby species (genotypes) are expected to coexist when intraspecific (genotypic) competition is greater than interspecific (genotypic) competition (Tilman, 1982; Silvertown and Charlesworth, 2001). Tradeoff-based theories of interspecific competition have been tested extensively at the species level (Tilman, 2004, and references therein) as well as at the genotype level (Turkington, 1979; Antonovics and Ellstrand, 1984; Aarssen and Turkington, 1985; Kelley and Clay, 1987; Crutsinger et al., 2008). Because cytotypes can be thought of as just a different kind of genotype, classical competition theory can also be applied to cytotypes, in that coexistence between cytotypes is expected when intracytotypic competition is greater than intercytotypic competition, but,

¹Manuscript received 5 February 2011; revision accepted 16 September 2011.

The authors thank E. Tarbush, A. Bourqui, and N. Imambocus for help in the greenhouse, P. Mráz for commenting on the manuscript and for providing information and seed material for the mixed-ploidy populations, and U. Schaffner for commenting on the manuscript. This project was funded by the Swiss National Science Foundation (grant no. 31003A_125314) and the National Centre of Competence in Research (NCCR) ‘Plant Survival’ (both to H.M.S.). R. Naderi thanks the Iranian Ministry of Science Research and Technology and the University of Fribourg for financial support of his stay at the University of Fribourg as part of his Ph.D. fulfillment.

⁴Author for correspondence (e-mail: robin.collins@unifr.ch)

to our knowledge, this has rarely been tested (Maceira et al., 1993; Tilman, 2004).

Competitive interactions may also be affected by the locality where genotypes are collected. Competitive effects driven by the identity of plant neighbors have been recognized (Turkington, 1979; Kelley and Clay, 1987; Fridley et al., 2007) and have been shown to influence plant fitness at small spatial scales (Antonovics and Ellstrand, 1984; Aarssen and Turkington, 1985; Crutsinger et al., 2008). For example, Aarssen and Turkington (1985) collected neighboring pairs of *Lolium perenne* L. and *Trifolium repens* L. from four different localities, planted them in all possible pairwise combinations, and found that each clover-genet-type generally produced more dry weight when grown with the grass-genet-associate with which it was collected than with the grasses collected from other localities. Likewise, mixed-cytotype populations (i.e., in which both cytotypes occur in proximity) may have more balanced competitive abilities than single-ploidy populations (i.e., populations that contain only one cytotype) because in a population where polyploids are able to establish there may be increased selection on diploids to have more balanced competitive abilities with tetraploids in order to coexist. Measuring competitive differences between cytotypes in both mixed- and single-cytotype populations will allow us to determine whether the same biotic specialization occurs at the cytotype level.

An ideal species for examining the extent of competition differences between cytotypes and whether these competitive differences change between single- and mixed-cytotype populations is *Centaurea stoebe* L. (Asteraceae), or spotted knapweed. *Centaurea stoebe* is native to Europe, where it occurs in two cytotypes, namely diploids ($2n = 18$) and tetraploids ($2n = 36$), and is highly invasive in North America, where so far it has been found to occur in only the tetraploid form. In the native range, populations primarily exist as single-cytotype populations; however, some mixed-cytotype populations have been identified and are most common in Eastern Europe (Španiel et al., 2008; Treier et al., 2009; P. Mráz et al., unpublished data). In mixed-cytotype populations cytotypes are generally spatially separated and, thus, compete only along the diploid-tetraploid boundary (P. Mráz et al., unpublished data). Triploid hybrids are extremely rare and perform poorly (Mráz et al., 2011), which points to diploids and tetraploids being functionally isolated. On the basis of their recent morphometric analyses—which showed a clear separation of $2\times$ and $4\times$ plants—and further evidence from ecological and genetic studies, Mráz et al. (2011) propose to treat the two cytotypes as different species and, thus, confirm an earlier suggestion of Ochsmann (2000) to treat the two cytotypes as different taxonomic entities.

We used a greenhouse experiment to determine the small-scale competitive interactions between diploid and tetraploid cytotypes collected from both single- and mixed-cytotype populations of *C. stoebe* along a longitudinal gradient. We assessed the competitive ability of *C. stoebe* cytotypes by (1) measuring the extent of intra- and intercytotypic competition over short time scales and (2) measuring traits that have been shown to affect competitive ability over longer time scales (e.g., number of accessory rosettes, proportion of bolting plants in the first year). Specifically, we ask (1) whether tetraploids have greater competitive ability than diploids over short and long time scales, (2) whether cytotypes from mixed-cytotype populations have more balanced competitive abilities than those from single-cytotype populations, and (3) whether competitive interactions change along a longitudinal gradient. Tetraploids have been shown to produce greater aboveground biomass in the absence of competition (Ridenour et al., 2008). Thus, we hypothesize that tetraploids will be able to outcompete diploids when grown in competition with one another. Given that tetraploids are invasive in North America and have traits that allow for faster early growth and long-term persistence (i.e., polycarpy; Henery et al., 2010), we hypothesize that tetraploid cytotypes will also be stronger competitors than diploids over longer time scales. Furthermore, we expect that tetraploids collected from single-cytotype populations may have greater competitive ability than tetraploids collected from mixed-cytotype populations because tetraploids and diploids may have more balanced competitive interactions when they naturally co-occur. Finally, given that a large-scale study of the geographic distribution of the two cytotypes revealed that mixed-cytotype populations occurred more frequently in Eastern Europe (Treier et al., 2009; P. Mráz et al., unpublished data), we expect that the competitive differences between diploid and tetraploid single-cytotype populations may be larger in Western Europe, where they are less likely to co-occur.

MATERIALS AND METHODS

Seed material and plant propagation—To test how competitive ability differed between single- and mixed-cytotype populations of *C. stoebe*, we collected seed material from three climatically similar diploid and tetraploid population pairs and from three mixed-cytotype populations. Seed material for the single-cytotype populations was collected in September and October 2009 in Switzerland (SW and CH), Germany (DE), and Hungary (HU) (Table 1). Candidate diploid and tetraploid population pairs were determined using an outlier mean index (OMI) analysis based on data collected from populations surveyed by Treier et al. (2009). To be sure that the populations were similar, we revisited 17 candidate populations and at each site collected data on the landscape (i.e., land use, topography, and soil properties [soil depth, pH,

TABLE 1. Origin, location, and site descriptions of the *Centaurea stoebe* populations from which seed material was collected for the competition experiment.

| Origin | Region | Population | Cytotypes | Altitude (m) | Location (°N, °E) | Habitat type | Soil type |
|--------|-------------|------------|-----------|--------------|-------------------|-----------------------|-----------------|
| Single | Switzerland | SW2 | 2× | 927 | 46.13, 7.09 | Seminatural grassland | Sandy clay |
| | | CH1 | 4× | 519 | 47.28, 8.15 | Ruderal | Silty sand |
| | Germany | DE1 | 2× | 269 | 47.66, 7.55 | Ruderal | No information |
| | | DE4 | 4× | 227 | 49.17, 11.97 | Seminatural grassland | No information |
| | Hungary | H3 | 2× | 173 | 46.91, 17.33 | Managed field | Sand |
| Mixed | Slovakia | H2 | 4× | 180 | 47.11, 17.44 | Ruderal | Sand |
| | | SK6 | 2×/4× | 138 | 48.20, 16.97 | Seminatural meadow | Sand |
| | Slovakia | SK8 | 2×/4× | 133 | 48.08, 17.15 | Seminatural meadow | Gravel sediment |

Note: “Single” indicates populations in which only a single cytotype occurred, and “mixed” indicates populations in which diploids and tetraploids co-occurred.

CaCO₃, color, texture]) and the plant community (abundance, size, and percent cover of different strata and size of *Centaurea* population). We then narrowed the 17 populations down to 6 populations that would be used in the competition experiment (3 diploid and tetraploid pairs, 6 populations total) based on four criteria: population pairs had (1) similar *Centaurea* plant density, (2) similar *Centaurea* stem height, (3) similar scores in a principal component analysis that included all of our soil data collected in 2009, and (4) similar scores in an OMI analysis based on climate data (Treier et al., 2009). At each site, ≥ 25 seed heads were collected from 20 mother plants along a 25-m transect such that each mother plant was ≥ 1 m apart from the next.

Seed material for the mixed-cytotype populations was collected from three populations in Slovakia (SK6, SK8, W; Table 1). All currently identified mixed-cytotype populations occur in Eastern Europe so we could not include populations across the same longitudinal gradient as for the single-cytotype populations. In each of the three mixed-cytotype populations, seeds and leaf tissue were collected from ≥ 100 randomly selected mother plants that were ca. 1 m apart. Leaf tissue was assessed for ploidy level using flow cytometry (CY-S-1022 HR; Partec, Münster, Germany). Once ploidy could be successfully determined, we randomly selected seed material from 40 mother plants from each mixed-ploidy population (20 diploids and 20 tetraploids) to be used in the competition experiment.

One month prior to the start of the greenhouse experiment, 900 seeds (100 seeds per population) were sown into 2×2 cm seed trays and were watered every other day. The greenhouse conditions were set at 23°C:15°C (day:night), 16 h of light and natural humidity.

Competition experiment—The experiment was conducted in a greenhouse at the University of Fribourg, Switzerland (46.793°N, 7.156°E), from 1 March to 7 June 2010. In total, nine populations were used in the competition experiment (6 single-cytotype populations; i.e., 3 diploid–tetraploid pairs and 3 mixed-cytotype populations). For single-cytotype populations, the competition experiment only examined the competitive interactions between climatically similar pairs of populations. For example, for Switzerland, only cytotypes from CH1 (tetraploid) and SW2 (diploid) were used to establish the competition treatments. Accordingly, the competition treatments for mixed-cytotype populations were established such that only diploid and tetraploid plants that originated from the same mixed-cytotype population were placed in competition with one another.

In late February 2010, keeping density constant at two seedlings per pot, seedlings were placed into one of four competition treatments: (1) diploid monoculture, (2) diploid mixture, (3) tetraploid monoculture, or (4) tetraploid mixture (“monoculture” refers to both plants being of the same cytotypic, and “mixture” refers to each plant being of a different cytotypic). We measured only one plant in each pot (which we refer to as the “target” plant) for all growth and biomass measures. Thus, the composition of the mixed-diploid and mixed-tetraploid treatments were identical; however, in the mixed-diploid treatment we measured only the diploid plant and in the mixed-tetraploid treatment we measured only the tetraploid plant to achieve independence between measurements for our statistical analyses. Using the target plants, we were able to test for the extent of intra- and intercytotypic competition, whereby increased performance of monoculture competition treatment provides support for reduced intracytotypic competition and increased performance of the mixture competition treatment provides support for increased intercytotypic competition. Our design also allowed us to compare the growth of diploid and tetraploid target plants grown in mixture to determine which cytotypic had greater performance when placed in direct competition.

Seedlings were planted in 21 pots (13 cm diameter and 18 cm deep) with a 50:50 mixture (by weight) of TKS 1 potting soil (Floragard, Oldenburg, Germany) and sand. For both single- and mixed-cytotype populations, the pairs of seedlings used in each competition treatment were chosen at random using a random number table. The timing of seed germination was similar across all populations and, thus, all plants were similar in size at the time of planting. Analysis of variance (ANOVA) of the initial plant size measurement (length of longest leaf) taken 2 wk after planting revealed that there were no significant differences in plant size between ploidy levels ($F_{1,188} = 0.59$, $P = 0.44$).

Each competition treatment was replicated 10 times for each of the three single- and mixed-cytotype population pairs, resulting in a total of 240 pots. The experiment was spread out over five greenhouse benches with each treatment replicated twice per bench (48 pots per bench) and all pots were randomly assigned to a position on each bench. All plants that died within the first 2 wk after planting were assumed to have died from transplant shock and were replaced. No plants were replaced thereafter.

Each month, we measured the following traits for each target plant: survivorship, number of leaves, length of longest leaf, presence or absence of bolting, and presence or absence of accessory rosettes. After 14 wk, we harvested above- and belowground biomass of each target plant. Belowground biomass was washed and above- and belowground biomass was dried at 60°C for 48 h before being weighed. For belowground biomass, the fine roots were very difficult to harvest reliably because they were often intertwined with the other plant roots in the pot. Thus, we decided to measure only the main roots and taproot for each target plant.

Statistical analyses—Aboveground biomass and root:shoot ratio of target plants were analyzed using a linear mixed-effects model procedure (PROC MIXED) in SAS version 9.1.3 (SAS Institute, Cary, North Carolina, USA). In our model, “origin” refers to whether the seeds were collected from a single- or mixed-ploidy population and “region” refers to the particular pairs that were placed in competition with one another. For the single-cytotype populations this refers to the country of seed collection (CH, SW, DE, or HU) and for the mixed-cytotype populations this refers to the population of seed collection (SK6, SK8, or W). Origin, region, ploidy, competition treatment, and their interactions were treated as fixed effects, and population within origin was treated as a random effect. Residuals were checked for normality prior to the analysis using JMP version 8.0.2 (SAS Institute). Root:shoot ratio was square root transformed to achieve normality.

Survivorship, the presence or absence of flowering and accessory rosettes were analyzed using logistic regression in SAS (PROC LOGISTIC) with the same fixed effects and random effect as our linear mixed-effects model. With the exception of the survival analysis, if either the target plant or its competitor died during the course of the experiment it was excluded from the analysis.

To assess how competitive differences changed along a longitudinal gradient, we analyzed each sampling region separately using a linear mixed-effects model procedure in SAS (PROC MIXED). Ploidy, competition treatment, and their interaction were treated as fixed effects and population within origin was treated as a random effect.

RESULTS

Competitive ability over short time scales—There was a significant ploidy \times competition treatment interaction for aboveground biomass (Table 2). Tetraploid target plants produced significantly greater aboveground biomass when grown with a different cytotypic neighbor (mean \pm SE = 1.0 ± 0.062) than with a same genotype neighbor (0.77 ± 0.041), which indicates that intracytotypic competition was significantly greater than intercytotypic competition. There was no difference in aboveground biomass between diploid target plants when grown with a neighbor of a different (0.93 ± 0.047) or the same (0.92 ± 0.061) cytotypic. There was no difference in aboveground biomass between diploid and tetraploid target plants when grown in mixture.

There was no significant ploidy \times competition treatment interaction for root:shoot ratio and there was also no significant region \times ploidy \times treatment interaction for either aboveground biomass or root:shoot ratio, which indicates that cytotypes did not perform differently in competition treatments across the six regions that were sampled (Table 2).

There was a highly significant effect of region for both aboveground biomass and root:shoot ratio as well as a significant ploidy \times region interaction for aboveground biomass of target plants at final harvest ($F_{5,188} = 5.28$, $P < 0.01$; Table 2). Tetraploid plants tended to produce greater aboveground biomass than diploids in both the CH and DE regions; however, this result was statistically significant only for CH (Fig. 1). By contrast, in all other regions (HU, SK6, SK8, and W) diploid target plants tended to produce greater aboveground biomass than tetraploids, but this result was statistically significant only for the HU and SK8 region (Fig. 1).

TABLE 2. Analysis of variance results for aboveground biomass, and root:shoot ratio using diploid and tetraploid cytotypes of *Centaurea stoebe*.

| Source of variation | Numerator df | Denominator df | Aboveground biomass <i>F</i> | Root:shoot ratio <i>F</i> |
|------------------------------|--------------|----------------|------------------------------|---------------------------|
| Regions | 5 | 188 | 2.64* | 2.77* |
| Ploidy | 1 | 188 | 0.55 | 7.27** |
| Treatments | 1 | 188 | 6.16** | 6.04** |
| Region × ploidy | 5 | 188 | 5.28** | 1.76 |
| Ploidy × treatments | 1 | 188 | 7.03* | 0.080 |
| Region × treatments | 5 | 188 | 1.16 | 0.46 |
| Region × ploidy × treatments | 5 | 188 | 1.13 | 2.18 |

Notes: "Regions" refers to the 6 competition pairs that were used in the experiment (3 mixed-cytotype populations and 3 single-cytotype populations); "ploidy" refers to whether the plants were diploid or tetraploid; "treatments" refers to whether plants were grown in mixture (plants of different cytotypes) or monoculture (plants of the same cytotype). Seed origin (whether seeds were collected from single- or mixed-ploidy populations) was excluded from the model because we found no significant effect. * $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$.

There was no significant effect of ploidy level for aboveground biomass ($F_{1,188} = 0.55$, $P = 0.46$), but ploidy level had a significant effect on the root:shoot ratio of target plants at final harvest ($F_{1,188} = 7.27$, $P < 0.01$) (Table 2). Tetraploids allocated a greater proportion of their biomass to belowground roots than to shoots and, thus, had a higher root:shoot ratio than diploids (1.04 ± 0.029 for tetraploids vs. 0.93 ± 0.024 for diploids).

The survivorship of target plants across all treatments was high (95% surviving) and there was no difference in survivorship between ploidal level ($\chi^2 = 0.72$, $P = 0.40$) or the different regions that were used in the competition experiments ($\chi^2 = 2.63$, $P = 0.76$).

Competitive ability over longer time scales—Whether plants were grown with same or different cytotype neighbors had no significant effect on plant flowering and the production of accessory rosettes; however, there was a significant effect of ploidy level for both traits (Table 3). A higher proportion of the tetraploids than of the diploids flowered. On the final sampling date, 17 (15%) tetraploid target plants compared to only 6 (5%) diploid target plants had bolted and were in flower (Fig. 2A). Bolting was not only more frequent among tetraploids but also occurred earlier than in diploids. Forty-two days after transplanting, 13 tetraploid plants had bolted whereas only 3 diploid plants had bolted. Tetraploids also produced a greater number of accessory rosettes than diploids. Tetraploids had 85% more plants with at least one accessory rosette than diploids (Fig. 2B). There was no significant effect of region, competition treatment, or any interaction for both plant flowering and the production of accessory rosettes (Table 3).

Effect of origin and region of seed collection—Plants from single- and mixed-cytotype populations showed no significant difference in aboveground biomass of the target plants at final harvest ($F_{1,188} = 0.17$, $P = 0.69$) or, in fact, for any other trait. Thus, we removed this effect from the model and considered single- and mixed-cytotype populations simultaneously. Here, there was a highly significant effect of region for aboveground biomass ($F_{5,188} = 2.64$, $P < 0.05$). Target plants from the mixed-ploidy population W produced significantly more aboveground biomass than all other regions, with the exception of the single-cytotype population pair of HU. There was also a significant effect of region for the root:shoot ratio of target plants at final harvest (Table 2). The mixed-ploidy population SK6 had the greatest root:shoot ratio (1.12 ± 0.048) and was significantly greater than both other mixed-cytotype populations SK8 (0.95 ± 0.039) and W (0.91 ± 0.049). The single-ploidy region of CH

had the lowest root:shoot ratio and allocated the most resources toward shoots. This region also had the highest number of flowering plants compared with all other regions; however, this result was not statistically significant.

Competitive ability across a longitudinal gradient—Although we found no region × ploidy × treatment interaction, evidence of a strong region effect led us to examine the extent of intra- and intercytotypic competition within each region. With the exception of SK8, there was no significant difference in the aboveground biomass produced by diploid target plants when grown with a neighbor of the same or a different cytotype (Fig. 3). Thus, the extent of intra- and intercytotypic competition was equal across regions. SK8 was the only region where diploid target plants grew larger when planted with the same cytotype (inter > intracytotypic competition). Likewise, with the exception of the CH and DE regions, there was no significant difference in the aboveground biomass produced by tetraploid target plants when grown with a neighbor of the same or a different cytotype (intra = interspecific competition). The CH and DE regions had greater aboveground biomass when grown with a competitor of a different cytotype than when grown with a competitor of the same cytotype (intra > interspecific competition; Fig. 3A, B).

Interestingly, when we compare the performance of diploid and tetraploid target plants grown in mixture, all mixed-cytotype populations that were located in Slovakia (SK6, SK8, and W) had no significant difference in the aboveground biomass produced by diploid and tetraploid target plants (Fig. 3D–F). Likewise, the HU region (also located in Eastern Europe) had no significant difference in aboveground biomass between the mixed-cytotype treatments (Fig. 3C). Thus, intercytotypic competition was more balanced in Eastern Europe. By contrast, for the two Western European regions (CH and DE), tetraploid target plants that were grown in mixture produced significantly greater aboveground biomass than diploid target plants grown in mixture (Fig. 3A, B). Thus, tetraploids in these regions were stronger intercytotypic competitors.

DISCUSSION

Competitive ability over short and long time scales—Despite the recognition that different cytotypes can co-occur in the same population, few studies have quantified the small-scale competitive interactions that occur between cytotypes (cf. below). Our study shows that tetraploids of *C. stoebe* have traits that may make them better competitors over their diploid

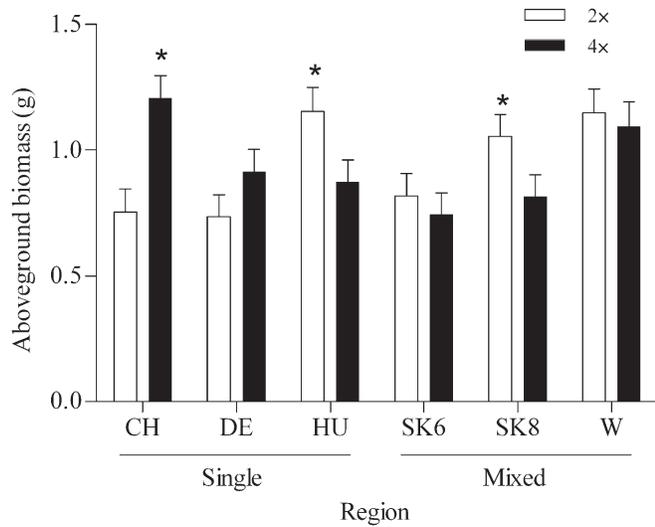


Fig. 1. Aboveground biomass of *Centaurea stoebe* diploids and tetraploids for each region of seed collection. “Mixed” refers to mixed-cytotype populations, in which diploid and tetraploid plants that co-occurred within the same population were placed in competition with one another (SK6, SK8, and W, Slovakia). “Single” refers to single-cytotype populations, in which climatically similar diploid and tetraploid populations from the same country were placed in competition with one another (CH, Switzerland; DE, Germany; HU, Hungary). Error bars indicate SEs and asterisks indicate significant differences ($P < 0.05$) between diploid and tetraploid pairs.

progenitors over longer time scales and, to a lesser extent, over shorter time scales. We measured competitive ability over short time scales by determining the extent of intra- and intercytotypic competition as measured by both aboveground biomass and root : shoot ratio. Plant aboveground biomass and root:shoot ratio have both been found to be suitable indicators of plant competitive ability, especially during early growth (Gaudet and Keddy, 1988). In competition treatments, tetraploids produced significantly greater aboveground biomass when planted in mixture than in monoculture (intra- > intercytotypic competition), whereas diploids produced equal aboveground biomass in both treatments (intra = intercytotypic competition; significant ploidy \times treatment interaction; Table 2). Diploids were more affected by intercytotypic competition than tetraploids, but this difference was not significant, which indicates that competitive differences between diploids and tetraploids across regions may be small enough that diploids and tetraploids can

TABLE 3. Logistic regression results of *Centaurea stoebe* for plant flowering and the presence of at least one accessory rosette.

| Source of variation | df | Plant flowering | | Accessory rosettes | |
|----------------------------|----|-----------------|--------------|--------------------|---------------|
| | | Wald χ | $P < \chi$ | Wald χ | $P < \chi$ |
| Regions | 5 | 5.45 | 0.36 | 8.33 | 0.14 |
| Ploidy | 1 | 4.60 | 0.032 | 7.07 | 0.0078 |
| Treatments | 1 | 2.83 | 0.093 | 0.53 | 0.47 |
| Ploidy \times treatments | 1 | 0.086 | 0.77 | 0.53 | 0.47 |

Notes: “Regions” refers to the 6 competition pairs that were used in the experiment (3 mixed-cytotype populations and 3 single-cytotype populations); “ploidy” refers to whether the plants were diploid or tetraploid; “treatments” refers to whether plants were grown in mixture (plants of different cytotypes) or monoculture (plants of the same cytotype).

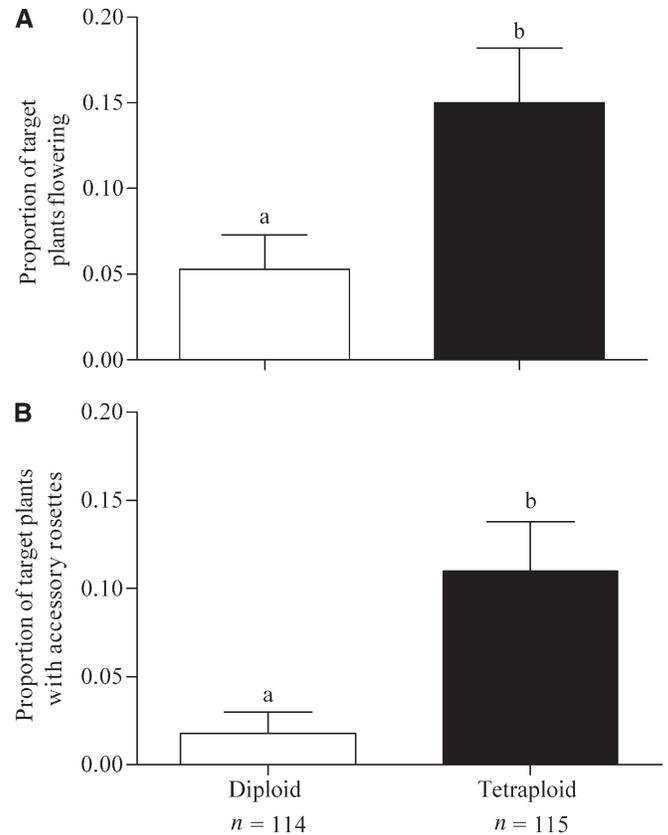


Fig. 2. Proportion of diploid and tetraploid *Centaurea stoebe* plants that were (A) flowering and (B) had at least one accessory rosette after 14 wk of cultivation. Different letters indicate statistical differences at the level of $P < 0.05$.

coexist. This is in contrast to the results obtained by Maceira et al. (1993), who examined the competitive interactions between two cytotypes of *Dactylis glomerata* and found that tetraploids had greater competitive ability than diploids and began to competitively exclude diploids over the course of only 2 yr.

Across competition treatments, tetraploids did not produce significantly more aboveground biomass than diploids. This is in contrast to a number of studies that have found that polyploids produce significantly greater aboveground biomass than diploids such as *Solidago gigantea* (Schlaepfer et al., 2010) and *Arrhenatherum elatius* (Petit and Thompson, 1997) over longer cultivation periods; however, it is consistent with the results of a study by Henery et al. (2010), who found no difference in aboveground biomass between diploid and tetraploid cytotypes of *C. stoebe* after 3 mo of growth. Tetraploids did, however, allocate a greater proportion of their biomass to belowground roots than to shoots. Schlaepfer et al. (2010) found a similar result for *Solidago gigantea*, in which North American tetraploids produced significantly more belowground rhizomes than North American diploids. Larger root systems can confer greater overall competitive ability in the presence of both root and shoot interactions (e.g., Gaudet and Keddy, 1988; Aerts et al., 1991) and, thus, may increase the competitive ability of tetraploids over diploids in both the short and the long term.

We measured competitive ability over longer time scales by measuring whether target plants bolted, whether they flowered, and whether they produced accessory rosettes. Tetraploid target

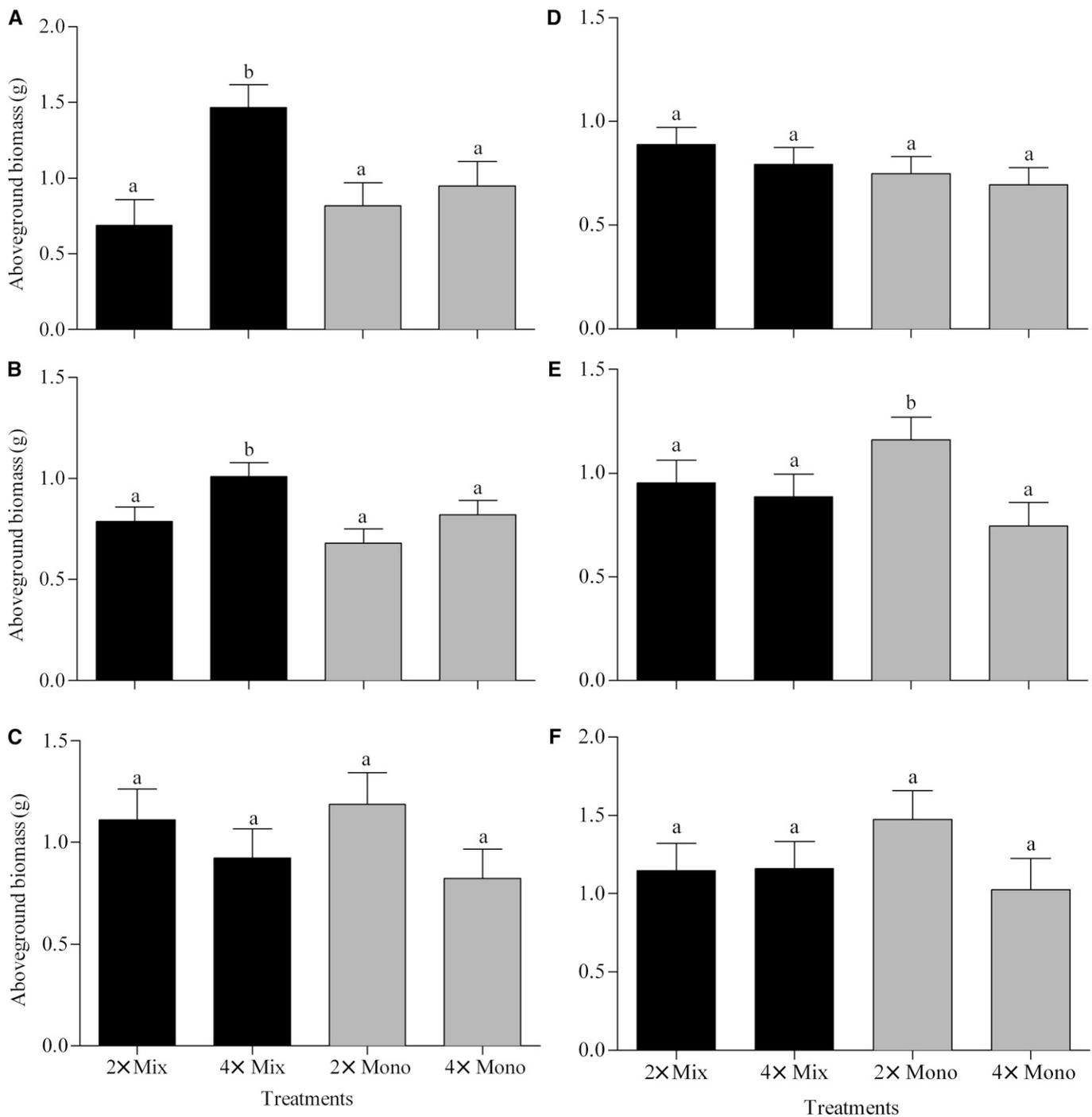


Fig. 3. Aboveground biomass of *Centaurea stoebe* by region for single-cytotype populations (A) CH, (B) DE, and (C) HU and mixed-cytotype populations (D) SK6, (E) SK8, and (F) W grown under different competition treatments at final harvest (after 14 wk). Competition treatments refer to whether target plants were grown with a competitor of a different (2x Mix and 4x Mix, black bars) or the same (2x Mono and 4x Mono, gray bars). Error bars indicate SE, and different letters indicate statistical differences ($P < 0.05$).

plants bolted earlier, flowered more often, and produced more accessory rosettes than diploid target plants, regardless of the competition treatment in which they were planted. Thus, the traits we measured for long-term persistence may overcome any effects of competition. Accessory rosette formation after flowering can be used as a proxy for perenniality (Henery et al., 2010). Therefore, our results also provide support for the

pronounced shift in life cycle between diploid and tetraploid cytotypes of *C. stoebe*, in which diploid cytotypes tend to have a monocarpic biennial life cycle and tetraploid cytotypes have a polycarpic life cycle (Boggs and Story, 1987; Müller, 1989; Ochsmann, 2000; Story et al., 2001; Španiel et al., 2008; Treier et al., 2009; Henery et al., 2010; Mráz et al., 2011). A polycarpic life cycle may ensure greater persistence and longevity

of tetraploid cytotypes because of a greater reproductive capacity (Broz et al., 2009; Treier et al., 2009; Henery et al., 2010; Mráz et al., 2011). Our results are also in accordance with studies that have found that tetraploid cytotypes of *C. stoebe* bolt earlier and more frequently than diploid cytotypes (Mráz et al., 2011; Treier et al., 2009). Earlier bolting may allow tetraploids to outcompete diploids by producing tall flowering stems that can intercept more light and easily shade out diploid rosettes. Greater performance of traits associated with competitive ability and long-term persistence may allow tetraploids to establish and outcompete diploids (Thébault et al., 2011) despite their initial minority cytotype disadvantage.

Effect of origin and region of seed collection—Contrary to our prediction, separate and mixed-cytotype populations did not show any significant difference in performance and competitive interactions. We had expected that mixed-cytotype populations, particularly if we assume that they are stable, might have more balanced competitive interactions to allow both diploid and tetraploid cytotypes to coexist at small spatial scales. This would have been visible in a significant treatment \times origin effect.

We found that target plants produced equivalent above-ground biomass in all mixed-cytotype populations, with the exception of SK8, where diploid target plants produced significantly greater above-ground biomass when grown in monoculture (Fig. 3). Our data suggest that sampling region is more important than population origin and that competitive differences between diploid and tetraploid cytotypes may change along a longitudinal gradient, though greater replication across the longitudinal gradient is needed. Differences in performance between diploid and tetraploid cytotypes when grown in mixture were larger in Western Europe (CH, DE regions) than in Eastern Europe (HU, SK6, SK8, W). A reduction or lack of competitive differences when grown in mixture in Eastern Europe may increase the ability for both cytotypes to coexist, whereas the competitive superiority of tetraploids in Western Europe may lead to the competitive exclusion of diploids. This prediction is partially supported by a large-scale study of the geographic distribution of diploid and tetraploid cytotypes of *C. stoebe* across Europe: of the 236 populations sampled and analyzed thus far (Treier et al., 2009; P. Mráz et al., unpublished data), only 13 (or 5.5%) were found to be mixed-cytotype populations, and all of those populations were located in Eastern Europe. No mixed-cytotype populations have been found west of Austria thus far. Therefore, mixed-cytotype populations may establish in Western Europe but are most likely transient, such that introduced tetraploids will eventually outcompete resident diploids. Tetraploids are thought to have originated in Southeastern Europe (the Balkans, Ukraine, and southeastern Russia) and have since spread across Western Europe (Ochsmann, 2000). Thus, the extended period of potential co-occurrence of the cytotypes in Eastern Europe may have selected for increased competitiveness in diploids, because the diploid progenitors are expected to be one of the primary competitors of novel tetraploids. This may explain the more balanced intercytotypic interactions observed in the East but not in the West (Figs. 1 and 3).

To our knowledge, the present study is the first to examine the small-scale competitive interactions between the two cytotypes of *C. stoebe* in the native range. A better understanding of how differences in competitive ability between cytotypes will influence mixed-cytotype populations in the field will require manipulating both plant density and plant size. Furthermore, demographic models examining cytotype growth rate under

different competitive neighborhoods will lend insight to the stability of mixed-cytotype populations over longer time scales. Nonetheless, our work provides an important contribution to the study of mixed-cytotype populations by showing that competitive differences between diploid and tetraploid cytotypes may allow tetraploids to establish despite their initial minority disadvantage and that the competitive interactions between cytotypes are not consistent across the native range but rather may change along a longitudinal gradient. Future work should investigate the competitive interactions between cytotypes under field conditions and between the native and invasive ranges. In this way, we could determine how different cytotypes respond to their conspecific neighbors, particularly over longer time scales, and gain insights into how competitive interactions may change during the invasion process.

LITERATURE CITED

- AARSSSEN, L. W., AND R. TURKINGTON. 1985. Biotic specialization between neighboring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *Journal of Ecology* 73: 605–614.
- AERTS, R., R. G. A. BOOT, AND P. J. M. VANDERAART. 1991. The relation between aboveground and belowground biomass allocation patterns and competitive ability. *Oecologia* 87: 551–559.
- ANTONOVICS, J., AND N. C. ELLSTRAND. 1984. Experimental studies of the evolutionary significance of sexual reproduction. 1. A test of the frequency-dependent selection hypothesis. *Evolution* 38: 103–115.
- BAACK, E. J. 2004. Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). *American Journal of Botany* 91: 1783–1788.
- BOGGS, K. W., AND J. M. STORY. 1987. The population age structure of Spotted Knapweed (*Centaurea Maculosa*) in Montana. *Weed Science* 35: 194–198.
- BRETAGNOLLE, F., AND J. D. THOMPSON. 1996. An experimental study of ecological differences in winter growth between sympatric diploid and autotetraploid *Dactylis glomerata*. *Journal of Ecology* 84: 343–351.
- BROZ, A. K., D. K. MANTER, G. BOWMAN, H. MÜLLER-SCHÄRER, AND J. M. VIVANCO. 2009. Plant origin and ploidy influence gene expression and life cycle characteristics in an invasive weed. *BMC Plant Biology* 9: 33.
- CRUTSINGER, G. M., L. SOUZA, AND N. J. SANDERS. 2008. Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters* 11: 16–23.
- FELBER, F. 1991. Establishment of a tetraploid cytotype in a diploid population—Effect of relative fitness of the cytotypes. *Journal of Evolutionary Biology* 4: 195–207.
- FELBER-GIRARD, M., F. FELBER, AND A. BUTTLER. 1996. Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*. *New Phytologist* 133: 531–540.
- FOWLER, N. L., AND D. A. LEVIN. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *American Naturalist* 124: 703–711.
- FRIDLEY, J. D., J. P. GRIME, AND M. BILTON. 2007. Genetic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species-rich grassland. *Journal of Ecology* 95: 908–915.
- GAUDET, C. L., AND P. A. KEDDY. 1988. A comparative approach to predicting competitive ability from plant traits. *Nature* 334: 242–243.
- HENERY, M. L., G. BOWMAN, P. MRÁZ, U. A. TREIER, E. GEX-FABRY, U. SCHAFFNER, AND H. MÜLLER-SCHÄRER. 2010. Evidence for a combination of pre-adapted traits and rapid adaptive change in the invasive plant *Centaurea stoebe*. *Journal of Ecology* 98: 800–813.
- HUSBAND, B. C., AND D. W. SCHEMSKE. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *Journal of Ecology* 88: 689–701.
- JOHNSON, M. T. J., B. C. HUSBAND, AND T. L. BURTON. 2003. Habitat differentiation between diploid and tetraploid *Galax urceolata*

- (Diapensiaceae). *International Journal of Plant Sciences* 164: 703–710.
- KELLEY, S., AND K. CLAY. 1987. Interspecific competitive interactions and the maintenance of genotypic variation within 2 perennial grasses. *Evolution* 41: 92–103.
- LEVIN, D. A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.
- LEVIN, D. A. 1983. Polyploidy and novelty in flowering plants. *American Naturalist* 122: 1–25.
- MACEIRA, N. O., P. JACQUARD, AND R. LUMARET. 1993. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia—Implications for the establishment of novel polyploid populations. *New Phytologist* 124: 321–328.
- MEERTS, P. 1992. An experimental investigation of life-history and plasticity in 2 cytotypes of *Polygonum aviculare* L. susp. *aviculare* that coexist in an abandoned arable field. *Oecologia* 92: 442–449.
- MIRÁZ, P., R. BOURCHIER, U. TREIER, U. SCHAFFNER, AND H. MÜLLER-SCHÄRER. 2011. Polyploidy in phenotypic space and invasion context: A morphometric study of *Centaurea stoebe* s.l. *International Journal of Plant Sciences: in press*.
- MÜLLER, H. 1989. Growth-pattern of diploid and tetraploid Spotted Knapweed, *Centaurea maculosa* Lam (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L) (Lep, Cochyliidae). *Weed Research* 29: 103–111.
- OCHSMANN, J. 2000. Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. *Dissertationes Botanicae* 324.
- OTTO, S. P. 2007. The evolutionary consequences of polyploidy. *Cell* 131: 452–462.
- PETIT, C., AND J. D. THOMPSON. 1997. Variation in phenotypic response to light availability between diploid and tetraploid populations of the perennial grass *Arrhenatherum elatius* from open and woodland sites. *Journal of Ecology* 85: 657–667.
- RAMSEY, J., AND D. W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.
- RIDENOUR, W. M., J. M. VIVANCO, Y. L. FENG, J. HORIUCHI, AND R. M. CALLAWAY. 2008. No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. *Ecological Monographs* 78: 369–386.
- RODRIGUEZ, D. J. 1996. A model for the establishment of polyploidy in plants. *American Naturalist* 147: 33–46.
- SCHLAEFFER, D. R., P. J. EDWARDS, AND R. BILLETER. 2010. Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: A common garden comparison of ploidy levels. *Oecologia* 163: 661–673.
- SCHÖNSWETTER, P., M. LACHMAYER, C. LETTNER, D. PREHSLER, S. RECHNITZER, D. S. REICH, M. SONNLEITNER, ET AL. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* 120: 721–725.
- SILVERTOWN, J., AND D. CHARLESWORTH. 2001. Introduction to plant population biology. Blackwell Science, Oxford, United Kingdom.
- ŠPANIEL, S., K. MARHOLD, I. HODÁLOVÁ, AND J. LIHOVÁ. 2008. Diploid and tetraploid cytotypes of *Centaurea stoebe* (Asteraceae) in Central Europe: Morphological differentiation and cytotype distribution patterns. *Folia Geobotanica* 43: 131–158.
- STORY, J. M., L. SMITH, AND W. R. GOOD. 2001. Relationship among growth attributes of spotted knapweed (*Centaurea maculosa*) in western Montana. *Weed Technology* 15: 750–761.
- THÉBAULT, A., F. GILLET, H. MÜLLER-SCHÄRER, AND A. BUTTLER. 2011. Polyploidy and invasion success: Trait trade-offs in native and introduced cytotypes of two Asteraceae species. *Plant Ecology* 212: 315–325.
- TILMAN, D. 1982. Resource competition and community structure. *Monographs in Population Biology* 17.
- TILMAN, D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences, USA* 101: 10854–10861.
- TREIER, U. A., O. BROENNIMANN, S. NORMAND, A. GUISAN, U. SCHAFFNER, T. STEINGER, AND H. MÜLLER-SCHÄRER. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* 90: 1366–1377.
- TURKINGTON, R. 1979. Neighbour relationships in grass-legume communities. IV. Fine scale biotic differentiation. *Canadian Journal of Botany* 57: 2711–2716.
- VANDIJK, P., AND R. BIJLSMA. 1994. Simulations of flowering time displacement between 2 cytotypes that form invariable hybrids. *Heredity* 72: 522–535.
- VINKENOOG, R., C. BUSHELL, M. SPIELMAN, S. ADAMS, H. G. DICKINSON, AND R. J. SCOTT. 2003. Genomic imprinting and endosperm development in flowering plants. *Molecular Biotechnology* 25: 149–184.