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STEFAN ANDERSSON and PATRIK WALDMANN

Systematic Botany, Department of Ecology, Lund University, Lund, Sweden

Plants from a population of *Scabiosa canescens*, a locally rare species with a narrow ecological amplitude, were raised under uniform growth conditions to examine the phenotypic effects of one generation selfing and outcrossing. Particular attention was given to direct components of fitness (seedling biomass, rosette leaf number, head number, flower number per head), but two morphological characters (plant height, flower size) were also considered. Estimates of inbreeding depression (δ), adjusted for maternal effects and lack of balance, were compared and tested for significance using randomization and bootstrap procedures. Inbreeding significantly depressed several characters during both early and late stages of the life cycle, with δ ranging from 0.14 (flower size) to 0.37 (seedling biomass). Based on these and other results, we propose that *S. canescens* is susceptible to inbreeding and that the genetic basis of inbreeding depression varies across life stages.

Stefan Andersson, Systematic Botany, Department of Ecology, Lund University, SE-223 62 Lund, Sweden. E-mail: stefan.andersson@sysbot.lu.se

Many rare and threatened plants occur in small and isolated populations due to the increasing fragmentation of their natural habitats. Such populations risk extinction from a variety of demographic and genetic factors, including the unmasking of deleterious recessive genes (inbreeding depression) as well as the fixation of new detrimental mutations (LANDE 1995). However, although the role of drift and inbreeding has become a major topic in conservation biology (PRAY et al. 1994; FRANKHAM 1995; HEDRICK and KALINOWSKI 2000), relatively few attempts have been made to quantify the effect of inbreeding on fitness in natural populations of rare and declining plant species (VAN TREUREN et al. 1993; WIDÉN 1993; FERDY et al. 2001).

Previously, we assessed allozyme diversity and quantitative genetic variation within Swedish populations of the locally rare plant *Scabiosa canescens* and the more common relative *S. columbaria* (WALDMANN and ANDERSSON 1998). Despite a more than 100-fold difference in population size, there was no detectable effect of population size on levels of marker gene diversity and quantitative genetic variation, nor was there any tendency for *S. columbaria* to possess higher levels of quantitative genetic variation than its rare and threatened congener. However, populations of *S. canescens* display significant dominance variance for several fitness-related characters (WALDMANN 2001), enhancing the potential for inbreeding depression to influence the long-term survival of this rare and endangered species.

In this study, we have collected family-structured data from an extensive crossing experiment to compare the phenotypic effects of one generation of self- and cross-fertilization in a population of *S. canescens*. As well as estimating the coefficient of inbreeding depression (δ) for a wide range of characters, we also examined whether the level of inbreeding depression for each of the maternal parents was correlated across characters, i.e. whether the genetic basis of inbreeding depression varies across the life cycle. Estimates of inbreeding depression were obtained with statistical methods that accounted for maternal effects and lack of balance, and tested for significance using randomization and bootstrap procedures to avoid assumptions underlying parametric analyses.

MATERIAL AND METHODS

The diploid, perennial herb *Scabiosa canescens* W. & K. (Dipsacaceae) flowers from July to September, when the basal leaf rosettes develop a variable number of branched stems with blue, insect-pollinated flowers arranged in dense, terminal inflorescences (heads). The flowers are bisexual and self-compatible but protandrous, enhancing the potential for cross-pollination. *S. canescens* is restricted to steppe-like habitats in central and western Europe, extending northwards to the southernmost part of Sweden (Skåne). The species is classified as 'vulnerable' in the Swedish red list of threatened plant species (ARONSSON 1999).

The present investigation is based on plant material from a natural population used in previous surveys of allozyme diversity and quantitative genetic variation (WALDMANN and ANDERSSON 1998; WALDMANN 2001). This population represents a locality near the village of Hällestad in the easternmost part of Skåne. Although the current number of plants in the source population is small (ca 25), historical evidence indicates a larger population size in the past (L. Knutsson pers. comm.). Judging from allozyme data (WALDMANN and ANDERSSON 1998), genotypic frequencies of this and other Swedish populations are close to their Hardy–Weinberg expectations, confirming the outbreeding nature of *S. canescens*.

One flower head with ripe seeds and one leaf rosette were collected from each of the flowering plants in the Hällestad population. The leaf rosettes were used to obtain tissue extracts for the allozyme analyses (see above), while the seeds were stored in separate paper bags. In 1994, five randomly chosen seedlings from each maternal family were planted in separate pots and placed in a random pattern in an unheated greenhouse (for details, see WALDMANN and ANDERSSON 1998). We have no information on the mating history of the seed parents, except that genotypic frequencies at marker loci conformed to random-mating expectations (see above).

In 1995, a set of ten plants, assigned as 'pollen donors', was mated to a set of eight 'recipients' in a factorial crossing design. Levels of inbreeding were minimized by emasculating each recipient head before outcrossing and by restricting matings to plants in different maternal families. The resulting progeny provided data for quantitative genetic analysis (WALDMANN 2001) and served as an outbred base population for the present study.

A controlled crossing program was used to produce selfed and outcrossed progeny from the base population. Two randomly chosen heads on each of 61 randomly selected individuals were subjected to one of two treatments: (1) self-pollination; or (2) emasculation followed by cross-pollination with pollen from a randomly chosen (nonsib) plant. In September 1997 a maximum of 20 seeds per full-sib family were planted in plastic trays consisting of 5×5 cm square pots with sandy soil. After 50 days, we planted five seedlings from each family in separate 2-liter pots, and arranged the plants in a randomized pattern in the greenhouse. The remaining seedlings were harvested for determination of mean seedling weight after drying at 60°C for 7 days. The plants were overwintered in an unheated greenhouse to enhance flowering in 1998.

In addition to seedling weight, we obtained phenotypic data on five variables chosen to represent vari-

ous aspects of the life-cycle, such as overall resource status (rosette leaf number), plant architecture (maximum plant height), reproductive allocation (the number of flower heads, the number of flowers per head) and pollination (flower size; for details see WALDMANN and ANDERSSON 1998). Measures of rosette size (leaf number) tend to be positively correlated with viability and seed production (ANDERSSON 1992), while head number and flower number per head contribute directly to the number of seeds produced by an individual. Plant height has been found to show higher levels of between-population variation than putatively neutral allozyme markers in *S. columbaria* indicating a potential for geographically varying selection on this character (WALDMANN and ANDERSSON 1998). Measures of flower size determine the plant's visual display to pollinators and have been shown to be under strong positive selection in a wide variety of plant species (BELL 1985).

Exclusion of plants with missing data reduced the sample size for each treatment group from 305 (61 maternal parents $\times$ 5 progeny) to 279 outbred and 237 inbred individuals, the mean number of offspring per family being 4.8 for outbred progeny and 4.4 for inbred progeny (range 0–5 in both groups).

Family-level analyses using conventional least-square approaches may lead to inaccurate estimates of inbreeding depression when data are unbalanced (JOHNSTON and SCHOEN 1994; LYNCH and WALSH 1998). To account for unbalance in the present study, we subjected the data to general mixed-model analyses using the REML directive in GENSTAT 5 (1998) and the following statistical model:

$$Y_{ijk} = \mu + t_i + m_j + (tm)_{ij} + e_{ijk}$$

where Y_{ijk} is the kth phenotype of the ith treatment (inbreeding or outcrossing) and jth mother, μ is the overall mean, t_i is the fixed effect for the ith treatment, m_j is the random effect of the jth mother, $(tm)_{ij}$ is the random effect of the interaction between treatment i and mother j, and e_{ijk} is the residual error. Fixed effects were estimated by a generalized least square (GLS) procedure and random effects by a restricted maximum likelihood (REML) procedure (for details, see THOMPSON and WELHAM 1993; MORRELL 1998). As for seedling weight, which was estimated at the family level, it was necessary to exclude the interaction term from all models. Residual plots confirmed that all variables were approximately normally distributed.

Our mixed-model analyses also provided predicted means for the calculation of inbreeding depression coefficients (CHARLESWORTH and CHARLESWORTH 1987):

Table 1. *The effect of maternal plant and its interaction with cross type, tested as random effects with REML analysis. Values denote the change in deviance when the factor is removed from the model*

Trait	Mother	Mother × Cross type
Seedling weight (g)	3.9*	–
Leaf number	48.9***	18.4***
Head number	19.2***	26.0***
Flowers per head	24.6***	2.3 ns
Plant height (mm)	103.7***	8.4**
Flower size (mm)	7.9**	3.5 ns

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$$\delta = 1 - (z_s/z_o)$$

where z_s and z_o are the predicted means for selfed and outbred plants, respectively (for details, see THOMPSON and WELHAM 1993, p. 559). The statistical precision of δ was evaluated by constructing 95 percent confidence intervals (CI) of each estimate using the REML directive in GENSTAT 5 (1998) in a bootstrap procedure with 2000 random samples from the original data set (using maternal plants as sample units) (EFRON and TIBSHIRANI 1993).

The relationships in inbreeding depression among characters was assessed by performing a series of REML analyses in which treatment, mother, and their interaction, were considered as fixed effects. Maternal estimates of δ, calculated from the predicted means of the selfed and outcrossed progeny from each mother plant, were subjected to a product-moment correlation analysis to quantify the correlated response to inbreeding (CR) for each pair of traits. The 95 percent CI of each CR value was estimated from 2000 bootstrap samples (using maternal plants as sample units).

RESULTS AND DISCUSSION

The response to inbreeding differed among maternal families (as reflected by the significance of the mother-by-cross type term), especially for characters related to leaf and inflorescence production, and there was a significant effect of 'mother' for all variables (Table 1). We have no evidence as to the cause of this variation (non-genetic maternal effects, variation in inbreeding history, recurrent mutation, etc), but note that similar effects have been documented in other studies (DUDASH 1990; FISHMAN 2001). After accounting for maternal sources of variation and the lack of balance resulting from variable family sizes, we found inbred offspring to produce smaller leaf rosettes, shorter stems, fewer heads, fewer flowers per head and smaller flowers, than progeny from outcross pollinations ($P < 0.001$ in all cases, GLS). The coefficients of inbreeding depression for these characters ($\delta = 0.14$–0.37, Table 2) are comparable to estimates reported from more common and widespread plant species (HUSBAND and SCHEMSKE 1996), including *S. columbaria* (VAN TREUREN et al. 1993), a close relative of *S. canescens*.

Although our analyses focused on characters that should be under positive selection irrespective of growth conditions, there is no reason to believe that all variables are linearly related to fitness in all habitats. Based on this consideration and the detection of inbreeding depression in plant height, for which the optimum phenotype may be habitat-specific (WALDMANN and ANDERSSON 1998), no attempt was made to estimate lifetime inbreeding depression based on the multiplicative effects of all variables (CHARLESWORTH and CHARLESWORTH 1987). In this context, we also emphasize that all progenies experienced favourable growth conditions, a factor that usually decreases the detectable effect of inbreeding; hence, the present investigation probably underestimates the true level of inbreeding depression (DUDASH 1990; PRAY et al. 1994).

Our bivariate analyses revealed a tendency for the level of inbreeding depression in plant height to increase with the level of inbreeding depression in flower number per head and flower size, while other estimates of correlated response (CR) were too small to reach significance (Table 3). On the basis of these findings, it seems that inbreeding depression in floral

Table 2. *Predicted means and inbreeding depression coefficients (δ) for different characters*

Trait	Predicted means		Level of inbreeding depression	
	Cross	Self	δ	95 % CI
Seedling weight (g)	0.0773	0.0485	0.372	0.277, 0.459
Leaf number	37.8	31.1	0.176	0.106, 0.246
Head number	12.8	10.0	0.218	0.109, 0.315
Flowers per head	92.0	73.1	0.205	0.166, 0.244
Plant height (mm)	387	292	0.245	0.198, 0.295
Flower size (mm)	10.3	8.82	0.143	0.110, 0.175

Table 3. *Estimates of the correlated response (CR, above) and their 95 percent CI (below), for each character combination*

	Leaf number	Head number	Flowers per head	Plant height	Flower size
Leaf number	1				
Head number	0.281	1			
	−0.012, 0.554				
Flowers per head	−0.110	−0.019	1		
	−0.416, 0.220	−0.310, 0.281			
Plant height	0.104	0.093	0.292	1	
	−0.193, 0.375	−0.250, 0.451	0.058, 0.503		
Flower size	0.003	0.000	0.184	0.542	1
	−0.318, 0.326	−0.342, 0.368	−0.081, 0.443	0.327, 0.709	

and architectural characters can be attributed to detrimental alleles expressed during flower and stem development and not solely to loci influencing general plant vigour. Hence, our results provide further evidence that the heritable basis of inbreeding depression varies across the life cycle (HUSBAND and SCHEMSKE 1996).

From a conservation perspective, the current study indicates that small, isolated populations can possess a considerable genetic load and that inbreeding depression could act as a constraint on the long-term survival of species or populations (FRANKHAM 1995; BYERS and WALLER 1999; WANG et al. 1999). When combined with the relatively high heritabilities of vegetative and reproductive characters in both small and large populations of *S. canescens* (WALDMANN and ANDERSSON 1998), our results also lead to the prediction that inbreeding depression will be of more immediate importance than the loss of adaptive potential in the long-term survival of this species. Further, given that deleterious genes are character-specific in their expression, our results also emphasize the importance of considering several aspects of the life-cycle before any broad generalizations are made regarding the magnitude of total inbreeding depression and the importance of genetic factors in determining extinction probabilities.

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