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Genetic differentiation, phenotypic plasticity and adaptation in a hybridizing pair of a more common and a less common *Carex* species

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Abstract: Phenotypic variation may be genetically determined or reflect phenotypic plasticity. More common plants are expected to be less differentiated between and within regions and more adapted than less common ones. However, such differences might not develop in hybridizing species which cannot evolve completely independently. We collected 311 genets of *Carex flava*, 215 of *C. viridula* and 46 of their hybrid *C. × subviridula* from 42 natural populations in cold temperate Estonia, mild temperate Lowland Switzerland and alpine Highland Switzerland. Three plantlets from each genet were planted to three experimental gardens, one in each region. We measured survival, growth, reproduction and morphological traits. The experimental transplants showed strong plasticity and grew smallest in the alpine garden. The less common *C. viridula* was slightly more differentiated between regions of origin than the more common *C. flava* and the hybrid. However, this depended on the experimental garden. Significant origin-by-garden-by-taxon and taxon-by-garden interactions suggest differential adaptation among populations and taxa. Regional differed from non-regional plants in several traits indicating both adaptations and, especially for *C. viridula*, maladaptations to the home regions. For *C. flava*, plant seed production was higher when mean annual temperature and precipitation were more similar between population of origin and garden, suggesting local adaptation to climate. Hybrids were intermediate between parental taxa or more similar to one of them, which was retained across gardens. We conclude that plasticity, genetic variation and genotype–environment interactions all contributed to regional differentiation of the closely related species. Hybridization did not completely align evolutionary patterns, and the less common species showed slightly more genetic differentiation between populations and more maladapted traits than the more common one.

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3 Genetic differentiation, phenotypic plasticity and adaptation in a hybridising pair of a more common and a less
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16
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Abstract

Phenotypic variation may be genetically determined or reflect phenotypic plasticity. More common plants are expected to be less differentiated between and within regions and more adapted than less common ones. However, such differences might not develop in hybridising species which cannot evolve completely independently. We collected 311 genotypes of *Carex flava*, 215 of *C. viridula* and 46 of their hybrid *C. x subviridula* from 42 natural populations in cold temperate Estonia, mild temperate Lowland Switzerland and alpine Highland Switzerland. Three plantlets from each clone were planted to three experimental gardens, one in each region. We measured survival, growth, reproduction and morphological traits. The experimental transplants showed strong plasticity and grew smallest in the Alpine garden. The less common *C. viridula* was slightly more differentiated between regions of origin than the more common *C. flava* and the hybrid. Significant origin-by-garden-by-taxon and taxon-by-garden interactions suggest differential adaptation among populations and taxa. Regional differed from non-regional plants in several traits indicating adaptations to the home regions. Plant seed production was higher when mean annual temperature and precipitation were more similar between population of origin and garden, suggesting local adaptation to climate. Hybrids were intermediate between parental taxa or more similar to one of them, which was retained across gardens. We conclude that plasticity, genetically determined variation and genotype-environment interactions all contributed to regional differentiation of the closely related species. Hybridization did not completely align evolutionary patterns, but the less common species showed more genetic differentiation between populations than the more common one.

Keywords: *Carex flava* group, genotype-by-environment interaction, performance, regional adaptation, transplant experiment, variability

Introduction

Species' natural environments are highly heterogeneous across distribution areas. Populations are under different selection pressures, which induce within-species, between-population phenotypic variation. This phenotypic variation may be heritable or non-heritable, depending on whether it results from genetic differences between populations or plastic responses (Schmid, 1992; Briggs and Walters, 1997; West-Eberhard, 2003). The degree of plasticity can also differ between species and populations (Fischer et al., 2004). This variability of plasticity is equivalent to genotype-by-environment (GxE) interaction (Schlichting, 1986; Des Marais et al., 2013). There is much experimental evidence of variability in plant morphology and performance along latitudinal and elevational gradients (Galloway and Fenster, 2000; Santamaría et al., 2003; Becker et al., 2006; Hall et al., 2007; Volis et al., 2016). Knowing the degree of variation of an organism is fundamental to our understanding of the process of plant adaptation and formation of biological diversity (Abbott et al., 2013) and allows us to estimate how plants may respond to predicted climate change (Stenström et al., 2001). Therefore, studying geographic variation and differentiation between and within species makes is valuable for other disciplines, including conservation programmes (Ennos et al., 2005).

(alpine environment and plant adaptations there)

Environmental gradients in abiotic and biotic conditions in alpine systems occur over relatively short distances and affect plants (Körner, 2016; Seguí et al., 2018). The heterogeneity of alpine landscapes together with low temperatures, long persistence of snow cover and short growing season impose constraints for reproduction and establishment (Premoli, 2003). At higher altitudes besides light intensity, UV radiation, precipitation and wind velocity are increased. Furthermore, soil microbial activity and nutrient availability are reduced causing stronger resource competition among plants (Körner, 2003, 2007, 2016). With decreasing temperatures, allocation to flowers at higher altitudes decreases in some species (Pluess and Stöcklin, 2005), but increasing number of flowering shoots or larger flowers are found in other species (Fabbro and Körner, 2004; Arx et al., 2006; Maad et al., 2013). Reduced plant size and earlier reproduction are typical adaptive responses to cooler and shorter growing seasons at higher latitudes (Clevering et al., 2001; Olsson and Ågren, 2002). Plants from high altitudes also often have smaller growth than low-altitude morphs of the same taxa (Schmid, 1992; Blionis and Vokou, 2002; Guo et al., 2010; Štátná et al., 2012), differences may not remain when plants are transplanted to lower elevations. Populations from high alpine environments could show increased phenotypic plasticity due to high thermal variability in comparison with populations from low altitudes (Molina-Montenegro and Naya, 2012), and this has been interpreted as a possible strategy for survival and persistence (Stöcklin et al., 2009; Frei et al., 2014). However, some studies (e.g. Gugger et al., 2015) found opposite results. At lower altitudes, climate conditions are more optimal for plant growth but more species grow together, increasing competition. Thus biotic pressures play a stronger role at lower altitudes (Emms and Arnold, 1997). Reciprocal transplants between altitudes enable us to compare plant phenotypic and genetic responses and help us to predict plant responses to altered climatic conditions (e.g. Gonzalo-Turpin and Hazard 2009; Hassel et al., 2005; Byars et al. 2007; Hautier et al. 2009; Scheepens et al. 2010; Hamann et al. 2017).

Differential adaptation of plant populations within species along environmental gradients is common (Kawecki and Ebert, 2004; Byars et al., 2007; Colautti et al., 2009; Montesinos-Navarro et al., 2011), as revealed by transplant experiments (Turesson, 1922; Clausen et al., 1948; Joshi et al., 2001). High differentiation is expected between environmentally very different regions, e.g. for large differences in latitude or altitude. Seguí et al. (2018) detected different reproductive and physiological responses in plants across alpine gradient. Populations across the elevation may differ in flowering time which leads to further differentiation (Ohsawa and Ide, 2008). The populations experience different temperature, precipitation or soil conditions not only across elevational gradients but also across latitudinal gradients, which could lead to regional differentiation. Besides, common species are expected to be genetically less differentiated, due to their larger and more connected populations, than less common ones (Slatkin, 1987; Hamrick and Godt, 1996). Neutral differentiation of the sparser species, with small populations is expected to be stronger as distance among populations grows and effects of genetic drift relative to effects of gene flow increase (Ellstrand and Elam, 1993).

While local or regional adaptation appears to be quite common for species with large populations, it is not for species with small populations (Leimu and Fischer, 2008), where genetic differentiation may more depend on random genetic drift (Baur and Schmid, 1996; Whitlock, 2003). However, in common taxa higher gene flow can hinder local adaptation, by homogenizing allele frequencies and restraining responses to selection (Kawecki and Ebert, 2004; Hereford, 2009; Blanquart et al., 2012). The relative contribution of these factors to adaptation in common vs. rare taxa is still poorly understood, because transplant experiments were mostly used to study single taxa (e.g. Santamaría et al., 2003; Becker et al., 2006; Sambatti and Rice, 2006; Janyszek et al., 2008; Ågren and Schemske, 2012; Anderson et al., 2015; Samis et al., 2016; Hamann et al., 2017).

In groups of closely related species, taxa are usually not evolving independently due to hybridization and backcrossing (Harper et al., 1961; Abbott et al., 2013; Abbott, 2017). Given that hybridization is common in flowering plants (Arnold, 1992), it is surprising that despite an increasing amount of literature on local or regional

adaptation and plasticity, few studies investigated whether and how differentiation, plasticity and GxE interactions differ between hybridizing parental taxa (but see Wang et al., 1997; Campbell and Waser, 2001; Miglia et al., 2005; Hamilton et al., 2013). Hybrids were found morphologically intermediate between parental taxa (Lamont et al., 2003; Lowe and Abbott, 2003) or having transgressive phenotypes (Rieseberg and Willis, 2007), but changes in hybrid phenotypes in different environments are understudied.

In the present study, we examined the roles of phenotypic plasticity, genetic differentiation and adaptation in explaining phenotypic variation of the more common *Carex flava* L., the less common *C. viridula* Michx. var. *viridula* (henceforth *C. viridula*) and their hybrid *C. x subviridula* Fernald. The taxa belong to the *C. flava* L. species group (*Cyperaceae*). So far, most studies of the *C. flava* group were taxonomically motivated and based on field-collected fresh or herbarium material (Stoeva and Stepankova, 1990; Hedrén, 2002; Blackstock and Ashton, 2010; Więclaw, 2014), while studies of species relationships and their responses in common gardens are very scarce and included material only from small regions (Davies, 1953a; Schmid, 1984a, 1986). Therefore, we did a large 3-year transplant experiment across three regions: Estonia and Lowland and Highland Switzerland (Fig. S1). We collected 572 genotypes from 43 natural populations of *C. flava*, *C. viridula* and *C. x subviridula* from those three regions, performed morphological and genetic differentiation analyses (Schmidt et al., 2017; Schmidt et al., 2018) and then propagated and transplanted cuttings of each genotype to pots in three gardens, one in each of the three regions. The regions differ in major environmental factors, including temperature, precipitation and day length. We recorded morphological and performance traits in the two years after planting.

We asked the following questions: i) how strongly are phenotypic plasticity and genetic variation reflected in transplant morphology and performance? ii) Is genetic differentiation more pronounced in the less common taxon *C. viridula*? iii) Do hybrids differ from each other and parental taxa in similar ways in different transplant gardens? iv) Do patterns of GxE interactions suggest regional adaptation and is this more pronounced in the more common *C. flava*? v) Is plant performance in the garden related to climatic differences between gardens and populations of origin in a way that would suggest local adaptation?

Material and Methods

Studied species and sites — In the studied regions, the *C. flava* group comprises the four taxa *C. flava*, *C. viridula*, *C. lepidocarpa* Tausch and *C. demissa* Hornem. (Schmid, 1981; Toom et al., 2016). In Estonia the two varieties *C. viridula* var. *pulchella* (Lönnr.) B. Schmid and var. *bergrothii* (Palmgr.) B. Schmid are also found (Toom et al., 2016). These perennial tussock-forming sedges grow in damp, unshaded localities like lakeshores, ditches, fens, bogs and wet meadows (Davies, 1953b; Crins and Ball, 1989a).

Carex flava and *C. viridula* are distributed across Europe. While both taxa have scattered distributions, *C. flava* usually occurs in large populations, whereas *C. viridula* typically occurs in small and isolated populations (Davies, 1953b; Vonk, 1979; Schmid, 1981, 1984b, 1986; Crins and Ball, 1989b). Populations of *C. viridula* are mostly found at early-successional microsites, which tend to be small, ephemeral, highly variable and subject to repeated local extinction and colonization (Kuchel and Bruederle, 2000). It is considered a good colonizer but weak competitor in dense vegetation, thus its occurrence depends on water fluctuations, where flooding keeps the vegetation open (Pykälä and Toivonen, 1994). *Carex flava*, on the other hand, is the most K-selected taxon in the group and a better competitor, but tolerates less environmental variability (Schmid, 1984a), and has been found to become rarer due to drainage of wetlands e.g. in Finland (Pykälä and Toivonen, 1994). *Carex flava* flowers generally earlier than *C. viridula*, but their flowering times can overlap and hybridization is possible where both taxa grow together (Vonk, 1979). Flowering time varies along the elevational gradient, as both taxa flower earlier at lower elevations in Switzerland and Estonia and later at higher elevations in Switzerland. Seeds of both taxa are potentially transported by biotic, e.g. birds, mammals, invertebrates, and abiotic agents, e.g., water and wind (Schmid, 1984a; Crins and Ball, 1989b).

Sedges of the *Carex flava* group form tussocks, which consist of genetically identical shoots or ramets and can thus be considered genotypes, different tussocks being derived from different sexually produced seeds. In each population, the number of genotypes studied varied depending on population size. Whenever possible at least three ramets (single reproductive shoots, hereafter called individuals) per genotype were collected. In Highland Switzerland we collected 121 genotypes of *C. flava* and 16 of *C. viridula*. In Lowland Switzerland we collected 80 genotypes of *C. flava*, 81 of *C. viridula* and 15 of *C. x subviridula*. In Estonia we collected 110 genotypes of *C. flava* and 118 of *C. viridula* and 31 of *C. x subviridula*. As hybrids we classified sterile individuals that were pale in color and often more robust in growth (Schmidt et al., 2017). Hybrids were not found in Highland Switzerland, however. More details of characteristics of populations and an analysis of morphological variation in natural populations are provided in Schmidt et al. (2017).

In 2012 and 2013 we sampled 22 natural populations of *C. flava*, 16 populations of *C. viridula*, and 5 populations of their hybrids *C. x subviridula*. Plants were collected from 11 sites in Estonia up to 100 m a.s.l., five in Lowland Switzerland between 250 and 1000 m a.s.l and 10 in Highland Switzerland between 1000 and 2000 m (Table S1, Fig S1). The northernmost region Estonia (57°–59° N) is situated in a transition zone between maritime

and continental temperate climates and has predominant summer precipitation with moderate temperatures during the 5–6 months of vegetation season. The second study region Switzerland (45°–47° N) is divided into Low- and Highland due to prominent differences in elevation and climate. In Lowland Switzerland below 1000 m a.s.l. the vegetation season is long and freezing temperatures occur only during a short period in winter, whereas in Highland Switzerland the vegetation season is much shorter (Körner, 1999).

Reciprocal transplant experiment — Each of the collected genotypes was divided into three ramets (individuals). In summer 2013, three common gardens (transplant sites) were established, each planted with one ramet of each of the clones. Because few individuals did not survive the transport in plastic bags between countries, the number of planted individuals differed between the gardens (Table S1). One garden was established in Highland Switzerland (Schynige Platte, 1976m; 46° 39' 11.80" N), one in Lowland Switzerland (Zurich, 503m; 47° 23' 45.96" N) and one in Northern Europe in Estonia (Tartu, 36m; 58° 23' 7.63" N). Ramets were transplanted into pots containing equal amounts of commercial soil and clay pebbles. Plants were weeded and watered when needed. Plants were covered with a fleece at the 1976-m elevation site and at the Northern 36-m elevation site during the snowy winter.

In our study regions, temperature is highest in Lowland Switzerland and in Highland Switzerland the precipitation is highest. In Estonia rainfall is smallest and annual temperature higher than in Highland Switzerland (Fig. 1). The length of growing season is longest in Lowland and shortest in Highland Switzerland (Körner, 1999). The three transplant sites were located in regions where the study taxa occur naturally. The climate at the three transplant sites is similar to the climate of the respective study regions (Fig. 1).

The two years of the experiment (2014 and 2015) differed in their weather conditions, especially in Switzerland. In 2014, there was a short heatwave in June, July had frequent rain and August was relatively cold. In contrast, in 2015 the summer was quite hot, 2–2.5° C above the norm (annual weather report, MeteoSwiss, 2015). In Estonia, in both years annual temperature was 1.5° C above the norm and rainfall was below usual (annual weather report, Ilmateenistus, 2015).

Morphological and performance traits were measured in two subsequent years (2014 and 2015) for the adult plants at each transplant site at the beginning of July in Zurich and in Tartu. In Schynige Platte, plants were measured one month later because the mountain vegetation season was delayed. For every individual eleven morphological traits (i.e. plant height, stem curvature, leaf length, leaf width, bract length, male spike length, male spike peduncle length, male spike deflection, pistillate spike length, utricle length, utricle width) and seven performance traits (i.e. number of vegetative shoots, number of reproductive shoots, total number of shoots, aboveground plant biomass, number of seeds per spike, percentage of utricles with nut per spike, survival) were measured. Morphological and reproduction-related performance traits were measured in the year 2014, whereas growth-related performance traits in the year 2015. Plant height, considered as morphological and performance trait, was measured in both years and showed similar tendencies in the two years (Table S2), thus we selected only the height measured in second year for the analyses. Stem curvature and male spike deflection were qualitatively measured traits. Utricles were scanned using an Epson perfection V700 photo scanner and five utricles per individual were measured with the image analysis system WINSEEDLE. Aboveground biomass was collected by cutting shoots (vegetative and reproductive) 2 cm above the soil surface, then it was dried at 70°C and weighed. All other traits were quantitative and either counted or measured with a ruler with an accuracy of 1 mm.

Data analysis — To describe environmental differences among locations quantitatively, we extracted eleven climatic variables from the WORLDCLIM database for each population (Hijmans et al., 2005), five measures of temperature and six related to precipitation (data from the period of 1950–2000 at 1 x 1 km resolution, the list of climatic variables is provided in the caption of Fig. 1) and performed a Principal Component Analysis (PCA) to illustrate the climatic differences between regions.

For analyses of variance, 15 traits were selected, seven weakly correlated ($r < 0.70$) morphological traits (i.e. leaf length, leaf width, bract length, male spike length, male spike peduncle length, pistillate spike length and utricle length) and eight performance traits, of which seven were weakly correlated ($r < 0.70$, i.e. number of vegetative shoots, number of reproductive shoots, plant height, biomass, total number of seeds per spike, % of seeds with nut and survival). Number of shoots and number of reproductive shoots were strongly correlated ($r = 0.74$), but because at the 1976-m elevation site all the plants had in general fewer reproductive shoots we found it useful to add the total number of shoots for among-garden comparisons.

Differences in 15 morphological and performance traits were tested with mixed-model analyses of variance (ANOVA) with taxa, region of origin and garden as fixed factors, genet, population and population in interaction with garden as random factors. Since genets and populations were chosen randomly to represent variation within and between populations at large, we calculated variance components to determine how much of the variance in the experiment could be attributed to the individual and population differences and how much to random error. If necessary, dependent variables were log-transformed prior to analyses to better meet the ANOVA assumptions of normally distributed residuals and homoscedasticity. To meet independence of predictors, collinearity of the main

fixed factors in mixed models was predicted by calculating a variance inflation factor (VIF) for each predictor (Zuur et al., 2010). Analysis of variance was implemented using the lme4 package (Bates et al., 2015) applying the lmer function with the exception of binomial survival data, for which the glmer function was applied. The Satterthwaite approximation, implemented in the lmerTest package (Kuznetsova et al., 2016), was used to obtain the degrees of freedom and p-values from lmer. Likelihood-ratio tests (where the reduced model was compared with the full model including all effects) were used to obtain the Chisq and p-values for glmer.

Separate linear mixed models were fitted with data of each taxon alone to test whether morphology and performance depended on the origin of plants, with region of origin and garden as fixed effects, and genet, population and population in interaction with garden as random factors. Another set of separate linear mixed-models ANOVA for each taxon was performed where the contrast regional (home) vs. foreign (away) (thereafter Regional) and transplant garden were fixed effects, origin a co-variate, and genet, population and population in interaction with garden were random factors. With these models we tested whether plants replanted to their home region showed different morphology and performance than plants transplanted to other regions (i.e. regional vs. foreign; Kawecki and Ebert, 2004) and whether the effect differed among gardens. The direction of regional vs. foreign effect was displayed with the R-package effects (John Fox, 2003).

Variance components were calculated for all traits firstly for *C. flava* and *C. viridula* together by fitting genetic effects (taxon, origin), environmental effects (garden) and their interaction as random factors and secondly for *C. flava*, *C. viridula* and *C. x subviridula* separately by fitting origin, garden and their interaction as random factors. Additionally population, genet and their interaction were in the models also as random factors similarly to the original models.

To visualize the variation underlying the first analyses just described, multivariate PCA was done with eleven morphological traits with taxonomic value (plant height, stem curvature, leaf length, leaf width, bract length, male spike length, male spike peduncle length, male spike deflection, pistillate spike length, utricle length, utricle width), using the morphotools function in R (Koutecký, 2015). Tukey HSD multiple-comparison tests were applied to test differences between species and regions based on the first two principal components, using multcomp (Hothorn et al., 2008) and the lme4 package.

Lastly, we tested whether plant performance depended on the climatic differences between transplant sites and origin sites. Of the eleven climatic variables, we used the two that were least correlated with each other, mean annual temperature and mean annual precipitation ($r < 0.70$). We fitted separate models for each taxon and tested for intraspecific differences across sites using a model with fixed effects of “Garden”, “ΔTemp” (difference in mean annual temperature between garden and site of origin of populations) and “ΔPpt” (difference in mean annual precipitation between garden and site of origin of population) and their quadratic terms. Garden was factorially crossed with the “ΔTemp”, “ΔPpt” and quadratic terms of the sites of origin of populations. For hybrids, two models, one with temperature and the other with precipitation, were fitted.

All analyses were performed in R 3.3.2 (R Development Core Team., 2016).

Results

Our results show that *C. flava*, *C. viridula* and *C. x subviridula* plants from all studied regions of origin can grow over a wide range of latitudes and elevations within Europe. Survival differed only slightly and non-significantly between gardens and taxa (Table S2). Survival after the second year of growth was slightly higher in the Zurich garden (90.3%) than in the Schynige garden (87.6%) and in the Tartu garden (88.1%). The hybrid *C. x subviridula* plants survived slightly better (95.6%), than *C. viridula* (89.1%) or *C. flava* (87.7%).

Genetic differences between and within taxa — *Carex flava* and *C. viridula* differed from each other in nine of 15 traits (taxon, Table 1a), with *C. flava* growing taller with longer and wider leaves, longer bracts, pistillate spikes and utricles, and with *C. viridula* growing more vegetative and reproductive shoots (Fig. 3, Table S2). With hybrids in the model and only two regions of origin, a significant taxon effect was detected in 13 of 15 traits (Table 1b). The finding was supported by Tukey multiple-comparison tests and ANOVA performed with principal components PC 1 and PC 2 (Table 2). The PCA of eleven morphological traits clearly separated *C. flava* and *C. viridula* in the experimental gardens, except the ones originating from Highland Switzerland (Table 2; Fig. 2).

On average, hybrids were intermediate in four traits (leaf width, pistillate spike length, utricle length and plant height) and in nine traits showed extreme values higher than either of the parents (leaf length, bract length, male spike length and male-spike-peduncle length and the performance-related traits biomass, total number of seeds, total number of shoots as well as number of vegetative and reproductive shoots; Table S2). The first two principal components explained 32–37% and 13–16% of the variation among the eleven morphological traits for all individuals of hybrids and parental species in the three experimental gardens (Fig. 2). Based on the two first principal components, in the Tartu garden hybrids from Estonia were intermediate between parental taxa but hybrids from Lowland Switzerland were indistinguishable from *C. flava* (Fig. 2a). In Zurich, hybrids from Estonia were similar to *C. viridula* and hybrids from Lowland Switzerland were similar to *C. flava* (Fig. 2b). In Schynige

Platte, hybrids from both origins were similar to *C. viridula* and different from *C. flava* (Fig. 2c). These results were significant only for hybrid differences from *C. viridula* in Tartu (Table 2). The hybrids originating from the two geographically distant regions differed significantly only in one of 15 traits (origin; Table 3c). Based on PCA ordination they were different from each other in each garden, but not based on Tukey multiple comparison tests (Table 2, Fig. 3). These results suggest that different selection pressures have shaped hybrids in the different regions or that they were different due to different selection pressures on their parental populations.

The regions of plant origin significantly affected four of the 15 traits (origin; Table 1a), and with hybrids in the model and two regions of origin, significant origin effects were found in two of 15 traits (origin; Table 1b). This indicates that *C. flava*, *C. viridula*, and their hybrids have evolved some regional differences in parallel, e.g. shorter heights of plants of *C. flava* and *C. viridula* originating from Highland Switzerland (Table S2). However, a significant interaction between taxon and region of origin in seven of 15 traits (Table 1a) and in two of 15 traits with hybrids in the model (Table 1b) indicated that regional differentiation was only partly parallel in the three taxa. In single-taxon models, we found significant origin effects in six of 15 traits for *C. flava*, in four of 15 traits for *C. viridula* and in one of 15 traits for *C. x subviridula* (Table 3). Genets of *C. flava* and *C. viridula* varied significantly among each other in six and five traits, respectively, indicating that there was significant within-population genetic variation (Table 3).

Plasticity — Significant garden effects indicated phenotypic plasticity in all examined traits, except in survival, with two parental taxa in the model (Table 1a) and in twelve of 15 traits with hybrids in the model (Table 1b). For all traits, except survival, environmental garden effect explained more trait variability than genetic origin effect (Table S5). These results suggest that phenotypic plasticity allowed most plants to survive equally well under the different environmental conditions. All three taxa grew best in terms of aboveground biomass, height, number of reproductive shoots and produced most seeds per shoot in Tartu (Fig. 3, Table S2), i.e. the northernmost garden at lowest altitude. On average, all taxa grew shorter with fewer reproductive and more vegetative shoots in the alpine Schynige Platte garden (Fig. 3, Table S2). Plants had significantly longer male spikes and peduncles at the intermediate altitude garden in Zurich. Also, peduncles of male spikes of all taxa were shorter in the alpine garden than in both other sites, indicating a possible tendency to higher self-fertilization in extreme weather conditions.

Taxon-by-garden and origin-by-garden interactions — Significant taxon x garden interactions, detected in two of 15 traits (Table 1a) and four of 15 traits with hybrids in the model (Table 1b), suggested that differences between taxa depended on the environment, i.e. transplant garden. Plant height, for example, differed less among the short *C. viridula*, taller *C. flava* and hybrid taxa in the alpine garden, where all plants grew shorter (Fig. 3, Table S2). Utricle lengths, an important taxonomic character, was constant across the different gardens in *C. viridula* but not in *C. flava*, for which utricles were longest in the Tartu garden.

Significant origin x garden interactions, detected in nine of 15 traits (Table 1a), respectively six of 15 traits with hybrids in the model (Table 1b), indicated that plants of different origins differed in their response to the different transplant gardens. Moreover, taxa x origin x garden interactions were found for nine of 15 traits (Table 1a), respectively for five of 15 traits with hybrids in the model (Table 1b), indicating that differences in the response of plants from different origins depended on the taxon. Consequently, separate models for individual taxa (Table 3) revealed significant origin x garden effects in four of 15 traits for *C. flava*, in ten of 15 for *C. viridula* and in four of 15 for *C. x subviridula*. This results is corroborated by variance components: for *C. viridula* origin x garden interactions explained variability in all 15 traits, for *C. flava* in 10 and for *C. x subviridula* in nine of 15 traits (Table S6). Thus, responses to gardens differed more often among *C. viridula* populations from different origin regions than they did among *C. flava* or hybrid populations.

Regional adaptation — Evidence for regional adaptation was tested with “regional vs. foreign” contrasts in single-species models. We did not detect stronger signals of regional adaptation for *C. flava* than for the less common *C. viridula* or hybrid (regional, Table 4). For example, regional *C. flava* plants had on average 5% higher survival at regional gardens; regional *C. viridula* male spikes were on average 2.75 mm longer and regional hybrids were on average 4.5 cm taller (comparison of plant height 2015 in two gardens) than foreign ones (Table S2). Significant “regional vs. foreign” x garden interactions were found for *C. flava* in three of 15 traits, for *C. viridula* in seven of 15 traits and for hybrids in two of 15 traits (Table 4), indicating that effects of regional origin on the morphology and performance also differed among gardens. The direction of these contrasts was generally suggesting better performance of regional plants, but for some traits maladaptive signals were also found (Table 4, Fig. 3, Table S2). For *C. flava* the direction of “regional vs. foreign” x garden interactions showed adaptation in two cases and maladaptation in one case. For *C. viridula* we observed adaptation in four cases and maladaptation in eight cases. For *C. x subviridula* we observed adaptation in three cases and maladaptation in none.

Regional *C. x subviridula* plants showed on average higher values for performance and morphology than foreign ones in 70% of 30 regional vs. foreign comparisons, *C. flava* in 46.6% of 45 such comparisons and *C. viridula* only in 24.4% of 45 such comparisons (Table S2). Comparisons of average values differ from the ANOVA

results because the number of populations analysed is not enough for statistically significant results. For example, in the alpine garden regional *C. viridula* plants had more vegetative shoots (18.2 vs. 12.5) and a higher survival than foreign ones (100% vs. 82.7%), while this was not found for the other gardens. In the garden in Zurich, regional *C. flava* plants were taller than foreign ones (17.7cm vs. 11.2cm) and had higher number of seeds with nuts (81.9% vs. 77.4%). In the garden in Tartu, *C. x subviridula* regional plants had more reproductive shoots (20.2 vs. 11.0) and had higher survival (100% vs. 77.8%; Table S2). Although not significant due to small sample size, regional hybrid plants generally showed a tendency to outperform the foreign ones (Table 3c).

We hypothesised climatic factors (mean annual temperature and precipitation) to be a major cause of origin x garden effects. However, when we fitted linear and quadratic terms of differences of climatic variables between gardens and sites of origin of population, only difference in precipitation had a significant effect on the trait “seeds with nut” for *C. flava* (Table S4, Fig S2), suggesting that there were no differences in regional adaptation among the different populations within regions or that the signal for regional adaptation we detected had been due to factors other than temperature and precipitation.

Discussion

Plastic and genetic response to transplantation — Our results indicate large environmentally-induced variation in the morphology and performance of all studied taxa (garden effect; Table 1). The detected high plasticity in morphological and performance traits may enable plants of the *C. flava* agg. to grow over a wide range of conditions (Vonk, 1979; Schmid, 1983, 1984a; Crins and Ball, 1989a). High levels of environmentally induced variation is reported for the common aquatic plant *Potamogeton pectiatus* (Santamaría et al. 2003) in all growth and reproduction-related characters and the direction showed reduced fitness at higher latitudes. In a transplant study with the alpine species *Geum reptans* Hamann et al. (2017) detected high plasticity and little evidence for local adaptation. They suggested that the capacity to respond plastically to local environmental conditions, could be a way to maximise plant performance in heterogeneous alpine environment. Kaplan (2002) found in a common-garden experiment that even most of the morphological characters considered in previous studies of *Potamogeton* as suitable for distinguishing taxa were under strong environmental influence and most of the earlier identified *Potamogeton* taxa that were not genetically differentiated. Of the taxa investigated in the present study, all plants grown in the northernmost, low-elevation garden in Estonia produced more seeds, grew taller and produced more reproductive shoots, regardless of their origin (Fig. 3). In the Schynige Platte alpine garden with more stressful conditions for growth, all taxa produced fewer reproductive shoots and seeds, but more vegetative shoots (Fig. 3), which fits with the expectation that at higher altitudes plants reproduce more vegetatively (Blionis and Vokou, 2002; Pluess and Stöcklin, 2005; Stöcklin et al., 2009; Gabel et al., 2017). Based on our data, we cannot distinguish, whether the reduced plant growth at 1976-m garden with colder temperature was an adaptively plastic response or a non-adaptive reaction to environmental stress, which limits growth. However, plants from different origins showed consistent responses in this high-elevation garden, suggesting that it could be adaptive. Similar reductions in growth with increasing altitude were found for many taxa (Freeman et al., 1999; Trtikova et al., 2010; Haider et al., 2012; Štašná et al., 2012). In the alpine landscape plastic responses to growth conditions are considered crucial for the survival of plants (Stöcklin et al., 2009; Trunschke and Stöcklin, 2017), though smaller degree of phenotypic plasticity is found in high-elevation species (Gugger et al., 2015). All taxa showed similarly strong responses to the environment (taxon x garden; Table 1). This suggests that the occurrence of “general purpose” genotypes (Schmid, 1992) enables *Carex flava* and *C. viridula* to adjust to different environmental conditions by phenotypic plasticity.

Genetically determined variation was observed among taxa and origins. In addition, the genetic origin effect varied by transplant site in 60% of the observed traits (significant origin x garden interaction, Table 1a, Fig. 3), which indicates that the different species evolved responses to environmental variation, which were similarly different among origins; this similarity in responses could in part be due to an effect of hybridization on species evolution and a possible trend of amalgamation of the hybridizing taxa through interspecific gene flow. Because plants grew in the experimental gardens for two seasons we suppose that the described differences between origins were indeed due to genetic differences and not maternal carry-over effects. Moreover, maternal effects are found to become insignificant after germination or over time (Bischoff and Müller-Schärer, 2010 and references therein).

Carex viridula* showed higher regional genetic differentiation than *C. flava — The difference between the two parental taxa depended on the origin region in 46.6% of the traits (taxon x origin interaction; Table 1a). Moreover, in 60% of the traits we found that the two parental taxa differed in their GxE (significant taxon x origin x garden interaction; Table 1a), indicating that to some extent taxa also differed in their genetically determined plastic response to the environment. Single-species models revealed higher genetic differentiation in *C. viridula* (significant origin x garden interaction; Table 3), in agreement with our expectation for this less common species. *Carex viridula* plants differed in overall growth, seed production and survival in transplant sites and this depended on the source of populations, indicating a genetic basis for trait differences among origin regions. Our finding is

consistent with previous studies done with wind-pollinated predominantly selfing species (Gonzalo-Turpin and Hazard, 2009; Ågren and Schemske, 2012). A genetic basis has also been found for altitudinal differentiation in widespread taxa, e.g. the grass *Poa hiemata* (Byars et al., 2007), or latitudinal differentiation for *Phragmites australis* (Clevering et al., 2001). Janyszek et al. (2008) performed a common-garden study with the common sedge *C. spicata* in Europe and detected differences in the traits of generative organs for different origins and guessed they were ecotypes. The relatively strong origin effect found in our study and the known presence of varieties in *C. viridula* could indicate that the *C. viridula* populations are also in an evolutionary active phase and follow different development paths in different regions.

Morphology and performance of hybrids as well as their dissimilarity from parental taxa depended on the experimental environment

— We found that hybrid intermediacy or similarity to either parent was not the same in different regions; it depended on the transplant garden in which they were grown (Table 2; Fig. 2). In terms of growth, hybrids were fit in new environments (Fig. 3). The hybrids showed morphological difference from each other in all three gardens, which suggests their independent local origin from different regional parental lineages. In a long-term transplant study with *Artemisia* species complex, hybrid fitness has been found to increase over time. Wang et al. (1997) found in an *Artemisia* species complex that after two years of growth hybrids were more fit only at hybrid sites, and in the same experiment after five years (Freeman et al., 1999) the hybrids grew at least as vigorously as the parental taxa at the parental sites and in the hybrid site surpassed both parental taxa. These results are coherent with other studies showing good performance of plant hybrids in different environments (Emms and Arnold, 1997; Campbell and Waser, 2001; Miglia et al., 2005; Fritz et al., 2006). Emms and Arnold (1997) suggested that through clonal growth hybrids can be as successful as parents and that it may ensure long-term survival and allow the establishment of introgressed populations, which may also be the case in the *C. flava* group (Schmid 1982). On the other hand, Johansen-Morris and Latta (2006) found lower mean fitness of hybrids compared with parental taxa, but detected also few hybrid genotypes capable of outperforming parental taxa. The fitness of hybrids is also found to depend on the frequency of plants on the plots (Hauser et al., 2003). We found intermediacy between parental taxa for some traits in our experimental gardens, but also traits indicating novel evolutionary potential of hybrids with more extreme phenotypes than parental taxa, suggesting the ability to cope with changes in environmental conditions. This is relevant for the further evolution of the species complex, because hybrids are partially male fertile and thus can introgress their genes into parent taxa via backcrossings (Schmid, 1982).

Genotype-by-environment interactions indicating regional adaptation were more pronounced in *C. viridula*

— Transplant studies often seek to find differences in performance and phenotype of “local” vs. “foreign” transplants as indications of local or regional adaptation (Kawecki and Ebert, 2004; Sambatti and Rice, 2006; Colautti et al., 2009; Gonzalo-Turpin and Hazard, 2009; Montesinos-Navarro et al., 2011). In our study we found interactions of genotypes with environments (taxon x garden and origin x garden interactions; Table 1), which encouraged us to study regional adaptation of different taxa. We found regional plants of the two parental taxa and hybrids to show significantly higher performance than foreign plants in some traits (regional; Table 4).

Especially for *C. viridula*, the morphology and performance of regional plants also depended on the garden under study. The region x garden interaction was significant in 46.6% and 20% of traits for *C. viridula* and *C. flava*, respectively (Table 4). Based on the comparisons of averages, *C. flava* regional plants were outperforming the foreign ones in more cases than in *C. viridula* (Table S2). For the hybrid, higher performance of regional plants was observed in 70% of the comparisons, although these were not significant (but see the smaller sample sizes; Table S2). Galloway and Fenster (2000) performed transplant experiments with self-compatible, predominantly outcrossing *Chamaecrista fasciculata* with limited gene flow and still detected local adaptation at the furthest spatial scales (2000 km), which were similar to the distance between Estonia and Switzerland.

Hybrids are in several studies found to be maladapted in parental environments, which is a way to maintain phenotypic divergence between two species in the face of gene flow (e.g. Brennan et al., 2016). An advantage of foreign populations in environments under study is not rare (Hereford, 2009). We found on the other hand in most cases significant advantage of regional plants over nonregional ones (Table 4). Only number of shoots in *C. viridula* and number of vegetative shoots in hybrids showed marginally significant maladaptation. Maladaptation may become more apparent as climate change proceeds and genotypes from warmer, lower-elevation populations gain a fitness advantage over local genotypes (Anderson 2015).

In small populations the effect of genetic drift could prevent local adaptation (Whitlock, 2003), as the ability to adapt may be compromised due to reduced genetic diversity (Pertoldi et al., 2007). We detected relatively high genetic diversity for *C. viridula*, despite its generally small population sizes (Schmidt et al., 2018) and we also found evidence for its adaptation to regional environments. Also for *Arabis fecunda* and *Carlina vulgaris* adaptation to local conditions was found despite small, isolated or fragmented populations (McKay et al., 2001; Becker et al., 2006). Our results indicate some regional advantage of both parental taxa and also a possible evolutionary trend of hybrids for adaptation to regional environments.

Climatic differences between garden and population of origin were not a major cause for local adaptation

— Relating geographical, environmental or ecological distances of plant origins to the relative fitness of plants is recommended as a more mechanistic measure of local or regional adaptation than general local vs. foreign comparisons (Galloway and Fenster, 2000; Weißhuhn et al., 2012; Bucharova et al., 2016). Accordingly, we tested whether differences between performances of plants from different populations arose from adaptation to local climates. When relating differences between temperature and precipitation at populations of origin and transplant garden to plant fitness traits, we found significantly higher numbers of seeds with nuts for *C. flava* when the precipitation in the garden was similar to the precipitation of the populations of origin (Table S4). This suggests a certain degree of adaptation to local climates.

The degree of regional adaptation detected in our study may have been an underestimate. Firstly, we used shoots instead of seeds, but seedling establishment is considered as one of the most environmentally sensitive life stages of plants (Fritsche and Kaltz, 2000; Giménez-Benavides et al., 2007). Hence we might have missed a possible differential performance at the seedling stage. Secondly, biotic interactions or other climatic factors, such as range and variability of temperature, regional microbial communities or soil and land use (Raabová et al., 2007; Rice and Knapp, 2008; Bucharova et al., 2016), not considered in our study, could also affect local or regional adaptation.

Conclusion — We observed considerable and significant plasticity, genetic variation and GxE interactions in the studied taxa of the *C. flava* group. Plant systematic studies need to take all three sources of variation into account. The two taxa showed some common evolutionary patterns where they co-occur in the same region, but also differences suggesting a high degree of independent evolution. Genetic differentiation was stronger in the less common *C. viridula*, as expected. Against expectation, we found that evidence for regional adaptation was not stronger in the more common *C. flava* than in the less common *C. viridula*. Hybrids also showed pronounced plasticity, GxE interactions and the tendency of regional adaptation. As a result, hybrid intermediacy or similarity to parental taxa differed across the regional environments represented by the three experimental gardens. Overall, our results indicate that plasticity, genetically determined variation and GxE interactions all contribute to regional differentiation of the closely related study species. Hybridization did not lead to completely parallel evolutionary patterns, but the less common species showed more genetic differentiation between populations than the more common species.

Compliance with Ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Figure captions

Fig. 1 Principal component analysis of ten climatic variables. Plotted are gardens (grey) and populations from three origin regions: Estonia (green), Switzerland lowland (yellow) and Switzerland highland (blue). Climate in the gardens is similar to climate at populations in the same region. Above are PCA loadings, arrows indicate the loadings of each climate variable. Temperature: MATemp, mean annual temperature (°C); MaxTemp, max temperature of warmest month (°C); MinTemp, min temperature of coldest month (°C); MWeQTemp, mean temperature of wettest quarter (°C); MWaQTemp, mean temperature of warmest quarter (°C). Precipitation: MAPpt, mean annual precipitation (mm); MaxPpt, mean precipitation of the wettest month (mm); MinPpt, mean precipitation of the driest month (mm); WeQPpt, precipitation of wettest quarter (mm); WaQPpt, precipitation of warmest quarter (mm)

Fig. 2 PCA of eleven morphological traits of *C. flava* (CF), *C. viridula* (CV) and *C. x subviridula* (FxV) populations in three transplant sites, in Estonia (a), in Lowland Switzerland (b) and in Highland Switzerland (c). The centroid of the phenotypic distribution of the taxa per origin is indicated by names in a rectangle to which each individual of that taxon and origin is connected by lines. Loadings of PC1 and PC2 are presented in Table S3

Fig. 3 Effect of origin (Estonia, Low- and Highland Switzerland) and gardens (Tartu in Estonia, Zurich in Lowland Switzerland and Schynige Platte in Highland Switzerland) on performance of *C. flava*, *C. viridula* and *C. x subviridula*. Plotted are mean and SE of number of vegetative shoots (a-c), number of reproductive shoots (d-f), total number of shoots (g-i), number of utricles per shoot (j-l), seeds with nut (m-o), plant height (p-r) and biomass (s-u). Different letters indicate interspecific significant differences of pairwise comparisons with a post-hoc test (Tukey's HSD; does not apply across vertical or horizontal panels).

Figure 1. Locations of *C. flava*, *C. viridula* and *C. x subviridula* populations sampled in Estonia (green), Lowland Switzerland (red), Highland Switzerland (dark green) and the three gardens Tartu, 36 m; Zurich 503 m; Schynige Platte, 1976 m (grey). Abbreviations follow Table S1.

1
2 **Figure 2.** Principal component analysis of ten climatic variables. Plotted are gardens (grey) and populations from
3 three origin regions: Estonia (green), Switzerland lowland (yellow) and Switzerland highland (blue). Climate in
4 the gardens is similar to climate at populations in the same region. Above are PCA loadings, arrows indicate the
5 loadings of each climate variable. Temperature: MATemp, mean annual temperature (°C); MaxTemp, max
6 memperature of warmest month (°C); MinTemp, min memperature of coldest month (°C); MWeQTemp, mean
7 temperature of wettest quarter (°C); MWaQTemp, mean temperature of warmest quarter (°C). Precipitation:
8 MAPpt, mean annual precipitation (mm); MaxPpt, mean precipitation of the wettest month (mm); MinPpt, mean
9 precipitation of the driest month (mm); WeQPpt, precipitation of wettest quarter (mm); WaQPpt, precipitation of
10 warmest quarter (mm).

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Figure 3. PCA of 11 morphological traits of *C. flava* (CF), *C. viridula* (CV) and *C. x subviridula* (FxV) populations in three transplant sites, in Estonia (a), in Lowland Switzerland (b) and in Highland Switzerland (c). The centroid of the phenotypic distribution of the taxa per origin is indicated by names in a rectangle to which each individual of that taxon and origin is connected by lines.

Figure 4. Effect of origin (Estonia, Low- and Highland Switzerland) and gardens (Tartu in Estonia, Zurich in Lowland Switzerland and Schynige Platte in Highland Switzerland) on performance of *C. flava*, *C. viridula* and *C. x subviridula*. Plotted are mean and SE of number of vegetative shoots (a-c), number of reproductive shoots (d-f), total number of shoots (g-i), number of utricles per shoot (j-l), fecundity (m-o), plant height (p-r) and biomass (s-u). Letters indicate interspecific significant differences of pairwise comparisons with a post-hoc test (Tukey's HSD).

Table 1.

Table 1. Summary of linear mixed-model ANOVAs evaluating the effect of taxon, region of origin, garden and their interaction on plant phenotype and performance with a) *C. flava* and *C. viridula* from three regions and b) *C. flava*, *C. viridula* and *C. x subviridula* from two regions. The table reports F, Chisq (X^2) and significances.

a) *C. flava* and *C. viridula* from three regions

	Taxon (T)	Origin (O)	T*O	Garden (G)	T*G	O*G	T*O*G	Pop [R]	Genet [R]	G*Pop [R]	Resid.
	(df = 1)	(df = 2)	(df = 2)	(df = 2)	(df = 2)	(df = 4)	(df = 4)	df= (36)	(df= 482-533)	(df= 102 - 108)	n= 873- 1186
Morphological traits:	F	F	F	F	F	F	F	v.c.	v.c.	v.c.	
Leaf length	27.2 ***	2.54	5.58 *	41.5 ***	1.61	2.01	0.47	11.7 ***	17.5 ***	5.19 **	65.6
Leaf width	143.4 ***	0.89	4.52 *	23.9 ***	0.01	3.13 *	2.19	5.56 **	35.2 ***	0.00	59.3
Bract length	10.9 **	5.89 **	4.20 *	70.8 ***	1.08	2.49	0.76	7.97 **	2.99	3.33	85.7
Male spike length	0.65	1.50	1.92	23.4 ***	1.07	6.90 ***	8.65 ***	3.59 .	10.1 *	1.90	84.4
Male sp. ped. length	0.15	1.90	2.96 .	56.0 ***	5.94 **	4.70 **	7.49 ***	6.56 **	2.72	0.84	89.9
Pistillate spike length	32.5 ***	0.82	0.82	25.9 ***	2.41 .	2.21 .	2.50 *	10.2 ***	5.33	0.00	84.4
Utricle length	136.5 ***	1.22	6.04 *	65.8 ***	14.5 ***	4.36 **	3.07 *	14.0 ***	24.9 ***	1.99	59.2
Performance traits:											
No. veg. shoots	30.1 ***	3.39 *	3.87 *	54.4 ***	1.59	3.37 *	3.52 *	1.83	7.05 *	1.84	89.3
No. rep. shoots	41.0 ***	5.00 *	0.27	122.0 ***	1.53	1.87	2.76 *	2.53	5.00	2.06	90.4
No. shoots	46.9 ***	2.27	0.80	33.2 ***	1.92	2.87 *	4.36 **	2.41	5.71 .	2.76	89.1
Plant height	24.2 ***	3.79 *	4.83 *	166.0 ***	1.05	10.5 ***	1.76	13.1 ***	17.9 ***	1.21	67.9
Biomass	1.65	0.51	1.49	101.7 ***	1.86	1.97	3.10 *	13.6 ***	16.4 ***	0.31	69.6
Seeds	3.67	1.63	1.02	45.9 ***	2.87 .	2.58 *	0.57	2.22	4.83	2.51	90.4
Fecundity	0.00	1.05	1.12	12.0 ***	0.45	1.78	2.98 *	0.00	9.80 *	9.12 ***	81.1
	X^2	X^2	X^2	X^2	X^2	X^2	X^2	v.c.	v.c.	v.c.	v.c.
Survival	1.04	4.13	7.45 *	2.88	3.63	33.2 ***	5.52	0.0	98.71 ***	1.21	-

For F and X^2 statistics; . $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

b) *C. flava*, *C. viridula* and *C. x subviridula* from two regions

	Taxon (T)	Origin (O)	T*O	Garden (G)	T*G	O*G	T*O*G	Genet (df = 379 - 440)	G*Pop [R] (df = 58)	Pop [R] (df = 29)	Resid. n = 576- 816
	(df = 2)	(df = 1)	(df = 2)	(df = 1)	(df = 2)	(df = 1)	(df = 2)				
Morphological traits:	F	F	F	F	F	F	F	v.c.	v.c.	v.c.	
Leaf length	42.3 ***	0.24	0.04	72.3 ***	0.48	3.23 .	1.61	18.5 **	6.73 **	7.33 .	67.4
Leaf width	89.9 ***	5.99 *	0.99	36.3 ***	2.01	11.2 **	3.04 *	39.4 ***	0.00	7.01 **	63.6
Bract length	13.1 ***	0.21	1.49	102.7 ***	0.00	9.99 **	0.52	0.00	5.67 .	2.97	91.4
Male spike length	7.67 **	0.00	11.5 ***	2.10	1.02	13.1 **	6.14 **	6.22	1.85	0.77	91.2
Male sp. ped. length	2.31	0.21	7.03 **	59.1 ***	3.09 .	2.17	1.65	0.86	1.87	7.41 .	89.9
Pistillate spike length	26.8 ***	1.13	1.64	0.76	1.80	1.63	3.63 *	0.00	0.00	10.5 **	89.5
Utricle length	117.4 ***	0.30	2.76 .	53.4 ***	16.5 ***	11.5 **	2.35 .	24.7 ***	0.78	13.5 **	61.0
Performance traits:											
No. veg. shoots	19.9 ***	0.12	0.78	12.6 **	10.3 **	1.29	3.46 *	6.45	2.77	0.63	90.2
No. rep. shoots	22.4 ***	0.01	3.06 .	238.4 ***	1.05	0.21	3.44 .	7.40	1.48	4.83	86.3
No. shoots	41.1 ***	0.14	2.85 .	48.2 ***	5.12 *	0.21	4.45 *	10.1 .	5.67 .	0.38	83.8
Plant height	30.5 ***	16.7 ***	1.10	307.6 ***	0.69	23.8 ***	1.65	18.7 **	0.35	13.5 **	67.5
Biomass	6.46 **	0.27	2.44	205.1 ***	4.89 **	0.74	2.20	17.5 **	0.00	12.5 ***	70.0
Seeds	4.58 *	1.71	0.71	107.2 ***	2.05	3.74 .	1.24	11.1	1.00	5.04	82.9
Fecundity	139.6 ***	0.68	0.51	0.16	1.73	8.0 **	0.05	0.00	13.6 ***	0.00	86.4
	X ²	X ²	X ²	X ²	X ²	X ²	X ²	v.c.	v.c.	v.c.	v.c.
Survival	0.04	0.05	1.6	6.7 **	1.79	2.90 .	1.87	96.3 ***	3.7 **	0.00	-

For F and X² statistics; . P < 0.10; *P < 0.5; ** P < 0.01; ***P < 0.001.

Table 2.

Table 2. Morphological differentiation of *C. flava*, *C. viridula* and *C. x subviridula* a) between species within region of origin and b) within species between regions of origin in three transplant sites, based on PCA with 11 morphological traits. Table entries are p-values, which derive from Tukey HSD multiple comparison tests with principal components 1 and 2, using population means.

a) Between species within region of origin

	Transplant site					
	Tartu, 36 m		Zurich, 503 m		Schynige, 1976 m	
	$n_{pop} = 45$		$n_{pop} = 44$		$n_{pop} = 36$	
	PC1	PC2	PC1	PC2	PC1	PC2
Estonia (EST)						
CV - CF	0.000	0.145	0.000	1.000	0.000	0.996
CF - FxV	0.739	0.147	0.279	0.885	0.056	0.881
CV - FxV	0.005	0.994	0.548	0.972	0.749	0.363
Switzerland lowland (CHL)						
CV - CF	0.000	0.184	0.000	1.000	0.009	0.065
CF - FxV	0.465	0.726	0.119	0.875	0.995	0.182
CV - FxV	0.003	0.997	0.246	0.990	0.296	1.000
Switzerland highland (CHH)						
CV - CF	1.000	0.626	0.858	0.246	1.000	0.052

b) Within species between regions of origin

<i>C. flava</i> (CF)						
CHL - CHH	0.002	0.686	0.004	0.977	0.683	0.981
EST - CHH	0.126	1.000	0.745	0.875	0.009	0.009
EST - CHL	0.746	0.784	0.312	1.000	0.331	0.114
<i>C. viridula</i> (CV)						
CHL - CHH	0.069	0.186	0.964	0.962	0.608	0.998
EST - CHH	0.023	1.000	0.601	0.974	0.939	1.000
EST - CHL	1.000	0.015	0.996	1.000	0.984	0.993
<i>C. x subviridula</i> (FxV)						
EST - CHL	0.997	0.163	0.909	1.000	1.000	0.958

Table 3.

Table 3. Summary of linear mixed-model ANOVAs evaluating the effect of region of origin, garden and their interaction on plant phenotype and performance. Analyses run for each taxa separately a) *C. flava*, b) *C. viridula* and c) *C. x subviridula*. The table reports F, Chisq (X^2) and percentage of variance components (v.c. for random factors [R]) and significances.

a) *C. flava*

	Origin (O)	Garden (G)	O*G	Pop [R]	Genet [R]	G*Pop [R]	Resid.
	(df = 2)	(df = 2)	(df = 4)	(df = 22)	(df = 291 - 334)	(df = 61 - 66)	(df = 386 - 805)
Morphological traits:	F	F	F	v.c.	v.c.	v.c.	
Leaf length	12.5 ***	41.8 ***	1.16	15.5 **	11.6 .	6.82 **	66.0
Leaf width	4.55 *	16.5 ***	0.50	6.38 .	25.4 ***	2.39	65.8
Bract length	14.3 ***	53.8 ***	1.64	9.9 **	8.4	2.75	79.0
Male sp. length	0.03	11.8 ***	6.23 ***	5.04	21.6 ***	2.90	70.4
Male sp. ped. length	0.47	85.8 ***	7.21 ***	7.84 *	5.9	0.15	86.1
Pistillate sp. length	1.30	21.5 ***	1.95	7.62 **	10.6	0.00	81.8
Utricle length	1.85 .	98.1 ***	2.81 *	7.60 *	23.8 ***	3.63	65.0
Performance traits:							
No. veg. shoots	0.35	57.3 ***	1.04	1.92	1.8	3.85 ***	92.4
No. rep. shoots	2.97 .	107.5 ***	0.15	1.87	5.3	5.04	87.8
No. shoots	1.03	43.5 ***	1.61	3.75	4.0	4.27 .	88.1
Plant height	7.18 **	183.7 ***	4.44 *	13.6 *	4.2	2.58	79.7
Biomass	2.30	71.4 ***	0.56	15.1 ***	15.9 *	0.83	68.2
Seeds total	0.20	18.8 ***	1.24	2.31	0.1	3.77	93.8
Seeds with nut	3.23 *	8.93 ***	2.24	0.00	15.1 **	6.19 *	78.7
	X^2	X^2	X^2	v.c.	v.c.	v.c.	v.c.
Survival	7.48 *	1.90	20.1 ***	0.0	97.4 ***	2.61	-

For F and X^2 statistics; . $P < 0.10$; * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$.

b) *C. viridula*

	Origin	Garden	O*G	Pop [R]	Genet [R]	G*Pop [R]	Resid.
	(df = 2)	(df = 2)	(df = 4)	(df = 14 - 15)	(df = 188 - 215)	(df = 41 - 45)	(df = 388 - 549)
Morphological traits:	F	F	F	v.c.	v.c.	v.c.	
Leaf length	0.56	13.9 ***	0.94	6.88 .	22.9 ***	3.02	67.2
Leaf width	1.73	6.22 **	3.76 **	4.12 **	42.8 ***	0.00	53.1
Bract length	2.07	26.5 ***	1.17	3.03	0.00	5.33	91.6
Male sp. length	3.72 *	11.8 ***	9.43 ***	3.08	2.52	1.38	93.0
Male sp. ped. length	4.42 *	14.7 ***	2.45 .	4.03	0.00	2.27	93.7
Pistillate sp. length	1.33	6.40 **	1.93	11.0 **	0.07	0.00	89.0
Utricle length	2.86 .	10.2 ***	3.00 *	24.5 ***	27.1 ***	0.68	47.7
Performance traits:							
No. veg. shoots	5.22 *	14.8 ***	4.15 **	0.37	15.7 **	0.00	84.0
No. rep. shoots	2.45	50.1 ***	4.25 **	2.49	5.65	0.21	91.6
No. shoots	1.98	9.87 ***	5.30 **	0.16	8.58	0.90	90.4
Plant height	5.72 *	51.7 ***	7.11 ***	12.5 **	24.8 ***	0.89	61.8
Biomass	0.71	45.9 ***	3.60 ***	10.7 **	16.4 **	0.00	72.9
Seeds total	2.43	28.6 ***	2.87 *	2.13	10.1 .	1.18	86.6
Seeds with nut	1.00	2.46	4.03 *	3.35	1.48	10.46 **	84.7
	X^2	X^2	X^2	v.c.	v.c.	v.c.	v.c.
Survival	0.73	6.69 *	17.9 **	1.61	98.4	0.00	-

For F and X^2 statistics; . $P < 0.10$; * $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$.

c) *C. x subviridula*

	Origin (O)	Garden (G)	O*G	Pop [R]	Genet [R]	G*Pop [R]	Resid.
	(df = 1)	(df = 2)	(df = 2)	(df= 4)	(df= 43 - 46)	(df= 14)	(df= 96 - 114)
Morphological traits:	F	F	F	v.c.	v.c.	v.c.	
Leaf length	0.53	16.8 ***	4.48 *	18.3	5.41	0.00	76.3
Leaf width	2.04	3.44 *	7.91 ***	19.3	8.53	0.00	72.2
Bract length	0.19	15.7 ***	3.86 *	0.00	17.6	0.00	82.4
Male sp. length	2.21	6.10 *	3.34	0.00	1.23	6.27	92.5
Male sp. ped. length	1.43	14.2 ***	0.57	0.00	0.00	0.00	100.0
Pistillate sp. length	0.18	4.23 *	2.79	25.7	0.00	0.00	74.3
Utricle length	0.13	8.7 ***	2.25	37.8	15.8	0.00	46.4
Performance traits:							
No. veg. shoots	0.17	4.54 *	1.46	0.22	12.0	0.00	87.8
No. rep. shoots	2.54	43.7 *	1.05	4.10	0.00	2.73	93.2
No. shoots	3.67	12.8 ***	0.38	0.32	16.6	0.00	83.1
Plant height	10.1 **	66.7 ***	7.82 ***	0.00	15.5	0.00	84.5
Biomass	0.45	33.6 ***	0.09	1.85	18.4	0.00	79.7
Seeds total	4.71	8.1 *	1.21	0.00	19.6	5.30	75.1
Seeds with nut	0.17	0.98	1.98	0.00	5.8	16.8	77.4
	X ²	X ²	X ²	v.c.	v.c.	v.c.	v.c.
Survival	3.53	0.38	2.69	NaN	NaN	NaN	-

For F and X² statistics; . P < 0.10; *P < 0.5; ** P < 0.01; ***P < 0.001.

Table 4.

Table 4. Summary of linear mixed-model ANOVAs for evaluating the effect of being regional or foreign and the interaction between regional vs. foreign and transplant garden on plant phenotype and performance. Analyses run for each taxa separately a) *C. flava* and b) *C. viridula*. The table reports F, Chisq (X^2) and percentage of variance components (v.c. for random factors [R]) and significances.

a) *C. flava*

	Reg. (R)	Origin (O)	Garden (G)	R*G	Pop [R]	Genet [R] (df = 266 - 290)	G*Pop [R] (df = 60)	Resid. n = 387 - 513
	(df = 1)	(df = 2)	(df = 2)	(df = 2)	(df = 21)			
Morphological traits	F	F	F	F	v.c.	v.c.	v.c.	v.c.
Leaf length	0.41	7.75 **	28.2 ***	0.56	16.0 **	11.2 .	7.16 **	65.6
Leaf width	0.37	2.53 .	14.6 ***	0.28	6.73 .	25.7 ***	1.87	65.7
Bract length	1.42	7.47 **	39.3 ***	1.41 * $\uparrow$ TA	10.2 **	8.16	2.86	78.8
Male sp. length	0.06	0.07	25.6 ***	1.51	2.46	20.1 ***	6.10 *	71.3
Male sp. ped. length	1.28	0.21	58.2 ***	2.54	3.34	5.99	7.95 *	82.7
Pistillate sp. length	0.03	2.27	32.7 ***	3.10 * $\downarrow$ TA	7.64 **	10.2	0.00	82.2
Utricle length	7.60 $\uparrow$ *	0.81	91.0 ***	1.29	8.20 *	23.1 ***	2.92	65.8
Performance traits								
No. veg. shoots	0.35	1.09	51.2 ***	1.67	2.08	1.73	3.59	92.6
No. rep. shoots	0.59	1.45	79.3 ***	0.03	2.26	4.84	4.48	88.4
No. shoots	0.65	2.26	31.3 ***	2.32	3.77	3.96	4.12 .	88.1
Plant height	0.00	1.59	132.2 ***	5.97 * $\uparrow$ ZH	12.3 *	2.62	5.00	80.1
Biomass	0.36	2.76 .	61.5 ***	0.88	16.2 ***	15.9 **	0.61	67.2
Seeds total	3.93 $\uparrow$.	0.57	15.9 ***	0.63	2.71	0.10	3.27	93.9
Seeds with nut	0.86	1.25	19.3 ***	0.83	0.00	13.6 *	8.19 *	78.2
	X^2	X^2	X^2	X^2	v.c.	v.c.	v.c.	v.c.
Survival	9.39 *** $\uparrow$	9.85 **	3.95	4.60	0.00	92.2 ***	7.79	-

For F and X^2 statistics; . $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

b) *C. viridula*

	Reg. (R)	Origin (O)	Garden (G)	R*G	Pop [R]	Genet [R]	G*Pop [R]	Resid.
	(df = 1)	(df = 2)	(df = 2)	(df = 2)	(df = 14)	(df = 200 - 207)	(df = 42)	n = 405 - 480
Morphological traits:	F	F	F	F	v.c.	v.c.	v.c.	v.c.
Leaf length	0.15	2.16	34.4***	2.37	7.26.	23.1***	2.87	66.8
Leaf width	1.12	3.85	38.3***	3.34*↓SP	5.72.	40.7***	0.00	53.6
Bract length	0.95	0.26	42.0***	0.33	2.44	0.00	5.57	91.9
Male sp. length	5.47↑*	1.12	12.9***	1.85	3.61	2.25	1.32	92.8
Male sp. ped. length	0.04	3.93*	22.8***	1.42	3.95	0.00	2.85	93.2
Pistillate sp. length	0.62	2.71.	2.24*	0.85	9.5*	0.00	0.00	90.5
Utricle length	1.89	5.82*	8.99**	3.27*↓ZH	21.3***	28.1***	1.21	49.4
Performance traits:								
No. veg. shoots	1.94	5.85**	45.4***	3.85*↓TA	0.51	15.6**	0.00	83.9
No. rep. shoots	6.32↑*	2.67.	109.7***	3.81*↓TA	2.63	6.26	0.03	91.1
No. shoots	3.32↓.	2.73.	7.65**	4.20*↓TA	0.11	8.89.	1.10	89.9
Plant height	4.42↑*	5.19*	123.9***	9.89***↑ZH, ↓SP,TA	11.8*	24.7***	1.66	61.9
Biomass	0.02	2.05	83.1***	3.01.↑TA	10.4**	16.3**	0.28	72.9
Seeds total	5.21↑*	1.18	62.4***	0.02	2.28	10.3.	1.08	86.4
Seeds with nut	2.04	1.88	1.02	1.50	0.00	1.02	16.4***	82.6
	X ²	X ²	X ²	X ²	v.c.	v.c.	v.c.	v.c.
Survival	0.71	0.59	4.29	8.43*↑SP,TA, ↓ZH	0.00	88.2	11.80	-

For F and X² statistics; . P < 0.10; *P < 0.5; ** P < 0.01; ***P < 0.001.

b) *C. x subviridula*

	Reg. (R)	Garden (G)	R*G	Pop [R]	Genet [R] (df = 42 - 44)	G*Pop [R] (df = 14)	Resid. n =96 - 108
	(df = 1)	(df = 1)	(df = 1)	(df = 4)			
Morphological traits:	F	F	F	v.c.	v.c.	v.c.	v.c.
Leaf length	7.17 ↑*	23.0 ***	0.17	13.4	2.61	0.00	83.9
Leaf width	12.7 ↑***	3.75 .	2.66	19.1	0.00	0.00	80.9
Bract length	9.81 ↑**	33.0 ***	0.00	0.00	34.0 .	0.00	66.0
Male sp. length	3.79	0.01	0.16	0.00	1.30	8.72	89.9
Male sp. ped. length	0.85	7.52 **	0.57	0.00	0.00	0.00	100.0
Pistillate sp. length	3.74 ↑.	0.03	0.58	26.9	0.00	0.00	73.2
Utricle length	4.37 ↑*	13.7 ***	0.17	33.6	16.8	0.00	49.7
Performance traits:							
No. veg. shoots	2.82 ↓.	0.47	0.00	0.00	5.81	0.00	94.2
No. rep. shoots	0.81	42.6 *	3.34	4.02	0.00	7.02	88.9
No. shoots	0.08	24.9 ***	4.25 *↑TA	0.00	14.7	0.00	85.3
Plant height	14.2 ↑***	84.5 ***	6.34 *↑TA,ZH	0.00	17.6	0.00	82.4
Biomass	0.03	59.9 ***	0.57	0.00	12.3	0.00	87.7
Seeds total	0.00	20.3 ***	1.10	10.9	6.71	0.00	82.4
Seeds with nut	3.33	0.59	0.02	0.00	0.00	18.4	81.6
	X ²	X ²	X ²	v.c.	v.c.	v.c.	v.c.
Survival	0.83	0.17	0.00	0.00	100.0	0.00	-

For F and X² statistics; . P < 0.10; *P < 0.5; ** P < 0.01; ***P < 0.001.

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Supplementary material

Additional supporting information in the online version of this article contains the following:

ESM_1 - **Table S1**: Overview of location and climatic variables of studied populations of *C. flava* (a), *C. viridula* (b) and *C. x subviridula* (c).

ESM_2 - **Table S2**: Mean and standard errors of morphological and performance traits of *C. flava* (a), *C. viridula* (b) and *C. x subviridula* (c).

ESM_3 - **Table S3**: Loadings of principal component scores (PC1 and PC2) from PCA of 11 morphological traits with *C. flava*, *C. viridula* and *C. x subviridula* in three transplant gardens.

ESM_4 - **Table S4**: Summary of linear mixed-model ANOVAs for evaluating the effects of climatic variables (temperature and precipitation) on performance of *C. flava* (a), *C. viridula* (b) and *C. x subviridula* (c).

ESM_5 – **Table S5**: Variance components (%) of genetic effects (Taxon, Origin, Taxon x Origin), environmental effects (Garden) and genotype x environment interaction effects (Taxon x Garden, Origin x Garden, Taxon x Origin x Garden) extracted for seven morphological and eight performance related traits from 22 *C. flava* and 16 *C. viridula* populations

ESM_6 – **Table S6**: Variance components (%) of genetic effects (Origin), environmental effects (Garden) and genotype x environment interaction effects (Origin x Garden) extracted for seven morphological and eight performance related traits from 22 *C. flava* (a), 16 *C. viridula* (b) and 5 *C. x subviridula* (c) populations.

ESM_7 – **Fig. S1**: Locations of *C. flava*, *C. viridula* and *C. x subviridula* populations sampled in Estonia (green), Lowland Switzerland (red), Highland Switzerland (dark green) and the three experimental gardens (grey) in Tartu, 36 m; Zurich 503 m and Schynige Platte, 1976 m. Abbreviations follow Table S1