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Invasion success in polyploids: the role of inbreeding in the contrasting colonization abilities of diploid versus tetraploid populations of *Centaurea stoebe* s.l.

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Summary

1. As a consequence of founder effects, inbreeding can hamper colonization success: First, in species with self-incompatibility controlled by an *S*-locus, inbreeding may decrease cross-compatibility, mainly due to the sharing of identical *S*-alleles between closely related mating partners. Secondly, inbreeding can reduce fitness of inbred relative to outbred offspring (i.e. inbreeding depression). Polyploids often show reduced inbreeding depression compared to diploids, which may contribute to the overrepresentation of polyploids among invasive species. This is the first study that tests how the effects of inbreeding differ between geocytotypes (i.e. ploidy levels within a given range).

2. Our model organism, *Centaurea stoebe*, is strictly self-incompatible and comprises three geocytotypes: diploids are more frequent than tetraploids in the native range, while only tetraploids occur in the invasive range. We conducted a breeding experiment (sib-mating vs. outcrossing) with 14 native diploid, 13 native tetraploid and 15 invasive tetraploid populations. We recorded cross-compatibility and estimated a cumulative index for offspring fitness. Since frequent inbreeding can result in purging of genetic load responsible for inbreeding depression, our analyses included a metric for within-population relatedness, based on eight microsatellite markers, to assess the effect of purging.

3. Inbreeding was found to reduce cross-compatibility, which was similarly pronounced in diploids and tetraploids. It also caused inbreeding depression in cumulative fitness, which was significant in diploids but not in tetraploids. No evidence of purging was observed as inbred fitness was not affected by within-population relatedness.

4. *Synthesis.* Our results provide new insights into the contrasting invasion success of the cytotypes of *C. stoebe*. As the effects of cross-compatibility and purging were comparable between cytotypes, both processes can be ruled out to affect the colonization success of diploids versus tetraploids. Our findings are consistent with the hypothesis that polyploidy increases the masking of recessive mutations, which maintains high fitness in inbred tetraploids and may thus facilitate colonization of new ranges. We highlight that reduced inbreeding depression may add to previously acknowledged advantages of polyploids in range expansions, a mechanism that may hitherto have been underestimated due to a lack of data on variation in inbreeding depression across geocytotypes.

Key-words: coefficient of relationship, founder effects, genetic bottleneck, geocytotype, inbreeding depression, invasion ecology, purging, *S*-alleles, sporophytic self-incompatibility, spotted knapweed

Introduction

Polyploid plants are more likely to become invasive than their diploid counterparts (Pandit, Pocock & Kunin 2011). Consequently, growing emphasis is being placed on elaborating the mechanisms that lead to higher colonization capabilities in polyploid species (Pandit, White & Pocock 2014; Bock *et al.* 2015; Suda *et al.* 2015). Although these recent studies have generated remarkable progress (reviewed in te

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Beest *et al.* 2011), the potential influence of ploidy level on the consequences of founder events is still understudied (Rosche *et al.* 2016a). This is surprising since evidence has accumulated that the negative impacts of founder effects may play a major role in invasion dynamics by considerably reducing establishment success, population growth and, ultimately, spread rates (e.g. Murren & Dudash 2012; Mullarkey, Byers & Anderson 2013; Szűcs *et al.* 2014; Schrieber & Lachmuth 2016). During initial colonization and subsequent range expansion at the leading edges (Slatkin & Excoffier 2012; Hufbauer *et al.* 2013; Dlugosch *et al.* 2015), small and isolated founder populations may undergo frequent mating among relatives, which increases homozygosity (i.e. inbreeding). In strictly self-incompatible species, such biparental inbreeding may reduce population growth for two reasons: (i) cross-compatibility may decrease, mainly as a consequence of the sharing of identical *S*-alleles between closely related mating partners; and (ii) offspring fitness can be reduced (i.e. inbreeding depression).

Self-incompatibility is a genetically controlled pollen–pistil cell–cell recognition system to avoid self-fertilization that is realized by different mechanisms across plant taxa (Franklin-Tong 2008). For instance, in Asteraceae, sporophytic self-incompatibility is controlled by an *S*-locus consisting of a pistil-expressed and a pollen-expressed *S*-gene, which together form a non-recombining *S*-haplotype (i.e. the *S*-allele; Brennan, Harris & Hiscock 2013). In addition to inhibiting self-pollination, crosses between mating partners are prevented when partners share identical *S*-alleles. Mating among close relatives increases the risk of *S*-alleles being shared, as such, inbreeding and/or a stochastic loss of *S*-alleles in bottlenecked populations may reduce cross-compatibility among mating partners (Brennan, Harris & Hiscock 2006; Wagenius, Lonsdorf & Neuhauser 2007). However, strong negative frequency-dependent selection favours high *S*-allele diversity, and dominance interactions can conceal recessive *S*-alleles in systems with sporophytic self-incompatibility (Busch, Witthuhn & Joseph 2014). Both phenomena increase cross-compatibility among mating partners (Brennan, Harris & Hiscock 2013), which facilitates reproduction in small populations but also allows biparental inbreeding, which can lead to inbreeding depression.

Inbreeding depression is predominantly based on the homozygous expression of recessive deleterious alleles with some contribution from heterozygote advantage (reviewed in Charlesworth & Willis 2009). The genomewide portion of deleterious recessive alleles is defined as the genetic load. Non-deleterious alleles are usually dominant and mask the genetic load (Hedrick, Hellsten & Grattapaglia 2016). Due to their multiplied chromosome sets and consequently higher number of alleles per locus, polyploids may mask recessive deleterious mutations more efficiently than diploids resulting in lower inbreeding depression (reviewed in Soltis & Soltis 2000). However, when approximating the mutation–selection equilibrium over a longer time period, a more efficient masking of recessive deleterious mutations in polyploids can lead to a greater accumulation of deleterious alleles in polyploid than in diploid genomes (Ronfort 1999). Among the few studies addressing inbreeding in polyploid complexes, some have confirmed lower levels of inbreeding depression in polyploids (e.g. Eliášová *et al.* 2013), while others found comparable inbreeding depression between diploids and polyploids (e.g. Galloway & Etterson 2007).

In addition to ploidy level, the demographic history of populations can affect the present-day degree of inbreeding depression. Population bottlenecks induce genetic drift and inbreeding, both of which can augment within-population relatedness and, at the same time, can reduce inbreeding depression. In particular, successive generations of inbreeding can expose deleterious mutations to selection in recessive homozygotes, which can lead to their selective removal (i.e. purging; Crnokrak & Barrett 2002). Purging in turn reduces genetic load in the gene pools of populations and increases fitness of inbred progeny. More specifically, during colonization processes that involve repeated demographic bottlenecks, purging may successively diminish inbreeding depression (Pujol *et al.* 2009). As such, demographic disequilibria in the course of biological invasions may result in lower inbreeding depression in invasive than native populations (Facon *et al.* 2011).

For the present study, we investigated the consequences of inbreeding on cross-compatibility and fitness in spotted knapweed (*Centaurea stoebe* L. s.l.; Asteraceae; syn. *C. maculosa* Lam.), a strictly self-incompatible polyploid complex consisting of the diploid *C. stoebe* ssp. *stoebe* and the tetraploid *C. stoebe* ssp. *micranthos* (Mráz *et al.* 2011). A fundamental cytotype shift between its invasive and native range makes *C. stoebe* an excellent model for studying the relevance of polyploidy in colonization success (te Beest *et al.* 2011): whereas diploids represent the majority cytotype in the native Eurasian range (Broennimann *et al.* 2014), exclusively tetraploids became established in North America (Mráz *et al.* 2011). Thus, three geocytotypes (GCTs) can be defined by ploidy level and range: native diploids (EU2x), native tetraploids (EU4x) and invasive tetraploids (NA4x). We performed inbred and outbred crosses in 42 *C. stoebe* populations varying in range, cytotype and degree of natural inbreeding (i.e. within-population relatedness). We then assessed the cross-compatibility of inbred versus outbred crosses and recorded inbreeding depression in offspring fitness.

The consequences of inbreeding on cross-compatibility or offspring fitness have never been investigated in a model system comprising distinct GCTs. This first study in that context was driven by the following hypotheses: (i) Cross-compatibility is reduced in inbred crosses; (ii) outcrossed progenies outperform inbred progenies, which is taken as an indicator for inbreeding depression; (iia) inbreeding depression decreases with increasing degree of natural inbreeding (i.e. within-population relatedness) due to purging; (iib) inbreeding depression is weaker in NA4x than in EU4x as a result of purging during the colonization of the invaded range; and (iic) inbreeding depression is lower in tetraploids than in diploids due to the higher probability of deleterious recessive mutations being masked in tetraploids.

Materials and methods

THE STUDY SYSTEM *CENTAUREA STOEBE* S.L

The two cytotypes of *C. stoebe* are reproductively isolated due to the strongly reduced viability of progeny of interploidy crosses because of unbalanced ratio between male and female genomes in the endosperm (i.e. triploid block; Marks 1966), and the infertility of extremely rare triploid hybrids (Mráz *et al.* 2012a). The tetraploid cytotype is considered to be a very young neopolyploid that originated from the hybridization between the diploid cytotype and an as yet unknown but closely related parental taxon (Mráz *et al.* 2012b). Although allopolyploidy may lead to the independent segregations of the two parental genomes (i.e. disomic inheritance; Barcaccia *et al.* 2014), tetraploid *C. stoebe* shows tetrasomic inheritance of microsatellite alleles (Rosche *et al.* 2016a).

Diploids and tetraploids are similar in their morphology (Mráz *et al.* 2011) and have comparable ecological amplitudes: both occupy dry, (semi)-natural (e.g. rocks, steppe slopes, dry grasslands) and ruderal habitats (Ochsmann 2000). The most important difference is that tetraploids are polycarpic, whereas diploids are predominantly monocarpic (Mráz *et al.* 2011). Both cytotypes are strictly self-incompatible; they are mainly pollinated by Hymenoptera (Mráz *et al.* 2012a) and disperse their achenes via barochory (Hahn *et al.* 2013). These gene dispersal agents generate moderate levels of natural inbreeding within populations in all three GCTs (Rosche *et al.* 2016a). The protandric florets of *C. stoebe* open successively towards the capitulum centre. Pollen is available for 1–4 days in each capitulum. Each floret exposes pollen for about 24 h; afterwards, the stigma becomes receptive for about 12–36 h (personal observations).

Tetraploids were introduced to North America in the late 19th century. Following a lag phase of 50 years (Broennimann *et al.* 2014), tetraploid *C. stoebe* has become one of the most noxious weeds in North America (Maron *et al.* 2013) and causes enormous economic damage (Corn, Story & White 2006).

SAMPLING

We sampled 14 populations of EU2x, 13 of EU4x and 15 of NA4x (Fig. 1; Table S1 in Supporting Information), where we collected matured capitula from four plants per population (i.e. four seed families). To reduce the probability of sampling close relatives, seed families were chosen in such a way so as to maximize the spatial distance between them. Sufficient replication at the population level with broad environmental gradients among populations is required to representatively assess inbreeding depression at the GCT level, because inbreeding depression may vary substantially across populations depending on the history of natural inbreeding and prevailing environmental conditions (Leimu, Kloss & Fischer 2008). Thus, we aimed at increasing the number of populations rather than seed families per population. Data on the ploidy level of the seed families (based on flow cytometry) and population genetic data (based on eight microsatellites) were available from Rosche *et al.* (2016a). Within each GCT, we calculated pairwise coefficients of relationship between all individuals (ρ_{ij}) using SPAGEDI 1.4 (Hardy & Vekemans 2002). We then averaged ρ within populations to establish an estimate of within-population relatedness with a view to describing the history of genetic drift and natural inbreeding within each population (see Table S1 for ρ of each study population). The ρ-statistic approach was used as it provides estimates that are the most comparable across different ploidy levels (Dufresne *et al.* 2014).

BREEDING EXPERIMENT

In October 2012, 10 achenes per seed family were germinated on water-filled Petri dishes. Emerged seedlings were placed on planting trays and grown in the glasshouse (25/15 °C day/night with a 16-h photoperiod). After 6 months, plants were transferred to the Botanical Garden in Halle (51.49° N; 11.96° E) where they received ample water supply and phosphorous fertilization in order to promote flowering (Kamasol Brilliant Rot, Compo Expert, applied according to manufacturer's instructions).

To avoid adverse environmental conditions, crosses were conducted in the glasshouse. Three individuals per seed family were used in hand-pollination breeding treatments with two levels: biparental inbreeding via sib-mating (at least half-sib) versus within-population outbreeding. Each plant of the parental generation was crossed with two plants from the same seed family and two from another seed family. Thus, four crosses per individual were conducted resulting in 12 inbred and 12 outbred lineages per population (Fig. S1). Where necessary, the breeding design was adjusted according to the availability of flowering plants per seed family (see Table S2). To prevent cross-pollination by insects, capitula were covered with mesh bags before anthesis and remained bagged until achenes had ripened. Crosses were conducted reciprocally by rubbing the respective capitula together with each plant serving as a pollen donor (paternal) and as a pollen acceptor (maternal). Each cross was initiated when pollen developed on both mating capitula and was carried out daily until stigmas were no longer receptive. Where necessary, other available pollen donor capitula from the same donor plant were used to ensure exhaustive pollination. A total of 1780 crosses were achieved. Thirty capitula from 30 separate individuals kept in closed mesh bags were checked for autogamous selfing. None of them set any fruit, indicating strict self-incompatibility in both diploid and tetraploid *C. stoebe*.

SURVEY OF CROSS-COMPATIBILITY

In October 2013, we recorded cross-compatibility among individuals. Partners were considered cross-compatible when at least one floret produced a fertile achene (i.e. darkish and full-sized). It is noted that in addition to sharing *S*-alleles, cross-compatibility may also be influenced by inbreeding depression, which may reduce the number of fertile achenes during early seed development (Harder, Hobbhahn & Richards 2012). A total absence of fertile achenes is, nonetheless, rather unlikely to be attributed to inbreeding depression, because each achene exhibits a different recombination of the involved parental genomes and not all combinations should result in the abortion of a fertilized ovule due to inbreeding depression. Therefore, our results for cross-compatibility may be attributed to the effects of self-incompatibility.

SURVEY OF FITNESS COMPONENTS

From all crosses that yielded achenes (i.e. cross-compatible crosses), we choose up to six inbred and six outbred lineages per population to estimate offspring fitness (482 lineages; 76 EU2x inbred, 80 EU4x inbred, 85 NA4x inbred, 81 EU2x outbred, 80 EU4x outbred, 87 NA4x outbred). For each population, we chose lineages that represented each of the parental seed families as equally as possible. The remaining lineages that were created in our breeding experiment were consequently not involved in any further observations. The seeds from the chosen lineages were then used for a germination experiment. Where available, ten achenes per lineage were germinated in

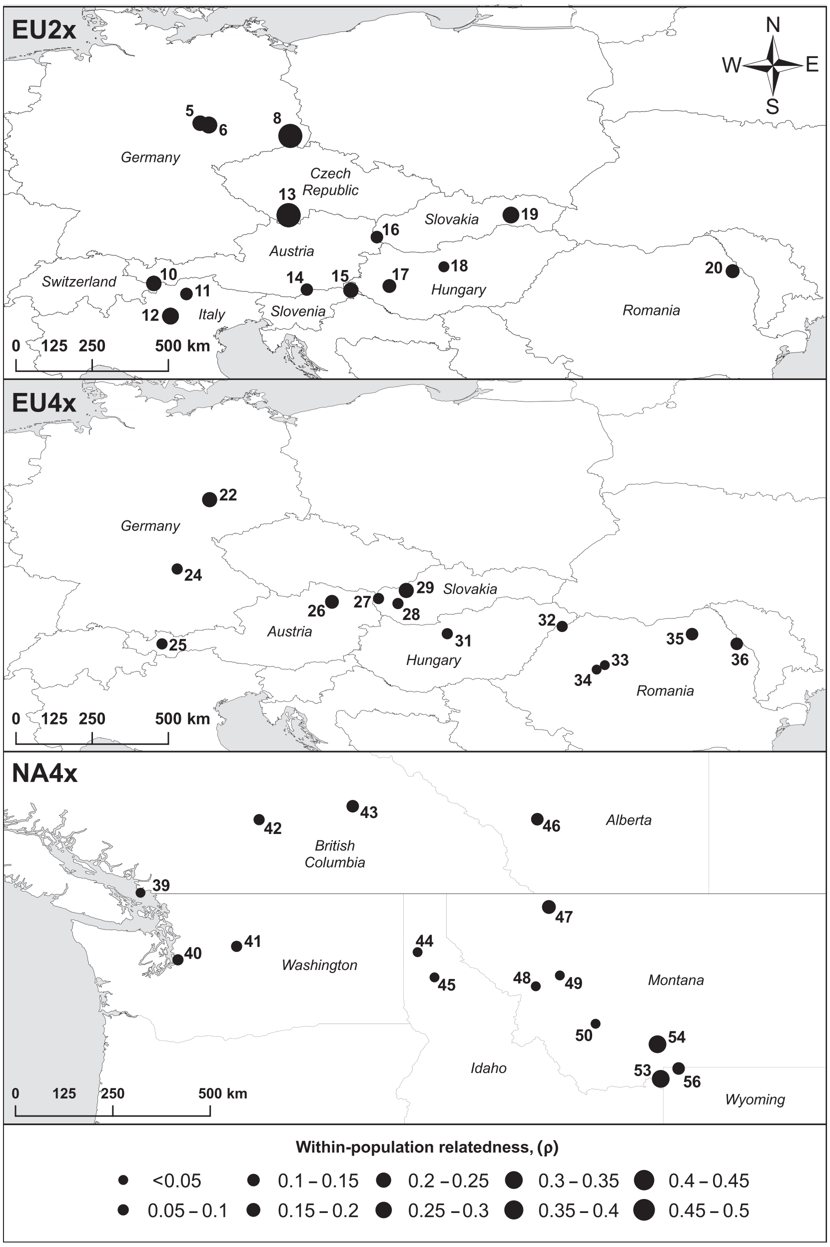

Fig. 1. Distribution of the *Centaurea stoebe* populations involved in the crossing experiments. The figure is divided into three maps, each of which corresponds to a geocytotype (EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid). The size of the dots refers to the within-population relatedness inferred from eight microsatellite markers. Further population characteristics can be found in Table S1.

water-filled Petri dishes placed in germination chambers (20 °C/10 °C with a 12-h photoperiod). For logistical reasons, we split the germination experiment into three separate runs: germination of the first cohort was initiated on October 20, the second on October 27 and the third on November 11. GCTs and breeding treatments were distributed equally across cohorts (Table S2). We recorded the total number of seeds that germinated within 12 days (i.e. germination success).

The seedlings of 430 lineages belonging to the first two germination cohorts were pricked out on planting trays immediately upon germination and moved to the glasshouse (25 °C/15 °C with a 12-h photoperiod). Five planted seedlings from each lineage were then randomly chosen for the subsequent assessment of fitness components, with the remaining seedlings serving as backups and grown under the same conditions. Within the first 5 days, all dead individuals were replaced with randomly chosen backup seedlings of the same lineage (where available). Juveniles were then repotted into 0.8-L pots after 3 weeks and into 2.2-L pots after 9 weeks.

In April 2014, the pots were transferred to the experimental site in the Botanical Garden in Halle. To simulate ruderal habitats typical of initial colonization stages (i.e. less competition, high radiation, drought exposure, low soil depth; Davis, Grime & Thompson 2000), we excluded competitors every 3 weeks, exposed the plants to full sunlight, watered them only in times of extreme drought and used thick plastic planes to avoid attachment of roots with ground soil. In August 2014, we recorded whether plants had survived and whether they had flowered, in which case we counted the number of capitula per plant (including flowering capitula, matured capitula and buds >3 mm according to Mráz *et al.* 2011). Throughout the experiment, the Petri dish and pot positions were frequently randomized. In accordance with Oakley & Winn (2012), we calculated a composite index of fitness over one vegetation period, that is cumulative fitness as a product of germination success, survival, flowering probability and number of capitula. We are, however, well aware that this proxy of fitness cannot unequivocally describe invasiveness. Nonetheless, this estimate allows comparing the relative performance of the different GCTs in habitats that are similar to the conditions we investigated (which simulated the typical habitats of initial colonization; see above).

STATISTICAL ANALYSES

Statistical analyses were performed using the LME4-package (Bates *et al.* 2014) implemented in R 3.1.2 (R Development Core Team 2014). For responses with Gaussian error distribution, we used linear mixed-effects models. Decisions on the transformation of variables followed graphical assessment of variance homogeneity and normality of errors (Crawley 2014). For responses with binomial or Poisson error distribution, we used generalized linear mixed-effects models. All models were fitted with a maximum-likelihood approach. In order to identify the minimal adequate models, we removed non-significant fixed effects in a stepwise backward manner based on chi-square tests.

We analysed the following response variables (see Table 1): cross-compatibility (binomial) and the fitness components of the offspring [germination success (binomial), survival (binomial), flowering probability (binomial), $\log_e$ number of capitula (Gaussian), square-root cumulative fitness (Gaussian)]. The models for all responses included an interaction of breeding treatment and GCT, an interaction of breeding treatment and $\log_e \rho$ (centred and scaled) and the respective main effects. In addition, latitude (centred and scaled) was included to account for the fact that *C. stoebe* shows adaptive differentiation along latitudinal environmental clines (Mráz, Tarbush & Müller-Schärer 2014). Germination cohort and the nested factors of pollen donor individual within donor seed family within donor population as well as of pollen acceptor individual within acceptor seed family within acceptor population were set as random effects.

Results

INBREEDING AND CROSS-COMPATIBILITY

Cross-compatibility, expressed as a probability to produce at least one well-developed achene per capitulum after crosses, was significantly influenced by the interaction of within-population relatedness (ρ) and breeding treatment ($\chi^2_{(1)} = 4.77$, $P < 0.05$, Table 1): overall, cross-compatibility was predominantly higher in outbred than inbred crosses, and it decreased with increasing ρ. This decline of cross-compatibility was more pronounced in outbred than in inbred crosses (Fig. 2). Moreover, cross-compatibility differed significantly between GCTs ($\chi^2_{(1)} = 6.46$, $P < 0.05$). The cross-compatibility of EU2x significantly exceeded that of NA4x, while the other GCT combinations did not show any differences. The means of cross-compatibility over breeding treatment × GCT are given in Table S3.

INBREEDING AND FITNESS COMPONENTS

All fitness components along with the cumulative fitness were lower in inbred than in outbred progeny (Fig. S2). The strongest inbreeding depression among the fitness components was found for the number of capitula. The factors that influenced the degree of inbreeding depression differed among the fitness components considered (Table 1).

Table 1. Analyses testing for the interactive effects of breeding treatment and geocytotype and the interactive effects of breeding treatment and within-population relatedness on cross-compatibility and fitness components of *Centaurea stoebe*. The table gives parameter estimates from the respective minimal adequate mixed-effects models for each fixed effect (with the second level of factors subscripted). Parameter estimates of significant fixed effect terms are bold. Parameter estimates of main effects involved in significant interaction are provided, but their significance has not been tested (Crawley 2014). Where geocytotype or its interactions were significant, the significance level does not correspond to the particular pairwise comparison, but to the overall fixed effect. Note that $\log_e \rho$ and latitude were scaled and centred in the models. Variance estimates are given for random effects, and the number of groups at each random effect level is given in parentheses

	Cross-compatibility	Germination success	Survival	Flowering probability	$\log_e$ no. capitula	Sqrt cumulative fitness
Fixed effects						
(Intercept)	0.331	2.134	−0.432	0.48	2.1	1.102
Latitude	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$\log_e \rho$	−0.133[a]	n.s.	n.s.	n.s.	−0.003[a]	0.006[a]
$\mathrm{BT}_{\mathrm{Outbred}}$	0.35[a]	2.1[a]	0.481[a]	**0.356***	0.079[a]	0.868[a]
$\mathrm{GCT}_{\mathrm{EU4x}}$	−**0.243***	0.207[a]	0.488[a]	**1.457*****	n.s.	0.647[a]
$\mathrm{GCT}_{\mathrm{NA4x}}$	−**0.716***	0.534[a]	0.52[a]	**1.465*****	n.s.	0.675[a]
$\mathrm{BT}_{\mathrm{Outbred:}}$ $\log_e \rho$	−**0.233***	n.s.	n.s.	n.s.	**−0.12***	**−0.209***
$\mathrm{GCT}_{\mathrm{NA4x:}}$ $\mathrm{BT}_{\mathrm{Outbred}}$	n.s.	**−1.389*****	**−0.516***	n.s.	n.s.	**−0.739****
$\mathrm{GCT}_{\mathrm{EU4x:}}$ $\mathrm{BT}_{\mathrm{Outbred}}$	n.s.	**−0.631*****	**−0.481***	n.s.	n.s.	**−0.594****
Random effect variances						
Acceptor: Population	0.15 (42)	0.00 (42)	0.00 (37)	0.03 (37)	0.00 (37)	0.00 (37)
Acceptor: Population/SF	0.22 (146)	0.58 (136)	0.03 (120)	0.00 (120)	0.02 (120)	0.00 (120)
Acceptor: Population/SF/Ind	0.19 (476)	0.00 (312)	0.00 (278)	0.00 (267)	0.00 (254)	0.00 (279)
Donor: Population	0.11 (42)	0.00 (42)	0.00 (37)	0.09 (37)	0.00 (37)	0.00 (37)
Donor: Population/SF	0.05 (146)	0.14 (137)	0.00 (120)	0.05 (119)	0.00 (118)	0.02 (120)
Donor: Population/SF/Ind	0.11 (472)	0.74 (316)	0.00 (281)	0.00 (270)	0.00 (257)	0.00 (281)
Germination cohort	n.t.	0.00 (3)	0.00 (2)	0.00 (2)	0.00 (2)	0.00 (2)
Residuals	n.e. (1787)	n.e. (481)	n.e. (1927)	n.e. (971)	0.5 (793)	0.83 (432)

GCT, geocytotype; NA4x, invasive tetraploid; EU4x, native tetraploid; BT, breeding treatment; SF, seed family; Ind, individual; sqrt, square-root; ρ, within-population relatedness; n.s., not significant; n.e., not estimated; n.t., not tested; [a], main effect in significant interaction; significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

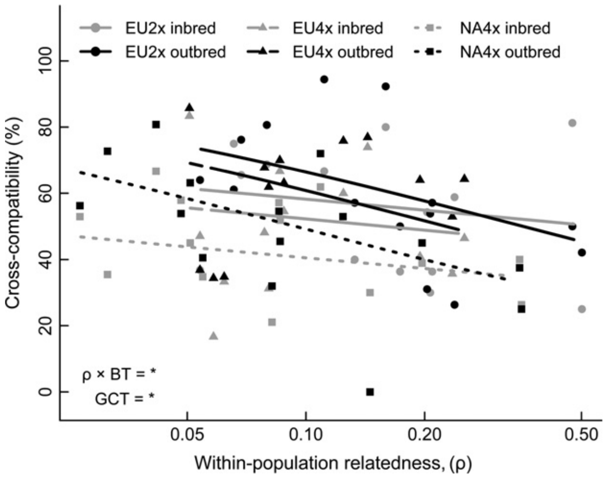

Fig. 2. Cross-compatibility as a function of geocytotype and of breeding treatment in interaction with within-population relatedness (ρ). Observation points represent means over populations and breeding treatments, and lines represent predictions of the minimal adequate model. EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid; ρ × BT, interaction of within-population relatedness and breeding treatment; GCT, geocytotype; significance level: *$P < 0.05$.

Flowering probability was the only fitness component that was exclusively affected by main effects and not by any of the interactions. Flowering probability was higher in outbred than in inbred individuals ($\chi^2_{(1)} = 4.5$, $P < 0.05$), and it differed among GCTs ($\chi^2_{(1)} = 31.18$, $P < 0.001$): both tetraploid GCTs showed a higher flowering probability than diploids, while there were no differences between tetraploid GCTs.

The interaction of breeding treatment with ρ significantly influenced the number of capitula ($\chi^2_{(1)} = 5.25$, $P < 0.05$) and the cumulative fitness ($\chi^2_{(1)} = 4.82$, $P < 0.05$). The number of capitula was slightly higher in outbred than in inbred offspring (Fig. 3a). While for outbred individuals the number of capitula decreased significantly with increasing ρ, inbred individuals were almost unaffected by ρ. Cumulative fitness was predominantly lower in inbred than outbred progeny (Fig. 3b). The magnitude of inbreeding depression became smaller with increasing ρ, but such decreasing difference between inbred and outbred fitness could not be ascribed to purging, because the fitness of inbred progeny did not increase with increasing ρ.

The interaction of GCT and breeding treatment significantly influenced germination success ($\chi^2_{(1)} = 11.41$, $P < 0.01$), survival ($\chi^2_{(1)} = 6.04$, $P < 0.05$) and cumulative fitness ($\chi^2_{(1)} = 11.54$, $P < 0.01$). The germination success of outbred progeny did not markedly differ among GCTs. In EU2x and in EU4x, the germination success of outbred progeny was significantly higher than that of inbred progeny, but there was no such difference in NA4x (Fig. 4a). Survival was similar across GCTs. While outbred individuals showed significantly higher survival than inbred individuals in diploids, both tetraploid GCTs did not reveal this pattern (Fig. 4b). Outbred cumulative fitness did not differ between GCTs. In contrast, inbred fitness differed among GCTs, with both tetraploid GCTs outperforming diploids. In particular, for diploids, inbred fitness was significantly lower than outbred fitness, which was not the case for both tetraploid GCTs (Fig. 4c). Mean values for the cumulative fitness and each fitness component divided by breeding treatment × GCT are given in Table S3. Within GCTs, population means varied strongly (Fig. 3b, Table S3).

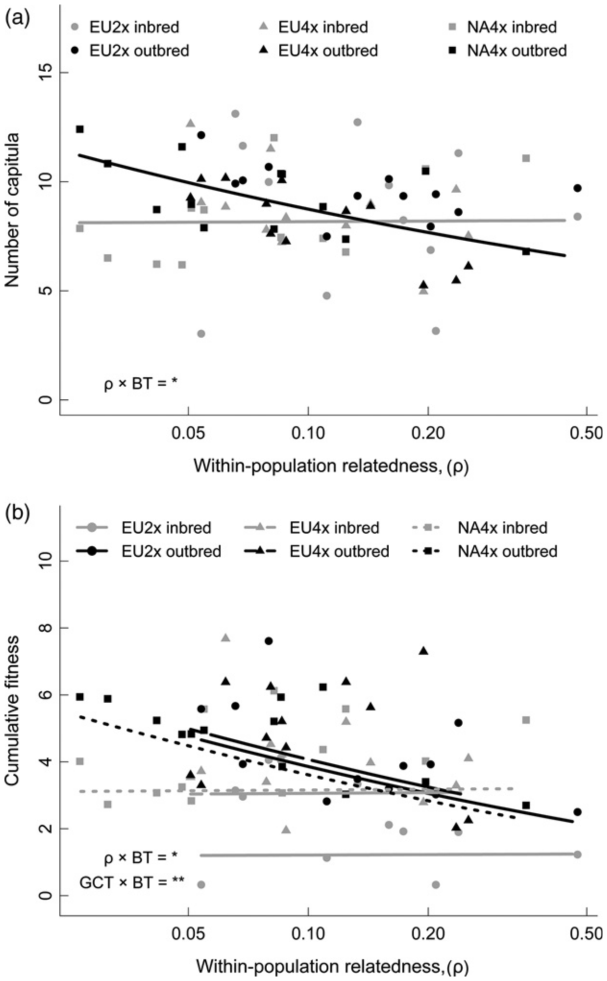

Fig. 3. Interactive effects of within-population relatedness and breeding treatment on (a) number of capitula and (b) cumulative fitness. Observation points represent means over populations and breeding treatments and lines represent predictions of the minimal adequate model. Note that in (a), lines reflect the predictions of the minimal adequate model for inbred (grey) and outbred (black) individuals, while in (b), lines reflect the predictions of the minimal adequate model for inbred and outbred individuals within each geocytotype (see legend). EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid; ρ × BT, interaction of within-population relatedness and breeding treatment; GCT × BT, interaction of geocytotype and breeding treatment; significance levels: *$P < 0.05$; **$P < 0.01$.

Discussion

IMPACT OF INBREEDING ON CROSS-COMPATIBILITY

Experimental inbreeding (breeding treatment) as well as increasing natural inbreeding (within-population relatedness) reduced cross-compatibility. The latter effect was significantly more pronounced in outbred than in inbred offspring, because with increasing relatedness among mating partners,

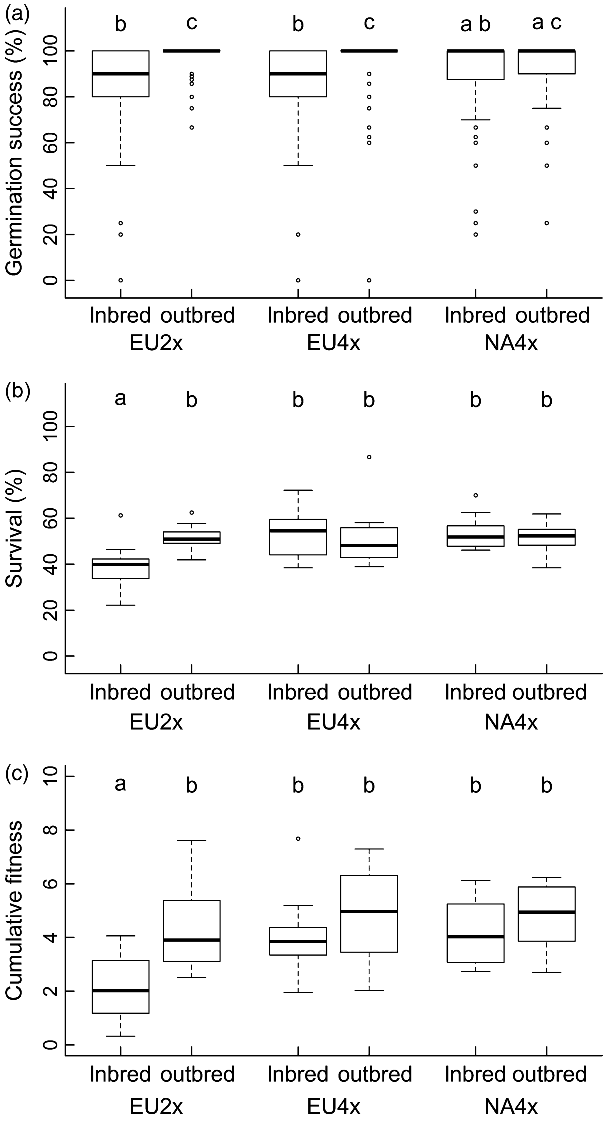

Fig. 4. Interactive effects of geocytotype and breeding treatment on (a) germination success, (b) survival and (c) cumulative fitness. Groupings (lower case letters) are based on Tukey's *post hoc* tests (pairwise comparisons with a significance level of $P < 0.05$). EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid.

outcrossing becomes more similar to sib-mating (Angeloni, Ouborg & Leimu 2011). In particular, for the *C. stoebe* populations that exhibited the highest within-population relatedness, we found nearly identical cross-compatibility in inbred as compared to outbred crosses. Previous studies confirmed that small populations with closely related individuals show reduced cross-compatibility (Willi, Van Buskirk & Fischer 2005; Young & Pickup 2010) and that reduced *S*-allele pools can considerably decrease colonization success (Wagenius, Lonsdorf & Neuhauser 2007).

Cross-compatibility did not differ between EU2x and EU4x, and the response of cross-compatibility to breeding treatment did not differ between GCTs. Consequently, our results on cross-compatibility cannot contribute to explain the cytotype shift between the ranges of *C. stoebe*. So far, exclusively Pickup & Young (2007) investigated self-incompatibility within a polyploid complex in Asteraceae (*Rutidosis leptorrhynchoides*) and they also found comparable cross-compatibility in different cytotypes.

In general, we found an overall moderate cross-compatibility, even in populations with high within-population relatedness and following inbred crosses. The reasons for the rather high cross-compatibility in inbred crosses may be extensive dominance interactions among *S*-alleles (as commonly observed in Asteraceae; see Brennan, Harris & Hiscock 2013) and maintenance of high *S*-allele diversity through negative frequency-dependent selection (see Busch, Witthuhn & Joseph 2014). During founder events, the high cross-compatibility in inbred crosses may have positive demographic effects due to increased reproduction (Barrett 2015), but it may also have negative consequences with respect to the occurrence of inbreeding depression.

IMPACT OF INBREEDING ON OFFSPRING FITNESS

Inbreeding reduced fitness, which was largely consistent across the investigated fitness components. Since composite fitness indices are more informative than single-stage fitness proxies (Oakley & Winn 2012), the following focuses on inbreeding depression in cumulative fitness. Differences in cumulative fitness between inbred and outbred progeny decreased significantly with increasing within-population relatedness. However, as opposed to being positively related to within-population relatedness, inbred fitness remained constant across the range of within-population relatedness. This finding refutes the hypothesis that purging represents the mechanism that accounts for the decreasing inbreeding depression. Several studies suggested that substantial genome-wide effects of purging occur only under specific conditions, such as intermediate and constant bottlenecks over several generations (e.g. Keller *et al.* 2012; Kennedy *et al.* 2014). Moreover, no purging can be expected for loci at which the underlying mechanism of inbreeding depression is heterozygote advantage (Charlesworth & Willis 2009). As such, the observed reduction in inbreeding depression in our study may have resulted from random genetic drift, which may fix mildly deleterious alleles, and the resultant drift load reduces the fitness of outbred individuals to a similar extent than of inbred individuals (Leimu *et al.* 2006; Oakley & Winn 2012; Willi, Griffin & Van Buskirk 2013). In addition, drift increases relatedness among mating partners. The higher the within-population relatedness, the more similar the random mating (i.e. outcrossing) to mating among relatives (i.e. inbreeding) becomes. Therefore, outcrossing can result in similar homozygosity as sib-mating (Angeloni, Ouborg & Leimu 2011), which aligns with the comparable cumulative fitness observed between outbred and inbred individuals in aforementioned populations of high within-population relatedness.

Furthermore, inbreeding depression in cumulative fitness was not affected by range. In fact, we expected lower inbreeding depression due to frequent purging in NA4x, if invasive populations had experienced severe colonization bottlenecks in their invasion history (Facon *et al.* 2011).

However, we found purging to be rather inefficient in *C. stoebe* (see above). Moreover, recent microsatellite data suggest that colonization bottlenecks in NA4x were counteracted by multiple introductions (Rosche *et al.* 2016a). Thus, frequent population admixture apparently restored genetic diversity, obviously including genetic load. In addition, Broennimann *et al.* (2014) demonstrated that parts of the 'native' range of EU4x (i.e. Central Europe) were colonized mainly within the last century along ruderal sites, a situation that corresponds to the colonization history of NA4x, which may have led to partly comparable population histories in both ranges.

Nevertheless, the reduction in germination success was significantly smaller in NA4x as compared to EU4x. This may result from range-specific purging of genetic load, which may be amplified to reflect a strong *r*-selection in favour of fast and successful recruitment (Lachmuth, Durka & Schurr 2011; Colautti & Lau 2015). However, within both tetraploid GCTs, we found a high among-population variation in both cumulative fitness and inbreeding depression. Divergent local adaptation can result from provenance–environment interactions (Zenni, Bailey & Simberloff 2014) and inbreeding–environment interactions (Schrieber & Lachmuth 2016). Both mechanisms may potentially increase invasiveness of certain genotypes in distinct environments. Therefore, further research is needed to test the generality of our results in different environments.

In accordance with our last hypothesis, inbreeding depression in cumulative fitness was significant in diploids but not in tetraploids, with survival having contributed most to this effect. Increased homozygosity of deleterious recessive alleles may have resulted in increasing mortality in the inbred diploids, whereas the whole-genome duplication obviously increased the masking of genetic load in the polyploids. Most, but not all, of the previous studies that have tested variation in inbreeding depression between cytotypes are concordant with our results (reviewed in Soltis & Soltis 2000). According to Galloway & Etterson (2007), two processes in polyploid speciation may lead to inconsistent results among inbreeding studies in polyploid complexes. First, under perfect tetrasomic inheritance, the probability of becoming homozygote is reduced by 50% in tetraploids compared to diploids (Bever & Felber 1992), whereas disomic inheritance of two rather independent genome parts would not lead to such effective masking of recessive deleterious alleles (Barringer & Geber 2008). Secondly, with increasing evolutionary age, polyploids accumulate detrimental alleles (Ronfort 1999). The large reductions in inbreeding depression in tetraploid *C. stoebe* may therefore have resulted from both its tetrasomic inheritance (Rosche *et al.* 2016a) and its evolutionary rather young polyploid origin (Mráz *et al.* 2012b).

POTENTIAL CONSEQUENCES OF POLYPLOIDY IN COLONIZATION CAPACITY

Our results clearly demonstrate that inbreeding reduces cross-compatibility and fitness in *C. stoebe*, which may partly explain the 50 years of lag phase during the species' invasion of North America (Broennimann *et al.* 2014). Subsequently, the reported population admixture in the invasion history of NA4x (Rosche *et al.* 2016a) may have led to genetic rescue of genetically depleted populations (Frankham 2016). Population admixture following multiple introductions has repeatedly been shown to boost invasion success (reviewed in Rius & Darling 2014). The influx of novel alleles into invasive populations creates unique opportunities for genetic interactions among previously isolated gene pools (Dlugosch *et al.* 2015). This may increase adaptive potential and alleviate both reduced cross-compatibility and inbreeding depression.

The effects of inbreeding on cross-compatibility did not differ between cytotypes. However, the polycarpic life cycle may enable tetraploids to outlast flowering seasons in the absence of compatible mating partners. In subsequent flowering seasons, polycarpic tetraploids may, in contrast to predominantly monocarpic diploids (Mráz *et al.* 2011), reproduce successfully when immigrating diaspores enlarge the *S*-allele pool. This may, in concert with the higher first year flowering probability (our results; Mráz *et al.* 2011) and higher lifetime seed output of tetraploids versus diploids (Broz *et al.* 2009), have enhanced the colonization success of tetraploid *C. stoebe* populations. In fact, the demographic consequences of the different life cycles might represent a key determinant of increased colonization capabilities in tetraploids compared to diploids. Interestingly, in a common garden study with the three GCTs, population growth rates were comparable between diploid and tetraploid *C. stoebe* (Hahn, Buckley & Müller-Schärer 2012). However, the artificial founder populations in that study comprised individuals from several populations per GCT and thus the populations experienced no genetic depletion, a scenario, which seems unlikely at least for the early stages of biological invasions (Theoharides & Dukes 2007).

Most importantly, our results show that polyploidy significantly counteracts inbreeding depression, which in turn provides a strong indication of a reduction in the negative demographic consequences of inbreeding in tetraploid founder populations. Thus, tetraploid founder populations may have been more likely to outlast critical early invasion phases until the influx of new genetic material allowed the sudden spread of the species across North America. Diploids, in contrast, face substantial inbreeding depression, and they cannot easily endure seasons with *S*-allele-mediated restrictions of mate availability. During phases of demographic disequilibrium, such disadvantages may ultimately exclude small diploid founder populations from a novel range, even before their gene pools can be restored via multiple introductions.

Future studies should aim at disentangling the relative contribution of self-incompatibility and inbreeding depression on reproductive output. Such studies should apply *S*-locus genotyping combined with parent diallel-estimations across a large number of within-population crosses in order to estimate the number, segregation and dominance interactions of *S*-alleles (see Brennan, Harris & Hiscock 2013). Empirical estimations of population growth rates with artificial founder populations (see Hahn, Buckley & Müller-Schärer 2012) may be conducted with individuals of differing breeding background and under varying environmental scenarios (see Szűcs *et al.* 2014), which can help to disentangle the relative importance of longevity and inbreeding depression on population growth. Moreover, demographic modelling (e.g. Smallegange & Coulson 2013) may reveal how inbreeding interacts with contrasting life histories to shape demographic rates and population growth in the three GCTs.

Since the effects of polyploidy on founder effects are most likely not the only explanations for the invasion success of NA4x, more ecoevolutionary research seems necessary to fully resolve the cytotype shift phenomenon in *C. stoebe*. For example, the potential of diploids versus tetraploids to form high-performance genotypes (Matesanz & Sultan 2013) and beneficial provenance–environment interactions (Zenni, Bailey & Simberloff 2014) through past evolutionary divergence or rapid evolutionary changes may clearly deepen our mechanistic understanding of the invasion success of NA4x (Colautti & Lau 2015). Moreover, the specific role of allopolyploidy needs further investigation, since interspecific hybridization can remarkably trigger evolutionary change due to transgressive segregation (Colautti & Lau 2015; Dlugosch *et al.* 2015).

Conclusions

Despite growing awareness of the significance of both polyploidy and inbreeding in biological invasions, this is the first study to address the consequences of mating among relatives in a GCT system. While cross-compatibility did not differ between GCTs, reduced inbreeding depression in tetraploids helps explain the drastic cytotype shift between the native and invasive ranges of *C. stoebe*, and in addition, the recent spread of tetraploids in the native range (Mráz, Tarbush & Müller-Schärer 2014). More generally, reduced inbreeding depression in polyploids may add an important keystone to the explanation for the overrepresentation of polyploids among invasive species. However, single case studies cannot identify complex mechanisms of global phenomena, and thus, more studies on polyploid complexes with polyploids of varying age since speciation and differing mode of inheritance are urgently required to test the representativeness of our results.

Authors' contributions

C.R. and S.L. conceived the idea and designed this study with significant contributions of I.H. and P.M.; C.R. and M.H. gathered data. Population genetic analyses were done at the laboratory of W.D.; C.R. and S.L. performed the analyses. C.R. drafted the manuscript with substantial contributions from all co-authors.

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Data accessibility

All data are available at the Dryad Digital Data Repository http://dx.doi.org/10.5061/dryad.pq6ts (Rosche *et al.* 2016b).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Scheme of the experimental crosses performed within each population.

Fig. S2. Relative performances of inbred vs. outbred progeny in four fitness components and in the cumulative fitness.

Table S1. Populations involved in the breeding experiment.

Table S2. Inbred and outbred lineages from which cross-compatibility and fitness components were obtained.

Table S3. Means and standard deviations of the cross-compatibility, of the fitness components and of the cumulative fitness over geocytotype × breeding treatment combinations.