BES Grant Report:

Studying rapid evolution in plants using resurrection ecology

Year: 2024

Published by: British Ecological Society

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Section 1 - Scientific Research Report

What was the overall aim of the work supported by this grant?

This project investigates the occurrence of contemporary evolution in two natural populations of an arable weed, Centaurium erythraea, using the resurrection method. Specifically, I grew side-by side ancestral genotypes and their contemporary descendants in a common garden and genotyped all individuals with ddRAD SNPs to test whether phenotypic trait shifts have occurred in response to changes in the natural environment of the populations over the past 30 years, and whether the observed trait shifts are consistent with contemporary evolution of natural populations in response to climate change and pollinator decline. The project design establishes protocols for future resurrection studies using the same method on different arable species. The seeds produced by within generation-within population open pollination will serve as a “refreshed” F1 generation (with reduced storage and maternal effects) for future resurrection experiments.

Have the overall aims of the project been met?

All planned data, protocols and materials have been successfully acquired, specifically: - phenotypic data (flowering phenology, floral attractivity traits, morphological traits, fitness traits), - ddRAD SNP data (5805 SNPs with a read depth ≥ 10 detected in 164 individuals), - seeds from 40-60 maternal plants per accession stored at +4°C, and - an experimental protocol as developed on C. erythraea and further tested on Mattiola tricuspidata in an independent resurrection experiment at the Goethe University of Frankfurt. Phenotypic data have been analysed to detect patterns of trait shifts in a common garden, showing trait differentiation is consistent with natural population response to climate change and pollinator decline over the past 30 years. Genomic data were used to calculate population genomics descriptors, population differentiation, and GWAS are currently in progress. GWAS analyses have been slowed down because of limited computational power, with preliminary data available for three traits. Fst-Qst comparisons were not possible with the available plant material, as ancestral accessions did not have a known population structure (e.g. material harvested by maternal lines) allowing to estimate additive genetic variance. The F1 seeds however are grouped by half-sib families, which will enable Fst-Qst comparisons in a follow-up experiment (see personal impact).

Background and Rationale

A multitude of anthropogenic threats currently affects ecosystems and the species that underpin them. The cocktail of global warming, intensive agriculture, urbanization, and unintended species introductions has led to several altered and fast-paced stressors on natural populations (Green et al., 2005). As a result, populations of wild plants and animals have drastically decreased (Hallmann et al., 2017). In addition to the detrimental effects that global changes are having on biodiversity in general, one of the most eminent concerns of our time is the steady decline of pollinator species and their services which has been documented in all over the world since the 1950’s (Winfree et al., 2011). While considerable efforts have been made to preserve biodiversity and genetic diversity of crops and endangered species, arable weeds have often been overlooked in conservation policies despite their roles as ecosystem service providers (Edwards, 2015).

A major concern about human-mediated environmental changes is their fast pace to which species could not adapt quickly enough because of the inherently slow nature of evolutionary processes (Bell, 2013). New evidence shows, however, that evolution can occur at contemporary scales, i.e. over a handful of generations. Major life history traits (dispersal traits, mating system, phenology) can evolve over short time spans as a consequence of drivers such as urbanization, climatic shifts, or pesticide use (Brys & Jacquemyn, 2012; Franks et al., 2018). In plants in particular, shifts from outcrossing to reproducing mainly through selfing can evolve in as few as 5 to 10 generations (Brys & Jacquemyn, 2012; Cheptou et al., 2022). Provided that these shifts allow for a reproductive rate which can compensate for the demographic losses, contemporary evolution can potentially keep up with the fast pace of anthropogenic environmental modification and thus rescue populations otherwise doomed to extinction (evolutionary rescue (Bell, 2013)).

Even when occurring at contemporary scales, investigating adaptive responses is often time-consuming in plants (i.e. forward-in-time evolutionary experiments (Kawecki et al., 2012)), expensive (comparisons of genome divergence (Hansen et al., 2012)), and/or relies on heavy assumptions about the populations' evolutionary history (i.e. reciprocal transplants in time, space for time substitution models (Agren & Schemske, 2012)). Resurrection ecology addresses this issue using a backwards-in-time approach, which 'instantly' reveals past evolutionary changes in natural populations (Franks et al., 2018). By reviving ancestral plant genotypes sampled in natural populations and stored in seed repositories, and comparing them to their contemporary descendants in a common environment, the resurrection approach offers vast possibilities to explore whether and how plant floral traits and plant-pollinator interactions have evolved and adapted in their natural environment over contemporary timescales.

Resurrection ecology opens the possibility for the study of rapid evolution in arable weeds, which are exposed to new, intense selective pressures with the development of modern agriculture and global change. In particular, pollinator decline, coupled with phenological mismatches in flowering time due to temperature fluctuations, could lead towards increased self-pollination and a decreased attractivity of plants to pollinators (selfing syndrome). Evolution towards increased selfing and a selfing syndrome could indeed temporarily increase plant fitness in the absence of pollinators, however on the long term it can lead to reduction of plant adaptive potential and permanent decoupling of plant-pollinator interactions (Igic & Busch, 2013). Altogether, this can produce a self-enhancing feedback loop, where the anthropogenic pollinator decline selects for higher selfing rates in plants, which in turn accelerates pollinator decline. Alternatively, arable weeds could evolve towards increasing their attractivity to pollinators, thus maintaining pollinator interactions despite the reduction in pollination services. Empirical observation of contemporary evolution in arable weeds has produced data in line with both hypotheses (Acoca-Pidolle et al., 2024; Brys & Jacquemyn, 2012; Cheptou et al., 2022; Thomann et al., 2015; Valencia-Montoya et al., 2021), and it is likely that the direction of trait changes is species and maybe population-specific.

This project investigated the occurrence of contemporary evolution of floral and reproductive traits in an arable weed, Cetnaurium erythraea, using the resurrection approach. Using seed resources from the European Native Seed Conservation Network (ENSCONET), I compared ancestral and descendant genotypes to investigate whether trait shifts consistent with contemporary evolution in response to climate change and pollinator decline have occurred in natural populations of C. erythraea over the past 30 years. I estimated population genomic parameters relative to historic selfing rates, population differentiation, and genotype-phenotype association using anonymous ddRAD SNPs markers. This project sets up the groundwork for future interdisciplinary resurrection studies on C. erythraea and multispecies resurrection studies on other arable weeds.

Methods

Centaurium erythraea is an annual/biennial arable weed which flowers in July and August in temperate climates. The ability of the species to evolve at contemporary timescales has been demonstrated in response to pollinator decline, which resulted in decreased herkogamy after five generations (Brys & Jacquemyn, 2012). I studied two populations of C. erythraea: PL was originally sampled in southeastern Polland in 2003, and BE in Belgium in 1992. In both countries, pollinator decline has been documented for various taxa (Duchenne et al., 2020; Kosior et al., 2007). Both populations were resampled in 2020. All seed accessions were preserved in a seed repository at -4°C with regulated humidity until the start of the study in 2022.

On the 5th of May 2022, up to 200 seeds per accession were germinated and a month later up to 60 seedlings per accession were transplanted into individual pots. The pots were kept outdoors at the Botanical Garden of the University of Ostrava until November 2022, overwintered in a non-regulated greenhouse, and were taken outside in April 2023. Each accession was kept in a separate mesh-cage to isolate it from pollinators, and every week one cage was opened to allow within-accession open pollination. No other plants of C. erythrae have been observed within a 1km radius from the Botanical Garden, thus the seeds produced from within-accession openpollinations can be used as a 'refreshed' F1 generation (i.e. seeds with decreased storage/environment of origin effects) in future resurrection experiments.

Starting the 21st of June 2023, I recorded newly flowering plants every other day (flowering phenology), and number of flowers on each plant were counted weekly (floral display). On the 30.06 and 11.07 one flower per plant was scanned with the goal to perform analyses of petal colour intensity. The flower was preserved in a 1:1 ethanolo:glycerol mixture. In October 2023 all preserved flowers were dissected, mounted, photographed, and the photographs were analysed with ImageJ. I measured petal length and width and anther and pistil length. I calculated petal surface as π*length*width, and heterostyly as (anther length-pistil length)/max(angther length, pistil length).

At the end of the season, the height of the tallest flowering stalk, the number of flowering stalks, and the number of fruits were measured and counted. The DNA of all individuals was extracted using DNeasy 96 kits from Quiagen, and ddRAD libraries were prepared with ecoRI and mspI at the Helmholtz-Centre for Environmental Research (HZU). Libraries were sequenced at Novogene, and quality checks and data filtering were performed at HZU.

All phenotypic traits were analysed in R 4.3.2. Trait shifts were analysed using glm with population (BE, PL) and accession (ancestral, descendant) as fixed variables. As the descendants of BE were sampled by maternal line, we included maternal family as a fixed effect nested in population. Selection gradients were calculated as the linear and quadratic regression coefficients of traits over absolute differences of mean population and individual fitness following (Lande & Arnold, 1983). Basic population genetic parameters per accession (Hobs, Hexp, Fis, pairwise population Fst), were estimated with packages adegenet and dartR. To visualise grouping of genotypes we ran PCA and as implemented in adegenet and clustered pairwise individual Euclidian distances with the Neighbor-joining method. Genomewides association scans were performed with Fisher's exact tests and adjusted for multiple testing with Bonferroni corrections.

Results and discussion

Phenotypic traits

Germination rates were high in all accessions (80% in PL ancestral, 85-87% in all other accessions), suggesting no long-term storage effects on seed viability.

Due to warm vernalization of the plants and generally warm weather throughout 2023, flowering started about a month earlier than planned, and therefore the beginning and peak of flower production was not recorded for PL ancestors and descendants. Plotting weekly floral counts over time nevertheless shows that in both populations, descendants start flowering later than ancestors (Figure 1). Selection coefficients for phenology favoured delayed flowering in ancestors and descendants, but selection intensity was higher for the descendants (Table2, 3). This delay in flowering is consistent with adaptation of summer annuals to climate change – delayed flowering due to warmer vernalization temperatures could be a mean to avoid prolonged summer droughts (Tun et al., 2021)

I compared the floral display at the peak of flowering of ancestors and descendants only in BE, where the flowering peak was captured, showing that descendants had larger floral display than ancestors, although the differences were only marginally significant (Figure 2, Table 1). Selection for floral display was acting in opposite directions, favouring smaller display in ancestors and larger display in descendants (Table 2, 3). Petal surface was significantly larger in descendants compared to ancestors, and the difference between ancestors and descendants was more pronounced in PL (Figure 3, Table 1). Selection favoured increase in petal size both in ancestors and descendants, but with different intensity among populations and accessions (Table 2, 3).

The flowering stem was significantly taller in PL compared to BE, and significantly taller in BE descendants compared to ancestors (Figure 4, Table 1). Taller flowering stems can render the flowers more visible to pollinators and hence the plants more attractive (Cayenne Engel & Irwin, 2003). Selection coefficients, however, did not show consistent patterns among populations and accession – we detected stabilizing selection for PL ancestral BE descendant accessions, and directional selection favouring shorter stems in PL descendant and BE ancestral (Table 2, 3).

Fitness, estimated as the fruit set, differed significantly between populations but did not differ between ancestors and descendants within a population (Figure 5, Table 1). The general observed trait shifts towards larger floral displays, taller flowering stems and larger flowers are consistent with descendants evolving towards increased attractivity to pollinators. This is in line with the hypothesis of maintenance of pollinator interactions according to which contemporary populations can evolve more attractive traits to maintain pollinator interactions despite pollinator decline in their environment (Thomann et al., 2015). Several selection coefficients, notably those associated with flowering phenology and petal surface were consistent with the direction of the observed trait changes, whereas others showed more variable patterns among populations and accessions. The discrepancies between trait shifts and selection coefficients could be due to differences in the population/accession original environment and the common garden environment, or to genetic correlations with other traits under selection (Sgrò & Hoffmann, 2004). Evolving to maintain pollinator interactions means that the population mating strategy, notably the selfing rate and selfing ability should remain unchanged. In line with this, we did not detect significant changes in herkogamy between ancestors and descendants in both populations (Figure 6, Table 1). Regarding selection for herkogamy, we observe marginally significant divergent selection (positive quadratic coefficient) which is consistent with the idea of maintaining two floral morph (short style-long anthers and long style-short anthers; Table 2, 3).

Genomic analyses

After filtering out loci with read depth lower than 10 and more than 40% missing data, and eliminating individuals that were manifestly contaminated, we obtained 5805 polymorphic loci amplified in 164 individuals. All accessions had Fis ≤ 0; indicating heterozygote excess and historically high outcrossing rates (Bürkli et al., 2017), consistent with the maintenance of pollinator interactions in descendants. Fis was higher in descendants than in ancestors, especially for PL, suggesting that some increase in the selfing rate or other type of inbreeding could have occurred in descendants (Table 4).

Pairwise Fst estimates showed low differentiation between BE ancestors and descendants, but Fst between PL ancestors and descendants was as high as Fst between BE and PL (Table 4). In line with this, PCA plots and the neighbor-joning tree show that the two Belgian accessions are closely grouped together, whereas the Polish accessions are separate from one another (Figure 7, 8). This suggests the occurrence of major evolutionary or demographic changes in PL since 2003 such as migration, extinction and recolonisation, or possible high sampling bias for the ancestral accession which is not representative of the population genetic diversity in the past. This result illustrates a shortcoming of resurrection studies that is often acknowledged but rarely accounted for – with only two temporal points in a single population, it is difficult to disentangle adaptive evolutionary processes from stochastic variation in allelic frequencies due to random evolutionary processes or sampling bias (Etterson et al., 2016). As returning in the past to resample the population is not an option, testing for different evolutionary scenarios using approximate Bayesian computations (ABC) could bring insight into the population evolutionary past (Collin et al., 2021). This nevertheless requires genotyping neighbouring populations to be able to infer the probability that PL in 2020 is the direct descendant of the PL sampled in 2003 or of another population in its vicinity.

Genome-wide association scans were performed for three traits – floral display, number of fruits and height of the flowering stalk (Figure 9). After a conservative correction for multiple testing, we detected respectively 77, 195 and 345 loci that were significantly associated with trait variation. The GWAS analyses are only at their preliminary stage, and further steps would include testing for loci-trait association using different R packages to confirm the initial results, using BLAST to search for homologous sequences in species with a known reference genome, and identifying gene functions. Combining GWAS and plant resurrection ecology has only been attempted in model species such as Brassica rapa, and only in resurrection experiments that study plant populations under artificial selection so far (Frachon et al., 2023).

While our genetic data cannot unequivocally confirm that contemporary evolution produced the observed trait shifts in natural populations, the differentiation patterns between past and contemporary genotypes (i.e. putative ancestors and descendants) in both populations are consistent increased attractivity to pollinators in contemporary genotypes. This indicates that regardless of their historic origin, contemporary genotypes could have been selected to maintain pollinator interactions despite pollinator decline over the past 30 years. Although the general expectation is that pollinator decline would lead to increased autonomous selfing and a selfing syndrome in plants (Acoca-Pidolle et al., 2024; Brys & Jacquemyn, 2012; Cheptou et al., 2022), evolution towards increased attractivity to pollinators has also been observed in some species (Thomann et al., 2015), and divergent trends among populations in other species (Valencia-Montoya et al., 2021). The observed variation in the responses can be due to functional and ecological differences at the species or at the population level. To disentangle between unique population patterns and general evolutionary trends, a planned continuation of this work is to set up multispecies resurrection studies that will allow to detect convergent evolutionary trends of floral traits and mating strategies in arable weeds.

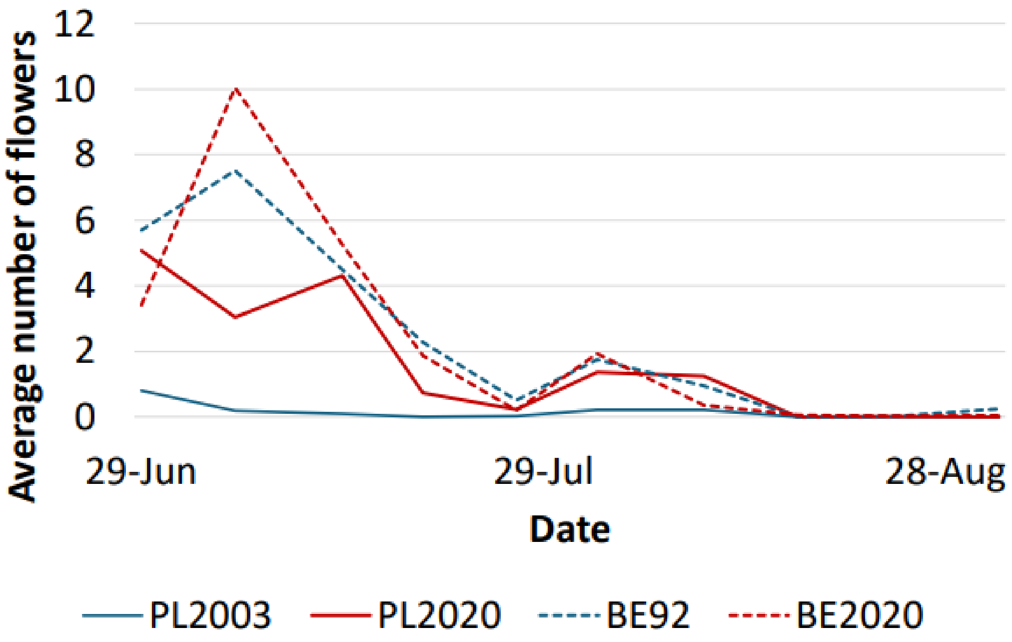


Figure 1. Average individual weekly flower count in the ancestral (blue) and descendant (red) accessions of Polish (full lines) and Belgian (dashed lines) populations. The flowering peak was not recorded for the Belgian populations

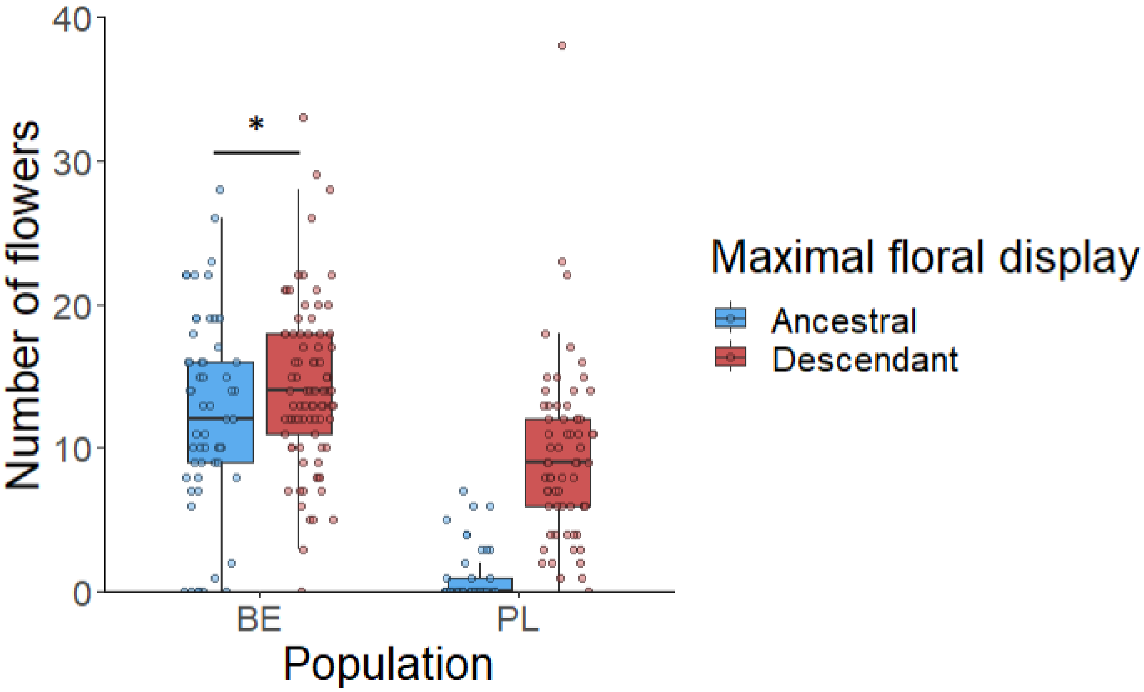


Figure 2. Boxplot of the maximal floral display, i.e. the weekly flower production on the week with highest average number of flowers in the accession. * denotes statistically significant differences between accessions according to post-hoc testing.. Values for the Polish population are considerably lower because the flowering peak was missed, and were not included in GLM analyses.

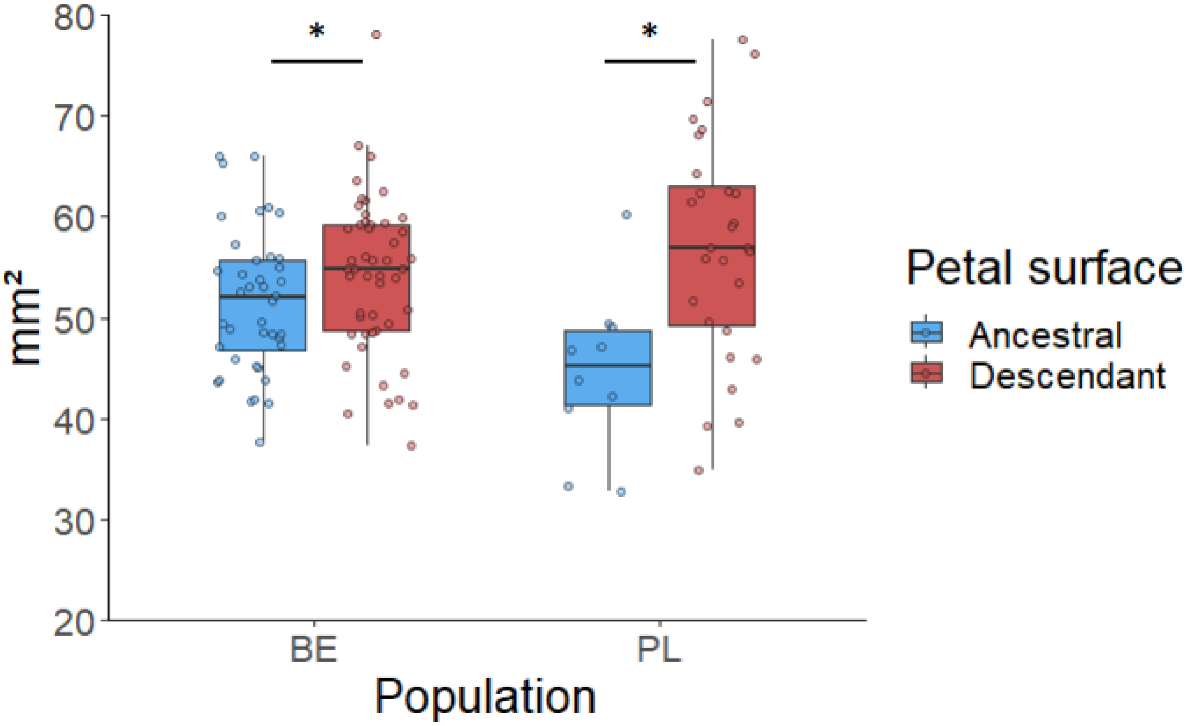


Figure 3. Boxplot of petal surface. * denotes statistically significant differences between accessions according to post-hoc testing.

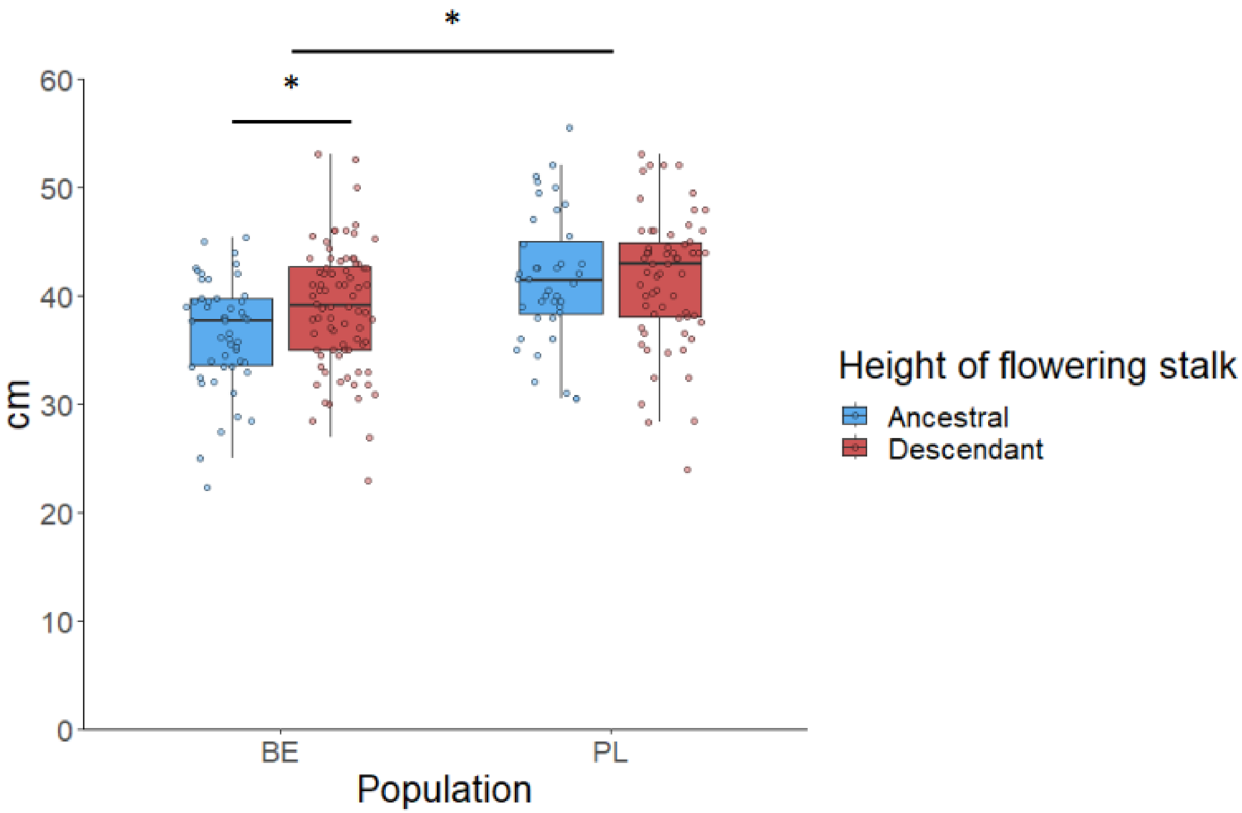


Figure 4. Boxplot of the height of the flowering stalk. * denotes statistically significant differences between accessions or populations according to post-hoc testing.

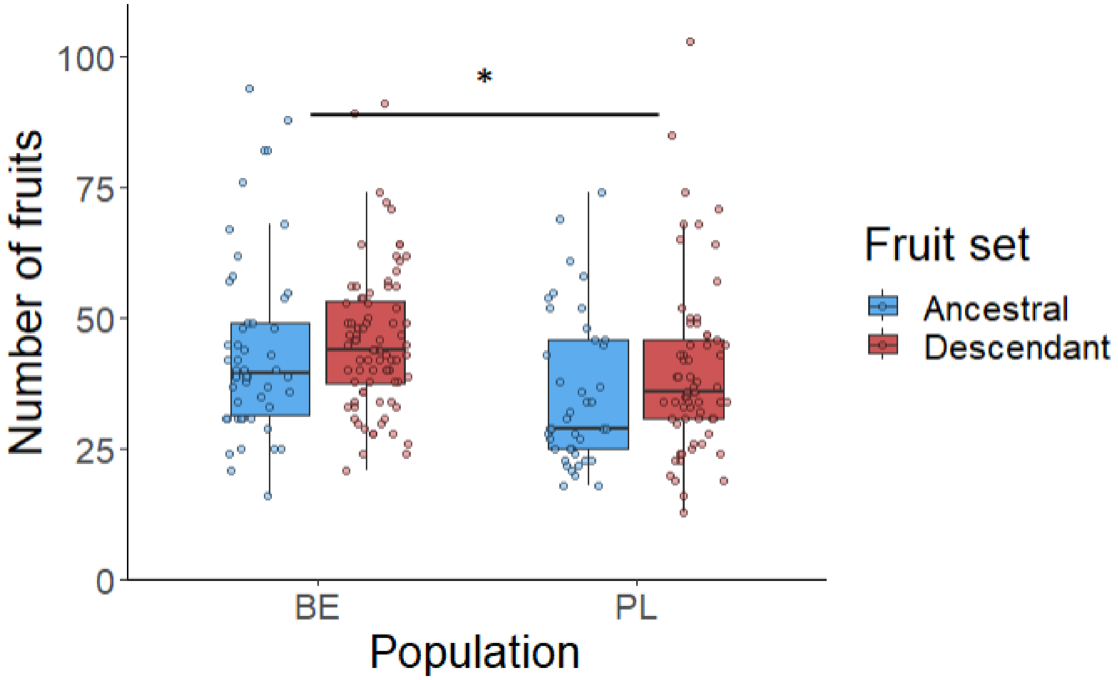


Figure 5. Boxplot of the fruit set.* denote statistically significant differences between populations according to post-hoc testing.

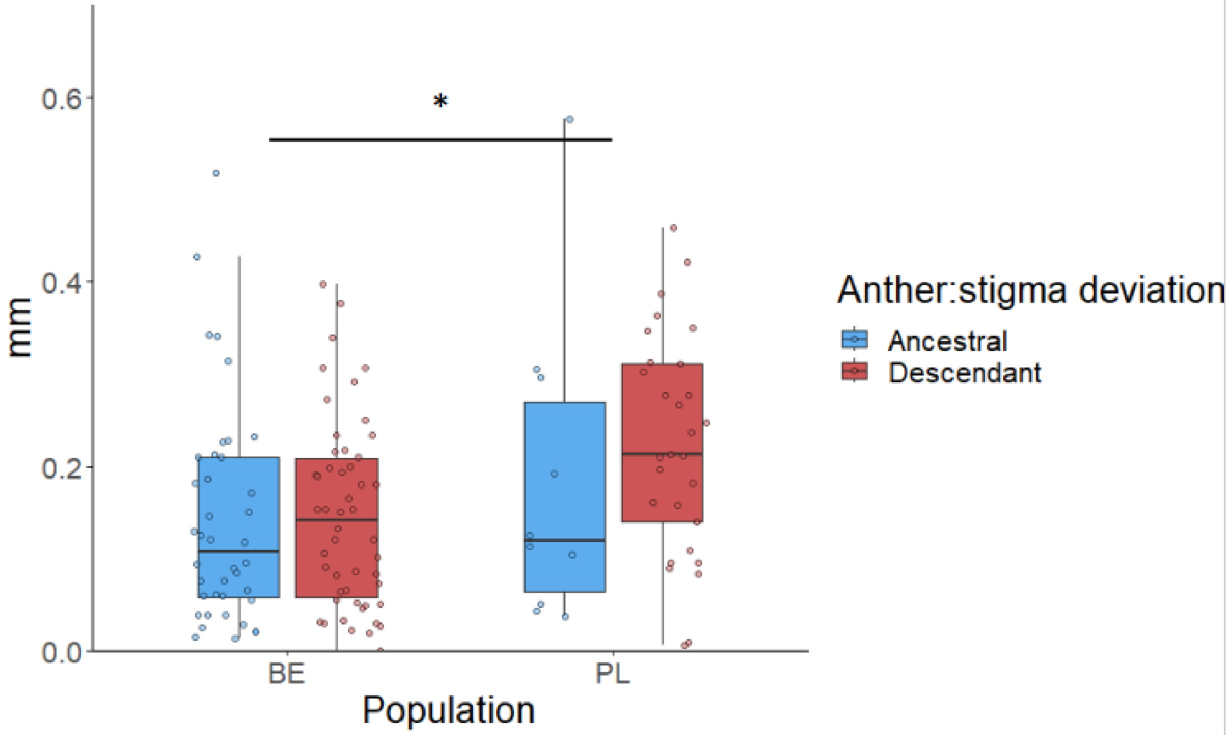


Figure 6. Boxplot of heterostyly estimated as the standardized anther-sitgma distance. * denotes statistically significant differences between populations according to post-hoc testing.

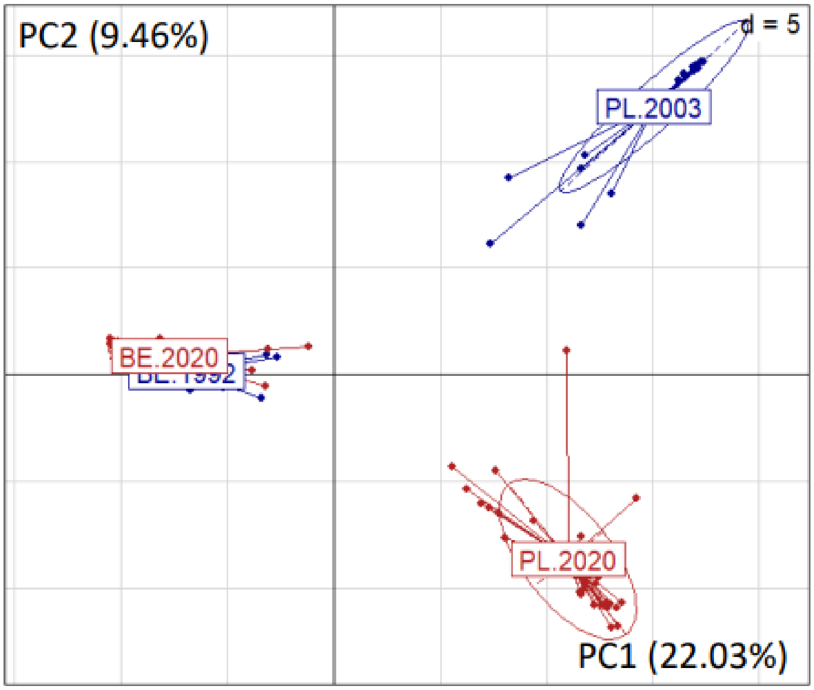


Figure 7. PCA analyses of 164 genotyped individuals. Clusters correspond to BE.1992 – ancestral Belgian, BE.2020 – descendant Belgian, PL.2003- ancestral Polish, PL.2020 – descendant Polish population

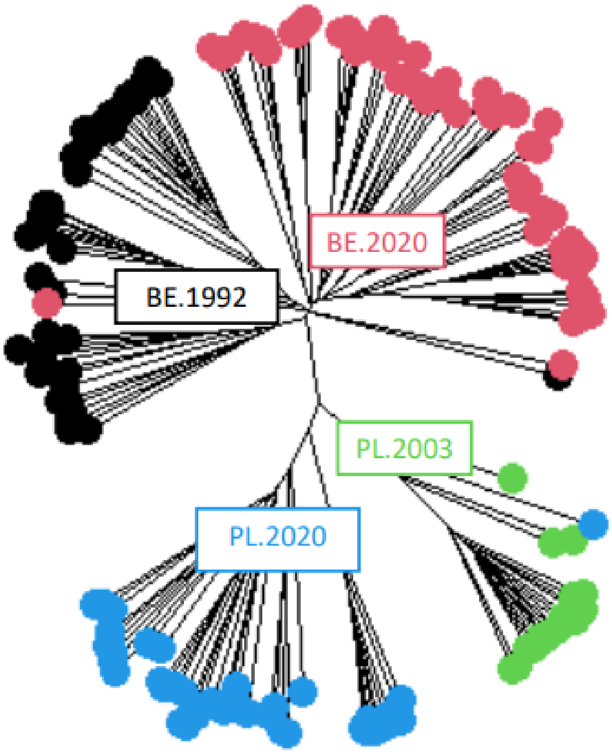


Figure 8. Neighbor-joining tree of pairwise genetic distances between individuals. Colour codes correspond to the provenance of the individual - BE.1992 – ancestral BE(lgian), BE.2020 – deccendant BE, PL.2003- ancestral Polish, PL.2020 – descendant Polish population

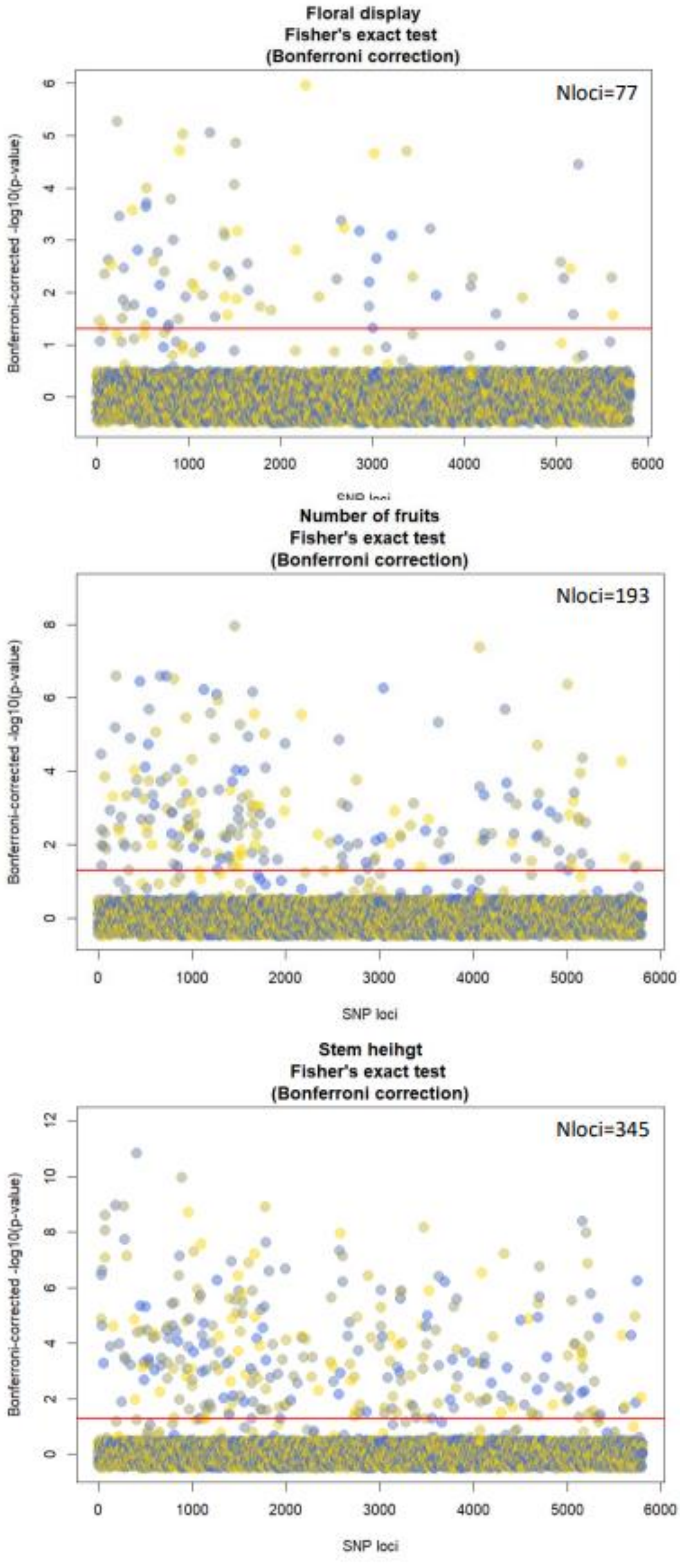


Figure 9. Manhattan plots of loci – trait association based on Fisher's exact tests after Bonferroni correction for multiple comparisons. The red line in each plot corresponds to the significant threshold. Colour codes are arbitrary and solely for the purpose of easier data visualisation

Variable	Flowering start (BE)			Floral display (BE)			Petal surface			Heterostyly			Stalk height			Total number of fruit		
	Df	Dev	F	Df	Dev	F	Df	Dev	F	Df	Dev	F	Df	Dev	F	Df	Dev	F
Population	1	0.001	1.121	1	125	**3.444·**	1	0.037	1.352	**1**	**0.196**	**9.100***	**1**	**709**	**23.33***	**1**	**2.289**	**17.25**
Accession							**1**	**0.164**	**5.969***	1	0.016	0.745	**1**	**92.68**	**3.052·**	1	0.280	2.114
Pop x Acc							**1**	**0.111**	**4.044***	1	0.012	0.550	1	72.93	2.401	1	0.078	0.590
Family (Pop)	**20**	**0.028**	**2.240**	**20**	**1124**	**1.552·**	19	0.378	0.725	19	0.479	1.170	**20**	**1125**	**1.853***	20	2.513	0.947
Total	125	0.092		140	5559		125	3.513		128	2.989		232	8347		236	33.42	

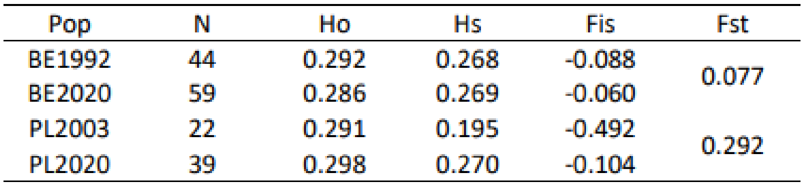
Table 1. GLM results for phenotypic traits. Flowering start and floral display were only analysed in population BE. Accession corresponds to ancestral or descendant genotypes. The descendants of population BE were sampled by maternal plant, whereas BE ancestral genotypes and both PL accessions were sampled in a bulk. We included family nested in population and accession to account for between family variation in the BE descendants. Significance levels: · ≤ 0.1, *≤0.05, **≤0.01, ***≤0.001

Model	Variable	Flowering phenology (BE)			Floral display (BE)			Petal surface			Heterostyly			Stalk height		
		Df	Dev	F	Df	Dev	F	Df	Dev	F	Df	Dev	F	Df	Dev	F
Full data	NULL	122	127.18		130	130		125	38114		125	38114		232	55403	
	Pop x Accession	1	0.745	0.733	1	1.345	1.572	3	1559	1.893	3	1559	1.708	3	132	0.217
	Linear	1	2.404	2.364	**1**	**14.66**	**17.14***	**1**	**2434**	**8.862**	1	5.240	0.017	**1**	**6760**	**33.28***
	Quadratic	1	0.130	0.128	1	1.802	2.107	1	2.700	0.010	**1**	**842**	**2.766·**	1	456	2.243
	Pop x Acc. x Linear	**1**	**4.147**	**4.077***	1	2.080	2.432	3	569	0.691	3	509	0.557	3	934	1.532
	Pop x Acc. x Quadratic	1	0.775	0.762	**1**	**3.201**	**3.742·**	**3**	**2241**	**2.720***	3	511	0.560	**3**	**2217**	**3.638***
BE1992	NULL	48	22398		50	22533		39	18054					49	15160	
	Linear	**1**	**1928**	**4.354***	**1**	**3343**	**9.134**	**1**	**1789**	**4.479***				**1**	**1266**	**4.284***
	Quadratic	1	101	0.229	**1**	**1622**	**4.433***	**1**	**1490**	**3.731·**				1	0.32	0.001
BE2020	NULL	73	14246		79	14754		46	8934.2					79	14754	
	Linear	1	3.098	0.016	**1**	**1321**	**7.585***	1	250	1.289				**1**	**1534**	**9.646**
	Quadratic	1	129	0.646	1	13.31	0.076	1	163	0.844				**1**	**972**	**6.112***
PL2003	NULL							9	3248.1					39	8271.1	
	Linear							1	39.60	0.106				**1**	**4092**	**47.71***
	Quadratic							1	586	1.566				**1**	**1005**	**11.72**
PL2020	NULL							28	6318.8					62	17085	
	Linear							**1**	**927**	**4.474***				**1**	**951**	**3.659·**
	Quadratic							1	1.400	0.007				1	545	2.100

Table 2. GLM results for selection gradient analyses. Fitness was calculated as the difference between population mean seed set and individual seed set. A quadratic regression was then fitted with each phenotypic trait as a dependent variable. Models where only linear effects are significant indicate directional selection. Models where quadratic effects are significant, regardless of the significance of the linear coefficient, indicate stabilizing or divergent selection. Accession and population were introduced as a single explanatory variable with four levels (BE ancestral, BE descendant, PL ancestral, PL descendant). Flowering start and floral display were only analysed in population BE. When a significant interaction between a linear or regression coefficient was detected (Petal surface, Stalk height), the selection gradient for that trait was calculated in a separate quadratic regression for each of the four accessions (lower part of the table). Significance levels: · ≤ 0.1, *≤0.05, **≤0.01, ***≤0.001

Pop	Coef	Phenology	Floral display	Petal surface	Heterostyly	Stalk height
BE1992	Intercept	-0.053 ± 0.199	0.275 ± 0.154	0.23 ± 0.195	-0.407 ± 0.233	0.077 ± 0.141
	Linear	**0.250 ± 0.175**	-	-	-	**-0.238 ± 0.127**
	Quadratic	-	**-0.292 ± 0.139**	**-0.249 ± 0.129**	**0.17 ± 0.135**	-
BE2020	Intercept	0.058 ± 0.171	-0.010 ± 0.126	-0.012 ± 0.177	-0.407 ± 0.233	0.177 ± 0.126
	Linear	0.043 ± 0.138	**0.309 ± 0.124**	0.149 ± 0.148	-	-
	Quadratic	-	-	-	**0.17 ± 0.135**	**-0.18 ± 0.073**
PL2003	Intercept			-0.859 ± 0.528	-0.407 ± 0.233	0.289 ± 0.128
	Linear			0.056 ± 0.451	-	-
	Quadratic			-	**0.17 ± 0.135**	**-0.278 ± 0.081**
PL2020	Intercept			0.262 ± 0.211	-0.407 ± 0.233	-0.153 ± 0.149
	Linear			**0.347 ± 0.164**	-	**-0.176 ± 0.13**
	Quadratic			-	**0.17 ± 0.135**	-

Table 3. Linear and quadratic coefficients associated with selection gradients. Coefficients in bold have significant linear or quadratic effect on fitness.



Pop	N	Ho	Hs	Fis	Fst
BE1992	44	0.292	0.268	-0.088	0.077
BE2020	59	0.286	0.269	-0.060	
PL2003	22	0.291	0.195	-0.492	0.292
PL2020	39	0.298	0.270	-0.104	

Table 4. Population genetic parameters estimated from 5805 polymorphic SNPs. N – number of genotyped individuasl, Ho – observed heterozygosity, Hs – expected heterozygosity, Fis – inbreeding index, Fst – pairwise temporal population differentiation.

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Section 2 - Monitoring and Evaluation

Regarding your BES award, which of the following do you feel to be true:

- Influenced you receiving funding from sources other than the BES
- Resulted in work that has the potential to influence policy
- Resulted in work that has potential to influence practice
- Improved communication and collaboration among ecologists
- Improved your access to equipment

Please provide any specific feedback or comments that you would like to make on your award: Having access to funds that would cover substantial costs of my research expenses was key for setting up and executing the project. Having flexible deadlines and logistic support for the implementation of the project also allowed to fulfill it in a manner that did not require to compromise on the quality of the research and the output in order to fulfil strict deadlines

Section 3 - Impact Report

Project Description.

Please describe the work funded by the BES grant in terms that would be understood by a member of the public. Please do not use detailed scientific terms.

Many flowering plants depend on pollinators to produce seeds. As the pollinator populations have steadily declined over the past few decades, plants are not able to efficiently reproduce unless they change their reproductive strategies. Luckily, plants can evolve within a handful of generations to adapt to these new environmental conditions, but this often comes at a cost for the plants and pollinator alike. For example, plants can evolve to increase their ability to self-pollinate, allowing them to reproduce even in the absence of pollinators. Although self-pollination is clearly advantageous when pollinators are scarce on the short term, on the long term it can further accelerate the loss of pollinators and can permanently decouple plant-pollinator interactions. Alternatively, plants can evolve to maintain their levels of interaction with pollinators, by producing showier and more numerous flowers to be more attractive. This requires investing more resources in reproduction but can maintain or slow down the decoupling of plant-pollinator interactions. I “resurrected” ancestral populations from seeds that were harvested 30 years ago and preserved in botanical seed banks and grew them side-by-side with their contemporary descendants. In this way I could directly observe which characteristics have evolved in natural populations over 30 years without any delays in time necessary for the populations to evolve. I show that the descendant plants are more attractive to pollinators – they produce showier, and more numerous flowers, and have not increased their ability to self-pollinate. Even though I cannot confirm the exact causes of the increased attractivity to pollinators, these are very encouraging results, showing that despite the global decline in pollinators, plant populations still invest in maintaining a certain level of cross pollination, leaving us more time to efficiently devise strategies to stop further pollinator loss.

Personal Impact.

What impact did receiving this grant have on you personally and the development of your career in ecology?

Establishing a base for resurrection research and a network of collaborators – as a scientist that only recently started her own independent research, the small research grant from BES was the ideal setting to establish a new, innovative research project that would provide proof of concept for the feasibility of more ambitious and long-term research projects. Following the award of the BES grant and the work on C. erythraea, I established collaborations with three research teams in Germany and Slovenia. Through this collaborative network I expanded my technical skills (performing floral morphometry analyses at the University of Primorska, genomewide association scans at the Helmholtz Environmental Center), and have set up the basis for long-term, multispecies resurrection experiments (Goethe University of Frankfurt). Developing new research directions – this research project produced materials ("refreshed" F1 seeds) that will reserve as the base for future interdisciplinary resurrection studies. The half-sib structure of the F1 seeds collected by maternal lines will be exploited in a subsequent experiment to calculate differentiation in traits under selection (Qst), and to estimate the evolutionary potential of ancestors and descendants (heritability, genetic constraints). As storage- and environment-of-origin effects are reduced in the "refreshed" F1 seeds, they are also suitable material for ecological epigenetic assays, which have not been carried out in plant resurrection studies so far. Altogether, the expertise and contacts I acquired during this project, combined with the well-established experimental design, have provided me with a jump start for international and interdisciplinary resurrection studies which would investigate convergent patterns of contemporary evolution in arable weeds using the resurrection method. The results of this study will back up my applications for national (German DFG, Czech GACR), and international (Biodiversa, ERC) research schemes.

Scientific Impact.

What impact did receiving this grant have on the research community?

A newly emerging discipline - While the term resurrection ecology has been coined in 1999 (Kerfoot et al., 1999), the development of plant resurrection ecology has only intensified after 2010 (Franks et al., 2018). We are thus at the key stage of the emergence of new plant science discipline which requires the development of robust protocols and methodologies allowing results comparisons among different resurrection studies. With this goal in mind, all data, script analyses and methodological protocols of this project will be made publicly available upon their finalization through Dryad, GitHub and protocols.io respectively. Resurrection genomics – Despite the omnipresence of genomics across ecological and evolutionary disciplines, plant resurrection genomics remains a largely underdeveloped field, likely because of the limited sequencing effort invested in genomics of non-model and non-cultivated plants. I used anonymous, ddRAD SNP markers, which are a cost-effective way to get an insight into population genetic structure. Although functional traits cannot be inferred from anonymous markers in species lacking a reference genome, ddRAD SNPs will nevertheless be useful into distinguishing between different evolutionary processes operating in natural populations (Fst-Qst comparisons), estimating the selfing rates in past and present, and identifying markers associated with traits under selection. Such contributions are still rare in resurrection ecology, with and exception of one study in the model species Brassica rapa (Frachon et al., 2023). Contemporary evolution does not always lead to decoupling of plant-pollinator interactions – The results of the study show that plants have evolved larger, more attractive flowers and larger floral displays, consistent with increased attractivity to pollinators. Provided that pollinators reciprocate to the plants' increased investment in the mutualistic relationship, this could slow down the decoupling of plant-pollinator interactions allowing more time for their effective conservation of plants, pollinators, and the ecosystem services they provide.

Wider Impact and Outcomes.

What impact and outcomes do you think the work funded by the BES may have within fields outside of academia? Please take into account all wider implications e.g. society/policy/public.

Arable weeds and conservation policies - Understanding how plants, pollinators, and their interactions are affected by rapidly occurring, human-mediated environmental changes is key for their preservation and the services they provide. In particular, arable weeds, due to their non-endangered and non-emblematic status have been largely neglected by conservation policies and priorities, including CBD and SDG. This project contributes to filling the knowledge gaps, with planned follow up projects that expand the same approach to multiple arable species with ancestral accessions readily available in European seed repositories. Knowledge about general trends of contemporary evolution in arable weeds will value their contribution to agroecosystems and can help implement evidence-based conservation policies of these largely neglected, yet key actors of agroecosystems. Advocating for the importance of botanical seed repositories - Botanical, non-commercial seed repositories across Europe remain largely underfunded, including renowned institutions such as Kew Botanical Gardens. This is in great part because of the perceived lack of commercial and societal value for non-endangered, noncultivated species such as arable weeds. Disseminating the results of resurrection studies to specialized and generalized audiences raises awareness about the necessity of ex situ preservation of extant (genetic) biodiversity of natural populations before it is irreparably lost. The sampling recommendations for ex situ genetic diversity preservation are perfectly compatible with sampling requirements for resurrection studies, i.e. sampling should be representative of the population genetic diversity, and should account for relatedness among individuals (e.g. by sampling by maternal plant). Thus promoting the resurrection approach among among conservation managers can directly contribute to the valuing of ex situ preservation of genetic resources of non-cultivated and non-endangered species.

Publications and Outputs.

Please provide us with a summary of any key outputs and publications. Have you published/are you intending to publish any papers relating to this work? (e.g. Published/submitted/in preparation)

Original research article - The resurrection method reveals contemporary evolution in response to pollinator decline and climage change in an arable weed, Centaurium erythraea (in preparation); Oral communications at the final meeting of COST Action ConservePlants (Izola, Slovenia, 2023), PopBio (Frankfurt, 2024), Poster communication at PopBio (Hohenheim, 2023), Invited seminars at the University of Grenoble (2023), Primorska University in Izola (2023), Masaryk University in Brno (2024). Data sets will be published in Dryad (phenotypic traits) and GenBank (ddRAD libraries), R scrips will be published on GitHub, and protocols on protocols.io. Refreshed F1 seed vouchers will be deposited at the Kew Millenial Seed Bank. All data and material will be made available upon submission of the manuscript.

Your Shout.

We'd love you to provide us with a testimonial. Testimonials will be used on various BES channels for new applicants to view and to promote our grants to the ecological community.

I was awarded the BES small research grant in 2022 for the study of contemporary evolution in arable weeds using the resurrection method and population genomics. The grant kick-started a new research axis in my career, allowing for the production of proof-of-concept results to support funding request for long-term, interdisciplinary and international research projects. I particularly appreciated the communication with the BES grant support team which was fast and efficient, and the funding deadlines were seamlessly adjusted to accommodate various constraints imposed by the ecological model. This allowed me to complete the research project as originally planned without having to adjust its objectives to accommodate administrative deadlines. The requirement to disseminate the research results among scientific and non-scientific audiences alike increases the visibility of the project beyond the scientific community, open possibilities for expansions of my research with implications in conservation biology and policy making.