Performance in the recruitment life stage and its potential contribution to invasive success in the polyploid invader *Centaurea stoebe*

Kevin Kožić[1,2], Matthias Hartmann[3], Ragan M. Callaway[4], Isabell Hensen[1,2], Dávid U. Nagy[1], Patrik Mráz[5], Mohammad M. Al-Gharaibeh[6], Svetlana Bancheva[7,8], Alecu Diaconu[9], Jiří Danihelka[10,11], David J. Ensing[12], Rita Filep[13], Zigmantas Gudžinskas[14], Avni Hajdari[15], Roxana Nicoară[16], Susanne Lachmuth[17], Chandra E. Moffat[12], Andriy Novikov[18], Dragica Purger[13], Mandy L. Slate[19], Agnieszka Synowiec[20], Ghizela D. Vonica[21], Annika M. Zuleger[1,2], Christoph Rosche[1,2]

1   Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
2   German Centre for Integrative Biodiversity Research (iDiv) Leipzig-Halle-Jena, Leipzig, Germany
3   Johann Heinrich von Thünen-Institute of Biodiversity, Braunschweig, 38116 Germany
4   Division of Biological Sciences and Institute on Ecosystems, University of Montana, Missoula, MT, USA
5   Herbarium and Department of Botany, Charles University, Prague, Czech Republic
6   Department of Plant Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan
7   Botanical Garden, Bulgarian Academy of Sciences, Sofia, Bulgaria
8   Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
9   "Stejarul" Research Centre for Biological Sciences, National Institute of Research and Development for Biological Sciences, Piatra Neamt, Romania
10 Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
11 Czech Academy of Sciences, Institute of Botany, Průhonice, Czech Republic
12 Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200, Summerland, BC, Canada
13 Institute of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Pécs, Hungary
14 Laboratory of Flora and Geobotany, Institute of Botany of the Nature Research Centre, Vilnius, Lithuania
15 Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina "Hasan Prishtina", Prishtinë, Kosovo
16 Institute of Biology - Bucharest, Romanian Academy, Bucharest, Romania
17 University of Maryland Center for Environmental Research, Appalachian Laboratory, Frostburg, USA
18 State Museum of Natural History of the NAS of Ukraine, Lviv, Ukraine
19 Department of Evolution, Ecology & Organismal Biology, The Ohio State University, Columbus, OH, USA
20 Department of Agroecology and Plant Production, University of Agriculture, Krakow, Poland
21 Brukenthal National Museum, Natural History Museum, Sibiu, Romania

Corresponding author: Kevin Kožić (k.kozic@web.de)

Abstract

The recruitment life stage, including germination and early seedling establishment, is the most vulnerable life stage of plants and has cascading effects on plant performance at later life stages. However, surprisingly little is known on the eco-evolutionary processes that determine the success of biological invasions at this life stage.

We performed germination experiments with and without simulated drought stress and monitored early seedling growth in diploid and tetraploid *Centaurea stoebe*. While diploids are the major cytotype in the native European range, only tetraploids became invasive in North America. Thus, *C. stoebe* is an excellent model species to simultaneously study both, pre-adaptive differences in the native range (diploids vs. tetraploids) and post-introduction evolution in the non-native range (native tetraploids vs. non-native tetraploids). To account for broad spatial-environmental variation within cytotypes and ranges, we germinated 23,928 seeds from 208 widely distributed populations.

Tetraploids germinated better than diploids. Within tetraploids, invasive populations outperformed native populations in germination. However, these differences were not evident under simulated drought stress. Seedlings of invasive tetraploids had a higher biomass and developed the first true leaf earlier than those from the native range, while the native cytotypes did not differ in these early seedling traits.

Our results suggest that a combination of pre-adaptation related to superior performance of polyploids (greater and faster germination) and post-introduction evolution towards higher performance in the invasive range (greater and faster germination, greater and faster accumulation of seedling biomass) may have contributed to the invasion success of tetraploid *C. stoebe* in North America.

Key words: Biological invasions, Cox-regression, geo-cytotypes, germination, pre-adaptation, post-introduction evolution, seedling establishment traits, spotted knapweed

Introduction

Germination and early seedling establishment comprise the recruitment life stage, which is likely the most vulnerable life stage of plants (Donohue et al. 2010; James et al. 2011; Winkler et al. 2024). Moreover, performance in the recruitment life stage has cascading influence on fitness, survival and competitive ability at later life stages (Donohue et al. 2010; Gioria and Pyšek 2017; D'Aguillo and Donohue 2023). Germination and early seedling establishment are particularly important for non-native plants because they are mostly introduced as seeds and their spread is often associated with effective dispersal and fast colonization of empty niches in ruderal habitats (Colautti et al. 2006; Pyšek and Richardson 2007; Theoharides and Dukes 2007). However, surprisingly little is known about the eco-evolutionary processes determining invasive success in the recruitment life stage (Jiménez-Alfaro et al. 2016; Saatkamp et al. 2019; Larson et al. 2020). In particular, we need more information on whether germination and early seedling traits exhibit 1) pre-adaptive differences between successful vs. non successful invaders (van Kleunen et al. 2010), and 2) differences between native vs. non-native populations due to post-introduction evolution (Parker et al. 2013).

With regard to pre-adaptation, polyploidy has been increasingly recognised as an advantage for invasion success because polyploid plants are more likely to become invasive than closely related diploids (Pandit et al. 2011; te Beest et al. 2012). Polyploids have, on average, higher competitive ability and fecundity than diploids (reviewed in Rutland et al. 2021) and they are also assumed to exhibit greater colonization success in ruderal environments (van Drunen and Johnson 2022). However, it remains unclear whether polyploidy confers benefits during the recruitment life stage, and if so, whether this may contribute to explaining the apparent overrepresentation of polyploids among invasive plants. The best models to test these relationships are polyploid complexes where the polyploid cytotype is more successful in the non-native range than its diploid relative (reviewed by te Beest et al. 2012).

Polyploids are also assumed to be better pre-adapted to stressful environments and show higher phenotypic variation than diploids (Chen 2007; Rice et al. 2019). More specifically, polyploids often exhibit faster germination under ambient conditions. This is especially important during range expansions, because fast germination and seedling growth could be advantageous to promote spread in ruderal habitats (Harper et al. 1970). At the same time, polyploids may show a more conservative germination behaviour than diploids under unfavourable conditions

(e.g., delaying germination while drought). Such conservative germination behaviour should increase their overall niche tolerance compared to diploids (Leitch and Leitch 2008; Stevens et al. 2020; Rutland et al. 2021) and ultimately increase survival probabilities of the emerging seedling (Saatkamp et al. 2019; Larson et al. 2020). However, studies on polyploid complexes in the context of biological invasions have not yet made this comparison. Therefore, we need to understand how germination differs between native and non-native populations under optimal and suboptimal conditions. With respect to post-introduction evolution, polyploidy may also enhance the potential for rapid evolutionary response to changing environmental conditions (Nagy et al. 2018; van Drunen and Johnson 2022). This is important for non-native species as they often experience climatic niche shifts (e.g., Bates and Bertelsmeier 2021) and encounter novel biotic interaction partners (e.g., Shah et al. 2014; Sheng et al. 2022) in their non-native ranges. Such altered environmental conditions may cause rapid post-introduction evolution, e.g., towards high-performance genotypes in the non-native ranges (Matesanz and Sultan 2013; Villasor et al. 2024), but information is lacking on whether this is also the case in the recruitment life stage (Nagy et al. 2024).

Finally, it is important to consider that populations from native and non-native ranges can show a pronounced among-population variation within either range (Rosche et al. 2019; Lucas et al. 2024). This variation should be associated with the population histories of the study populations such as the habitat type of source populations (e.g., between ruderal vs. (semi-)natural habitats) or may differ along climatic gradients such as aridity regimes in the population history (Baskin and Baskin 1998; Donohue et al. 2010; Al-Gharaibeh et al. 2024; Nagy et al. 2024). For example, more pronounced fluctuations in water availability in ruderal than (semi-)natural habitats may select for fast germination and seedling growth with a high investment in root mass allocation to quickly occupy open germination niches (Chapin et al. 1993; Larson et al. 2020). Nevertheless, most studies that investigated post-introduction evolutionary changes considered relatively few populations and underestimated variation within native and non-native ranges (reviewed by Lucas et al. 2024). Many studies have also examined different environmental gradients in native vs. non-native ranges, which may lead to false-positive differences between the ranges in the traits under investigation (reviewed by Colautti and Lau 2015). Similar to the study of post-introduction, assessing pre-adaptive differences between cytotypes requires a broad and representative sampling of populations within each cytotype (Rosche et al. 2018).

An example of a polyploid plant invader that shows evidence for post-introduction evolution is *Centaurea stoebe* L. (spotted knapweed; Asteraceae), a polyploid complex consisting of a diploid and a tetraploid cytotype. Both cytotypes are native to Europe, whereas only tetraploids are invasive in North America (Mráz et al. 2011). This cytotype shift between the ranges results in three so-called geo-cytotypes (GCTs): native European diploids (EU2x), native European tetraploids (EU4x) and non-native North American tetraploids (NA4x). Thus, *C. stoebe* is a suitable model to simultaneously test both pre-adaptive differences within the native range (EU2x vs. EU4x) and post-introduction evolution in the non-native range (EU4x vs. NA4x). Previous research found similar germination rates between the cytotypes (Hahn et al. 2013), but greater seedling root growth rates in tetraploids than diploids (Collins et al. 2013). Although these two studies provided important insights into the recruitment ecology of this polyploid complex,

they did not involve broad spatial-environmental gradients along the distribution ranges of the three GCTs, nor did they identify drivers of among-population variation within each GCT. There is also no information on whether the germination behaviour of GCTs differs across treatments, and there are no data on potential post-introduction evolution in early seedling traits between NA4x vs. EU4x.

Here, we studied germination and early seedling traits in 208 *Centaurea stoebe* populations that occupy a wide environmental variation in local aridity regimes across the three GCTs. We also differentiated between populations from ruderal vs. (semi-)natural habitats. We performed germination experiments with and without simulated drought stress and monitored early seedling development. Our study was directed by the following hypotheses: EU4x shows a greater performance in recruitment traits than EU2x which would indicate pre-adaptive differences to successfully invade North America; 2) NA4x outperforms EU4x in recruitment traits which would suggest evidence of post-introduction evolution in the non-native range; and 3) differences in recruitment traits can be explained by the population history (e.g., ruderal populations germinate faster than (semi-) naturals; mesic populations show higher germination than arid populations) which would identify drivers of among-population variation within the GCTs. By understanding these mechanisms, we expect to reveal factors that contribute to the successful invasion of tetraploid *C. stoebe* which could have broader implications for predicting future invasion dynamics. More generally, we anticipate to gain insights into the significance of the recruitment life stage in the context of biological invasions and how polyploidy can confer benefits during this life stage.

Materials and methods

Study species

Taxonomically, *Centaurea stoebe* L. is divided into two subspecies: the diploid *C. stoebe* subsp. *stoebe* ($2n$=18; 2x) and the tetraploid *C. stoebe* subsp. *micranthos* (Gugler) Hayek (2n=36; 4x). Tetraploids originated from allopolyploidization between diploids and a yet unknown, closely related, second parental species (Mráz et al. 2012a). In their sympatric range, diploids represent the major cytotype and prevail particularly in Central Europe, while tetraploids are more prevalent in South Eastern Europe (Broennimann et al. 2014; Rosche et al. 2016). The cytotypes have a similar morphology, but differ in their life cycle: diploids are short-lived and monocarpic, whereas tetraploids are perennial and polycarpic (Ochsmann 2000; Mráz et al. 2011). The increased longevity of tetraploids has been interpreted as an improved ability to colonize disturbed environments in comparison to diploids (Mráz et al. 2012b; Rosche et al. 2016, 2018). EU4x are thought to be pre-adapted to the drier summers in western North America than EU2x (Treier et al. 2009), because of characteristics like increased leaf dry matter content, reduced specific leaf area and faster root growth (Henery et al. 2010; Mráz et al. 2011; Collins et al. 2013). In contrast, other research found that drought tolerance in *C. stoebe* is more related to among-population variation within cytotypes than any general differences between the cytotypes (Collins et al. 2011; Mráz et al. 2014).

At the end of the 19th century, both cytotypes were likely introduced to North America, but only tetraploids established (Treier et al. 2009; Sun et al. 2016) and became a highly harmful invader causing immense ecological and economic damage

(Müller-Schärer 1991; van Driesche et al. 2002; Maron and Marler 2008). Their range expansion appeared to stimulate post-introduction evolutionary changes between EU4x and NA4x. Compared to EU4x, NA4x was shown to flower earlier (Hahn and Müller-Schärer 2013), have a more pronounced polycarpic life cycle (Henery et al. 2010), a higher competitive ability (Ridenour et al. 2008) and an increased population growth rate (Hahn et al. 2012). The increased population growth rate was related to an increased seed production (Hahn et al. 2012) and a higher seedling emergence (Hahn et al. 2013). In contrast to these findings supporting post-introduction evolution, phenotypic plasticity was similar between EU4x and NA4x (Hahn et al. 2012), and also leaf traits related to drought tolerance did not differ between but rather within EU4x and NA4x (Henery et al. 2010; Mráz et al. 2014).

Sampling

We sampled seeds from 208 populations (83 EU2x, 96 EU4x and 29 NA4x, Fig. 1, Suppl. material 1: table S1), covering broad spatial-environmental gradients in the sympatric native ranges of both cytotypes and also from both the western and eastern invasion routes of NA4x (sensu Broennimann et al. 2014). We collected
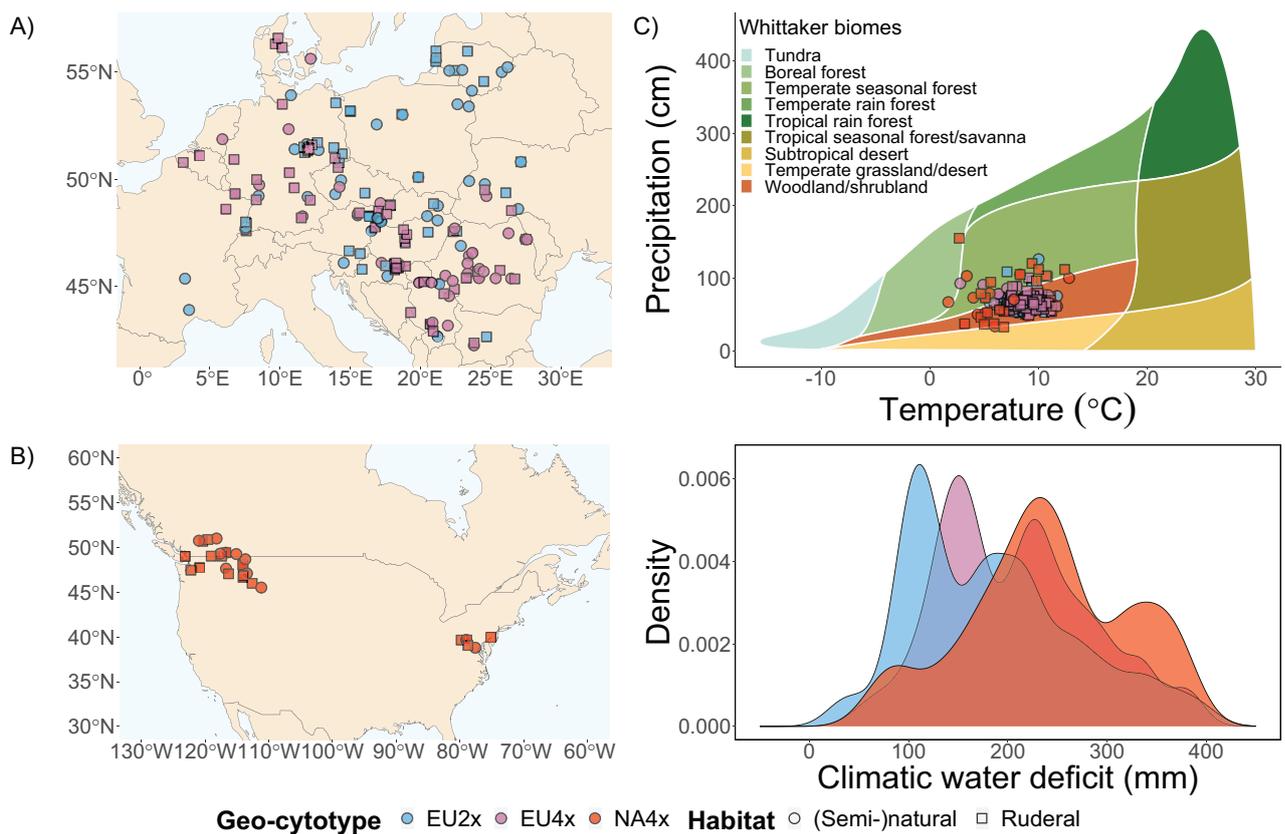
Figure 1. Overview of the sampled *Centaurea stoebe* populations across the three geo-cytotypes. The panels **A, B** show the geographical distribution of the populations across the native, European and the non-native, North American ranges, respectively. Circles represent populations from (semi-)natural habitats, whereas squares represent populations from ruderal habitats. In panel **C** populations are plotted according to their mean annual temperature and precipitation on a Whittaker diagram showing the classification of the main terrestrial biomes. Panel **D** shows the comparison of climatic water deficit among the three geo-cytotypes. Geo-cytotypes: native diploids (EU2x) = blue, native tetraploids (EU4x) = purple and non-native tetraploids (NA4x) = red. Climatic water deficit data was downloaded from the TerraClimate database (Abatzoglou et al. 2018). Annual precipitation and annual mean temperature data were downloaded from the CliMond database (Kriticos et al. 2012).

ripe capitula from < 20 individuals (seed families) distributed throughout every population with a minimum distance of 2 m between sampled individuals. To assess the cytotype of all populations, we pooled samples per population (one seed per seed family) and used flow cytometry following the same protocol as in Mráz et al. (2011). Seeds were stored at room temperature until the germination experiment was initiated in each autumn in the respective year of each sampling. Suppl. material 1: table S1 provides details on the sampling year and the number of seed families sampled within each population.

For each population, we distinguished between ruderal and (semi-)natural habitats following the methodology in Broennimann et al. (2014). Industrial and agricultural habitats were considered ruderal, whereas populations from rock outcrops, natural and semi-natural grasslands, natural and diluvial sediments were considered (semi-)natural. We sampled 46 (semi-)natural and 37 ruderal EU2x populations, 34 (semi-)natural and 62 ruderal EU4x populations and 11 (semi-)natural and 18 ruderal NA4x populations (Suppl. material 1: table S1). Population size was assessed by estimating the number of flowering individuals. To determine the local aridity patterns of sampled populations, we obtained seasonal data (1958–2022) on the climatic water deficit (CWD) from the TerraClimate dataset (Abatzoglou et al. 2018) at a spatial resolution of ca. 4 km². The CWD is calculated as the difference between the actual and potential evapotranspiration. From the obtained seasonal data, we calculated the annual mean CWD of each sampled population (Nagy et al. 2024).

Germination trials

We used only undamaged, healthy-looking seeds (e.g., black/brown coloured and well rounded). For each seed-family, 20 seeds were weighed using a fine scale. Ten of these 20 seeds were placed on a filter paper moistened with tap water in a 10–cm Petri dish (no replication of seed families within each treatment). Seeds were germinated in a growth chamber at 20:10 °C with a 12:12 h photoperiod; positions of Petri dishes were randomized daily. Germination was monitored daily over 14 days. Prolonging this germination trial may have resulted in further germination. However, previous studies on *C. stoebe* showed that the vast majority of seeds germinated by the end of this period (Hahn et al. 2013; Rosche et al. 2017). A seed was considered germinated once the radicle emerged and was removed from the Petri dish immediately after germination.

The remaining ten seeds per seed family were germinated under experimental drought. To simulate drought stress, we used D-Mannitol solution in place of water to moisten the filter paper and sealed Petri dishes with Parafilm®. Mannitol decreases osmotic pressure and therefore reduces the water availability, and is more stable than other osmotic agents such as polyethylene glycol (Thill et al. 1979). In order to identify a suitable drought treatment that would inhibit but not prevent germination, we randomly selected 97 seed families evenly distributed among the GCTs and germinated them for 14 days in tap water and in three different D-Mannitol solutions of -0.25 MPa (0.1 mol/l), -0.5 MPa (0.2 mol/l) and -1 MPa (0.4 mol/l). This preliminary experiment revealed that there was a significant difference only between the water and the -1 MPa treatment (F = 27.09, $p < 0.001$; Suppl. material 1: fig. S1). Thus, we applied two treatments in which the Petri dishes were either filled with tap water (hereafter referred to as 'mesic') or with a D-Mannitol solution of -1 MPa (hereafter referred to as 'dry').

Early seedling traits

To assess early seedling traits, we used the first seed per seed family that germinated from the mesic treatment. For each seed family, we transferred this seedling into a new Petri dish in the growth chamber (set up as described previously). At this point, we measured radicle length with ImageJ (Schneider et al. 2012). We then monitored the seedlings until the first sign of the first true leaf appeared. This developmental stage initiates the transition from auto- to heterotrophy (Harper 1977). We recorded the number of days from germination until the day of first true leaf appearance (DTL). We measured the radicle length again for root elongation rate (RER = (length at day of germination - length at day of first true leaf) / day of first true leaf). We then harvested the seedling, dividing it into above- and belowground biomass, dried at 60 °C for 48 h, and then calculated the root:shoot ratio (RSR = belowground biomass / aboveground biomass) and the relative growth rate (RGR = (belowground biomass + aboveground biomass) / day of first true leaf).

Statistical analysis

All statistical analyses were performed with R version 4.2.3 (R Core Team 2023). To assess germination probability through time, we used time-to-event analyses which are considered a powerful method for analysing germination data and meet more statistical assumptions than e.g., non-linear regression models (McNair et al. 2012; Romano and Stevanato 2020; Onofri et al. 2022). The time-to-event analyses based on mixed-effects Cox-regression models (Cox 1979), using the 'coxme' function of the R-package *coxme* ver. 2.2.18.1 (Therneau 2022a). These models apply Cox proportional hazards to identify significant predictors of the hazard ratio (HR). The HR indicates the probability to experience a germination event at every given point in time over the whole observation period compared to a reference group. Explanatory variables of our models were geo-cytotype (GCT) in interaction with habitat type and GCT in interaction with climatic water deficit (CWD). Our reference groups were: EU4x and (semi-)natural habitat type. We set population nested within year of germination trial as random effects. Furthermore, we included log_e-transformed seed mass and log_e-transformed population size as covariates to account for potential maternal and Allee effects, respectively. All numerical predictors were centred and scaled. We ran two separate mixed-effects models for both mesic and dry treatments. The statistical assumptions of the Cox proportional hazards models were checked based on Schoenfeld residuals and their graphical assessments (McNair et. al 2012). Moreover, we calculated the variance inflation factor to test for multicolinearity between our variables. We displayed the germination patterns using Kaplan-Meier curves (Kaplan and Meier 1992) with the 'survfit' function of the R-package *survival* ver. 3.5.3 (Therneau 2022b).

To identify significant predictors of germination success at the end of the 14-day period (binomial, cumulative values over population means), we used generalized linear mixed-effects models. We used the same explanatory variables as for the mixed-effects Cox-regression models, setting population nested within year of germination trial as random effects. Again, we ran two separate models for both mesic and dry treatments.

For the early seedling traits and the seed mass, we used linear mixed-effects models analysing the following traits as response variables: log_e-transformed RGR, log_e-transformed RER, log_e-transformed DTL, untransformed RSR and log_e-trans-

formed seed mass. Explanatory variables and random effects were identical to the generalized mixed-effect models on germination success. In all linear models, we assessed statistical significances of the predictors through stepwise backward model selection. The minimal adequate models were achieved using χ^2-tests in the R-package *lme4* ver. 1.1.32 (Bates et al. 2015).

Results

Seed mass

Seed mass differed among GCTs ($\chi^2 = 7.4$, p < 0.05). EU4x populations had higher seed mass than EU2x (p < 0.01, Suppl. material 1: table S2, fig. S2A) but there was no difference between NA4x and either native GCT (EU4x, EU2x). Moreover, seed mass was negatively correlated with CWD (p < 0.01, Suppl. material 1: fig. S2B).

Germination probability

We monitored germination probability – the chance of a germination event happening on successive days - across a total of 23,928 seeds (9,608 EU2x, 10,713 EU4x, 3,607 NA4x) from 208 *C. stoebe* populations. In the mesic treatment, germination patterns differed among geo-cytotypes (GCTs: $\chi^2 = 51.4$, $p < 0.001$, Fig. 2) with NA4x having an 86% greater germination probability compared to EU4x (HR = 1.86, $p < 0.001$, Fig. 2, Suppl. material 1: table S3) and EU2x having a 21% lower germination probability compared to EU4x (HR = 0.79, $p < 0.01$). However, the difference between the native cytotypes only occurred with seeds from (semi-)natural habitats ($p < 0.001$) but not with those from ruderal habitats ($p = 0.68$). Habitat type as a main effect also significantly affected germination probability ($\chi^2 = 33.8$, $p < 0.001$): overall, seeds from ruderal habitats showed 45% higher germination probability than those from (semi-)natural habitats (HR = 1.45). Furthermore, the Cox-regression model revealed that increasing seed mass affected germination probability positively (HR = 1.26, $\chi^2 = 49.5$, $p < 0.001$) while increasing climatic water deficit (CWD) had a negative effect (HR = 0.85, $\chi^2 = 8.7$, $p < 0.01$) There was also an interaction between GCT and CWD ($\chi^2 = 24.6$, $p < 0.001$), indicating that the relationship between germination probability and CWD was most pronounced in EU2x.

In the dry treatment, the germination probability of all GCTs declined (Fig. 2) and there was no difference among GCTs anymore ($\chi^2 = 3.4$, $p = 0.18$). However, germination was again affected by habitat type ($\chi^2 = 9.1$, $p < 0.01$): seeds from ruderal habitats had a 44% increased germination probability compared to seeds from (semi-)natural habitats (HR = 1.44). Still, seed mass affected germination probability positively ($\chi^2 = 36.9$, $p < 0.001$) but to a higher extent than in the mesic treatment (HR = 1.64). In contrast to the mesic treatment, the germination probability was no longer related to CWD ($\chi^2 = 0.1$, $p = 0.81$). However, population size affected germination probability positively (HR = 1.19, $\chi^2 = 6.8$, $p < 0.01$).

Total germination

In the mesic treatment, total germination differed among GCTs ($\chi^2 = 405.0$, $p < 0.001$; Fig. 3, Suppl. material 1: table S4) with NA4x seeds showing higher total germination than EU4x, whereas EU2x had lower total germination than
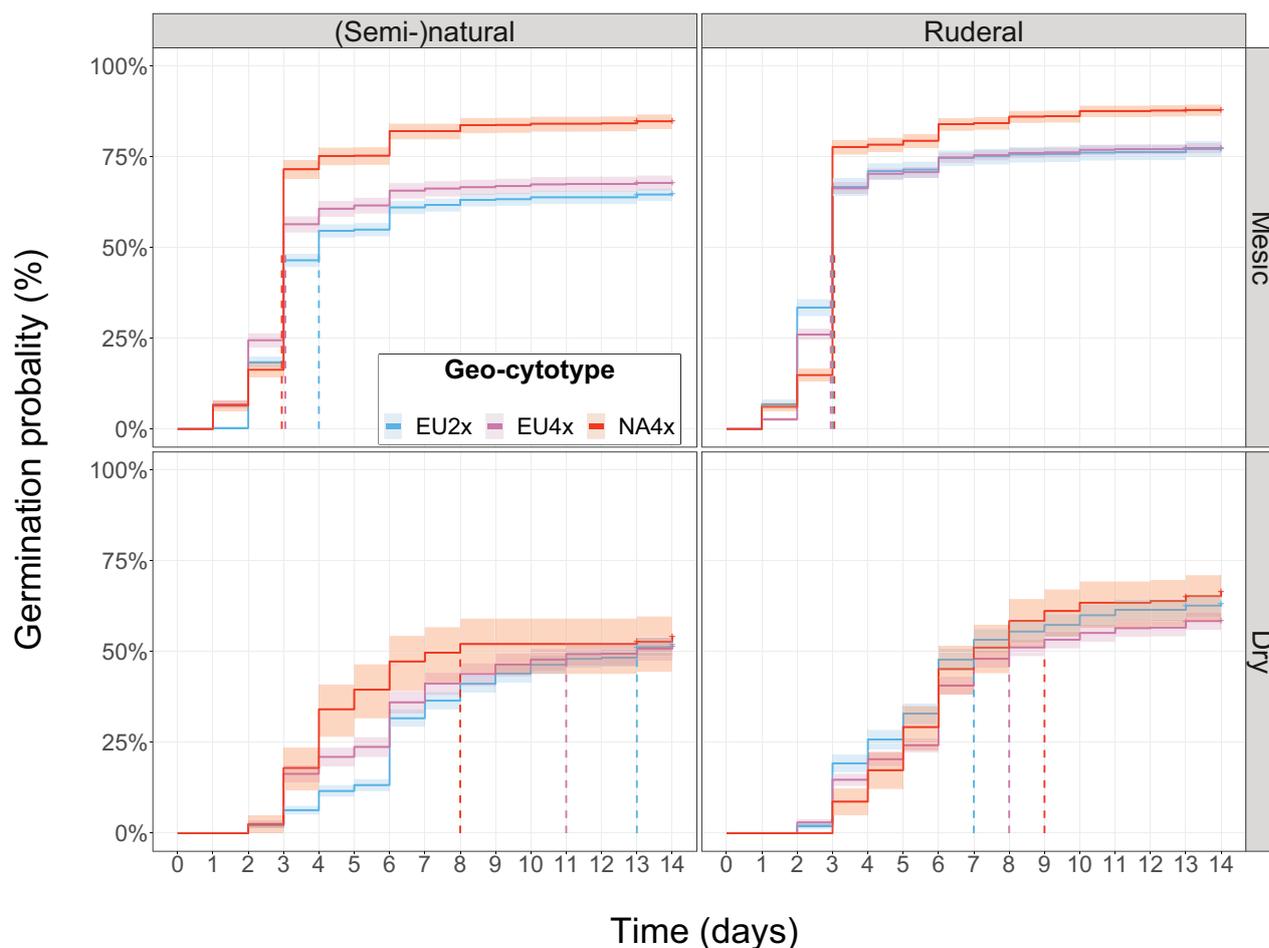
Figure 2. Kaplan-Meier curves of germination probability and their 95% confidence intervals for the three geo-cytotypes of *Centaurea stoebe* in (semi-)natural (left panels) and ruderal (right panels) habitats. Kaplan-Meier curves are presented for the mesic (upper panels) and the dry treatment (lower panels). Geo-cytotypes: native-range diploids (EU2x) = blue, native-range tetraploids (EU4x) = purple and non-native range tetraploids (NA4x) = red. The dotted lines represent the day at which 50% of the seeds that did germinate had germinated.

EU4x. There was also a difference between habitat types ($\chi^2 = 57.0$, $p < 0.001$). Populations from ruderal habitats had higher total germination success than those from (semi-)natural habitats. Moreover, CWD was negatively correlated with total germination ($\chi^2 = 308.2$, $p < 0.001$) and there was a significant interaction between GCT and CWD ($\chi^2 = 11.9$, $p < 0.01$), with the effect of CWD on germination more pronounced for EU2x than for the other two GCTs. Seed mass ($\chi^2 = 138.0$, $p < 0.001$) had a positive effect on total germination while population size had no effect ($\chi^2 = 0.53$, $p = 0.47$).

In the dry treatment, total germination again differed among GCTs ($\chi^2 = 20.8$, $p < 0.001$), but in contrast to the mesic treatment, total germination was comparable between EU4x and NA4x, whereas EU2x showed greater total germination than EU4x. Again, total germination differed between habitat types ($\chi^2 = 33.8$, $p < 0.001$) with populations from ruderal habitats showing greater germination success. Contrary to the mesic treatment, CWD was positively correlated with total germination ($\chi^2 = 5.8$, $p < 0.05$) and the effect of CWD differed among GCTs ($\chi^2 = 29.9$, $p < 0.001$), with a positive relationship evident only for NA4x. Moreover, seed mass ($\chi^2 = 102.4$, $p < 0.001$) and population size ($\chi^2 = 9.9$, $p < 0.01$) were positively correlated with total germination under dry conditions.

Early seedling traits

There was no difference in any of the early seedling traits between EU2x and EU4x (Suppl. material 1: table S5). In contrast, NA4x developed their first true leaf earlier than both native GCTs (χ^2 = 13.29, $p < 0.01$, Fig. 4A) and had a higher RGR (χ^2 = 13.89, $p < 0.001$, Fig. 4B). Early seedling traits did not differ between habitat types except for RSR. Ruderal populations had higher RSR than (semi-)natural ones, regardless of GCT, (χ^2 = 4.79, $p < 0.05$, Suppl. material 1: fig. S4). Seed mass had a significant effect on some early seedling traits investigated (Suppl. material 1: table S5, fig. S4).
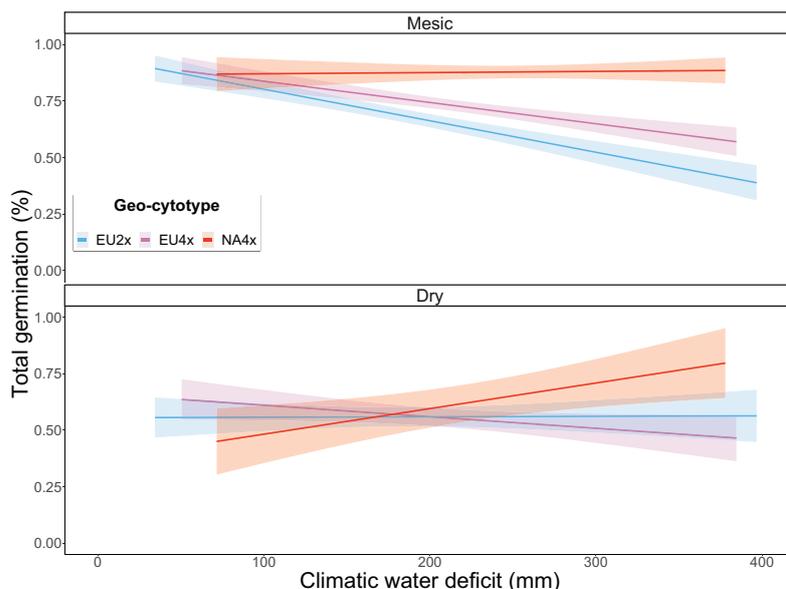
Figure 3. Relationship between climatic water deficit and total germination across 208 populations from the three geo-cytotypes of *Centaurea stoebe* in the mesic treatment (upper panel) and the dry treatment (lower panel). Geo-cytotypes: native diploids (EU2x) = blue, native tetraploids (EU4x) = purple and non-native tetraploids (NA4x) = red.
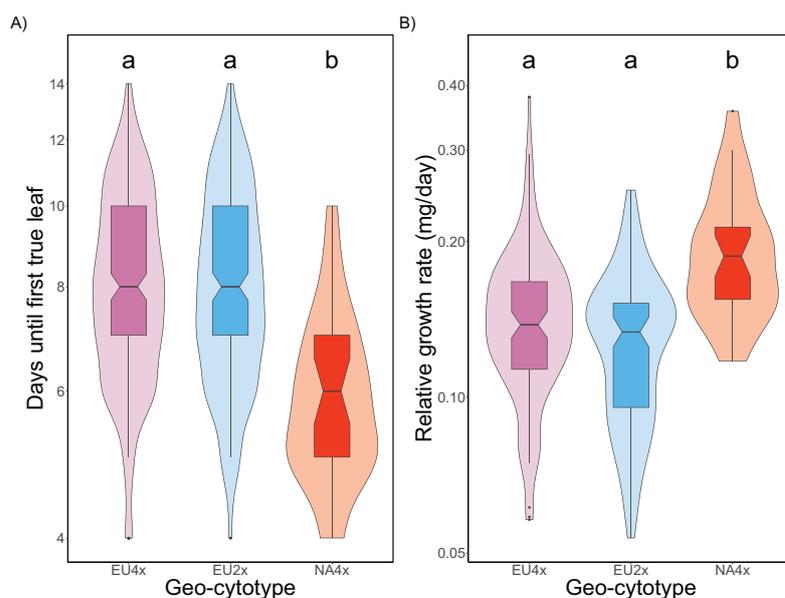
Figure 4. Differences among the three geo-cytoypes of *Centaurea stoebe* in early seedling traits. The panels **A, B** show the days needed to develop the first true leaf and the relative growth rate, respectively. Geo-cytotypes: native diploids (EU2x) = blue, native tetraploids (EU4x) = purple and non-native tetraploids (NA4x) = red. For model estimates see Suppl. material 1: table S5.

Discussion

Pre-adaptive differences between EU4x and EU2x

Under mesic conditions, EU4x seeds showed a higher germination probability through time and a greater total germination than seeds of EU2x. These findings contrast with previous results from Hahn et al. (2013) who did not find such differences between the cytotypes. We studied more populations covering broader environmental gradients than Hahn et al. (2013) and we used Cox-regression models instead of the linear models in Hahn et al. (2013). However, our results are consistent with other literature reporting that polyploidy can be associated with higher germination rates (e.g., Stevens et al. 2020; Selvi and Vivona 2021). This superior germination performance of polyploids has been linked to higher seed mass (Bretagnolle et al. 1995; Stevens et al. 2020). We indeed found that EU4x had heavier seeds than EU2x, and there was a strong, positive correlation between germination probability and seed mass, independent of GCT. Given the strong effects of germination success on population growth in founder populations of *C. stoebe* (Hahn et al. 2012), the superior germination performance of EU4x may display an important pre-adaptive advantage to colonize novel ranges compared with EU2x.

Under simulated drought, the superior germination performance of tetraploids disappeared. Instead, EU2x showed greater total germination than EU4x under dry conditions. Polyploids often shift their germination behaviour under unfavourable conditions (Hoya et al. 2007; Stevens et al. 2020; Rutland et al. 2021) and the seeds of tetraploid *C. stoebe* can remain viable in the seed bank for at least eight years (Davis et al. 1993). Delaying germination until better conditions may be thus a beneficial strategy for tetraploids colonizing non-native ranges with novel environmental conditions. Moreover, the positive effect of seed mass on germination probability almost tripled in the dry treatment, suggesting that seed size is more important under harsh conditions, which is consistent with evidence that heavier seeds cope better with environmental stress (Milberg and Lamont 1997; Paz and Martínez-Ramos 2003; Hallett et al. 2011; but see Yi et al. 2019).

Given the differences in germination probabilities and seed masses between the cytotypes, it is surprising that EU2x did not differ from EU4x in early seedling traits. Larger seeds are expected to result in faster seedling growth, especially in root mass allocation and seedling size (Bretagnolle et al. 1995; Kidson and Westoby 2000; Hallett et al. 2011; but see Norden et al. 2009; Tumpa et al. 2021). A previous study found that EU4x seeds performed better in all seedling traits they studied (Collins et al. 2013). However, they used five populations per cytotype and terminated their experiment after 16 days rather than in response to the developmental stage of seedlings as we did. A recent review demonstrated that such methodological differences can substantially affect the results of seedling trait studies (Winkler et al. 2024).

Post-introduction evolution between EU4x and NA4x

NA4x showed greater germination than EU4x, which contrasts with Hahn et al. (2013) who did not find such differences. Again, the contrasting results may be explained by the different study designs and analyses. In the dry treatment, however, the superior germination of NA4x over EU4x disappeared. This is surprising given the niche shift towards a drier climate tetraploids experienced during their

range expansion in North America (Broennimann et al. 2007). However, it may be possible that the novel conditions in the non-native range were selected for more conservative germination behaviour under drought.

Regarding early seedling traits, we found seedlings of NA4x to develop their first true leaf faster than those of EU4x while also accumulating more overall biomass in this shorter time period. This faster development and seedling growth is not only important because it marks the transition from auto- to heterotrophy (Harper 1977), but it may also increase seedling drought resistance (Larson et al. 2020) and might be advantageous regarding competitive interactions with resident species in the invaded range (Gioria et al. 2016). Indeed, NA4x seedlings were recently found to establish faster than resident species in the invaded plant communities in western USA (Slate et al. unpublished data). Considering the cascading effects of seedling performance on later life stages (Donohue et al. 2010; Gioria and Pyšek 2017), the faster seedling growth may also partly explain the common observation of increased adult plant performance of NA4x compared to EU4x populations under diverse common garden conditions (e.g., Blumenthal and Hufbauer 2007; Ridenour et al. 2008; He et al. 2010; Hahn and Müller-Schärer 2013). Such post-introduction evolution towards high performance genotypes in the non-native range is well-known for adult plant performance traits in numerous invaders (Parker et al. 2013) and could be a response to novel biotic and abiotic interactions in the non-native range (Bates and Bertelsmeier 2021; Sheng et al. 2022). However, so far this phenomenon could not be shown for the recruitment life stage. More experiments are needed to understand the eco-evolutionary processes selecting at this early life stage and if so, how this may affect adult plant growth.

Among-population variation within the three geo-cytotypes

The negative correlation between the climatic water deficit and germination probability could be due to reduced energy content of the seeds from dry populations, where mother plants likely faced drought stress during seed production (Hampton et al. 2013; Heinicke et al. 2016). However, in the dry treatment, total germination was positively affected by increasing climatic water deficit. Local adaptation in populations from drier regions may increase likelihood of germination under dry conditions (Godfree et al. 2017; Veselá et al. 2021; Gya et al. 2023). Seedling performance traits were not affected by climatic water deficit, although seedlings sampled from dry environments are assumed to show a more conservative growth, e.g., investing more into root than shoot biomass allocation (Larson et al. 2020; Gya et al. 2023). Because we did not investigate seedling performance traits in the dry treatment, our results rather explore the population level variability across CWD while future research is needed to understand seedling growth under experimental drought (Dhanda et al. 2004; Birkeli et al. 2023; Winkler et al. 2024).

The higher germination in ruderal than (semi-)natural habitats, indicates a selection toward genotypes that quickly occupy open niches in ruderal habitats whereas in (semi-)natural habitats there may be a selection for genotypes that show more conservative germination behaviour (Hufbauer et al. 2012). The higher root:shoot ratio in ruderal than (semi-)natural habitats may indicate adaptation to fluctuating water availability (Chapin et al. 1993). Ruderal habitats usually have less vegetation cover and tend to have greater evapotranspiration than (semi-)natural habitats (Bai et al. 2020). The significant differences we found in recruitment traits between hab-

itat types correspond to pronounced differences between ruderal vs. (semi-)natural *C. stoebe* populations in their adult plant growth performance (Rosche et al. 2018).

Our results support current concepts emphasizing that populations within native and non-native ranges (Colautti and Lau 2015) and within cytotypes (Rosche et al. 2018) may vary strongly in their population history. Such among-population variation within GCTs may be particularly pronounced for recruitment traits that are under strong natural selection (Baskin and Baskin 1998; Donohue et al. 2010; Cochrane et al. 2015). This means that the choice of populations can affect the outcome of experiments, particularly if habitat types and local aridity regimes were not sampled to similar extents among GCTs (Rosche et al. 2018; 2019). Moreover, with a view to ecological restoration, we strongly recommend choosing seed sources that correspond to the target habitat type to avoid maladapted germination and seedling growth behavior (Larson et al. 2020).

Conclusions

Our study is the first in the recruitment life stage to show that a biological invasion can be associated with a combination of pre-adaptive differences between cytotypes and post-introduction evolution of native and non-native populations. Because early seedling establishment is a crucial bottleneck in the life-cycle of plants and has cascading effects on all later individual performances, we call for further eco-evolutionary research at this life stage. Given that we recorded early seedling traits only under mesic conditions, we recommend that future research should investigate those traits also at suboptimal conditions. Furthermore, we emphasize that adaptive differentiation can be even more pronounced within than among GCTs. To avoid misleading conclusions from rather simplistic native vs. non-native or diploid vs. polyploid comparisons, future experimental studies should consider that not only the habitat type but also environmental gradients should be sampled to a broad and comparable extent between ranges and cytotypes.

Author contributions

C.R. and K.K. designed the study. K.K. wrote the manuscript with the help of C.R.. K.K. analyzed the data with the help of M.H. and C.R.. All authors contributed to the sampling, discussed the results and contributed to the final manuscript.

Author ORCIDs

Kevin Kožić https://orcid.org/0000-0003-4738-0242
Matthias Hartmann https://orcid.org/0000-0001-9721-0925
Dávid U. Nagy https://orcid.org/0000-0001-7742-4459
Patrik Mráz https://orcid.org/0000-0002-1415-070X
Mohammad M. Al-Gharaibeh https://orcid.org/0000-0001-9242-4262
Svetlana Bancheva https://orcid.org/0000-0001-7365-9971
Alecu Diaconu https://orcid.org/0000-0001-7030-4083
Jiří Danihelka https://orcid.org/0000-0002-2640-7867
David J. Ensing https://orcid.org/0000-0003-2903-1209
Rita Filep https://orcid.org/0000-0003-2777-3225
Zigmantas Gudžinskas https://orcid.org/0000-0001-6230-5924
Avni Hajdari https://orcid.org/0000-0001-5688-9679
Roxana Nicoară https://orcid.org/0000-0002-8191-5522
Susanne Lachmuth https://orcid.org/0000-0002-4027-7632
Chandra E. Moffat https://orcid.org/0000-0002-0357-9922
Andriy Novikov https://orcid.org/0000-0002-0112-5070
Dragica Purger https://orcid.org/0000-0003-2480-0777
Mandy L. Slate https://orcid.org/0000-0002-4026-7952
Agnieszka Synowiec https://orcid.org/0000-0001-6585-7759
Annika M. Zuleger https://orcid.org/0000-0003-4057-7595
Christoph Rosche https://orcid.org/0000-0002-4257-3072

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Abatzoglou JT, Dobrowski SZ, Parks SA, Hegewisch KC (2018) TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958–2015. Scientific Data 5(1): 170191. https://doi.org/10.1038/sdata.2017.191

Al-Gharaibeh MM, Bakhit HA, Masadeh S, Nagy D, Rosche C (2024) Resurrecting a locally extinct species from Jordan: Pre-Germination treatments affect seed dormancy, germination dynamics and seedling survival in *Rosa pulverulenta*. Global Ecology and Conservation 54: e03123. https://doi.org/10.1016/j.gecco.2024.e03123

Bai P, Liu X, Zhang Y, Liu C (2020) Assessing the impacts of vegetation greenness change on evapotranspiration and water yield in China. Water Resources Research 56(10): e2019WR027019. https://doi.org/https://doi.org/10.1029/2019WR027019

Baskin CC, Baskin JM (1998) Seeds: Ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-080260-9.X5000-3

Bates OK, Bertelsmeier C (2021) Climatic niche shifts in introduced species. Current Biology 31(19): R1252–R1266. https://doi.org/10.1016/j.cub.2021.08.035

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using Lme4. Journal of Statistical Software 67(1): 1–48. https://doi.org/10.18637/jss.v067.i01

Birkeli K, Gya R, Haugum SV, Velle LG, Vandvik V (2023) Germination and seedling growth of *Calluna vulgaris* is sensitive to regional climate, heathland succession, and drought. Ecology and Evolution 13(7): e10199. https://doi.org/10.1002/ece3.10199

Blumenthal DM, Hufbauer RA (2007) Increased plant size in exotic populations: A common-garden test with 14 invasive species. Ecology 88(11): 2758–2765. https://doi.org/10.1890/06-2115.1

Bretagnolle F, Thompson JD, Lumaret R (1995) The influence of seed size variation on seed germination and seedling vigour in diploid and tetraploid *Dactylis glomerata* L. Annals of Botany 76(6): 607–615. https://doi.org/10.1006/anbo.1995.1138

Broennimann O, Treier UA, Müller-Schärer H, Thuiller W, Peterson AT, Guisan A (2007) Evidence of climatic niche shift during biological invasion. Ecology Letters 10(8): 701–709. https://doi.org/10.1111/j.1461-0248.2007.01060.x

Broennimann O, Mráz P, Petitpierre B, Guisan A, Müller-Schärer H (2014) Contrasting spatio-temporal climatic niche dynamics during the eastern and western invasions of spotted knapweed in North America. Journal of Biogeography 41(6): 1126–1136. https://doi.org/10.1111/jbi.12274

Chapin FS III, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. American Naturalist 142: 78–92. https://doi.org/10.1086/285524

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annual Review of Plant Biology 58(1): 377–406. https://doi.org/10.1146/annurev.arplant.58.032806.103835

Cochrane A, Yates CJ, Hoyle GL, Nicotra AB (2015) Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography 24(1): 12–24. https://doi.org/10.1111/geb.12234

Colautti RI, Lau JA (2015) Contemporary evolution during invasion: Evidence for differentiation, natural selection, and local adaptation. Molecular Ecology 24(9): 1999–2017. https://doi.org/10.1111/mec.13162

Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: A null model for biological invasions. Biological Invasions 8(5): 1023–1037. https://doi.org/10.1007/s10530-005-3735-y

Collins AR, Naderi R, Müller-Schärer H (2011) Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (*Asteraceae*). American Journal of Botany 98(12): 1935–1942. https://doi.org/10.3732/ajb.1100063

Collins AR, Thalmann D, Müller-Schärer H (2013) Cytotypes of *Centaurea stoebe* found to differ in root growth using growth pouches. Weed Research 53(3): 159–163. https://doi.org/10.1111/wre.12010

Cox DR (1979) A note on the graphical analysis of survival data. Biometrika 66(1): 188–190. https://doi.org/10.1093/biomet/66.1.188

D'Aguillo M, Donohue K (2023) Changes in phenology can alter patterns of natural selection: The joint evolution of germination time and postgermination Traits. The New Phytologist 238(1): 405–421. https://doi.org/10.1111/nph.18711

Davis ES, Fay PK, Chicoine TK, Lacey CA (1993) Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. Weed Science 41(1): 57–61. https://doi.org/10.1017/S0043174500057581

Dhanda SS, Sethi GS, Behl RK (2004) Indices of drought tolerance in wheat genotypes at early stages of plant growth. Journal Agronomy & Crop Science 190(1): 6–12. https://doi.org/10.1111/j.1439-037X.2004.00592.x

Donohue K, de Casas RR, Burghardt L, Kovach K, Willis CG (2010) Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics 41(1): 293–319. https://doi.org/10.1146/annurev-ecolsys-102209-144715

Gioria M, Pyšek P (2017) Early bird catches the worm: Germination as a critical step in plant invasion. Biological Invasions 19(4): 1055–1080. https://doi.org/10.1007/s10530-016-1349-1

Gioria M, Pyšek P, Osborne BA (2016) Timing is everything: Does early and late germination favor invasions by herbaceous alien plants? Journal of Plant Ecology 11(1): 4–16. https://doi.org/10.1093/jpe/rtw105

Godfree RC, Marshall DJ, Young AG, Miller CH, Mathews S (2017) Empirical evidence of fixed and homeostatic patterns of polyploid advantage in a keystone grass exposed to drought and heat stress. Royal Society Open Science 4(11): 170934. https://doi.org/10.1098/rsos.170934

Gya R, Geange SR, Lynn JS, Töpper JP, Wallevik Ø, Zernichow C, Vandvik V (2023) A test of local adaptation to drought in germination and seedling traits in populations of two alpine forbs across a 2000 mm/year precipitation gradient. Ecology and Evolution 13(2): e9772. https://doi.org/10.1002/ece3.9772

Hahn MA, Müller-Schärer H (2013) Cytotype differences modulate eco-geographical differentiation in the widespread plant *Centaurea stoebe*. Ecology 94(5): 1005–1014. https://doi.org/10.1890/12-1393.1

Hahn MA, Buckley YM, Müller-Schärer H (2012) Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. Ecology Letters 15(9): 947–954. https://doi.org/10.1111/j.1461-0248.2012.01813.x

Hahn MA, Lanz T, Fasel D, Müller-Schärer H (2013) Increased seed survival and seedling emergence in a polyploid plant invader. American Journal of Botany 100(8): 1555–1561. https://doi.org/10.3732/ajb.1200540

Hallett LM, Standish RJ, Hobbs RJ (2011) Seed mass and summer drought survival in a Mediterranean-climate ecosystem. Plant Ecology 212(9): 1479–1489. https://doi.org/10.1007/s11258-011-9922-2

Hampton JG, Boelt B, Rolston MP, Chastain T (2013) Effects of elevated CO_2 and temperature on seed quality. Journal of Agricultural Science 151(2): 154–162. https://doi.org/10.1017/S0021859612000263

Harper JL (1977) Population biology of plants. Academic Press, London.

Harper JL, Lovell PH, Moore KG (1970) The shapes and sizes of seeds. Annual Review of Ecology and Systematics 1(1): 327–356. https://doi.org/10.1146/annurev.es.01.110170.001551

He WM, Thelen GC, Ridenour WM, Callaway RM (2010) Is there a risk to living large? large size correlates with reduced growth when stressed for knapweed populations. Biological Invasions 12(10): 3591–3598. https://doi.org/10.1007/s10530-010-9753-4

Heinicke S, Hensen I, Rosche C, Hanselmann D, Gudkova PD, Silanteva MM, Wesche K (2016) Fragmentation and environmental constraints influence genetic diversity and germination of *Stipa pennata* in natural steppes. Flora 224: 42–49. https://doi.org/10.1016/j.flora.2016.06.003

Henery ML, Bowman G, Mráz P, Treier UA, Gex-Fabry E, Schaffner U, Müller-Schärer H (2010) Evidence for a combination of pre-adapted traits and rapid adaptive change in the invasive plant *Centaurea stoebe*. Journal of Ecology 98(4): 800–813. https://doi.org/10.1111/j.1365-2745.2010.01672.x

Hoya A, Shibaike H, Morita T, Ito M (2007) Germination characteristics of native Japanese dandelion autopolyploids and their putative diploid parent species. Journal of Plant Research 120(1): 139–147. https://doi.org/10.1007/s10265-006-0034-3

Hufbauer RA, Facon B, Ravigné V, Turgeon J, Foucaud J, Lee CE, Rey O, Estoup A (2012) Anthropogenically induced adaptation to invade (AIAI): Contemporary adaptation to human-altered habitats within the native range can promote invasions. Evolutionary Applications 5(1): 89–101. https://doi.org/10.1111/j.1752-4571.2011.00211.x

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. Journal of Applied Ecology 48(4): 961–969. https://doi.org/10.1111/j.1365-2664.2011.02009.x

Jiménez-Alfaro B, Silveira FAO, Fidelis A, Poschlod P, Commander LE (2016) Seed germination traits can contribute better to plant community ecology. Journal of Vegetation Science 27(3): 637–645. https://doi.org/10.1111/jvs.12375

Kaplan EL, Meier P (1992) Nonparametric estimation from incomplete observations. In: Kotz S, Johnson NL (Eds) Breakthroughs in Statistics. Springer Series in Statistics. Springer, New York, 319–337. https://doi.org/https://doi.org/10.1007/978-1-4612-4380-9_25

Kidson R, Westoby M (2000) Seed mass and seedling dimensions in relation to seedling establishment. Oecologia 125(1): 11–17. https://doi.org/10.1007/PL00008882

Kriticos DJ, Webber BL, Leriche A, Ota N, Macadam I, Bathols J, Scott JK (2012) CliMond: Global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. Methods in Ecology and Evolution 3(1): 53–64. https://doi.org/10.1111/j.2041-210X.2011.00134.x

Larson JE, Anacker BL, Wanous S, Funk JL (2020) Ecological strategies begin at germination: Traits, plasticity and survival in the first 4 days of plant life. Functional Ecology 34(5): 968–979. https://doi.org/10.1111/1365-2435.13543

Leitch AR, Leitch IJ (2008) Genomic plasticity and the diversity of polyploid plants. Science 320(5785): 481–483. https://doi.org/10.1126/science.1153585

Lucas MS, Hensen I, Barratt CD, Callaway RM, Durka W, Lekberg Y, Nagy DU, Onstein RE, Shah MA, van Dam NM, Thoma AE, Rosche C (2024) Re-focusing sampling, design and experimental methods to assess rapid evolution by non-native plant species. Biological Invasions 26(5): 1327–1343. https://doi.org/10.1007/s10530-024-03249-x

Maron JL, Marler M (2008) Field-based competitive impacts between invaders and natives at varying resource supply. Journal of Ecology 96(6): 1187–1197. https://doi.org/10.1111/j.1365-2745.2008.01440.x

Matesanz S, Sultan SE (2013) High-performance genotypes in an introduced plant: Insights to future invasiveness. Ecology 94(11): 2464–2474. https://doi.org/10.1890/12-1359.1

McNair JN, Sunkara A, Frobish D (2012) How to analyse seed germination data using statistical time-to-event analysis: Non-parametric and semi-parametric methods. Seed Science Research 22(2): 77–95. https://doi.org/10.1017/S0960258511000547

Milberg P, Lamont BB (1997) Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. The New Phytologist 137(4): 665–672. https://doi.org/10.1046/j.1469-8137.1997.00870.x

Mráz P, Bourchier RS, Treier UA, Schaffner U, Müller-Schärer H (2011) Polyploidy in phenotypic space and invasion context: A morphometric study of *Centaurea stoebe* s.l. International Journal of Plant Sciences 172(3): 386–402. https://doi.org/10.1086/658151

Mráz P, Garcia-Jacas N, Gex-Fabry E, Susanna A, Barres L, Müller-Schärer H (2012a) Allopolyploid origin of highly invasive *Centaurea stoebe* s.l. (Asteraceae). Molecular Phylogenetics and Evolution 62(2): 612–623. https://doi.org/10.1016/j.ympev.2011.11.006

Mráz P, Španiel S, Keller A, Bowmann G, Farkas A, Šingliarová B, Rohr RP, Broennimann O, Müller-Schärer H (2012b) Anthropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. Annals of Botany 110(3): 615–627. https://doi.org/10.1093/aob/mcs120

Mráz P, Tarbush E, Müller-Schärer H (2014) Drought tolerance and plasticity in the invasive knapweed *Centaurea stoebe* s.l. (Asteraceae): Effect of populations stronger than those of cytotype and range. Annals of Botany 114(2): 289–299. https://doi.org/10.1093/aob/mcu105

Müller-Schärer H (1991) The impact of root herbivory as a function of plant density and competition: Survival, growth and fecundity of *Centaurea maculosa* in field plots. Journal of Applied Ecology 28(3): 759–776. https://doi.org/10.2307/2404206

Nagy DU, Stranczinger S, Godi A, Weisz A, Rosche C, Suda J, Mariano M, Pal RW (2018) Does higher ploidy level increase the risk of invasion? A case study with two geo-cytotypes of *Solidago gigantea* Aiton (Asteraceae). Journal of Plant Ecology 11(2): 317–327. https://doi.org/10.1093/jpe/rtx005

Nagy DU, Thoma AE, Al-Gharaibeh M, Callaway RM, Flory SL, Frazee LJ, Hartmann M, Hensen I, Jandová K, Damase PK, Lekberg Y, Pal RW, Samartza I, Shah MA, Sheng M, Slate M, Stein C, Tsunoda T, Rosche C (2024) Among-population variation in drought responses is consistent across life stages but not between native and non-native ranges. New Phytologist 243(3): 922–935. https://doi.org/10.1111/nph.19895

Norden N, Daws MI, Antoine C, Gonzalez MA, Garwood NC, Chave J (2009) The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. Functional Ecology 23(1): 203–210. https://doi.org/10.1111/j.1365-2435.2008.01477.x

Ochsmann J (2000) Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. Dissertationes Botanicae 324. Cramer in der Gebr.-Borntraeger-Verl.-Buchh, Berlin, Stuttgart.

Onofri A, Mesgaran MB, Ritz C (2022) A unified framework for the analysis of germination, emergence, and other time-to-event data in weed science. Weed Science 70(3): 259–271. https://doi.org/10.1017/wsc.2022.8

Pandit MK, Pocock MJO, Kunin WE (2011) Ploidy influences rarity and invasiveness in plants. Journal of Ecology 99(5): 1108–1115. https://doi.org/10.1111/j.1365-2745.2011.01838.x

Parker JD, Torchin ME, Hufbauer RA, Lemoine NP, Alba C, Blumenthal DM, Bossdorf O, Byers JE, Dunn AM, Heckman RW, Hejda M, Jarošík V, Kanarek AR, Martin LB, Perkins SE, Pyšek P, Schierenbeck K, Schlöder C, van Klinken R, Vaughn KJ, Williams W, Wolfe LM (2013) Do invasive species perform better in their new ranges? Ecology 94(5): 985–994. https://doi.org/10.1890/12-1810.1

Paz H, Martínez-Ramos M (2003) Seed mass and seedling performance within eight species of *Psychotria* (Rubiaceae). Ecology 84(2): 439–450. https://doi.org/10.1890/0012-9658(2003)084[0439:SMASPW]2.0.CO;2

Pyšek P, Richardson DM (2007) Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig W (Eds) Biological Invasions 193. Springer, Berlin, Heidelberg, 97–125. https://doi.org/https://doi.org/10.1007/978-3-540-36920-2_7

R Core Team (2023) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rice A, Šmarda P, Novosolov M, Drori M, Glick L, Sabath N, Meiri S, Belmaker J, Mayrose I (2019) The global biogeography of polyploid plants. Nature Ecology & Evolution 3(2): 265–273. https://doi.org/10.1038/s41559-018-0787-9

Ridenour WM, Vivanco JM, Feng Y, Horiuchi J, Callaway RM (2008) No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. Ecological Monographs 78(3): 369–386. https://doi.org/10.1890/06-1926.1

Romano A, Stevanato P (2020) Germination data analysis by time-to-event approaches. Plants 9(5): 617. https://doi.org/10.3390/plants9050617

Rosche C, Durka W, Hensen I, Mráz P, Hartmann M, Müller-Schärer H, Lachmuth S (2016) The population genetics of the fundamental cytotype-shift in invasive *Centaurea stoebe* s.l.: Genetic diversity, genetic differentiation and small-scale genetic Structure differ between cytotypes but not between ranges. Biological Invasions 18(7): 1895–1910. https://doi.org/10.1007/s10530-016-1133-2

Rosche C, Hensen I, Mráz P, Durka W, Hartmann M, Lachmuth S (2017) Invasion success in polyploids: The role of inbreeding in the contrasting colonization abilities of diploid versus tetraploid populations of *Centaurea stoebe* s.l. Journal of Ecology 105(2): 425–435. https://doi.org/10.1111/1365-2745.12670

Rosche C, Hensen I, Lachmuth S (2018) Local pre-adaptation to disturbance and inbreeding-environment interactions affect colonisation abilities of diploid and tetraploid *Centaurea stoebe*. Plant Biology 20(1): 75–84. https://doi.org/10.1111/plb.12628

Rosche C, Hensen I, Schaar A, Zehra U, Jasieniuk M, Callaway RM, Khasa DP, Al-Gharaibeh MM, Lekberg Y, Nagy DU, Pal RW, Okada M, Schrieber K, Turner KG, Lachmuth S, Erst A, Tsunoda T, Sheng M, Schmidt R, Peng Y, Luo W, Jäschke Y, Reshi ZA, Shah MA (2019) Climate outweighs native vs. nonnative range-effects for genetics and common garden performance of a cosmopolitan weed. Ecological Monographs 89(4): e01386. https://doi.org/10.1002/ecm.1386

Rutland CA, Hall ND, McElroy JS (2021) The impact of polyploidization on the evolution of weed species: Historical understanding and current Limitations. Frontiers in Agronomy 3: 626454. https://doi.org/10.3389/fagro.2021.626454

Saatkamp A, Cochrane A, Commander L, Guja LK, Jimenez-Alfaro B, Larson J, Nicotra A, Poschlod P, Silveira FAO, Cross AT, Dalziell EL, Dickie J, Erickson TE, Fidelis A, Fuchs A, Golos PJ, Hope M, Lewandrowski W, Merritt DJ, Miller BP, Miller RG, Offord CA, Ooi MKJ, Satyanti A, Sommerville KD, Tangney R, Tomlinson S, Turner S, Walck JL (2019) A research agenda for seed-trait functional ecology. The New Phytologist 221(4): 1764–1775. https://doi.org/10.1111/nph.15502

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7): 671–675. https://doi.org/10.1038/nmeth.2089

Selvi F, Vivona L (2021) Polyploidy in *Odontarrhena bertolonii* (Brassicaceae) in relation to seed germination performance and plant phenotype, with taxonomic implications. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology 156(4): 959–968. https://doi.org/https://doi.org/10.1080/11263504.2021.1985001

Shah MA, Callaway RM, Shah T, Houseman GR, Pal RW, Xiao S, Luo W, Rosche C, Reshi ZA, Khasa DP, Chen S (2014) *Conyza canadensis* suppresses plant diversity in its nonnative ranges but not at home: A transcontinental comparison. The New Phytologist 202(4): 1286–1296. https://doi.org/10.1111/nph.12733

Sheng M, Rosche C, Al-Gharaibeh M, Bullington LS, Callaway RM, Clark T, Cleveland CC, Duan W, Flory SL, Khasa DP, Klironomos JN, McLeod M, Okada M, Pal RW, Shah MA, Lekberg Y (2022) Acquisition and evolution of enhanced mutualism—An underappreciated mechanism for invasive success? The ISME Journal 16(11): 2467–2478. https://doi.org/10.1038/s41396-022-01293-w

Stevens AV, Nicotra AB, Godfree RC, Guja LK (2020) Polyploidy affects the seed, dormancy and seedling characteristics of a perennial grass, conferring an advantage in stressful climates. Plant Biology 22(3): 500–513. https://doi.org/10.1111/plb.13094

Sun Y, Müller-Schärer H, Schaffner U (2016) Neighbour origin and ploidy level drive impact of an alien invasive plant species in a competitive environment. PLoS One 11(5): e0155712. https://doi.org/10.1371/journal.pone.0155712

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesova M, Pyšek P (2012) The more the better? The role of polyploidy in facilitating plant invasions. Annals of Botany 109(1): 19–45. https://doi.org/10.1093/aob/mcr277

Theoharides KA, Dukes JS (2007) Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. The New Phytologist 176(2): 256–273. https://doi.org/10.1111/j.1469-8137.2007.02207.x

Therneau TM (2022a) Coxme: Mixed effects cox models. https://CRAN.R-project.org/package=coxme

Therneau TM (2022b) A package for survival analysis in R. https://CRAN.R-project.org/package=survival

Thill DC, Schirman RD, Appleby AP (1979) Osmotic stability of mannitol and polyethylene glycol 20,000 solutions used as seed germination media. Agronomy Journal 71(1): 105–108. https://doi.org/10.2134/agronj1979.00021962007100010027x

Treier UA, Broennimann O, Normand S, Guisan A, Schaffner U, Steinger T, Müller-Schärer H (2009) Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. Ecology 90(5): 1366–1377. https://doi.org/10.1890/08-0420.1

Tumpa K, Vidaković A, Drvodelić D, Šango M, Idžojtić M, Perković I, Poljak I (2021) The effect of seed size on germination and seedling growth in Sweet Chestnut (Castanea *sativa* Mill.). Forests 12(7): 858. https://doi.org/10.3390/f12070858

van Driesche R, Blossey B, Hoddle M, Lyon S, Reardon R (2002) Biological control of invasive plants in the eastern United States. USDA, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV. https://lccn.loc.gov/2003628938

van Drunen WE, Johnson MTJ (2022) Polyploidy in urban environments. Trends in Ecology & Evolution 37(6): 507–516. https://doi.org/10.1016/j.tree.2022.02.005

van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters 13(2): 235–245. https://doi.org/10.1111/j.1461-0248.2009.01418.x

Veselá A, Hadincová V, Vandvik V, Münzbergová Z (2021) Maternal effects strengthen interactions of temperature and precipitation, determining seed germination of dominant alpine grass species. American Journal of Botany 108(5): 798–810. https://doi.org/10.1002/ajb2.1657

Villasor C, Robertson K, Becker T, Cahill JF, Deák B, Hensen I, Otfinowski R, Rosche C, Borovyk D, Vakhlamova T, Valkó O, Wagner V (2024) Invasion success of three cool-season grasses in the northern prairie: A test of three hypotheses. Oikos 2024(3): e10266. https://doi.org/10.1111/oik.10266

Winkler DE, Garbowski M, Kožić K, Ladouceur E, Larson J, Martin S, Rosche C, Roscher C, Slate ML, Korell L (2024) Facilitating comparable research in seedling functional ecology. Methods in Ecology and Evolution 15(3): 464–476. https://doi.org/10.1111/2041-210X.14288

Yi F, Wang Z, Baskin CC, Baskin JM, Ye R, Sun H, Zhang Y, Ye X, Liu G, Yang X, Huang Z (2019) Seed germination responses to seasonal temperature and drought stress are species-specific but not related to seed size in a desert steppe: Implications for effect of climate change on community structure. Ecology and Evolution 9(4): 2149–2159. https://doi.org/10.1002/ece3.4909

Supplementary material 1

Supplementary information

Link: https://doi.org/10.3897/neobiota.95.127654.suppl1

Supplementary material 2

Raw data

Authors: Kevin Kožić, Matthias Hartmann, Ragan M. Callaway, Isabell Hensen, Dávid U. Nagy, Patrik Mráz, Mohammad M. Al-Gharaibeh, Svetlana Bancheva, Alecu Diaconu, Jiří Danihelka, David J. Ensing, Rita Filep, Zigmantas Gudžinskas, Avni Hajdari, Roxana Nicoară, Susanne Lachmuth, Chandra E. Moffat, Andriy Novikov, Dragica Purger, Mandy L. Slate, Agnieszka Synowiec, Ghizela D. Vonica, Annika M. Zuleger, Christoph Rosche

Data type: xlsx

Explanation note: Experimental data.

Link: https://doi.org/10.3897/neobiota.95.127654.suppl2