Evolutionary Ecology Research, 2007, **9**: 959–974

Inter-specific competitive stress does not affect the magnitude of inbreeding depression

Yvonne Willi,[1,2,3]* Stefan Dietrich,[4] Mark van Kleunen,[4,5,6]
and Markus Fischer[4,6]

[1]*Institute of Integrative Biology, ETH Zürich, Universitätstrasse 2, CH-8092 Zürich, Switzerland,* [2]*Centre for Environmental Stress and Adaptation Research CESAR, Zoology Department, University of Melbourne, Melbourne, VIC 3010, Australia,* [3]*Institute of Environmental Sciences, University of Zürich, CH-8057 Zürich, Switzerland,* [4]*Institute of Biochemistry and Biology, University of Potsdam, D-14471 Potsdam, Germany,* [5]*Centre for Invasion Biology, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg 3209, South Africa and* [6]*Institute of Plant Sciences, University of Bern, CH-3013 Bern, Switzerland*

ABSTRACT

Hypothesis: Stressful inter-specific competition enhances inbreeding depression.

Organisms: Creeping spearwort (*Ranunculus reptans* L.) and its common competitor, the creeping bentgrass (*Agrostis stolonifera* L.).

Field site: Outdoor common garden experiment at the University of Potsdam.

Methods: We collected plants of 12 natural populations of *R. reptans* differing in mean parental inbreeding coefficient (0.01–0.26). We performed within-population crosses for two generations and kept the offspring in a common environment. In the second generation, we raised plants in the presence or absence of the grass competitor *A. stolonifera*. After 2 months, we assessed survival and growth of two replicate plants per seed family and treatment. To compare our results with those of previous investigations, we compiled a list of studies on inbreeding and competition.

Conclusions: Both population mean inbreeding and inter-specific competition led to a significant reduction in plant performance (survival and growth) and were therefore sources of stress. However, inbreeding and competition did not interact in their effects on plant performance or developmental instability (estimated by within-family coefficient of variation in performance). Inter-specific competition did not enhance inbreeding depression, which is in line with the results of most previous studies.

Keywords: environment dependence, environmental stress, inbreeding depression, inter-specific competition, intra-specific competition.

---

* Author to whom all correspondence should be addressed. e-mail: yvonne.willi@agrl.ethz.ch

INTRODUCTION

Inbreeding depression is the decline in fitness caused by reproduction among close relatives or by selfing in hermaphrodites. The magnitude of inbreeding depression is important for designing conservation strategies and breeding programmes, and is an integral part of the evolutionary theory of dispersal strategies and mating systems (Armbruster and Reed, 2005). Ample empirical evidence documents that inbreeding depression occurs in domestic and laboratory-inbred individuals as well as in wild populations (Crnokrak and Roff, 1999; Crnokrak and Barrett, 2002; Keller and Waller, 2002; Kristensen and Sørensen, 2005). However, empirical studies also show that the magnitude of inbreeding depression can vary among different kinds of environments (Armbruster and Reed, 2005).

Based on these results, the general consensus has been that inbreeding depression is usually enhanced when the environment is stressful (Lynch and Walsh, 1998, pp. 273–274). Stressful conditions are commonly defined as conditions leading to lowered fitness relative to a more benign environment (Koehn and Bayne, 1989). Armbruster and Reed (2005) performed a systematic review of the empirical studies on inbreeding depression and environment dependence. They showed that only 48% of studies documented significantly increased inbreeding depression under stressful conditions. This leaves us with the open question of why there is increased inbreeding depression under stressful conditions in some studies, but not in others. To improve our understanding of the interaction between inbreeding depression and the environment, we need to establish a theoretical framework and move towards testing more specific hypotheses.

There are two genetic settings in which inbreeding depression is environment-dependent. First, simple genotype × environment (G × E) interactions could cause enhanced inbreeding depression under environmental stress. For example, an outbred individual may have high fitness over a wider range of ecological conditions than an inbred individual with homozygous recessive deleterious mutations. A second possibility is that there are higher-order G × E interactions such as in the case of environment-sensitive developmental (de-)canalization. Inbreeding and environmental stress have been shown to weaken canalization, causing increased phenotypic variation (Stearns *et al.*, 1995; Badyaev and Foresman, 2000). Inbreeding depression through de-canalization may be enhanced under environmental stress if the effects of inbreeding and external stress interact in a negative way.

The environmental sensitivity of inbreeding depression, and hence the existence of simple- or higher-order G × E interactions, may depend importantly on how rare an environmental state is. Assume that an inbred recessive deleterious mutant has a narrower fitness optimum over an environmental gradient than an outbred individual. If the environment moves frequently away from that optimum, the mutant is likely to be purged. Hence, if a certain stressful environment commonly occurs, only those recessive deleterious alleles will remain in the population that do not cause a more severe decline in fitness under stressful than under benign conditions. In line with this reasoning, Bijlsma *et al.* (1999) artificially purged deleterious recessive mutations of *Drosophila melanogaster* from a wild population under laboratory conditions. They then created inbred and outbred control flies and exposed them to new stressful environments. While inbred homozygous flies and outbred heterozygous flies had similar fitness under the conditions their ancestors had experienced, the inbred flies had on average 25% lower fitness than outbred control flies under novel, stressful conditions. This experiment illustrates that inbreeding × environment interactions are more likely when the stressful environment is rarely experienced and purging has not occurred.

In this study, we investigated whether the magnitude of inbreeding depression depends on the strength of interspecific competition, a common environmental stress for many plant species. The study system was the clonal *Ranunculus reptans* around Lake Constance (Central Europe) where populations vary in mean inbreeding coefficient (Willi *et al.*, 2005). Although the species is self-incompatible and tetraploid, the levels of inbreeding in some populations in nature suggest that biparental inbreeding has been frequent for at least several generations. Additionally, populations of *R. reptans* characterized by low gene diversity, a consequence of long-term small population size, have reduced mean clonal fitness partly because of inbreeding depression (Willi and Fischer, 2005; Willi *et al.*, 2005). Furthermore, clonal propagules of populations with low gene diversity show increased variation in performance traits, indicating the action of de-canalization (Fischer *et al.*, 2000).

Ranunculus reptans occurs on the gravelly shore between the winter low-water line and early-summer flood line. Plants that grow near the low-water line experience little competition, whereas plants that grow farther away from the lake usually compete with a dense stand of grasses and sedges. In many cases, rosettes grow within a few centimetres of their competitors. In our experiment, we used similar densities of the common grass competitor *Agrostis stolonifera* to create the stressful environment. The common occurrence of inter-specific competition in all populations and the naturally occurring variation in the intensity of inbreeding make *R. reptans* a good system for assessing environmental influence on the expression of inbreeding depression.

The goal of this study was to answer the following questions: Does stress caused by inter-specific competition enhance population inbreeding depression? And does competitive stress combined with inbreeding lead to an increase in within-family phenotypic variation, indicating developmental instability as a result of de-canalization and higher-order genotype × environment interactions?

METHODS

Plant material and crossing design

In spring 2002, we collected 149 plants from 12 populations of *R. reptans* at Lake Constance. Fourteen individuals were sampled at 5-m intervals along two parallel transects separated by 5 m at each site. In five populations, the band of *R. reptans* was so short that we could only sample 8–12 individuals. After collection, plants were grown in separate tubs in a growth room. Five of 149 field-collected plants died during propagation. We assumed that we dealt with individual genotypes as there were only four pairs of adjacent field-collected plants that were of the same multi-locus genotype at eight allozyme loci (data not shown).

To minimize maternal environmental effects from the source locations, we performed within-population crosses over two generations (Schmid and Dolt, 1994). To produce the first generation, each field-collected plant was crossed with two randomly chosen plants from the same population. The crosses were performed reciprocally: each plant served as a pollen donor and a pollen receptor, leading to two maternal seed families per cross combination. We germinated the seeds and raised one seedling per seed family in a common garden. After 2 months, we randomly chose 24 plants per population and used them for the production of the next generation of seed families. However, we ensured that each field-collected genotype was represented at least once as mother and once as father (pollen donor) so that no initial genetic variation was lost.

To produce the second generation, each of the 24 within-population cross plants was crossed reciprocally with another, randomly chosen plant of the same population. The random choice was restricted so that each cross originated from four different grandparental field-collected plants. This led to a total of 266 reciprocal crosses [12 populations × 24 crosses = 288, – 20 crosses (some plants produced no flowers and could not be crossed) – 2 crosses lost due to handling errors]. Seeds were harvested 1 month after crossing.

Outdoor common garden experiment

On 8 June 2004, we sowed all seeds of each maternal seed family into a cell of several multi-pot trays (77 cells of 3 × 3 × 5 cm per tray) containing a 3:1 mixture of horticultural soil and sand. Trays were distributed in the greenhouse, and re-randomized weekly. Six weeks after the start of germination, we randomly chose four individuals per seed family and planted each individual separately into a tub of 18 × 13 × 5 cm filled with a 1 : 2 mixture of horticultural soil and sand. We reared two replicate seedlings of each cross in each of two environments, one free of competitors and one in the presence of the grass *A. stolonifera*. Because some seed families produced fewer than four seedlings, the experiment included 670 instead of 1064 plants. Directly after planting, we sowed seeds of *A. stolonifera* into two of the four tubs per seed family. Those seeds germinated within 7 days and formed a dense stand, similar to densities in the natural habitat of *R. reptans*. We randomly assigned each tub to a position within one of six outdoor beds covered with 50%-shade cloth. The plants were watered daily, unless it rained. We checked survival after 1 and 2 weeks, and if a plant had died it was replaced by another representative of the same seed family. Twelve plants were replaced because they died after heavy rainfall on the first day in the garden, and a further 11 plants were replaced during the first 2 weeks. We re-randomized the positions of the tubs 1 month after the start of the outdoor experiment.

Post-seedling performance and population inbreeding coefficient

For each plant, we calculated multiplicative measures of clonal and flowering performance. Clonal performance is especially important for *R. reptans* in years of high lake water level, when plants do not flower and reproduce exclusively via above-ground stolons with roots on some nodes. Eight weeks after transplantation (20–24 September 2004), we counted the number of rooted rosettes, flowers, and fruits of each plant. We calculated clonal performance and flowering performance by multiplying survival (0 or 1) by the number of rooted rosettes or the total of flowers and fruits, respectively.

Inbreeding coefficients were calculated from a previous study in which all field-collected plants were genotyped at eight allozyme loci and kinship coefficients were estimated for all possible pairwise combinations within each population (Willi *et al.*, 2005). We defined the population mean inbreeding coefficient as the mean kinship coefficient of a random sample of pairs of field-collected plants, excluding pairs that were incompatible in the first round of crossing (i.e. they produced no seeds in either of the two reciprocal crosses). We chose this approach over a direct, marker-based estimate of inbreeding coefficients of field-collected plants because it reflected the true level of inbreeding in the experiment. We measured population mean inbreeding coefficients and not inbreeding coefficients of individual plants. This was appropriate in our case because the genetic composition of experimental plants was homogenized within populations by two generations of random mating among relatively few field-collected plants.

Statistical analysis

To determine whether differences in post-seedling performance depended on the population inbreeding coefficient and competition, we applied three-level mixed models with the random effects of population and maternal seed family nested within population (Singer, 1998; MIXED procedure in SAS: SAS Institute, 2002). The fixed effect of competition was a predictor on the level of the plant (level 1), and the population mean inbreeding coefficient was a covariate on the level of the population (level 3). As clonal and flowering performance were correlated ($N = 670$, $r = 0.51$, $P < 0.0001$), our dependent variable was the first component of a principal component analysis (PCA) on the covariance matrix of log-transformed clonal and flowering performance (explaining 89% of the variation in the two measures). We call this first component 'aggregate performance'. We also performed a separate analysis for clonal and flowering performance, and treated the latter variable as binary because many plants produced no flowers (GLIMMIX procedure: SAS Institute, 2006).

We applied two-level mixed models to test for differences in within-family phenotypic variation in post-seedling performance (Singer, 1998; MIXED procedure in SAS: SAS Institute, 2002). The explanatory variables were population inbreeding coefficient, competition, and the random effect of population. The dependent variables were coefficients of variation in performance based on cross combinations – that is, based on pooled reciprocal crosses between two plants in the two competition environments. As both clonal and flowering performance were correlated, the main analysis included the coefficient of variation of the first component from the principal component analysis. Denominator degrees of freedom for testing fixed effects were estimated using Satterthwaite's approximation in both types of models.

RESULTS

Aggregate performance (PC1) of *R. reptans* plants from inbred populations was lower than the performance of plants from more outbred populations (Table 1; slope of regression line ± standard error = -2.0 ± 0.8). This was mainly a consequence of lower clonal performance for inbred populations (Fig. 1). Inter-specific competition also caused lower aggregate performance, mostly by reducing its clonal component. However, there was no significant interaction between inbreeding and competition, indicating that inbreeding depression did not depend on the competitive environment. Results were similar when we did the analysis with relative performance measures; the proportional decline in performance as inbreeding increased did not differ significantly between competition environments, for either clonal or flowering performance ($P > 0.1$ for both).

Inbreeding did not affect phenotypic variation in aggregate performance within reciprocal seed families (Table 2). For clonal performance by itself, cross combinations from more inbred populations had somewhat higher coefficients of variation than those from less inbred populations (Table 2; slope of regression line ± standard error = 55.2 ± 54.9). Competition did not lead to increased variation in aggregate performance, and the interaction between inbreeding and competition was not significant, indicating that the combination of inbreeding and environmental stress did not enhance developmental instability.

Table 1. Results of mixed-model analysis examining the fixed effects of population mean inbreeding coefficient, competition (with or without a grass competitor), their interaction and the random effects of population, seed family, and their interaction with competition on the first component (PC1) of a principal components analysis on post-seedling clonal and flowering performance ($n = 670$)

		Post-seedling performance (PC1)			Clonal performance			Flowering performance		
Source of variation	d.f.$_{\text{Num}}$	d.f.$_{\text{Den}}$	F	P	d.f.$_{\text{Den}}$	F	P	d.f.$_{\text{Den}}$	F	P
Inbreeding coefficient (IC)	1	18.7	4.51	**0.047**	18.7	5.56	**0.030**	11.2	0.44	0.522
Competition	1	484	127.43	**<0.001**	165	128.57	**<0.001**	9.0	4.19	0.071
IC × competition	1	497	2.78	0.096	185	2.43	0.121	9.9	1.71	0.220
Random effects		VAR	LCL, UCL		VAR	LCL, UCL		VAR	LCL, UCL	
Population		0.004	$0.001, 2.1 \times 10^5$		0.004	$0.000, 6.0 \times 10^6$		0.068	−0.416, 0.552	
Population × competition		0	—		0	—		0.329	−0.311, 0.969	
Seed family		0.231	0.167, 0.339		0.209	0.148, 0.317		0.097	−0.331, 0.525	
Seed family × competition		0	—		0	—		0.313	−0.019, 0.645	

Note: P-values < 0.05 are printed in **bold**. Statistics for random effects include VAR (variance component), LCL (lower 95% confidence limit), and UCL (upper 95% confidence limit).

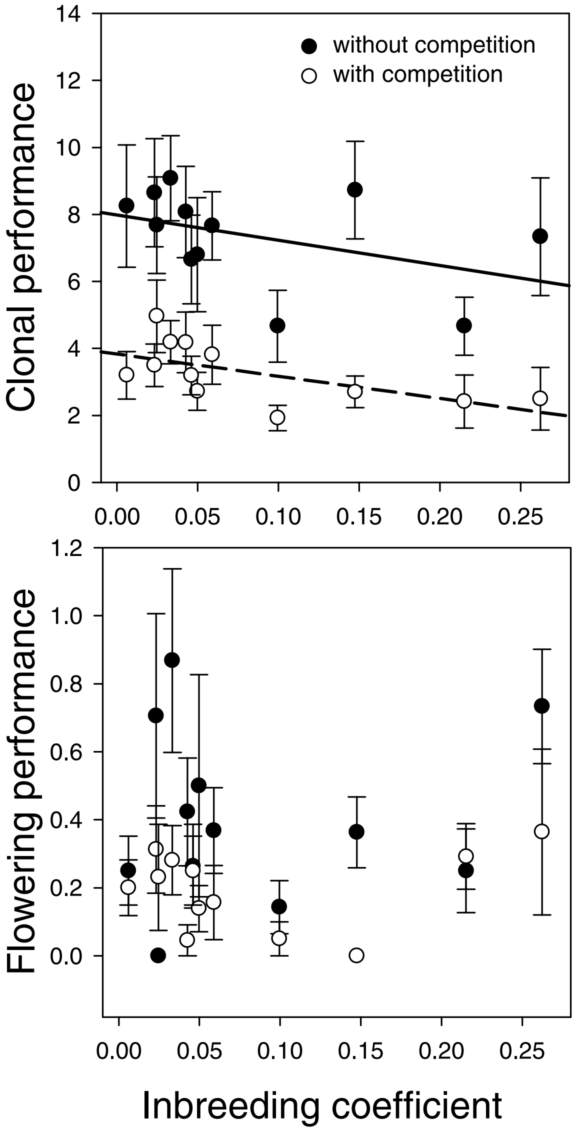

Fig. 1. Relationships between post-seedling clonal performance (top) and flowering performance (bottom) of *Ranunculus reptans* and the population mean inbreeding coefficient. Plants were raised in the absence (●; ±1 standard error) and presence (○) of the competitor grass species, *Agrostis stolonifera*. Clonal performance (survival × production of rooted rosettes) decreased significantly with increasing inbreeding coefficient. The separate regression lines for both environments reflect a significant difference between treatments (see Table 1). The decline in clonal performance was not different between the two competition treatments, indicating that the magnitude of inbreeding depression did not depend on environmental stress. Post-seedling flowering performance (survival × number of flowers) was not affected by inbreeding (Table 1).

Table 2. Results of mixed-model analysis examining the fixed effects of population mean inbreeding coefficient, competition (with or without a grass competitor), their interaction and the random effect of population and its interaction with competition on coefficients of variation (CV; based on reciprocal crosses) in the first component (PC1) of a principal components analysis on post-seedling clonal and flowering performance

		CV in post-seedling performance			CV in clonal performance			CV in flowering performance		
Source of variation	$d.f._{Num}$	$d.f._{Den}$	*F*	*P*	$d.f._{Den}$	*F*	*P*	$d.f._{Den}$	*F*	*P*
Inbreeding coefficient (IC)	1	21.7	0.53	0.473	205	4.14	**0.043**	12.5	1.00	0.335
Competition	1	197	0.68	0.411	205	0.39	0.533	12.7	0.15	0.706
IC × competition	1	197	0.04	0.847	205	0.29	0.588	17.8	0.60	0.447
Random effects		VAR	LCL, UCL		VAR	LCL, UCL		VAR	LCL, UCL	
Population		9.3×10^4	$2.2 \times 10^4, 1.5 \times 10^7$		0	—		19.2	—	
Population × competition		0	—		0	—		154.4	$18.6, 5.1 \times 10^{11}$	

Note: $n = 206$ for CV in PC1, $n = 205$ for CV in clonal performance, $n = 87$ for CV in flowering performance. *P*-values < 0.05 are printed in **bold**. Statistics for random effects include VAR (variance component), LCL (lower 95% confidence limit), and UCL (upper 95% confidence limit).

DISCUSSION

The main outcome of our study is that inbreeding depression is not magnified under stressful inter-specific competition, neither in post-seedling performance nor in measures of variation in performance. According to Bijlsma *et al.* (1999), the explanation for this pattern may be that *R. reptans* experiences competition with other species frequently, so that purging has been just as effective in environments with inter-specific competitors as in environments without competitors.

In line with our results, previous studies of inbreeding under *inter-specific* competition, including those using experimentally inbred individuals or inbred specimens derived from natural populations, mainly report no effect of competition on the magnitude of inbreeding depression in life-history measures associated with fitness (Appendix 1a). Studies on inbreeding and *intra-specific* competition in plants, involving trials with inbred, outbred or in- and outbred (mixed) competitors, reveal a similar picture. Sixteen of 20 studies of plants report no (overall) pattern of increased inbreeding depression under competitive stress (Appendix 1b). In contrast, about half of the studies of animals have found evidence for enhanced inbreeding depression under competitive stress, particularly when imposed by outbred conspecifics. This might support Bijlsma and colleagues' 'rare stress' hypothesis if exposure of inbred individuals to outbred conspecifics is relatively rare, so that purging has not occurred in this context. However, few studies have compared levels of competitive stress with those experienced in the past or in nature, which is necessary to evaluate the hypothesis properly.

Our observation that within-family phenotypic variation did not increase under the combined influence of inbreeding and competitive stress is in line with previous findings (Delph and Lloyd, 1996; Fowler and Whitlock, 2001). Recent research on heat-shock proteins, which – among others – assist in proper protein folding and are up-regulated during both inbreeding and environmental stress (Kristensen *et al.*, 2002; Sørensen *et al.*, 2003), indicate that de-canalization is unlikely to enhance the magnitude of inbreeding depression under stress. Kristensen *et al.* (2006) hypothesized that functional heat-shock proteins may become limiting under combined inbreeding and stress, and therefore compared genome-wide gene expression in inbred and outbred lines of *Drosophila melanogaster* under benign and high temperatures. They found that inbreeding and stress both independently and synergistically affected gene expression patterns. However, the genes affected by combined inbreeding and temperature stress were metabolic rather than heat-shock proteins, which suggests that the synergistic effect of inbreeding and stress decreases metabolic efficiency but not canalization. The pattern emerging from our study and others is that de-canalization is not further enhanced under the combined action of inbreeding and stress, although more studies are needed (Willi *et al.*, 2006).

Based on our results and those of others, models of mating-system evolution and optimal dispersal distance need not include aspects of inter-specific competition when considering the effect of inbreeding depression. Similarly, intra-specific competition seems to be irrelevant for the magnitude of inbreeding depression in many species of plants. In the context of conservation, it should be noted that even though inbreeding depression is not stronger under inter-specific competition, both inbreeding and suboptimal conditions can dramatically decrease individual fitness and growth rates (Fig. 1). Hence, effective conservation measures may operate at two levels: overcoming inbreeding by genetic restoration (Willi *et al.*, 2007) and reduction of competitive stress caused by non-target organisms.

ACKNOWLEDGEMENTS

We thank L. Blischke, J. Kaufmann, F. Kloß, V. Pasqualetto, and D. Raudnitschka for technical assistance and J. Van Buskirk for constructive comments on the manuscript. We received financial support from the Swiss National Science Foundation.

REFERENCES

Armbruster, P. and Reed, D.H. 2005. Inbreeding depression in benign and stressful environments. *Heredity*, **95**: 235–242.

Badyaev, A.V. and Foresman, K.R. 2000. Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proc. R. Soc. Lond. B*, **267**: 371–377.

Berg, H. and Redbo-Torstensson, P. 1999. Offspring performance in three cleistogamous *Viola* species. *Plant Ecol.*, **145**: 49–58.

Berg, H. and Redbo-Torstensson, P. 2000. Offspring performance in *Oxalis acetosella*, a cleistogamous perennial herb. *Plant Biol.*, **2**: 638–645.

Bijlsma, R., Bundgaard, J. and Van Putten, W.F. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster. J. Evol. Biol.*, **12**: 1125–1137.

Carr, D.E. and Dudash, M.R. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity*, **75**: 437–445.

Cheptou, P.-O. and Schoen, D.J. 2003. Frequency-dependent inbreeding depression in *Amsinckia. Am. Nat.*, **162**: 744–753.

Cheptou, P.-O., Imbert, E., Lepart, J. and Escarré, J. 2000. Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant *Crepis sancta* (Asteraceae). *J. Evol. Biol.*, **13**: 522–531.

Cheptou, P.-O., Lepart, J. and Escarré, J. 2001. Inbreeding depression under intraspecific competition in a highly outcrossing population of *Crepis sancta* (Asteraceae): evidence for frequency-dependent variation. *Am. J. Bot.*, **88**: 1424–1429.

Christen, M., Kurtz, J. and Milinski, M. 2002. Outcrossing increases infection success and competitive ability: experimental evidence from a hermaphrodite parasite. *Evolution*, **56**: 2243–2251.

Crnokrak, P. and Barrett, S.C.H. 2002. Perspective: Purging the genetic load: a review of the experimental evidence. *Evolution*, **56**: 2347–2358.

Crnokrak, P. and Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity*, **83**: 260–270.

Daehler, C.C. 1999. Inbreeding depression in smooth cordgrass (*Spartina alterniflora*, Poaceae) invading San Francisco Bay. *Am. J. Bot.*, **86**: 131–139.

Delph, L.F. and Lloyd, D.G. 1996. Inbreeding depression in the gynodioecious shrub *Hebe subalpina* (Scrophulariaceae). *NZ J. Bot.*, **34**: 241–247.

Eckert, C.G. and Barrett, S.C.H. 1994. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution*, **48**: 952–964.

Fischer, M., van Kleunen, M. and Schmid, B. 2000. Genetic Allee effects on performance, plasticity and developmental stability in a clonal plant. *Ecol. Lett.*, **3**: 530–539.

Fowler, K. and Whitlock, M.C. 2001. Environmental stress, inbreeding, and the nature of phenotypic and genetic variance in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B*, **269**: 677–683.

Galeuchet, D.J., Perret, C. and Fischer, M. 2005. Performance of *Lychnis flos-cuculi* from fragmented populations under experimental biotic interactions. *Ecology*, **86**: 1002–1011.

Gallardo, J.A. and Neira, R. 2005. Environmental dependence of inbreeding depression in cultured Coho salmon (*Oncorhynchus kisutch*): aggressiveness, dominance and intraspecific competition. *Heredity*, **95**: 449–456.

Gilligan, D.M., Woodworth, L.M., Montgomery, M.E., Briscoe, D.A. and Frankham, R. 1997. Is mutation accumulation a threat to the survival of endangered populations? *Conserv. Biol.*, **11**: 1235–1241.

Goodwillie, C. 2000. Inbreeding depression and mating systems in two species of *Linanthus* (Polemoniaceae). *Heredity*, **84**: 283–293.

Gurevitch, J., Taub, D.R., Morton, T.C., Gomez, P.L. and Wang, I.-N. 1996. Competition and genetic background in a rapid-cycling cultivar of *Brassica rapa* (Brassicaceae). *Am. J. Bot.*, **83**: 932–938.

Haag, C.R., Hottinger, J.W., Riek, M. and Ebert, D. 2002. Strong inbreeding depression in a *Daphnia* metapopulation. *Evolution*, **56**: 518–526.

Keller, L.F. and Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.*, **17**: 230–241.

Keller, L.F., Grant, P.R., Grant, B.R. and Petren, K. 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. *Evolution*, **56**: 1229–1239.

Kéry, M., Matthies, D. and Spillmann, H.-H. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *J. Ecol.*, **88**: 17–30.

Koehn, R.K. and Bayne, B.L. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. *Biol. J. Linn. Soc.*, **37**: 157–171.

Koelewijn, H.P. 2004. Sibling competition, size variation and frequency-dependent outcrossing advantage in *Plantago coronopus*. *Evol. Ecol.*, **18**: 51–74.

Kristensen, T.N. and Sørensen, A.C. 2005. Inbreeding – lessons from animal breeding, evolutionary biology and conservation genetics. *Anim. Sci.*, **80**: 121–133.

Kristensen, T.N., Dahlgaard, J. and Loeschcke, V. 2002. Inbreeding affects Hsp70 expression in two species of *Drosophila* even at benign temperatures. *Evol. Ecol. Res.*, **4**: 1209–1216.

Kristensen, T.N., Sørensen, P., Pedersen, K.S., Kruhøffer, M. and Loeschke, V. 2006. Inbreeding by environmental interactions affect gene expression in *Drosophila melanogaster*. *Genetics*, **173**: 1329–1336.

Latter, B.D.H. and Mulley, J.C. 1995. Genetic adaptation to captivity and inbreeding depression in small laboratory populations of *Drosophila melanogaster*. *Genetics*, **139**: 255–266.

Lhamo, N., Ramsey, M. and Vaughton, G. 2006. Density- and frequency-dependent inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius*. *Evol. Ecol. Res.*, **8**: 717–730.

Lynch, M. and Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates.

Marr, A.B., Arcese, P., Hochachka, W.M., Reid, J.M. and Keller, L.F. 2006. Interactive effects of environmental stress and inbreeding on reproductive traits in a wild bird population. *J. Anim. Ecol.*, **75**: 1406–1415.

Meagher, S., Penn, D.J. and Potts, W.K. 2000. Male–male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. USA*, **97**: 3324–3329.

Pluess, A.R. and Stöcklin, J. 2004. Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size. *Conserv. Genet.*, **5**: 145–156.

Pujol, B. and McKey, D. 2006. Size asymmetry in intraspecific competition and the density-dependence of inbreeding depression in a natural plant population: a case study in cassava (*Manihot esculenta* Crantz, Euphorbiaceae). *J. Evol. Biol.*, **19**: 85–96.

Rowe, G. and Beebee, T.J.C. 2005. Intraspecific competition disadvantages inbred natterjack toad (*Bufo calamita*) genotypes over outbred ones in a shared pond environment. *J. Anim. Ecol.*, **74**: 71–76.

SAS Institute. 2002. *SAS OnlineDoc®*, Version 9.1. Cary, NC: SAS Institute.

SAS Institute. 2006. *The GLIMMIX Procedure*. Cary, NC: SAS Institute.

Schmid, B. and Dolt, C. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution*, **48**: 1525–1549.

Schmitt, J. and Ehrhardt, D.W. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. *Evolution*, **44**: 269–278.

Singer, J.D. 1998. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *J. Educ. Behav. Stat.*, **24**: 323–355.

Sørensen, J.G., Kristensen, T.N. and Loeschke, V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.*, **6**: 1025–1037.

Stearns, S.C., Kaiser, M. and Kawecki, T.J. 1995. The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.*, **8**: 539–557.

van Treuren, R., Bijlsma, R., Ouborg, N.J. and van Delden, W. 1993. The significance of genetic erosion in the process of extinction. IV. Inbreeding depression and heterosis effects caused by selfing and outcrossing in *Scabiosa columbaria*. *Evolution*, **47**: 1669–1680.

Waller, D.M. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution*, **38**: 427–440.

Willi, Y. and Fischer, M. 2005. Genetic rescue in interconnected populations of small and large size of the self-incompatible *Ranunculus reptans*. *Heredity*, **95**: 437–443.

Willi, Y., Van Buskirk, J. and Fischer, M. 2005. A threefold genetic Allee effect: population size affects cross-compatibility, inbreeding depression and drift load in the self-incompatible *Ranunculus reptans*. *Genetics*, **169**: 2255–2265.

Willi, Y., Van Buskirk, J. and Hoffmann, A.A. 2006. Limits to the adaptive potential of small populations. *Annu. Rev. Ecol. Evol. Syst.*, **37**: 433–458.

Willi, Y., van Kleunen, M., Dietrich, S. and Fischer, M. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proc. R. Soc. Lond. B*, **274**: 2357–2364.

Wolfe, L.M. 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. *Evolution*, **47**: 374–386.

APPENDIX 1

Studies of inbreeding under (a) inter- and (b) intra-specific competition

Species	Type of organism	Inbreeding[a]	Competition treatment: types of competitors[b]	Fitness traits	Inbreeding × competition[c]	More ID under stress[d]	Reference
(a) Inter-specific competition							
Brassica rapa	plant	self vs. outbred		biomass, survival	N.S.	–	Gurevitch *et al.* (1996)
Viola riviniana	plant	CL vs. CH		germination rate, survival, leaf number	N.S.	–	Berg and Redbo-Torstensson (1999)
Crepis sancta	plant	inbred vs. outbred		survival, biomass	N.S.	–	Cheptou *et al.* (2000)
				flower production	sign./N.S.	+/–	
Primula veris	plant	small vs. large pop.		biomass, flowering probability	N.S.	–	Kéry *et al.* (2000)
Scabiosa columbaria	plant	pop. H_e: 0.1–0.2		biomass	—	+	Pluess and Stöcklin (2004)
Lychnis flos-cuculi	plant	pop. H_o: 0.3–0.6		flowering probability, biomass	N.S.	–	Galeuchet *et al.* (2005)
Geospiza scandens	bird	inbred vs. outbred		juvenile and adult survival	N.S.	–	Keller *et al.* (2002)
Geospiza fortis	bird	inbred vs. outbred		juvenile and adult survival	N.S.	–	Keller *et al.* (2002)

(*continued*)

APPENDIX—*Continued*

Species	Type of organism	Inbreeding[a]	Competition treatment: types of competitors[b]	Fitness traits	Inbreeding × competition[c]	More ID under stress[d]	Reference
(b) Intra-specific competition							
Impatiens capensis	plant	CL vs. CH	mixed, several densities	biomass	sign./N.S.	–	Waller (1984)
Impatiens capensis	plant	CL vs. CH	same cross type vs. mixed	biomass	sign.	–	Schmitt and Ehrhardt (1990)
			none vs. same cross type/mixed	biomass	—	–/+	
Scabiosa columbaria	plant	self vs. outbred	none vs. mixed	biomass	N.S.	–	van Treuren *et al.* (1993)
Hydrophyllum appendiculatum	plant	self vs. outbred	none vs. with competitors	plant size	—	–	Wolfe (1993)
				growth rate	—	+	
Decodon verticillatus	plant	self vs. outbred	3 pot sizes	biomass	N.S.	–	Eckert and Barrett (1994)
Mimulus guttatus	plant	self vs. outbred	selfed vs. outbred	biomass	N.S.	–	Carr and Dudash (1995)
Hebe subalpina	plant	self vs. outbred	none vs. mixed	biomass	N.S.	–	Delph and Lloyd (1996)
Brassica rapa	plant	self vs. outbred	4 densities of competitors	biomass	sign./N.S.	+/–	Gurevitch *et al.* (1996)
				flower production	N.S.	–	
Viola hirta	plant	CL vs. CH	none vs. sibs	germination rate, survival, leaf number	—	–	Berg and Redbo-Torstensson (1999)
Viola mirabilis	plant	CL vs. CH	none vs. sibs	germination rate, survival, leaf number	—	–	Berg and Redbo-Torstensson (1999)
Spartina alterniflora	plant	self vs. outbred	none vs. other cross type	biomass	sign.	+	Daehler (1999)
Oxalis acetosella	plant	CL vs. CH	none vs. sibs	germination rate, survival, leaf number	N.S.	–	Berg and Redbo-Torstensson (2000)

Linanthus bicolor	plant	self vs. outbred	none vs. inbred	biomass	N.S.	–	Goodwillie (2000)
Linanthus jepsonii	plant	self vs. outbred	none vs. mixed	biomass	N.S.	–	Goodwillie (2000)
Crepis sancta	plant	self vs. outbred	same cross type vs. mixed	total fitness	sign.	+	Cheptou *et al.* (2001)
			2 densities	total fitness	N.S.	–	
Amsinckia douglasiana	plant	self vs. outbred	mixed (replacement series)	biomass, flower production	N.S.	–	Cheptou and Schoen (2003)
Amsinckia gloriosa	plant	self vs. outbred	mixed (replacement series)	biomass	sign./N.S.	–	Cheptou and Schoen (2003)
				flower production	N.S.	–	
Plantago coronopus	plant	self vs. outbred	3 densities of sibs	biomass	sign.	–	Koelewijn (2004)
				flowering probability	N.S.	–	
Hibiscus trionum	plant	self vs. outbred	same cross type, 2 densities	biomass	N.S.	–	Lhamo *et al.* (2006)
				seed production	sign.	–	
Manihot esculenta	plant	inbred vs. outbred	mixed, various densities	survival	—	+	Pujol and McKey (2006)
Schistocephalus solidus	tapeworm	self vs. outbred	same cross type vs. mixed	4 host infection traits	—	+ (3 ×)	Christen *et al.* (2002)
Daphnia magna	crustacean	inbred vs. outbred	same cross type vs. mixed	fitness	sign.	+	Haag *et al.* (2002)
Drosophila melanogaster	insect	inbred vs. outbred	same cross type, 2 densities	competitive ability	—	–	Latter and Mulley (1995)
Drosophila melanogaster	insect	inbred vs. outbred	none vs. outbred	offspring production	—	–	Gilligan *et al.* (1997)
Drosophila melanogaster	insect	inbred vs. outbred	same cross type, 2 densities	survival	N.S.	–	Fowler and Whitlock (2001)

(*continued*)

APPENDIX—*Continued*

Species	Type of organism	Inbreeding[a]	Competition treatment: types of competitors[b]	Fitness traits	Inbreeding × competition[c]	More ID under stress[d]	Reference
Oncorhynchus kisutch	fish	inbred vs. outbred	mixed, 2 densities	survival	—	+	Gallardo and Neira (2005)
				2 growth traits	N.S.	–	
Bufo calamita	toad	inbred vs. outbred pop.	same cross type vs. mixed	larval survival	—	+	Rowe and Beebee (2005)
Geospiza scandens	bird	inbred vs. outbred	mixed, various densities	juvenile survival	N.S.	–	Keller *et al.* (2002)
				adult survival	N.S./sign.	–/ +	
Geospiza fortis	bird	inbred vs. outbred	mixed, various densities	juvenile and adult survival	N.S.	–	Keller *et al.* (2002)
Melospiza melodia	bird	inbred vs. outbred	mixed, various densities	male mating success	N.S.	–	Marr *et al.* (2006)
Mus domesticus	mouse	inbred vs. outbred	none vs. mixed	male offspring production	sign.	+	Meagher *et al.* (2000)
				female offspring production	N.S.	–	

[a] Types of inbreeding: CL = cleistogamous; CH = chasmogamous; H_e = expected population heterozygosity; H_o = observed population heterozygosity.
[b] Types of competitors: mixed = inbred and outbred competitors combined; sibs = competitors are from the same family as the target individual.
[c] Test result for the inbreeding × competition treatment interaction: sign. = significant; N.S. = not significant; — , not reported.
[d] Evidence for enhanced inbreeding depression (ID) under stress based on test statistics and/or treatment means and variances: +, positive; –, no evidence. Some studies described only verbally which environment was stressful.