Plant Ecology and Evolution 150 (1): 67–75, 2017
https://doi.org/10.5091/plecevo.2017.1167

REGULAR PAPER

Establishment of a natural floral variant of Shepherd's purse in the wild: analysis of life-history traits in '*Capsella apetala*' (Brassicaceae)

Steffen Hameister[1,*] & Barbara Neuffer[2]

[1] Institute of Botany, Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Applied Life Sciences, Gregor Mendel-Straße 33, A-1180 Vienna, Austria
[2] Department of Botany, University of Osnabrueck, Barbarastraße 11, D-49076 Osnabrueck, Germany
*Author for correspondence: steffen.hameister@boku.ac.at

Background and aims – Molecular studies in model systems have pushed forward our understanding of floral developmental genetics, but the evolutionary significance of such modifications in natural populations is rather unexplored. To improve our knowledge in this field, the sympatric occurrence of two floral variants of *Capsella bursa-pastoris* (L.) Medik. in the wild appears to be an ideal model system. In our study, wild-type plants showing small white petals and the homeotic variant '*Stamenoid petals*' (*Spe* also known as *Capsella apetala* Opiz), in which petals are replaced by additional stamens, will be compared to evaluate potential ecological differentiation.
Material and methods – Progenies from field collections were used in common garden experiments to detect possible differences in several life-history traits involved in the reproductive fitness of both variants. A second experiment was intended to shed light on the relative hybridization rate among floral variants, using the enzyme aspartate aminotransferase (AAT) as a molecular marker.
Results – Comparing fitness revealed that the two variants invested differently into their progeny. Wild-type plants showed more fruits per plant, whereas *Spe* showed higher investment in seeds per fruit. However, the overall reproductive output (seeds/plant) is almost equal. Wild-type donates more pollen for cross-fertilization, because floral visits are more common in this variant. Furthermore, both variants are separated in the onset of flowering, with *Spe* having a significant later onset of flowering.
Conclusion – We conclude that the maintenance of the floral variant within a broad wild-type population is driven by complementary mechanisms including high rates of self-fertilization and ecological differentiation. Taking into account that fitness is not reduced in *Spe*, the floral variant might in fact have the potential to be a promising model to study speciation at an early stage.

Key words – Floral homeotic variant, fitness, outcrossing, field experiment, flower visitors, *Capsella*.

INTRODUCTION

The transformation of one organ structure into another (homeosis) is certainly one of the most eye-catching examples for alterations in morphological body plans. Non-natural homeotic mutants have been used in numerous studies and have comprehensively improved our knowledge about origin and organ development of the angiosperm flower (e.g. Coen & Meyerowitz 1991, Theißen 2001, Theißen & Saedler 2001, Krizek & Fletscher 2005 and literature therein, Bowman 2006). Most studies have focused on three angiosperm model systems: *Antirrhinum majus* L., *Arabidopsis thaliana* (L.) Heynh. and *Petunia hybrida* E.Vilm. However, little is known about the occurrence of natural homeotic mutants in the wild, nor about their genetic differentiation and the potential to establish in natural stands. Just a few studies have shown that such striking morphological innovations are able to establish in wild populations. Well-known examples are the *bicalyx* variant of *Clarkia concinna* (Fisch. & C.A.Mey.) Greene (Ford & Gottlieb 1992), the peloric *Linaria vulgaris* Mill. (Cubas et al. 1999) and flower reversion in *Psophocarpus tetragonolobus* (L.) DC. (Benya 1995, Benya & Windisch 2007).

In context of the evolutionary relevance of such morphological novelties, a homeotic variant of *Capsella bursa-pastoris* (L.) Medik. has been (re)discovered (Reichert 1998) and might represent a promising model system (Theißen 2000, Hintz et al. 2006, Nutt et al. 2006). This variant is characterized by an increased number of stamens (10 instead of 6), as a consequence of homeotic transformation of pet-

All rights reserved. © 2017 Botanic Garden Meise and Royal Botanical Society of Belgium
ISSN: 2032-3913 (print) – 2032-3921 (online)

als (fig. 1A & B), and the modified phenotype was originally termed "decandric" (Opiz 1821). Decandric flowers in *C. bursa-pastoris* were reported for the first time almost 200 years ago in only a few locations throughout Europe (Opiz 1821, Trattinnick 1821, Wiegmann 1823, Becker 1828), and were recognized at species level at that time (*Capsella apetala* Opiz). More recently, it was rediscovered in vineyards in Southwest Germany (Reichert 1998). In his observation, Reichert (1998) found that the number and distribution of decandric plants is quite stable. More recent field surveys revealed that, *C. bursa-pastoris* is the predominant species in single rows of vine plantation with tens of thousands of individuals and the variant occurs with a frequency of about 10% (Hameister et al. 2009). Another population was discovered in Central Germany (Nutt et al. 2006). Interestingly, the variant is more common in locations where it has been first described, like ruderal sites in Vienna, Prague and Brno, and can be found additionally in vineyards of Lower Austria and southern Moravia, where it occurs in dozens of populations (Hameister, University of Natural Resources and Applied Life Sciences, Austria, unpubl. data). Heritability of the floral trait was already mentioned in early reports (Opiz 1821, Schlechtendahl 1823, Dahlgren 1919). Recent crossing experiments and a linkage map analysis (Hameister et

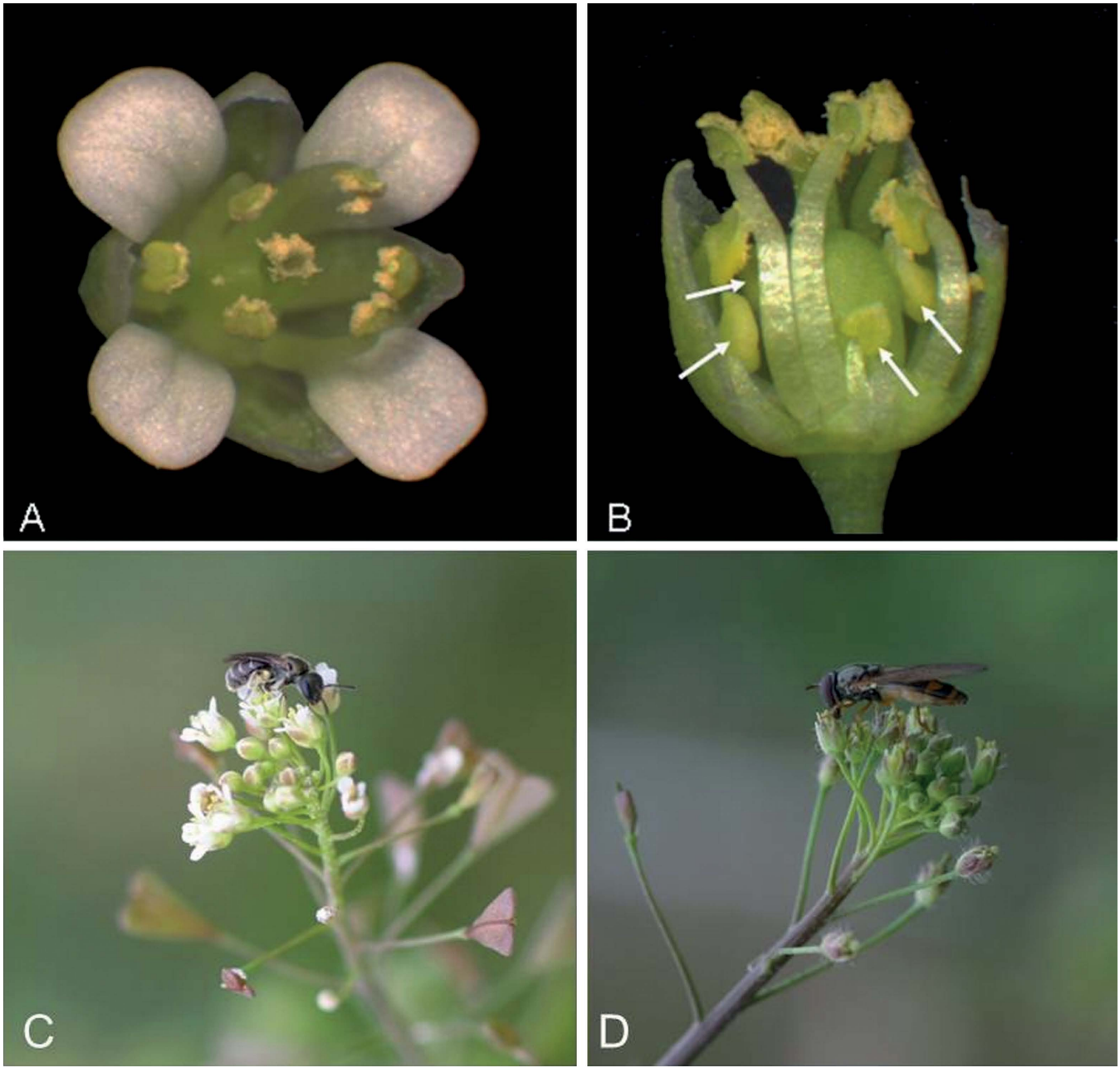

Figure 1 – In contrast to the wild-type flower of *C. bursa-pastoris* (A), all petals are replaced by additional stamens in the floral variant (B; arrows). Solitary bee visiting a wild-type inflorescence (C) and a hoverfly on a decandric inflorescence (D). Photographs: A & B, S. Hameister; C & D, B.v. Höveling.

al. 2013) suggest that the decandric phenotype is most likely caused by a single co-dominant inherited locus named '*Stamenoid petals*' (*Spe*; Nutt et al. 2006). There is evidence that the variant with four showy petals (hereafter wild-type) and the mutant *Spe* are ecologically differentiated according to a later onset of flowering in *Spe* leading to prezygotic reproductive isolation (Hameister et al. 2009). The decandric flower shape does not affect floral symmetry, but the lack of petals might change the potential pollinator community. For instance, a lesser attraction of the flower might reduce the number of flower visits that may result in higher rates of self-fertilization within the *Spe* sub-population compared with wild-type. The homeotic replacement of petals into additional stamens may involve increased investment into pollen production, which might affect the competitive capacity of the floral novelty under natural conditions. However, the sympatric occurrence in at least one natural population is shown for over twenty years in Southwest Germany (Reichert 1998, Hameister et al. 2009). Considering the massive dominance of wild-type plants, the question arises of how the long-time co-existence is accomplished.

In this paper, we studied different phenotypic traits and outcrossing behaviour as possible factors that might have promoted the successful establishment of *Spe* plants within a wild-type population. To evaluate such mechanisms, progenies from field collections were used in common garden experiments. A first approach was performed to detect possible differences in several life-history traits involved in the reproductive fitness of both variants. The second experiment was intended to shed light on the relative hybridization rate among floral phenotypes using enzyme aspartate aminotransferase (AAT) as a molecular marker to detect heterozygotes in the F1 progeny.

MATERIAL AND METHODS

Plant material

Seed material for this study was collected from the only two known populations in Germany. In both locations, the *Spe* variant co-exists with wild-type plants. A well-established population of the *Spe* variant is located in intensively managed vineyards close to Gau-Odernheim in southwestern Germany (Reichert 1998). The second population is located on a basalt hill close to Warburg, North-Rhine Westphalia (Nutt et al. 2006). Occurrence of *Spe* individuals is entirely restricted to the hilltop within an area of 200 m^2 (c. 25 individuals). Mature seeds were collected in the field, raised under controlled conditions and the progeny used for our various analyses. In addition to the German populations, a single wild-type individual from a distant population was chosen to ensure high polymorphisms among parental lineages in the crossing experiment (OSBU-740, Sierra Nevada, California, USA). Progenies were raised from seeds collected in the field and seedlings were cultivated under controlled conditions in a climate chamber to gain selfed progenies for the experiments. Therefore, all analyses were done with second-eneration material except for the analysis of crossing events at the natural stand (first-generation).

Fitness evaluation under field conditions

For each variant, 50 second-generation individuals from the Gau-Odernheim collection were cultivated in a randomized common garden experiment (12 May to 15 July 2007) in the Botanical Garden of the University of Osnabrueck (Lower Saxony, Germany). Sowing was done in an unheated, not artificially illuminated greenhouse. Eight individuals did not survive the juvenile stage. Thus, 92 individuals were analysed in total for the five different traits. The total number of *fruits per plant* was counted at the end of the vegetation period. Underdeveloped fruits (i.e. fruits that did not show full seed set) occurred only in the uppermost part of the inflorescence and were not counted.

Seeds per fruit were averaged on ten mature fruits of the lower main inflorescence (shortly before fruit dehiscence) per individual and the total *amount of seeds* was extrapolated from both measurements. The *onset of flowering* was recorded in days after sowing as opening of the first flower bud was observed. The *plant height* (cm) of the main inflorescence axis was measured at the end of its flowering period.

Data analysis

Mean values, standard deviation (s), range (r) and coefficients of variation (cV) were calculated for wild-type and *Spe* individuals. A multivariate analyses of variance (MANOVA) was performed to assess whether there was an overall difference between the variants. In order to examine whether trait means of the two morphological groups (i.e. wild-type and *Spe*) were statistically different, independent-samples *t*-Test were calculated for each trait. As a prerequisite for *t*-Test, the normal distribution was proven by Kolmogorov-Smirnov test. All analyses were performed with SPSS 24.0. The Pearson correlation analysis was performed, to provide evidence whether there is any linear dependence between the measured traits. As both floral variants most likely represent two separated subpopulations, the correlation analysis was carried out for each floral phenotype independently.

Outcrossing events

A progeny screening of aspartate aminotransferase allozymes (AAT; EC 2.6.1) was carried out to estimate the degree of crossing events. This enzyme system has been well studied in *Capsella* and has proven high polymorphism rates (Hurka & Neuffer 1997). Due to the tetraploid genome of *C. bursa-pastoris*, all loci are duplicated (six isozymes) and allozymes within one locus have been serially numbered. Further details on genetics of this isozyme system are given in Hurka et al. (1989).

In a first approach, F1 plants from Gau-Odernheim were screened for AAT genotypes, to detect gene flow events in a natural stand. For that, seeds from 13 wild-type and 15 *Spe* mother plants (hereafter families) were collected in the field. For each family, eleven individuals (F1 plants) were cultivated under controlled greenhouse conditions (12h light/day: min 14°C to max 30°C; night: min 10°C; 308 individuals in total). After ten weeks of growth, 0.7 g of rosette leaves were harvested and stored at -80°C until preparation. Leaf mate-

Table 1 – **Comparative fitness evaluation of wild-type (*Wt*) and decandric (*Spe*) individuals.**
Plants were cultivated under natural conditions in a common garden field experiment. All differences among variants were significant, except the *plant height*. std = standard deviation; cV = coefficient of variance; * $P \leq 0.5$; ** $P < 0.01$; *** $P < 0.001$.

Trait	Type	N	Mean (± std)	cV (%)	*Wt* vs. *Spe*		
					T	df	*P*
onset flowering (days after sowing)	*Wt*	44	55.9 (± 7.23)	12.9			
	Spe	48	67.0 (± 7.24)	10.8	7.39	89	0.000***
plant height (cm)	*Wt*	48	54.4 (± 17.5)	32.2			
	Spe	44	55.4 (± 15.8)	28.5	0.29	87	n.s.
fruits / plant	*Wt*	48	1314.2 (± 648.2)	49.3			
	Spe	44	894.1 (± 629)	70.4	-3.15	90	0.002**
seed / fruits	*Wt*	48	21.2 (± 4,7)	22.2			
	Spe	44	24.6 (± 6,7)	27.2	2.83	76	0.006**
total seeds / plant	*Wt*	48	28518.5 (± 15999.1)	56.1			
	Spe	44	21633.9 (± 16576.7)	76.6	-2.02	89	0.046*

rial was ground on ice. Extracts were stored at -28°C until processing.

In our second approach, an experimental parent generation with known AAT genotypes was cultivated in the Botanical Garden of Osnabrueck. We used selfed individuals from very distant populations to ensure that both parents differ significantly in their allozyme genotype. Sufficient variation in all three loci were given in one *Spe* plant from Warburg population (AAT genotype: 1111 1144 1155) and a wild-type individual from a population in Nevada, USA (AAT genotype: 2244 1111 3355). As differentiation in flowering time has been reported (Hameister et al. 2009), all plants were vernalized to assure synchronized flowering. Parental individuals were cultivated in five plots (A–E). In every plot, eight individuals of each floral phenotype were randomly planted in square by a distance of 0.25 m.

For each plot, mature fruits of two central plants per flower type were harvested. These F1 plants were cultivated in a climate chamber (10°C/20°C, 12 h photoperiod) for subsequent screening of AAT genotypes. Analyses of AAT genotypes were intended for 60 progenies from each of these central plants (= families). Due to space limitations in growth chambers, only three out of the five plots were randomly chosen (plot A, D, E). Fresh leaves were harvested and processed as described above. In total, 288 *Spe* F1 plants and 318 F1 wild-type plants were analysed.

AAT genotyping – For native gel electrophoresis, 50 µl samples were loaded on 7.5% polyacrylamide gels. After 0.5 h of pre-run at 35 mA, electrophoresis was performed at 4°C for 3.5 h at constant amperage of 70 mA following basically Stegemann (1979). Overnight staining of enzyme was done according to Wendel & Weeden (1989). Further experimental details are given in Hurka et al. (1989). In case that complete selfing occurred in the parental generation, the offspring would represent inbred lines with identical genotypes. Identification of more than one AAT genotype in the analysed progeny would indicate crossing events of different AAT genotypes in the parental generation.

Survey of flower visitors

A qualitative survey of the potential pollinator community of *C. bursa-pastoris* was done in the natural stand in Gau-Odernheim. Floral visitors observed on *C. bursa-pastoris* inflorescences were captured by net during the main flowering period of both variants (May). Sampling was carried out from 11:00 till 14:00 on two following days for 30 min at five sites. The flower type was denoted for each plant on which insects were collected. Specimens were frozen until determination. Specimens were identified to genus level and determination of insects was carried out by one of the authors (SH).

RESULTS

Fitness evaluation

The assumption of equality of covariance matrices for MANOVA was satisfied and the multivariate analysis revealed statistically significant differences in the phenotypic data ($P < 0.001$). The Kolmogorov-Smirnov test showed that all measured morphological traits correspond to the assumption of a normal distribution. Independent-samples *t*-Test showed significant differences in mean scores of three measured traits under field conditions (table 1). Wild-type plants exhibited significantly more *fruits*/*plant* compared to *Spe* plants (1,314.2 and 894.1 respectively). In contrast, the *Spe v*ariant provided the higher amount of *seeds*/*fruit* (24.6) than wild type (21.2). Extrapolating the data of both measurements, wild-type individuals showed sparsely more seeds in total than the *Spe* variant but this tendency was proven with low statistical assurance ($P = 0.046$). Wild-type plants started to flower significantly earlier, at an average of 55.9 days after sowing, while the floral variant started at an av-

Table 2 – **Number of identified heterozygotes in the allozyme progeny analysis.**
AAT screening revealed three heterozygotes in progenies of wild-type plants and eleven in the decandric variant (in bold). Mother plants were cultivated in a common garden experiment. No. Ind. = number of individuals; No. Het. = number of heterozygotes.

Plot	wild-type			decandric variant		
	No. Ind.	**No. Het.**	% Het.	No. Ind.	**No. Het.**	% Het.
A	107	**3**	2.8	120	**5**	4.16
D	93	**0**	0	92	**2**	2.17
E	105	**0**	0	76	**4**	5.26

erage of 67.1 days ($P < 0.001$). The wild-type subsample showed a range in the onset of flowering of 32 days, starting on the 12th of May 2007 and lasting until June 13th. In the *Spe* subsample, the onset of flowering covered a period of 47 days. A first individual started to flower on the 15th of May, however, the majority of plants started not before May 23rd. The latest beginning was recorded on the 1st of July. No statistical differentiation was obtained for the morphological trait *plant height*. In both variants, the total number of fruits (*seeds*/*plant*) was positively correlated with the *plant height* and showed an inverse correlation with the *onset of flowering* (electronic appendix 1).

Outcrossing events

Allozyme screening of F1 individuals has been carried out for 308 progenies from field collection (165 *Spe* / 143 wild-type), and 606 individuals from the common garden experiment (288 *Spe* / 318 wild-type). Reduced sampling size in the common garden experiment resulted from low germination capacity. Based on AAT genotype patterns, both studies revealed differences in the outcrossing potential of the wild-type and *Spe* sub-sample. Within-family heterogeneity of AAT genotypes from wild-type families was higher than that of AAT genotypes of *Spe* plants (fig. 2). More than 50% of the analysed wild-type families had more than one AAT genotype in the progeny analysis. Four families showed two different genotypes and three additional families showed four different genotypes. In the *Spe* sub-sample only three out of fifteen families revealed two genotypes per family (fig. 2). In the wild-type subsample, eleven different genotypes were assigned. The two most common genotypes occur with frequencies of 39.9% and 28.7% respectively. The remaining genotypes have a frequency of less than 10%. In the *Spe* subsample only four genotypes were identified with one dominant genotype (96.4%).

Determination of heterozygote offspring in the second experiment was straightforward due to known homozygote parental AAT genotypes. In total, 353 of wild-type and 288 *Spe* individuals were screened for AAT heterozygotes (table 2). In the wild-type progeny, three out of 104 analysed individuals were heterozygotic in plot A (2.8%). No further heterozygotes were detected in the wild-type subsample. In the *Spe* subsample, heterozygotes were detected in all three plots varying between 4.2% (plot A), 2.2% (plot D) and 5.5% (plot E). On average, 0.85% of the analysed wild-types plants and 3.8% of the *Spe* variant were heterozygotes.

Flower visitors

Diversity of insects visiting the plants in the field was surprisingly high. A total of 65 different visitors were observed during the period of three successive years. They belong to Hymenoptera, Diptera or Coleoptera. Survey of smaller insects (e.g. thrips) was not intended. Wild bees (Apidae) are the most frequent visitors (41.5%) of *C. bursa-pastoris* inflorescences followed by hoverflies, Syrphidae (30.8%). Within wild bees, ten species from three genera were detected. In hoverflies, only three species from three genera were recorded. *Sphaerophoria scripta* was the dominant species in

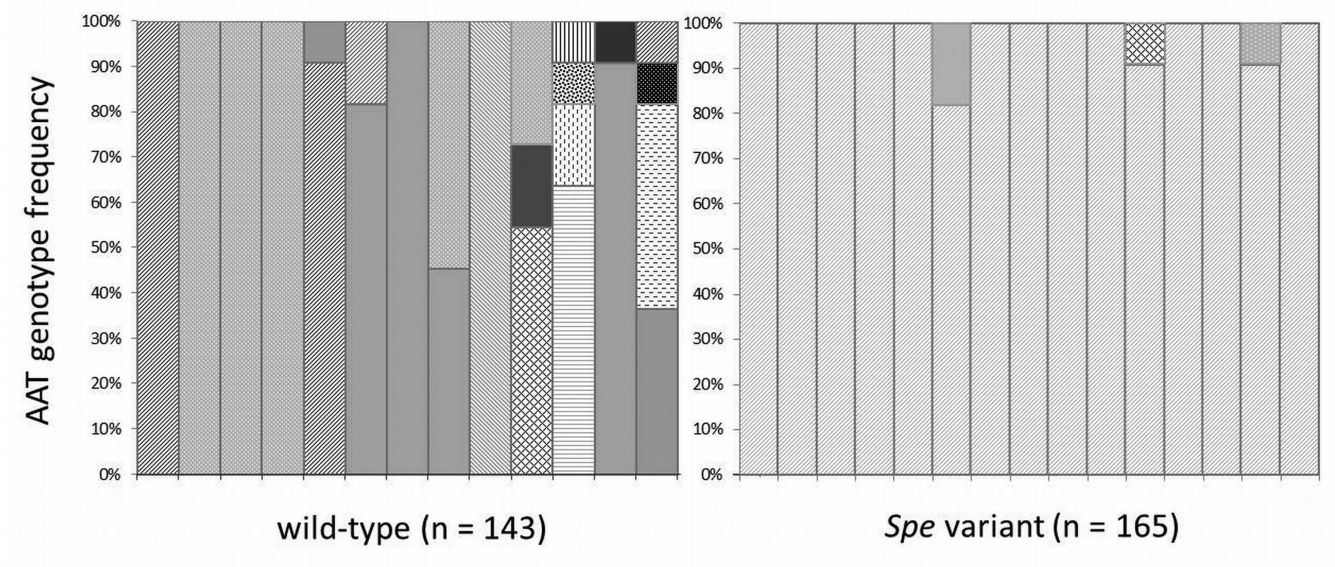

Figure 2 – Frequency of AAT genotypes in offspring analyses of 13 wild-type families (left) and 15 families of the *Spe* variant (right).

this group of visitors (35%). Further taxa from the Diptera and Coleoptera were observed (fig. 3). Two-thirds (66.2%) of all specimens (n = 43) were collected on wild-type plants, including 21 wild bees (48.8%). Among 22 flower visitors detected on the *Spe* variant (33.8%), only six wild bees were recorded.

DISCUSSION

The establishment of evolutionary innovations in natural populations is clearly related to the ability to compete under local conditions. Based on the outcome of the present study, we showed that the persistence of decandric *C. bursa-pastoris* within a wild-type population is accomplished by complementary means. Both floral variants invested differently into the progeny, but the overall reproductive fitness (*seeds*/*plant*) is almost equal. This suggests that under the given conditions the reproductive success of *Spe* is not negatively affected by the lack of petals.

The origin of *Spe* is most likely based on just one single mutant individual within a massive numerical dominance of wild-type plants. Beside compensated fitness, it is therefore reasonable to assume supporting mechanism for a long-term establishment of the novel phenotype. *Capsella bursa-pastoris* is generally known for its high colonization ability due to preadaptation and ecotypic differentiation (Hurka & Neuffer 1997, Neuffer & Hurka 1986, Linde et al. 2001). To ensure persistence of the initial colonization stage, the isolation barrier between wild-type and variant is assumed to function highly successfully. Common factors that cause an initial barrier of gene flow are high rates of selfing (Levin 1971) and a differentiation in flowering phenology among floral variants (Weis & Kossler 2004). Both mechanisms bring about prezygotic isolation. They might have played a key role in the continuing co-existence of wild-type and *Spe* and might explain a flower-type dependent population structure revealed in Gau-Odernheim (Hameister et al. 2009). *Capsella bursa-pastoris* is known to be predominantly autogamous,

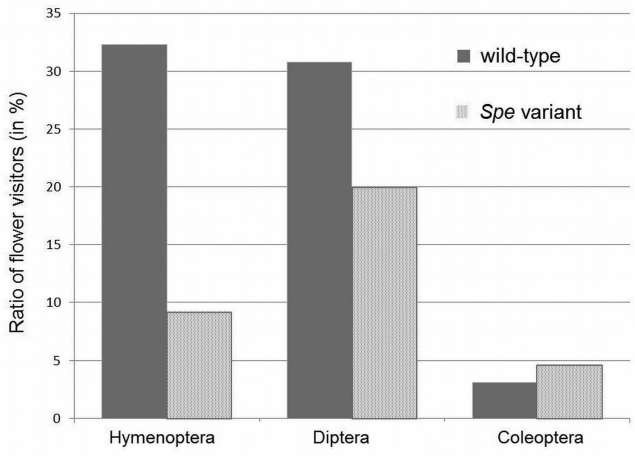

Figure 3 – Percentage of flower visitors recorded on the two floral phenotypes of *C. bursa-pastoris*. Insect visitations are twice as often in wild-type (*Wt*) than in plants with stamenoid petals (*Spe*) and effective pollinators are more frequent on wild-type inflorescences.

but selfing rates are not constant and differ within and among populations. The species is proterogynous, which generally favors outcrossing and the ratio of outcrossing *vs*. selfing is influenced by weather conditions, with dry and sunny situations favoring outcrossing (Hurka et al. 1976). The chance of cross pollination is also promoted by the fact that the length of flowering may expand over a period of more than two months in *C. bursa-pastoris* (Ianetta et al. 2007). Despite the later onset of flowering in *Spe* the flowering time overlaps between variants and gene flow is likely to occur. We assume that insect pollinations are the primary source for outcrossing, since flowering behaviour corresponds to requirements of entomophily (Hurka et al. 1976) and effective pollinators are known to use *C. bursa-pastoris* as food source (Westrich 1989). Indeed, we found a quite diverse spectrum of visiting insects, including wild-bees and hoverflies. This was in accordance with a former observation in Gau-Odernheim (Reichert 1998) and results from a common garden experiment (Ziermann et al. 2009). A quite similar species assemblage was reported for the closely related and also predominantly selfing *Arabidopsis thaliana* (Hoffmann et al. 2003).

On the one hand, floral visits of pollinators are related to flower size (Martin 2004), and the number of flowers of one individual might increase the amount of floral visitations (Conner & Rush 1996 and literature cited therein). Both is relevant for pollination and outcrossing pattern in the *Spe* variant, as the corolla is apparently smaller and the number of flowers are reduced, compared to wild-type plants (according to the number of fruits in table 1). Indeed, visiting insects were observed twice as often on wild-types plants, and bees were recorded more often on wild-type than on *Spe*, whereas hoverflies and beetles preferred *Spe* plants (Ziermann et al. 2009). Changes in flower morphology like in *Spe* might not only cause shifts in the frequencies and assemblage of visiting insects. Alteration in flower morphology can also cause differences in the quantity of cross-fertilization (Holsinger 2000). Our results of AAT allozymes progeny analyses from field collections support this hypothesis. The field experiment indicated that the decandric *C. bursa-pastoris* is more often recipient of wild-type pollen than *vice versa*. Because important traits for pollinator attraction exist only in wild-type plants (petals/scents; Ziermann et al. 2009), we hypothesize that occasional flower visits of effective pollinators on *Spe* inflorescences occur as a by-product of wild-type attraction in mixed stands. This was supported by our observation, that most insects do not visit more than three individual plants in a row and more than two-thirds of such visitations start on wild-type inflorescences (data not shown). Reduced pollinator attraction due to the altered flower shape has caused nearly complete autogamy in the *Spe* subsample. Additionally, rare outcrossing events of *Spe* might occur in a flower-type specific manner due to the shifted flowering phenology. Both effects have certainly facilitated the establishment of *Spe* as an independent entity within a wild-type population.

However, beside environmental conditions or flowering time differentiation, the amount of gene flow is also influenced by the actual pollination success. A pollination experiment showed that pollen tube growth is delayed in case of cross-pollinations among flower types compared to self-pollination (Neuffer & Paetsch 2013). This would shift the ratio

of outcrossing *vs*. selfing in favour of selfing. When wild type plants were pollen donor in these crossing experiments, the pollen tube growth occurred more rapidly (Neuffer & Paetsch 2013). This would suggest that the contribution to cross-pollination is higher in the wild-type subsample than in *Spe* and may explain our results from AAT progeny screening. Apart from biological factors, the anthropogenic influence in the intensively managed vineyards is highly relevant for the persistence of *Spe*, too. The agricultural processing affects *C. bursa-pastoris* in multiple ways: ploughing could resurrect seeds from the soil seed bank and may enhance the genetic diversity (Bosbach & Hurka 1981). Due to the mucilaginous seed layer (Hurka & Haase 1982), the mechanical processing certainly promotes seed dispersal within vineyards. Open soils and high colonization ability may then lead to a dominance of *C. bursa-pastoris* in vineyard vegetation. Due to the parcelling of vineyard properties to different owners, spreading of plant or seed material in adjacent vineyards can be supposed in the region. Mowing in-between vine rows might negatively affect reproductive output if plants are cut before full seed maturity. This might promote selection on early or late flowering ecotypes.

Evolutionary relevance

Based on our results, the *Spe* variant serves as a recent example for the persistence of morphological novelties in natural populations, in line with well-known examples like *Linaria* (Ford & Gottlieb 1992) or *Clarkia* (Cubas et al. 1999). Until now, we provided no evidence that the assumed single allele responsible for the homeotic mutation has also caused the shift to late flowering (Hameister et al. 2013). Most likely this is a case of genetic hitch-hiking. The differentiation in flowering time, however, is one important factor for local adaptation (Neuffer & Hurka 1986, Hall & Willis 2006) and might also impact on life-cycle strategy (van Kleunen 2007). Both might help *Spe* to establish an independent evolutionary lineage and mark an early stage of sympatric speciation. Considering the drastic morphological change in *Spe*, the flower shape might be under strong selection (Conner & Rush 1996, Gómez et al. 2006). However pollinator-mediated selection is certainly highly relevant in species that rely on insect pollination but rather negligible in a predominantly selfing species like *C. bursa-pastoris*. Additionally, in highly disturbed habitats like the vineyards in Gau-Odernheim, selection pressure is known to be reduced in general (Bosbach & Hurka 1981). Apart from increased pollen donation in *Spe* due to the enhanced male function (additional stamens, higher male fitness), there are no further evidences for an assumed tendency to wind pollination as argued in Nutt et al. (2006). Although anemophily is reported for Brassicaceae, e.g. in *Pringlea antiscorbutica* R.Br. ex Hook.f. (Al-Shebaz 1984) or *Hormathophylla spinosa* (L.) P.Küpfer (Gómez & Zamora 1996), key adaptations to wind pollination like altered pollen structure or stigma surface have not been revealed in *C. bursa-pastoris* so far.

Conclusion

Studies of the decandric *Capsella* have improved general understanding of mechanisms that might be involved in the long-term persistence of evolutionary novelties in the wild. The existence of this naturally occurring and persistent mutant underlines the evolutionary relevance of homeotic mutants as proposed by Theißen (2006). Besides compensated reproductive fitness in general, the establishment of the *Spe* variant is accomplished by interacting mechanisms involving high rates of self-fertilization and anthropogenic disturbance facilitating (seed) dispersal. Although the differentiation in flowering time is statistically significant its role should be classified as of minor importance at this stage, due to the sufficient overlap in flowering. However the difference in the time until flowering might be increased in the future. Apart from environmental conditions, farming activity might act as another factor of selection (Neuffer & Hurka 1986), for instance the agricultural processing in vineyards intensifies during May, which also is the main time of flowering of *C. bursa-pastoris*. This could favour very early and late flowering ecotypes and thus sharpen the separation of early (wild-type) and late (*Spe*) flowering. As a result, flower-type specific mating would be promoted. As the variant has been identified in a few other locations too, molecular studies are needed to analyse the genetic relationship among these occurrences.

First efforts revealed genetic clusters in accordance with their geographical origin indicating a repeated evolution of the decandric phenotype independently in the different habitats (Hameister, unpublished data). In future studies it will be interesting to cultivate both variants from different populations under various environmental conditions, to test whether additional ecological differentiations exist. Such studies may prove for any selective (dis)advantage of the novelty. Clearly, the *Spe* variant of *C. bursa-pastoris* is a rare and meaningful example of a naturally occurring homeotic mutant that has successfully established in the wild.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of the following: (1) Pearson correlation coefficient for fitness components; and (2) Individual records of floral visitors on decandric (*Spe*) and the wild-type (*Wt*) inflorescences of *Capsella bursa-pastoris*.

ACKNOWLEDGEMENTS

The authors wish to thank L. Schmitz and B. von Höveling for performing of field experiments, and U. Coja and R. Grupe for technical assistance. We are grateful to G. Theißen and J.Ziermann for helpful discussion on unpublished data and H. Hurka for valuable comments on the manuscript. We thank the local authorities of Rhineland-Pfalz for permission to collect insect specimens and N. Exeler for support on wild bee determination. We also thank two anonymous reviewers for helpful comments on an earlier version of the manuscript. Financial support by the Deutsche Forschungsgemeinschaft DFG (NE314/7-1 and –2; TH417/4-1 and -2) is gratefully acknowledged.

REFERENCES

Al-Shehbaz I.A. (1984) The tribes of Cruciferae (Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum 65: 343–373.

Becker J. (1828) Flora der Gegend um Frankfurt am Main. Brede, Offenbach.

Benya E.G.F. (1995) Genetic aspects of flower reversion in the winged bean (Psophocarpus tetragonolobus (L.) DC.). Acta Biologica Leopoldensia 17: 65–72.

Benya E.G.F, Windisch P.G. (2007) A phylloid ground state of reverted floral specimens of Psophocarpus tetragonolobus (L.) DC. (Fabaceae): cancelled floral meristem and continued floral organ identity. Flora 202: 437–446. https://doi.org/10.1016/j.flora.2006.09.004

Bosbach K., Hurka H. (1981) Biosystematic studies on Capsella bursa-pastoris (Brassicaceae): enzyme polymorphisms in natural populations. Plant Systematics and Evolution 137: 73–94. https://doi.org/10.1007/BF00983207

Bowman J.L. (2006) Molecules and morphology: comparative developmental genetics of the Brassicaceae. Plant Systematics and Evolution 259: 199–215. https://doi.org/10.1007/s00606-006-0419-8

Coen E.S., Meyerowitz E.M. (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37. https://doi.org/10.1038/353031a0

Conner J.K., Rush S. (1996) Effects of flower size and number on pollinator visitation to wild radish, Raphanus raphanistrum. Oecologia 105: 509–516. https://doi.org/10.1007/BF00330014

Cubas P., Vincent C., Coen E. (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401: 157–161. https://doi.org/10.1038/43657

Dahlgren K.V.O. (1919) Erblichkeitsversuche mit einer dekandrischen Capsella bursa-pastoris (L.). Svensk Botanisk Tidskrift 13: 48–60.

Ford V.S., Gottlieb L.D. (1992) Bicalyx is a natural homeotic floral variant. Nature 358: 671–673. https://doi.org/10.1038/358671a0

Gómez J.M., Zamora R. (1996) Wind pollination in high-mountain populations of Hormathophylla spinosa (Cruciferae). American Journal of Botany 83: 580–585.

Gómez J.M., Perfectti F., Camacho J.P.M. (2006) Natural selection on Erysimum mediohispanicum flower shape: insights into the evolution of zygomorphy. The American Naturalist 168: 531–545. https://doi.org/10.1086/507048

Hall M.C., Willis J.H. (2006) Divergent selection on flowering time contributes to local adaptation in Mimulus guttatus populations. Evolution 60: 2466–2477. https://doi.org/10.1111/j.0014-3820.2006.tb01882.x

Hameister S., Neuffer B., Bleeker W. (2009) Genetic differentiation and reproductive isolation of a naturally occurring floral homeotic mutant within a wild-type population of Capsella bursa-pastoris (Brassicaceae). Molecular Ecology 18: 2659–2667. https://doi.org/10.1111/j.1365-294X.2009.04216.x

Hameister S., Nutt P., Theißen G., Neuffer B. (2013) Mapping a floral trait in Shepherds purse – 'Stamenoid petals' in natural populations of Capsella bursa-pastoris (L.) Medik. Flora 208: 641–647. https://doi.org/10.1016/j.flora.2013.09.008

Hintz M., Bartholmes C., Nutt P., Ziermann J., Hameister S., Neuffer B., Theißen G. (2006) Catching a 'hopeful monster': shepherd's purse (Capsella bursa-pastoris) as a model system to study the evolution of flower development. Journal of Experimental Botany 57: 3531–3542. https://doi.org/10.1093/jxb/erl158

Hoffmann M.H., Bremer M., Schneider K., Burger F., Stolle E., Moritz G. (2003) Flower visitors in a natural population of Arabidopsis thaliana. Plant Biology 5: 491–494. https://doi.org/10.1055/s-2003-44784

Holsinger K.E. (2000) Reproductive systems and evolution in vascular plants. Proceedings of the National Academy of Sciences of the United States of America 97: 7037–7042. https://doi.org/10.1073/pnas.97.13.7037

Hurka H., Krauss R., Reiner T., Wöhrmann K. (1976) Das Blühverhalten von Capsella bursa-pastoris (Brassicaceae). Plant Systematics and Evolution 125: 87–95.

Hurka H., Haase R. (1982) Seed ecology of Capsella bursa-pastoris (Cruciferae): dispersal mechanism and soil seed bank. Flora 172: 35–46.

Hurka H., Freudner S., Brown A.H.D., Plantholt U. (1989) Aspartate aminotransferase isozymes in the genus Capsella (Brassicaceae): subcellular location, gene duplication, and polymorphism. Biochemical Genetics 27: 77–90. https://doi.org/10.1007/BF00563019

Hurka H., Neuffer B. (1997) Evolutionary processes in the genus Capsella (Brassicaceae). Plant Systematics and Evolution 206: 295–316. https://doi.org/10.1007/BF00987954

Hurka H., Paetsch M., Bleeker W., Neuffer B. (2005) Evolution within the Brassicaceae. Nova Acta Leopoldina 342: 113–127.

Ianetta P.P.M., Begg G., Hawes C., Young M., Russel J., Sqiure G.R. (2007) Variation in Capsella (shepherd's purse): an example of intraspecific functional diversity. Physiologia Plantarum 129: 542–554. https://doi.org/10.1111/j.1399-3054.2006.00833.x

Krizek B.A., Fletscher J.C. (2005) Molecular mechanisms of flower development: an armchair guide. Nature Reviews Genetics 6: 688–698. https://doi.org/10.1038/nrg1675

Levin D.A. (1971) The origin of reproductive isolating mechanisms in flowering plants. Taxon 20: 91–113. https://doi.org/10.2307/1218538

Linde M., Diel S., Neuffer B. (2001) Flowering ecotypes of Capsella bursa-pastoris (L.) Medik. (Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL) and molecular markers. Annals of Botany 87: 91–99. https://doi.org/10.1006/anbo.2000.1308

Martin N.H. (2004) Flower size preferences of the honeybee (Apis mellifera) foraging on Mimulus guttatus (Scrophulariaceae). Evolutionary Ecology Research 6: 777–782.

Neuffer B., Hurka H. (1986) Variation of development time until flowering in natural populations of Capsella bursa-pastoris (Cruciferae). Plant Systematics and Evolution 152: 277–296. https://doi.org/10.1007/BF00989433

Neuffer B., Paetsch M. (2013) Flower morphology and pollen germination in the genus Capsella (Brassicaceae). Flora 208: 626–640. https://doi.org/10.1016/j.flora.2013.09.007

Nutt P., Ziermann J., Hintz M., Neuffer B., Theißen G. (2006) Capsella as a model system to study the evolutionary relevance of floral homeotic mutants. Plant Systematics and Evolution 259: 217–235. https://doi.org/10.1007/s00606-006-0420-2

Opiz P.M. (1821) Capsella apetala Opiz. Eine neue merkwürdige Pflanze. Flora 4(II): 436–443.

Reichert H. (1998) Eine kronblattlose Sippe des Hirtentäschels (Capsella bursa-pastoris) seit Jahren bestandsbildend bei Gau-Odernheim/Rheinhessen. Hessische Floristische Rundbriefe 47: 53–55.

Schlechtendal D.E.L. (1823) Flora Berolinensis. Berlin, Duncker & Humblot.

Theißen G. (2000) Evolutionary developmental genetics of floral symmetry: the revealing power of Linnaeus' monstrous flower. Bioessays 22: 209–213. https://doi.org/10.1002/(SICI)1521-1878(200003)22:3<209::AID-BIES1>3.0.CO;2-J

Theißen G. (2001) Development of floral organ identity: stories from the MADS house. Current Opinion in Plant Biology 4: 75–85. https://doi.org/10.1016/S1369-5266(00)00139-4

Theißen G., Saedler H. (2001) Plant biology: floral quartets. Nature 409: 469–471. https://doi.org/10.1038/35054172

Theißen G. (2006) The proper place of hopeful monsters in evolutionary biology. Theory in Biosciences 124: 349–369. https://doi.org/10.1016/j.thbio.2005.11.002

Trattinnick L. (1821) Botanische Bemerkungen. Flora 4(II): 623–624.

van Kleunen M. (2007) Adaptive genetic differentiation in life-history traits between populations of Mimulus guttatus with annual and perennial life-cycles. Evolutionary Ecology 21: 185–199. https://doi.org/10.1007/s10682-006-0019-7

Weis A.E., Kossler T.M. (2004) Genetic variation in flowering time induces phenological assortative mating; quantitative genetic methods applied to Brassica rapa. American Journal of Botany 91: 825–836. https://doi.org/10.3732/ajb.91.6.825

Wendel F.J., Weeden N.F. (1989) Visualization and interpretation of plant isozymes. In: Soltis D.E., Soltis P.S. (eds) Isozymes in plant biology: 5–45. Dioscorides Press, Portland, Oregon. https://doi.org/10.1007/978-94-009-1840-5_2

Westrich P. (1989) Die Wildbienen Baden-Württembergs. Allgemeiner Teil: Lebensräume, Verhalten, Ökologie, Schutz. Stuttgart, Ulmer.

Wiegmann A.F. (1823) Correspondenz. Flora 19: 297.

Ziermann J., Ritz M., Hameister S., Abel C., Hoffmann M.H., Neuffer B., Theißen G. (2009) Floral visitation and reproductive traits of Stamenoid petals, a naturally occuring floral homeotic variant of Capsella bursa-pastoris (Brassicaceae). Planta 230: 1239–1249. https://doi.org/10.1007/s00425-009-1018-z

Manuscript received 28 May 2016; accepted in revised version 12 Dec. 2016.

Communicating Editor: Catarina Rydin.