Importance of population size for offspring fitness at conservation of the gene pool of cultivars

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Abstract

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Effective population size for conservation of cultivated plants was studied in *Coreopsis grandiflora* on populations in variants of 1, 5, 10, 30, 50 and 100 individuals. In five consecutive years these traits were studied on seeds originating from parental populations: seed number and weight per infructescence, thousand seed weight and germination. Plant height and flower size were investigated in a field trial. The influence of population size was expressed mainly in traits of early life-history stages. Inbreeding depression influenced germination already in the first year and was similar for the five years while seed weight was influenced from the second year. In the fifth year there were significant differences among variants of 30 individuals and less and variant of 50 individuals in all evaluated traits. The results document that mostly cross-pollinated cultivars of a population smaller than 50 individuals are exposed to impairment. The population of 10 individuals and less is at risk of extinction. This result will be influenced by the level of cross-pollination and degree of selection.

Keywords: inbreeding depression; cross-pollinated cultivars; *Coreopsis grandiflora*

The gene pool is a set of all alleles of all individuals of a given population (Acquaah 2007). The gene pool of plants comprises both the wild plants and the varieties of cultivated plants. The gene pool of cultivated plants is a valuable source of genetic variability for breeding. The objective of the conservation of cultivated plant gene pool is to maintain the original gene set of a given variety. The method of conservation of these varieties is related to the mode of their reproduction. In conventional varieties of cross-pollinated plants the genome is contained in a set of genotypes that form a population. Varieties-populations are represented mostly by older varieties. These are mainly grasses, vegetables and flowers. E.g. the gene pool of the Czech germplasm documentation system of cultivated plants EVIGEZ in the Czech Republic comprises more than 20% of these varieties (EVIGEZ 2012).

According to the Hardy-Weinberg law the condition of gene equilibrium in a population is a sufficient number of individuals with different genotypes. Effective population size (N_{ef}) is a critical indicator of the vulnerability of a population to allele loss via genetic drift, and it can also be used to assess the evolutionary potential of a population. Population with a very low N_{ef} is more susceptible to genetic drift and less able to respond to selection (Espeland, Rice 2010). Genetic variation within

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randomly mating populations is generally increased by mutation and dispersal, but decreased by drift and selection (Willi et al. 2006). Genetic drift may cause the loss of some alleles and as a consequence, the loss or change in some properties and traits in varieties-populations of cultivated plants may occur. In small populations a probability of mutual pollination of individuals carrying a common allele increases, which results in inbreeding. If lethal alleles that have been in heterozygous condition are coupled in this way, inbreeding depression occurs that is reflected in reduced fitness of individuals and/or in death in a different life stage. Another consequence of inbreeding is disorder of polygene balance and disorder of the fitness of individuals within a population (Charlesworth, Charlesworth 1999). The degree to which inbreeding depression will evolve, will however depend on the magnitude of inbreeding, the genetic basis of inbreeding depression (i.e. dominance vs. overdominance), the number of loci that contribute to inbreeding depression and the magnitude of effects of alleles at these loci (Charlesworth, Charlesworth 1987). Plants in large populations may show reduced fitness after inbreeding, whereas individuals of populations that have already been small during several generations may exhibit a much lower response to inbreeding because of purging of genetic load (Van Treuren et al. 1993; Husband, Schemske 1996; Byers, Waller 1999).

As stated by Klank et al. (2012), while many studies found positive effects between population size, neutral genetic diversity and plant fitness, the absence of such a relationship was also found for other species across a wide range of habitats. Furthermore, while reduced population size and decreased genetic variability often occur jointly, other studies did not find such relationships, indicating more complex mechanisms regulating plant fitness and performance. One of the cardinal problems of gene pool conservation is determination of the minimum size of effective population.

Shaffer (1981) proposed the concept of the minimum viable population (MVP). He defined the MVP as the smallest isolated population having a 99% chance of remaining in existence for 1,000 years, despite the foreseeable effects of demographic stochasticity, genetic drift, environmental stochasticity (random changes in the environment), and natural catastrophes. According to Franklin (1980) the population with inbreeding effective population size (N_{ef}) smaller than 50 individuals is at immediate risk of extinction and the population with variance effective size (N_{ev}) smaller than 500 individuals is at long-term risk of extinction (Harmon, Braude 2010). Inbreeding depression was reported in different life-history stages and in a wide variety of plant species (Van Treuren 1993; Husband, Schemske 1996).

The aim of the conservation of varieties-populations is to conserve the population fitness, and compared to natural populations, to conserve traits and properties characteristic of a variety. It is to assume that a number of alleles carrying deleterious mutations are eliminated during the breeding process. The success of this process depends on the genetic constitution of mutation and duration of selection against these alleles. In the *ex situ* conservation of varieties-populations by direct sowing the number of individuals is sufficiently high so that it encompasses the entire genome. The situation is different in varieties propagated from pre-grown planting material at spacings of tens of centimetres. In this case a higher number of individuals may substantially increase the size of plots for outplanting, thus increasing the cost of conservation. The majority of grown annual plants are cross-pollinated. For seed-production purposes stock plants are selected but their desirable numbers are not known. It is to note that their minimum numbers are crucial for appropriate conservation of a variety without reducing its fitness.

The objective of the present paper is to study the influence of population size differently large sets of individuals on offspring fitness in the cross-pollinated species *Coreopsis grandiflora* and to determine the minimum viable population necessary for conservation of the given variety.

MATERIAL AND METHODS

Material. *Coreopsis grandiflora* T. Hogg ex Sweet cv. Early Sunrise is an important annual plant native to North America; it belongs to the family Asteraceae and is insect-pollinated. The inflorescence is composed of golden-yellow flowers. The fruit is achene. Plants reach the height of 45 cm, flowers are 5 cm in diameter. Compact plants are of upright growth habit.

Experimental variants and description of experiment. The influence of differently large sets of individuals (populations) on offspring fitness was studied in five consecutive years. Six variants of populations were created: 1 (A), 5 (B), 10 (C), 30 (D), 50 (E) and 100 (F) of randomly selected individuals. The populations B and C were placed into a technically insulated and flowers were hand-pollinated with a mixture of pollen of the given population once a week until the end of August. Populations D and E were planted in spatially isolated beds, population F in the field. In variants D, E and F, a spontaneous pollination was realized. Plants of the populations D, E and F were planted in spacing 40 × 30 cm. Variant F, unlike the other variants, was not watered regularly. In the next year, respective variants were established from plants originating from populations of the same size. Infructescences were harvested gradually as seeds (achenes) were ripening. The maximum number of harvested infructescences from each variant was 30. Seed number and weight per infructescence, thousand seed weight and seed germination were evaluated in each variant. A portion of harvested seed was used to lay out a field experiment. The experiment was laid out with five replications by 12 to 20 plants. Plant height and flower diameter were evaluated.

Data analysis. Statistical evaluation was done by means of the statistical package Statistica 7.1 (StatSoft Inc., Tulsa, USA). The analysis of the effect of independent factors (variants; years) on dependent variables was carried out by analysis of variance, the post-hoc comparisons were made by Duncan's test at a significance level $P = 0.05$.

A decrease in the fitness was determined by the coefficient of inbreeding depression (δ):

$\delta = (W_o - W_i)/W_o$ (Lande, Schemske 1985), where: W_o is the set of individuals from outbreeding, W_i is the set of individuals from inbreeding. Variant E with the highest values of studied traits was chosen as W_o while variants B to D, in which an increased degree of inbreeding was assumed, were chosen as W_i.

Table 1. Differences among variants in the studied traits in particular years

Variant	2006	2007	2008	2009	2010
Seed number per infructestence					
B	25.4^{c}	3.8^{c}	10.1^{d}	5.3^{e}	2.5^{c}
C	18.6^{c}	6.1^{c}	23.4^{c}	18.2^{d}	17.3^{b}
D	52.8^{a}	42.9^{b}	44.4^{b}	41.6^{c}	–
E	36.2^{b}	49.0^{b}	50.8^{b}	52.3^{b}	52.8^{a}
F	35.2^{b}	62.4^{a}	63.5^{a}	64.0^{a}	54.0^{a}
Seed weight per infructestence (g)					
B	0.0732^{b}	0.0098^{c}	0.0191^{d}	0.0075^{e}	0.0029^{c}
C	0.0478^{c}	0.0173^{c}	0.0516^{cd}	0.0346^{d}	0.0287^{b}
D	0.1198^{a}	0.1132^{b}	0.1114^{bc}	0.0957^{c}	–
E	0.1120^{a}	0.1661^{a}	0.1721^{a}	0.1818^{a}	0.1485^{a}
F	0.0983^{a}	0.1280^{b}	0.1326^{b}	0.1412^{b}	0.1362^{a}
Thousand seed weight (g)					
B	2.88^{b}	2.61^{b}	1.90^{c}	1.41^{d}	1.16^{e}
C	2.57^{c}	2.85^{a}	2.21^{b}	1.90^{c}	1.66^{d}
D	2.27^{d}	2.64^{b}	2.51^{ab}	2.30^{b}	2.33^{c}
E	3.09^{a}	2.66^{b}	2.71^{a}	2.84^{a}	2.75^{a}
F	2.79^{b}	2.61^{b}	2.61^{a}	2.70^{a}	2.58^{b}
Seed germination (%)					
B	24.6^{d}	36.4^{d}	33.8^{e}	34.6^{d}	44.0^{d}
C	44.2^{c}	54.2^{c}	56.6^{d}	63.4^{c}	64.0^{c}
D	63.4^{b}	64.2^{b}	69.6^{c}	74.2^{b}	80.0^{b}
E	63.6^{b}	75.6^{a}	80.8^{b}	90.0^{a}	90.4^{a}
F	75.2^{a}	74.2^{a}	76.0^{a}	77.6^{b}	74.8^{b}
Plant height (cm)					
B	41.0^{b}	38.4^{a}	37.0^{a}	35.1^{d}	39.3^{c}
C	36.8^{c}	37.8^{a}	37.8^{a}	37.9^{c}	41.9^{b}
D	39.7^{ab}	38.6^{a}	38.6^{a}	36.4^{cd}	44.6^{a}
E	38.6^{ac}	37.7^{a}	37.9^{a}	44.9^{a}	44.3^{a}
F	39.9^{ab}	38.5^{a}	38.5^{a}	40.4^{b}	43.8^{a}

number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F); values followed by the same letter are statistically significant at $P = 0.05$

RESULTS

Influence of population size on fitness of components

In the first year the offspring of differently large populations produced seeds in all variants including variant A (Table 1). The lowest number of seeds per infructescence was observed in variants A, B and C (14.6, 25.4, 18.6), but it did not correlate with population size. In the second year variant A did not produce any seeds any longer and variants B and C produced significantly less seeds (3.8 and 6.1) compared to variants D, E and F (42.9 to 62.4). In variants B and C a significant decrease in seed number occurred also in the next years whereas in variants D, E and F the variability in seed number was in-

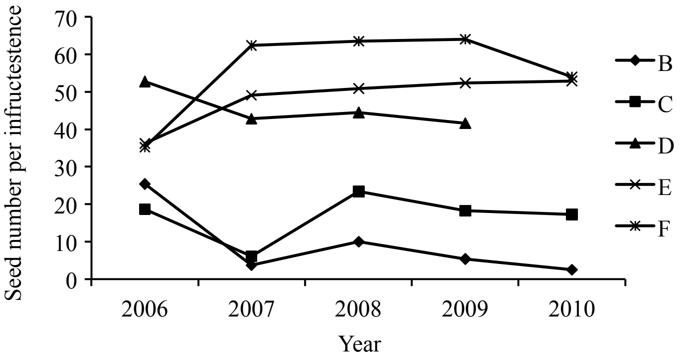

Fig. 1. Seed number per infructescence
Number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F)

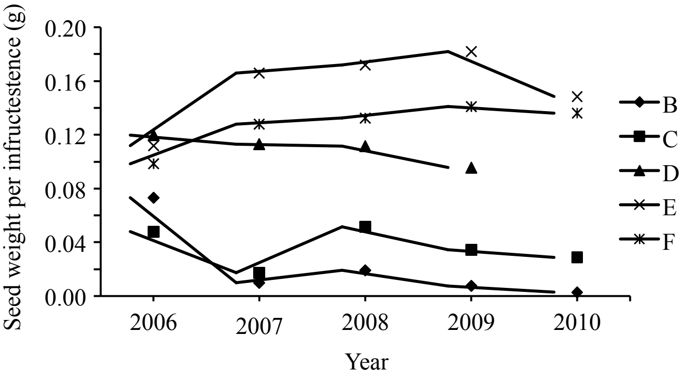

Fig. 2. Seed weight per infructescence
Number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F)

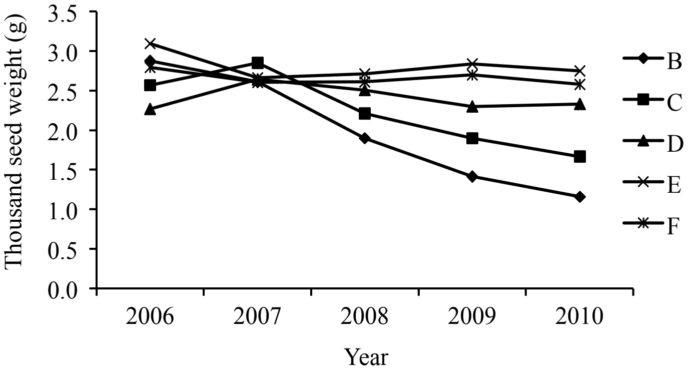

Fig. 3. Thousand seed weight
Number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F)

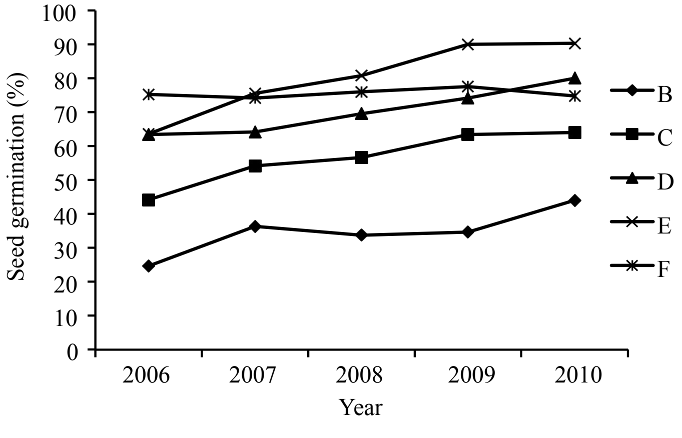

Fig. 4. Seed germination
Number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F)

significant. Seed number in variant D was significantly lower compared to variants E and F (Fig. 1). Similarly, in seed weight per infructescence, in the first year the size of this component varied without being related to the size of parent population even though variants B and C were significantly differ-

Table 2. The influence of population size on the fitness of components (determined by the coefficient of inbreeding depression (δ))

Year	B/E	C/E	D/E	B/E	C/E	D/E
	Number seed per infructestence			Seed weight per infructestence (g)		
2006	0.2983	0.4862	–0.4586	0.3466	0.5730	–0.0698
2007	0.9224	0.8755	0.1245	0.9408	0.8959	0.3186
2008	0.8012	0.5394	0.1260	0.8889	0.7000	0.3524
2009	0.8987	0.6520	0.2046	0.9586	0.8096	0.4736
2010	0.9527	0.6723	–	0.9803	0.8066	–
	Thousand seed weight (g)			Seed germination (%)		
2006	0.0680	0.1683	0.2654	0.6132	0.3050	0.0031
2007	0.0188	–0.0714	0.0075	0.5185	0.2831	0.1508
2008	0.2989	0.1845	0.0738	0.5817	0.2995	0.1386
2009	0.5035	0.3310	0.1901	0.6156	0.2956	0.1756
2010	0.5782	0.3964	0.1527	0.5133	0.2920	0.1150
	Plant height (cm)			Flower size (diameter, mm)		
2006	–0.0181	0.0466	–0.0285	0.0667	0.0444	0.0000
2007	–0.0186	–0.0027	–0.0212	0.1064	0.1064	0.0426
2008	0.0237	0.0026	–0.0185	0.0870	0.0870	0.0217
2009	0.2183	0.1559	0.1893	0.1636	0.1273	0.0182
2010	0.1129	0.0542	–0.0068	0.0727	0.0545	–0.0182

number of individuals in population (variants): 5 (B), 10 (C), 30 (D), 50 (E), 100 (F)

ent from the other variants. These differences increased over the years. In the fifth year seed weight per infructescence in variant E was 0.1485 g and in variant B it was only 0.0029 g. The lower value of variant F compared to variant E (similarly like in 1,000 seed weight) was probably caused by different conditions in which the parental stands were growing (Fig. 2). The influence of population size on thousand seed weight became obvious in the third year when there were significant differences among variants that correlated with population size. A significant decrease in this component occurred in variants B and C. In the fifth year thousand seed weight in variant B was 1.16 g while in variant E it was 2.75 g. The seed size was not significantly compensated by the lower number of seeds per infructescence (Fig. 3). Significant differences in seed germination in relation to the population size were clearly evident in the first year and they were also observed in subsequent years (Fig. 4).

Among the habit morphological traits flower size and plant height were evaluated. There were differences in flower size that corresponded with the size of parental population. A difference between variant F and B was 9% in the fifth year. Surprisingly, within each variant flower size was enlarged in all variants during the experimental years. Concerning plant height, differences among variants were observed in the fourth and fifth year of the experiment. In the fifth year differences were significant between variants B and C and among variants D, E and F. In the last year plant height was bigger in all variants, which was probably caused by higher rainfall amounts in that year.

Inbreeding depression

A decrease in plant fitness caused by inbreeding was expressed as a coefficient of inbreeding depression δ. This approach partly compensated potential differences among the years. Based on the value of δ, the evaluated traits can be divided into three groups. The trait of seed germination was included in group 1. Fitness decreased already in the first year while the highest decrease occurred in variant B and the lowest in variant D. In the next years, the value of δ was similar to that in the first year. Group 2 comprised traits describing the amount and size (weight) of developed seeds (seed number and weight per inflorescence and thousand seed weight). The fitness decrease was low in the first year but it accelerated in the next years, mainly in variant B or in variant C, being more intensive during the experimental years. Group 3 contained the traits of flower size (diameter) and plant height, i.e. the traits that were formed outside mother plants. An overall decrease in fitness in these traits was low, the lowest of all evaluated traits. Differences among variants and among years were also small (Table 2).

DISCUSSION

The influence of the parental population size on offspring fitness was differently manifested. While it was obvious in seed germination already in the first year, it was observed in the number and weight of developed seeds in the second year. In habit morphological traits it was manifested within the given population after several-year repeated mating.

Seed germination is related, *inter alia*, with the genome of the embryo which is a result of the coupling of maternal and paternal genetic information. This is the reason why an immediate expression of inbreeding is possible in this trait. The stability of differences among variants during five years are probably due to a multitude of mildly harmful polygenes with additive effects or to a combination of polygenes and major recessive genes rather than to one or a few major recessive genes alone (Levin 1991). An increase in germination in all variants during the years could be associated with gradual elimination of semi-lethal genes (Van Treuren et al. 1993; Husband, Schemske 1996).

A small decrease in fitness in seed number and weight was observed in all variants in the first year, and it was accompanied by different germination. It implies that the genotype of the original population was effective in genetic information that controlled seed formation. To the contrary embryos of these seeds probably carried different genetic load depending on the size of parental population.

The whole plants that were included in the experiment were subjected to preceding selection, i.e. genotypes whose seeds did not germinate and/or genotypes of seedlings with low fitness were eliminated. Genotypes with retarded growth and delayed development were excluded from the evaluation of the trait of flower size. It could explain small differences in fitness among the variants. A small influence of population size on traits studied in adult plants was also observed in wild populations (Shao et al. 2008). Similarly like in germination, gradual enlargement of flower size in all variants can be associated with the loss of semi-lethal genes. In the course of five generations no changes in morphological traits or in higher susceptibility to diseases were observed. The number of retarded plants and the number of plants with delayed development (delayed formation of inflorescence) were similar in all variants and in all years. Based on this observation, no genetic drift probably occurred in the above-mentioned traits.

Our study of the influence of population size on fitness of traits in the species *Coreopsis grandiflora* introduced into cultivation did not confirm the 50/500 rule (Franklin 1980). In the offspring of parental populations under study no mortality occurred within a population smaller than 50 individuals and on the contrary, the population of 50 individuals had the constant non-decreasing values of studied traits. It confirms the finding that wild populations have a higher degree of inbreeding depression compared to already selected populations (Crnokrak, Roff 1999).

A decrease in fitness is the most critical in early life-history stages (Levin 1991; Husband, Schemske 1996), which was manifested in small populations (one to ten individuals) by complete or gradual decrease in the number of produced seeds and by a reduction in their weight. On the contrary, across five generations populations of 50 and 100 individuals in size showed stability of both the traits related to the early life-history stages and traits characteristic of adult plants. So we are convinced that a set of 50 individuals and more in size is sufficient for the conservation of the studied cultivar.

CONCLUSION

The level of inbreeding depression is highly influenced by mating system, population size and history of population (Van Treuren et al. 1993). In a breeding process the cultivars-populations are size-limited sets of plants that have been exposed to negative selection for traits carrying genetic load for many generations. On the contrary, positive selection for desirable traits was realized in them. Hence, purging of genetic load and pressure against genetic drift occurred at the same time. Such a different history of cultivated populations from wild populations can explain different influence of population size on offspring fitness.

Results document that mostly cross-pollinated cultivars of the type of population from 50 individuals in size and more are exposed to inbreeding depression that can lead to their impairment. Populations of 10 individuals and less threaten the existence of a given variety. This result will be influenced by the degree of allogamy of a cultivar and by the degree of its selection.

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