
Regional Consequences of Local Population Demography and Genetics in Relation to Habitat Management in *Gentiana pneumonanthe*

SERGEI VOLIS,*‡ GIL BOHRER,† GERARD OOSTERMEIJER,* AND PETER VAN TIENDEREN*

*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94062, 1090 GB Amsterdam, The Netherlands

†Department of Civil and Environmental Engineering, Duke University, Durham, NC 27708, U.S.A.

Abstract: *A joint demographic and population genetics stage-based model for a subdivided population was applied to *Gentiana pneumonanthe*, an early successional perennial herb, at a regional (metapopulation) scale. We used numerical simulations to determine the optimal frequency of habitat disturbance (sod cutting) and the intensity of gene flow among populations of *G. pneumonanthe* to manage both population viability and genetic diversity in this species. The simulations showed that even small populations that initially had near-equal allele frequencies could, if managed properly through sod cutting every 6 to 7 years, sustain their high genetic variation over the long run without gene flow. The more the allele frequencies in the small populations are skewed, however, the higher the probability that in the absence of gene flow, some alleles will be lost and within-population genetic variation will decrease even under proper management. This implies that although local population dynamics should be the major target for management, regional dynamics become important when habitat fragmentation and decreased population size lead to the loss of local genetic diversity. The recommended strategy to improve genetic composition of small populations is the introduction of seeds or seedlings of nonlocal origin.*

Key Words: matrix population models, population viability analysis

Consecuencias Regionales de la Demografía y Genética de una Población Local en Relación con la Gestión de Hábitat de *Gentiana pneumonanthe*

Resumen: *Aplicamos un modelo combinado de demografía y genética poblacional basado en etapas a una población subdividida de *Gentiana pneumonanthe*, una hierba perenne de sucesión temprana, a una escala regional (metapoblacional). Utilizamos simulaciones numéricas para determinar la frecuencia óptima de perturbación de hábitat (corte de césped) y la intensidad de flujo genético entre poblaciones de *G. pneumonanthe* para manejar tanto la viabilidad poblacional como la diversidad genética de esta especie. Las simulaciones mostraron que, manejadas adecuadamente mediante cortes cada 6-7 años, aun las poblaciones pequeñas que inicialmente tenían frecuencias alélicas casi iguales podían mantener su alta variación genética a largo plazo sin deriva génica. Sin embargo, a mayor sesgo en las frecuencias alélicas de las poblaciones pequeñas, es mayor la probabilidad de que se pierdan algunos alelos y disminuya la variación genética intrapoblacional, aun bajo condiciones de manejo adecuadas. Esto implica que, aunque la dinámica poblacional local debiera ser el principal objetivo de manejo, la dinámica regional es importante cuando la fragmentación del hábitat y la reducción del tamaño poblacional conducen a la pérdida de diversidad genética local. La introducción*

‡Current address: The Institutes for Applied Research, Ben-Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel, email volis@bgumail.bgu.ac.il

Paper submitted December 8, 2003; revised manuscript accepted July 1, 2004.

de semillas o plántulas de origen fuereño es la estrategia recomendada para mejorar la composición genética de poblaciones locales.

Palabras Clave: análisis de viabilidad poblacional, modelos poblacionales matriciales

Introduction

The importance of both genetic and demographic effects on population viability has long been recognized by conservation biologists (e.g., Schonewald-Cox et al. 1983; Lande 1988). Estimating these effects on the short- and long-term viability of a population, and especially of a network of populations, however, is extremely difficult. This estimation requires (1) extensive data on population demography sampled during a sufficiently long period of time, (2) knowledge of the reproductive biology of the species, and (3) an assessment of the extent and structure of genetic variability and environmental and demographic effects on the reproductive biology. Studies with sufficient data to estimate genetic and demographic effects (either alone or simultaneously) on population viability are rare (reviewed in Oostermeijer et al. 2003). Numerical simulation performed on the basis of demographic, environmental, and genetic data is a powerful tool. With this tool it is possible to investigate the separate and joint effects of several parameters on long-term population viability, such as demographic and environmental stochasticity (Menges 1991, 1992), colonization and extinction (Valverde & Silvertown 1997), genetics (Burgman & Lamont 1992; Oostermeijer 2000; Young et al. 2000), and conservation and management strategies (Crone & Gehring 1998; Menges & Dolan 1998; Oostermeijer 2000; Bohrer et al. 2002; Doubledee et al. 2003; Osunkoya 2003). Population modeling is particularly useful for addressing issues of manipulated disturbances in ecosystems subject to periodic disturbance and subsequent recovery. Population viability analyses (PVA) make it possible to identify optimal frequency of burning (Gross et al. 1998; Drechsler et al. 1999; McCarthy et al. 2001; Quintana-Ascencio et al. 2003), and regimes of mowing, grazing (Hunt 2001; Lennartsson & Oostermeijer 2001), and sod cutting (Oostermeijer 2000). But these PVAs are rarely conducted for populations linked through migration, and metapopulation viability analyses that incorporate genetic information (reviewed in Menges 2000; Oostermeijer et al. 2003) are especially rare.

We used a numerical simulation model to determine the optimal management scheme for *Gentiana pneumonanthe* (marsh gentian), a perennial herb of early successional stages of heathlands and nutrient-poor mown grasslands. The low and relatively open vegetation characteristic of these two seminatural habitats has been ensured in The Netherlands for many centuries by annual mowing and haymaking, sod cutting, and grazing. Ongo-

ing abandonment of traditional farming leads to the rapid disappearance of these species-rich unique biotopes for which the marsh gentian flowers form a sort of "flagship." Oostermeijer et al. (1996) and Oostermeijer (2000) suggest that heathland populations of *G. pneumonanthe* should be managed with sod cutting because it resets succession. The demographic simulation model Oostermeijer (2000) set up in RAMAS/stage 1.4 (Ferson 1991) is based on regression equations for stage-transition probabilities that depend on time since disturbance (onset of secondary succession) and population size (number of reproducing adults). Transitions in this model are sensitive to small population size through a demographic "inbreeding depression" effect that reduces transition rates for the progeny of self-pollinated plants. We used Oostermeijer's (2000) parameterization approach in a simulation model that allowed incorporation of genetic drift and gene flow at specified rates among several populations in a metapopulation network. We reconsidered his management recommendations with a stricter goal of preserving not only the populations but also the genetic diversity within the populations.

We used our model to determine the optimal frequency of disturbance (in this case cutting sod on heathlands) and the optimal intensity of gene flow among populations of *G. pneumonanthe* that will maintain or increase population viability and population genetic diversity in this species.

Methods

Study Species and Demographic Data

Gentiana pneumonanthe is a predominantly outcrossing, self-compatible, iteroparous perennial herb characteristic of wet heathlands and hay meadows. It is a rare species because of various negative effects of human impact on its natural habitats and the lack of appropriate management in nature reserves (Oostermeijer et al. 1994).

We used data on a heathland population in Terschelling, where the demographic effects of succession after sod cutting on *G. pneumonanthe* have been monitored for 6 years. Demographic censuses on *G. pneumonanthe* have been done on six life stages (Oostermeijer et al. 1994, 1996), but for the transition probability matrix used in this study we distinguished only three stages to reduce computational complexity: (1) immatures, individuals with

cotyledons, and with or without a stem or an overwintering shoot (pooling the seedlings and juveniles described in Oostermeijer et al. 1994, 1996); (2) nonreproducing adults, individuals without cotyledons, with one or more flowerless stems, and with one or more overwintering shoots or plants in a dormant state without any organs visible above ground (pooling the vegetative adult and dormant stages described by Oostermeijer et al. 1994, 1996); and (3) reproducing (generative) adults, plants with one or more flowering stalks.

The Model

We created a numerical simulation program (written in R-script) that allows PVA to be conducted on a set of populations connected through predefined rates of migration of dispersing immatures. The simulation model incorporates population genetics (initial allele frequencies and heterozygosity, random genetic drift, and inbreeding), population demography, and migration among populations.

The model did not incorporate environmental stochasticity because directional time-related (i.e., successional) changes in population demography were more important in the heathland populations of gentian than changes caused by climate-related stochastic variability (Oostermeijer et al. 1996). In addition, the assumption of deterministic demographic processes made it easier to follow and interpret different effects on stochastically modeled genetic structures. Because our main objective was to understand how the population’s demography and genetics are affected by migration and sod-cutting management, adding stochastic environmental variability would have made the separation of effects more difficult. We therefore did not model stochastic variability and set the transitions at the observed means reported by Oostermeijer (2000).

The model deals with a spatially structured population (metapopulation) that occupies a finite (and relatively small) number of patches (12 populations in this case). Demography within each patch is described by a stage-genotype-structured transition matrix incorporating the three life stages described earlier in this paper and three genotypes (AA, AB, and BB) of a single locus with two codominant alleles, *A* and *B*. The metapopulation is represented by a vector, *N*. We define *n* as the dimension of *N*:

$$n = (12 \text{ populations}) * (3 \text{ life stages}) * (3 \text{ genotypes}) = 108. \tag{1}$$

Each “population element” in *N* represents the number of individuals of a particular genotype at a particular life stage in a population.

The model simulates three subprocesses: (1) demography that includes all life-stage transitions (excluding fecundity) and migration; (2) genetics that includes mating

relationships affecting the gene distribution in the offspring (fecundity); and (3) management. All demographic processes, the total number of offspring, and the portion of the population to survive management were determined by a set of deterministic equations (Table 1). The offspring genotypes and the management time were determined stochastically by drawing random numbers from defined distributions.

In general the model progresses in time through a series of transitions that are described by

$$N_{t+1} = M * [D(A_t) * (T(A_t, S_t) * N_t + F_t(A_t, S_t))], \tag{2}$$

where *T* is a matrix of transition rates that does not include fecundity; *F* is a fecundity vector; *D* is a management matrix; *M* is a migration matrix; *t* is the current time step; *S* is a vector of successional stages of each population element; and *A* is a vector of the number of adults in the population. The *A*, *F*, *N*, and *S* are vectors of dimension *n*, and *T*, *D*, and *M* are square matrices of dimension *n*².

Because the transition matrix *T* did not include fecundity and migration, it represented only survival and stage transition probabilities within each population. Transitions were functions of the successional stage *S* and the number of adults in a population (*A*). Figure 1 shows a scheme of possible transitions within a population in *T* and Table 1 gives the functions and coefficients of *T*.

Migration (represented by the *M* matrix) was possible only for individuals of the immature life stage, hence representing seed dispersal. Because each individual represented one of three genotypes (AA, AB, or BB), their movement changed the allele frequencies in the original and destination population. Migration among patches through seed dispersal occurred according to the “island” model, meaning that the emigrants from each origin population were distributed equally over all destination populations (i.e., all populations were equally spaced from each other). For all nonmigrating populations and life

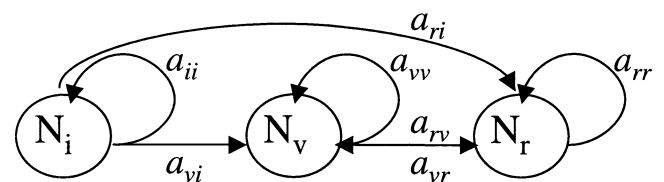


Figure 1. Scheme of population transitions in *G. pneumonanthe*, which is one block section of the total transition matrix (*T*), where *N* is the total number of individuals at the particular life stage in the population and *a* is the transition rate from one stage to another. The subscripts *i*, *v*, and *r* are the immatures, nonreproducing adults, and reproducing adults, respectively. The functions for transition rates are presented in Table 1. Fecundity is not included in the transition matrix.

Table 1. Parameterization used in the demographic-genetic stage-based model for *G. pneumonanthe*.*

Maximal transition probabilities matrix $T\{a(0)\}$ at succession stage $S = 0$			Equations for transitions related to time since sod-cutting coefficient, selfing rate, and population size			Sod-cutting intensity at different life stages as a function of <i>G. pneumonanthe</i> population size		
						no. of reproducing adults in a population	portion of individuals to survive sod cutting	
i	v	r		R	i	v	r	
<i>i</i>	0.030	0	0	$a_{vi} = (a_{vi}(0) - 0.107 * t_s) * ID * e^{-0.000001*(A)}$	<100	0.20	0.20	1.00
<i>v</i>	0.756	0.916	0.181	$a_{ri} = (a_{ri}(0) - 0.0134 * \ln(t_s)) * ID * e^{-0.000001*(A)}$	100-500	0.10	0.10	0.75
<i>r</i>	0.022	0.067	0.765	$a_{vv} = (a_{vv}(0) - 0.150 * \ln(t_s)) * ID$	500-1000	0.05	0.05	0.50
				$a_{rv} = (a_{rv}(0) - 0.033 * \ln(t_s)) * ID$	>1000	0.01	0.01	0.20
				$a_{rr} = (a_{rr}(0) - 0.2828 * \ln(t_s)) * ID$				
				$a_{vr} = 1 - 0.054 - a_{rr}$				
				$f_{ir} = \max(0, \text{round}(16 - 9.320 * \ln(t_s)))$	equations for sod-cutting stage and coefficient			
				$ID = 1 - (0.40 * sf)$	$t_s = \min(8, \max(1, (S - 4)))$			
				$sf = 0.898 - 0.148 * \ln(R)$	$S = t - t_{st} + 8 - \text{integer}((1 - [\text{portion } r \text{ survived}]) * 8)$			

*Key to variables and abbreviations: *i*, immature; *v*, vegetative adult; *r*, reproducing adult; **R**, **A**, number of reproducing adults in a population and the total number of adults (nonreproducing + reproducing), respectively; *a*, transition rates, first and second subscripts of a specify to what life stage and from which, respectively; *f*, fecundity; *ID*, inbreeding depression coefficient; *sf*, selfing rate coefficient; *t*, current time step; *t_{st}*, time step of last sod cutting in the population; *S*, succession stage; *t_s*, succession coefficient; integer, a function that rounds a real number down to the nearest integer; round, a function that rounds a real number to the nearest integer; min and max, functions that equal the minimum and maximum, respectively, of two values.

stages, **M** had unity diagonal values and zero off-diagonal values.

The fecundity vector **F** specified the number of offspring to be added to the population at the current time step. The **F** values for all life stages other than immature were set to zero. The total number of immatures produced by a population was a deterministic and successional-stage-dependent function (*f_{ir}* equation, Table 1). This total was distributed among the three possible genotypes, assuming random mating, production of infinite gametes, and survival of zygotes corresponding to the number of offspring per adult following Mendelian genetic rules. This random redistribution of the total among genotypes was done using one of the following procedures:

1. When the population was not large (<1000 offspring), the model used an individual-based approach. For each reproducing individual, the model drew a random number from the distribution of genotypes in the reproductive adults in the population to determine the genotype of its mating pair. Then the model drew another set of random numbers from the Mendelian distribution of expected offspring genotypes of the parental pair to determine the genotype of each offspring.
2. When the total number of expected offspring in the population was >1000, a statistical approach was adopted. This approach preserved the same genotype distributions as the individual-based approach but was more computationally efficient. The determined total number of offspring in each population

was distributed among genotypes according to the following equations:

$$\begin{pmatrix} F_{1AA} \\ F_{1BB} \end{pmatrix} = NF \begin{pmatrix} P\{A\}^2 \\ P\{B\}^2 \end{pmatrix} + \sqrt{NF} * \begin{pmatrix} \sqrt{\lambda_{C1}} & 0 \\ 0 & \sqrt{\lambda_{C2}} \end{pmatrix} * \begin{pmatrix} V_{C11} & V_{C21} \\ V_{C12} & V_{C22} \end{pmatrix} * \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \tag{3}$$

and

$$F_{1AB} = NF - F_{1AA} - F_{1BB}, \tag{4}$$

where *NF* is the total number of offspring produced by the population; *P{A}* and *P{B}* are the allele distributions of A and B; *X₁* and *X₂* are standard normal random numbers; and *λ_{C1}*, *λ_{C2}*, *V_{C1}*, and *V_{C2}* are the eigenvalues and corresponding eigenvectors, respectively, of the covariance matrix **C** of the genotype probabilities. The covariance matrix is

$$C = \begin{bmatrix} P\{A\}^2 (1 - P\{A\}^2) & -P\{A\}^2 * P\{B\}^2 \\ -P\{A\}^2 * P\{B\}^2 & P\{B\}^2 (1 - P\{B\}^2) \end{bmatrix}. \tag{5}$$

Parameterization and Simulations

Because *G. pneumonanthe* is a successional species, the transitions in the population matrix were dependent on time and population size. These dependences were introduced as linear and logarithmic functions as described in Oostermeijer (2000). A negative relationship between selfing rate and survival and fecundity of reproductive individuals in populations of *G. pneumonanthe* (Raijmann et al. 1994) was implemented in our model through

the “inbreeding depression” equation (ID in Table 1), which reduced stage transition rates in smaller populations. Transition from immature to adult was also affected by density dependence in large populations (equations in Table 1) and simulated the competitive suppression of seedlings by adult plants.

Succession affected transitions by changing the succession coefficient (t_s) in the transitions equations (Table 1). In a full course of succession (starting at succession time $S_t = 1$), there was no effect during the first 5 years and transitions were at a specified maximal level. Then the transitions logarithmically decreased for the next 8 years and from year 13 until the next sod cutting the transitions were kept at a constant minimum (Table 1). Succession time was a function of how long ago and how intensive the last sod cutting had been (Table 1). In principle, sod cutting is administered by the park managers in rotation between plots with a fixed time interval. But the actual year of sod cutting at each particular site varies because of logistic considerations (e.g., costs and availability of the machinery) and the order of the sites, which is decided arbitrarily. To simulate this variation, we sampled the year at which sod cutting was administered in our model and used a uniform distribution from 2 years before to 2 years after the target year (i.e., the year scheduled according to the planned cutting interval). In this way, sod cutting occurred at random, with an average at the scheduled time, and maximally 2 years off the target schedule.

Management by sod cutting removes vegetation and sets back succession, creating favorable conditions for germination and establishment of *G. pneumonanthe*. The cutting, however, has a side effect on the gentian populations because it inevitably kills some individuals. The

fraction of the population surviving after sod cutting was dependent on size (Oostermeijer 2000). In the first year after sod cutting, the succession stage (S) was set back as a function of this surviving population fraction and then increased by 1 for each time step until the next sod cutting (Table 1). The succession stage determined the succession coefficient (t_s) that affected transitions and fecundity (Table 1).

The number of simulations was set to 100 for each experimental scenario. In each simulation we ran the model for 100 generations. For the 100th generation, in addition to the number of adult plants, we calculated the proportion of populations that went extinct and the following genetic parameters: total genetic variation (H_T), among-population structure of genetic variation (F_{ST}), and heterozygosity (H_S).

We estimated the effects of initial population size, initial population allele frequencies, gene flow, variation in the start of sod cutting among populations, and frequency of sod cutting. Table 2 shows the initial population sizes and distributions for 12 populations connected through migration of newly recruited immatures (which also supply gene flow). The initial population size of a large population was set to 200 reproducing adults, which is representative of natural heathland *G. pneumonanthe* populations. The numbers for the other two stages were characteristic of “invasive” populations in an early successional stage (Oostermeijer et al. 1994). Nine flowering, nine nonreproducing, and no immature individuals comprised the small population, a population structure typical for “regressive” populations in a late successional stage. Large populations initially had an inbreeding coefficient of zero and equal allele frequencies. Small populations had (1) a

Table 2. The initial population sizes and stage/allele distributions of *G. pneumonanthe*, estimates of observed (H_i) and expected (H_s) within-population heterozygosity, population inbreeding coefficient (F_{IS}), and population subdivision (F_{ST}).

Stage	Genotype	Initial size*			
		small			large
		no differentiation	moderate differentiation	strong differentiation	no differentiation
<i>i</i>	AA	0	0	0	500
	AB	0	0	0	1000
	BB	0	0	0	500
<i>v</i>	AA	2	4	7	500
	AB	5	5	2	1000
	BB	2	0	7	500
<i>r</i>	AA	2	4	7	50
	AB	5	5	2	100
	BB	2	0	7	50
Population genetic parameters					
H_i		0.55	0.55	0.22	0.50
H_s		0.50	0.40	0.20	0.50
F_{IS}		-0.11	-0.38	-0.10	0
F_{ST}		0	0.20	0.60	0

*The scenarios of genetic composition assumed either one population type throughout the metapopulation under “no differentiation” or two population types in equal proportion under “moderate differentiation” and “strong differentiation.”

slight excess of heterozygotes and symmetrical and equal allele frequencies (i.e., no genetic differentiation as a result of past genetic drift, mimicking sudden fragmentation of a large, random mating population); (2) an excess of heterozygotes and a moderately unequal frequency of *A* and *B* alleles among populations (moderate genetic differentiation); or (3) a slight excess of heterozygotes and a very unbalanced frequency of *A* and *B* alleles among populations (strong genetic differentiation; Table 2). The moment of disturbance (sod cutting) was either synchronized (performed in the same year in all populations) or was randomly assigned to each population within a range of ± 2 years around the target time. Three scenarios of island-type model gene flow assumed either no (0), moderate (0.01), or high (0.1) migration (expressed as the proportion of immature originating from immigration).

Results

Effects of Management under No Migration

The demographic effect of the sod-cutting interval was evident. Frequent sod cutting improved the transition rates of the population by setting back succession. Because the current practice of sod cutting never kills all the reproductive adults, frequent sod cutting does not drive the population to extinction. Instead, it increases its viability via improved growth transition rates and higher fecundity. But there was also a distinct effect of initial population size on population performance (Fig. 2). Large pop-

ulations (200 flowering plants) did not go extinct when sod cutting was performed at least every 11 years. The initially small population of nine flowering plants, however, survived only if sod cutting was done at least every 7 years, and for an increase of the initial flowering population by a factor of 2 (36, 72, 144, 288), the minimal sod-cutting frequency could increase by 1 year (8, 9, 10, and 11 years, respectively). The effect of environmental stochasticity may change the optimal interval between subsequent sod-cutting events but not the general trend we observed (i.e., an increase in the optimal sod-cutting interval with an increase in initial population size).

Because of the deterministic settings of demographic processes in the model, the genetic composition of small populations did not influence the population growth rate (data not shown). Initial differences in allele frequencies had no or a very minor effect on the total genetic variation (H_T) but a strong effect on the among-population structure of genetic variation (F_{ST}) and heterozygosity (H_S). Heterozygosity decreased and population subdivision increased when allele frequencies in initial populations changed from equal to skewed (Table 3). These effects were observed under both synchronized and desynchronized sod-cutting management regimes but were more pronounced when there was variation in the start of sod cutting among populations (Table 3).

A comparison between the two disturbance scenarios (cutting in the same year in all populations or randomly assigned to populations within ± 2 years around the target time) revealed that introducing variation at the start of sod cutting across populations decreased the probability

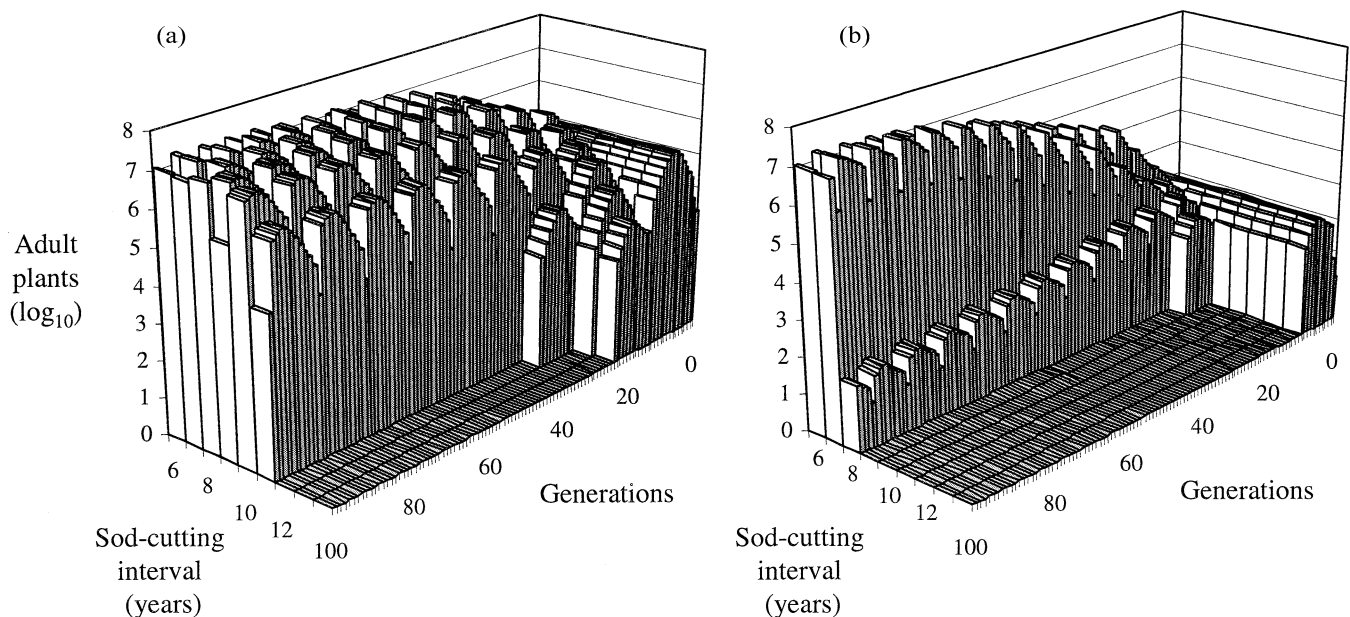


Figure 2. Performance of *G. pneumonanthe* metapopulation under synchronized sod-cutting managements ranging from every 6 to every 14 years when initial population sizes are (a) large (200 reproducing plants) and (b) small (9 reproducing plants).

Table 3. Genetic parameters (SD) calculated for *G. pneumonanthe* metapopulation composed of small populations (nine flowering plants) after 100 generations as an average of 100 simulations under different intensities of gene flow among populations, initial genetic differentiation, interval of sod cutting and synchronized versus variable application of sod cutting.

Genetic parameter	Sod-cutting interval ± variability (years)	Migration											
		0						0.1					
		differentiation			differentiation			differentiation			differentiation		
no	moderate	strong	no	moderate	strong	no	moderate	strong	no	moderate	strong		
F_{ST}	6 ± 0	0.007 (0.003)	0.219 (0.045)	0.909 (0.019)	0.005 (0.002)	0.147 (0.025)	0.608 (0.016)	<0.001	0.002 (0.001)	0.008 (0.001)	0.008 (0.001)	0.008 (0.001)	
	7 ± 0	0.012 (0.004)	0.256 (0.074)	0.924 (0.020)	0.007 (0.003)	0.160 (0.039)	0.643 (0.022)	<0.001	0.002 (0.001)	0.008 (0.001)	0.008 (0.001)	0.008 (0.001)	
H_S	6 ± 0	0.496 (0.001)	0.390 (0.023)	0.046 (0.010)	0.497 (0.001)	0.425 (0.013)	0.196 (0.008)	0.500 (0.001)	0.498 (0.001)	0.495 (0.001)	0.495 (0.001)	0.495 (0.001)	
	7 ± 0	0.494 (0.002)	0.371 (0.037)	0.038 (0.010)	0.496 (0.002)	0.419 (0.020)	0.178 (0.011)	0.499 (0.001)	0.498 (0.001)	0.494 (0.003)	0.494 (0.003)	0.494 (0.003)	
H_T	6 ± 0	0.500 (0.001)	0.499 (0.001)	0.500 (0.001)	0.500 (0.001)	0.499 (0.001)	0.500 (0.001)	0.500 (0.001)	0.499 (0.001)	0.499 (0.001)	0.499 (0.001)	0.499 (0.001)	
	7 ± 0	0.500 (0.001)	0.499 (0.002)	0.500 (0.001)	0.500 (0.001)	0.499 (0.001)	0.500 (0.001)	0.499 (0.001)	0.499 (0.001)	0.498 (0.003)	0.498 (0.003)	0.498 (0.003)	
F_{ST}	6 ± 2	0.011 (0.005)	0.243 (0.070)	0.922 (0.019)	0.005 (0.003)	0.103 (0.031)	0.435 (0.036)	<0.001	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	
	7 ± 2	0.012 (0.007)	0.203 (0.069)	0.913 (0.033)	0.005 (0.003)	0.089 (0.031)	0.437 (0.078)	<0.001	0.001 (0.001)	0.003 (0.002)	0.003 (0.002)	0.003 (0.002)	
	8 ± 2	0.007 (0.008)	0.079 (0.079)	0.586 (0.404)	0.004 (0.005)	0.060 (0.074)	0.479 (0.251)	<0.001	0.004 (0.007)	0.017 (0.032)	0.017 (0.032)	0.017 (0.032)	
	9 ± 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
H_S	6 ± 2	0.494 (0.003)	0.372 (0.036)	0.036 (0.009)	0.497 (0.002)	0.443 (0.016)	0.271 (0.018)	0.499 (0.001)	0.497 (0.003)	0.490 (0.011)	0.490 (0.011)	0.490 (0.011)	
	7 ± 2	0.493 (0.004)	0.386 (0.042)	0.038 (0.012)	0.497 (0.002)	0.442 (0.022)	0.245 (0.047)	0.494 (0.050)	0.488 (0.049)	0.466 (0.058)	0.466 (0.058)	0.466 (0.058)	
	8 ± 2	0.492 (0.010)	0.390 (0.095)	0.036 (0.030)	0.493 (0.018)	0.418 (0.044)	0.127 (0.058)	0.496 (0.006)	0.473 (0.023)	0.405 (0.078)	0.405 (0.078)	0.405 (0.078)	
	9 ± 2	0.489 (0.013)	0.444 (0.033)	0.029 (0.023)	0.493 (0.018)	0.348 (0.153)	0.015 (0.017)	0.485 (0.021)	0.413 (0.009)	0.392 (0.078)	0.392 (0.078)	0.392 (0.078)	
H_T	6 ± 2	0.499 (0.001)	0.490 (0.006)	0.471 (0.015)	0.499 (0.001)	0.494 (0.005)	0.479 (0.017)	0.499 (0.001)	0.498 (0.003)	0.491 (0.011)	0.491 (0.011)	0.491 (0.011)	
	7 ± 2	0.499 (0.001)	0.484 (0.031)	0.451 (0.040)	0.499 (0.001)	0.486 (0.019)	0.441 (0.070)	0.494 (0.050)	0.488 (0.049)	0.467 (0.058)	0.467 (0.058)	0.467 (0.058)	
	8 ± 2	0.495 (0.010)	0.427 (0.105)	0.240 (0.171)	0.495 (0.018)	0.446 (0.044)	0.308 (0.145)	0.497 (0.006)	0.475 (0.024)	0.413 (0.081)	0.413 (0.081)	0.413 (0.081)	
	9 ± 2	0.489 (0.012)	0.444 (0.033)	0.029 (0.023)	0.493 (0.008)	0.348 (0.153)	0.015 (0.017)	0.485 (0.021)	0.413 (0.010)	0.392 (0.078)	0.392 (0.078)	0.392 (0.078)	

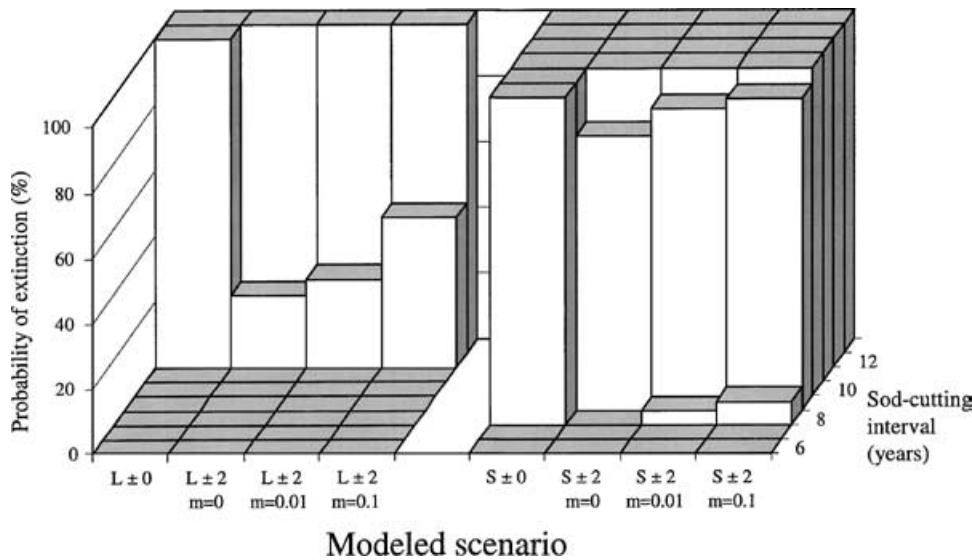


Figure 3. Probability of metapopulation extinction in 100 generations with different scenarios of initial population size (L, 200 reproducing plants; S, 9 reproducing plants), sod cutting (± 0 , synchronized—all populations in the same year; ± 2 , variable—randomly assigned to each population within ± 2 years), and gene flow ($m = 0\%$, 0.01% , and 0.1% of newly recruited immatures).

of metapopulation extinction, irrespective of initial population size or allele frequencies (Fig. 3). It did, however, decrease the number of surviving populations (Table 4).

Effect of Management with Migration

In a metapopulation with an island-type structure of gene flow (used here to simulate *G. pneumonanthe* dynamics) and under an assumption of synchrony in stage transitions of different populations, gene flow through migration cannot have any consequences for species growth rate and viability because immigration equals emigration. This corresponds to a situation of several semi-isolated populations at similar distance from each other that are of similar sizes, demographic structure, and dynamics, and for which management is carried out in the same year. Under such circumstances, all populations are equal in size and the number of individuals emigrating from a single population equals the sum of all immigrants from the other

populations. Desynchronization of stage transitions in different populations introduced by variation in the start of sod cutting among the populations, however, made migration important for both demography and genetics. Among populations, desynchronization of the management time enabled the whole metapopulation to survive at a lower sod-cutting frequency. In real populations, environmental stochasticity would add to this effect, amplifying the population differences caused by desynchronized management.

A metapopulation composed of small initial populations of nine flowering plants survived with variable sod-cutting frequency of at least every 9 years compared with every 7 years with synchronized sod cutting, and survived with minimal sod-cutting frequency of every 12 years compared with 11 years in large initial populations (Fig. 3).

When management was desynchronized, the effect of migration on overall population demography was

Table 4. Number of populations surviving (SD) in *G. pneumonanthe* metapopulation after 100 generations as an average of 100 simulations under different initial population sizes and allele frequencies and interval of sod cutting.*

Sod-cutting interval (years)	Large (200 reproducing adults)		Small (9 reproducing adults)					
	± 0	± 2	± 0			± 2		
	differentiation		differentiation			differentiation		
	no	no	no	moderate	strong	no	moderate	strong
6	12 (0)	12 (0)	12 (0)	12 (0)	12 (0)	11.98 (0.14)	11.83 (0.43)	11.90 (0.30)
7	12 (0)	12 (0)	12 (0)	12 (0)	12 (0)	9.71 (1.47)	8.57 (1.53)	9.09 (1.50)
8	12 (0)	12 (0)	1.56 (0.82)	0	0	3.30 (1.60)	2.63 (1.35)	3.16 (1.23)
9	12 (0)	12 (0)	0	0	0	1.06 (0.25)	1 (0)	1 (0)
10	12 (0)	11.69 (0.56)	0	0	0	0	0	0
11	12 (0)	7.45 (1.70)	0	0	0	0	0	0
12	0	1.81 (0.87)	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0

*Sod cutting: ± 0 , synchronized (all populations in same year); ± 2 , variable (randomly assigned to each population with ± 2 years).

negative (Fig. 3). This occurs because large early-successional populations with high transition rates are disproportionately losing juveniles that migrate to late-successional, low-transition populations. But this negative effect was rather small for both large and small initial populations, and an increase from no migration to high migration (i.e., 10% of the immature population each year are immigrants) caused an increase of no more than 20% in extinction probability (Fig. 3).

Gene flow (via migration) had no effect on the extent of genetic diversity in the total population (H_T). It did, however, have a great effect on the structure of genetic diversity when initial populations were small and allele frequencies skewed. Increase in gene flow increased H_S and decreased F_{ST} and eventually turned the whole metapopulation into a single panmictic population (Table 4). This effect was evident under both management regimes, with or without variation in the timing of sod cutting.

Discussion

Our results place the genetic and demographic consequences of small population size in *G. pneumonanthe* in a metapopulation context. The genetic and demographic effects of population size were tested by incorporating explicit genetic composition of populations and gene flow among the populations. Before considering the effect of management on *G. pneumonanthe* at the regional scale, however, we need to define what kind of a “population of populations” this species represents. The metapopulation of *G. pneumonanthe*—once widely distributed over wet heathlands and hay meadows—has become extremely fragmented in the last 50 years. Many populations became small and isolated from each other because of the intensification of human activities. The breeding system (insect-pollinated, self-compatible outcrosser) and seed dispersal (gravity) suggest some modest among-population gene flow through pollen exchange but very little exchange via seed dispersal. Small populations of few individuals may persist for long periods of time (more than 30 years; Chapman et al. 1989; Oostermeijer et al. 1992), but the chances of recolonization of areas in which *G. pneumonanthe* has become extinct are nearly zero. Freckleton and Watkinson (2002) called a population of populations of this kind a “regional ensemble” in which local dynamics prevails over regional dynamics, with a relatively low probability of local extinction and a very low probability of recolonization. Therefore, local population dynamics should be a major target for management in this type of species. Questions remain, however, about how important regional dynamics actually are and whether there is a need to design specific regional management plans.

Like Oostermeijer (2000), we found that small populations can be regenerated through disturbance management such as sod cutting of appropriate frequency. Small

populations, however, often have reduced levels of genetic diversity because of either inbreeding (reduced heterozygosity) or genetic drift (loss of alleles), and this can be prevented or restored only through gene flow. Our simulations showed that small populations that initially had near-equal allele frequencies could sustain their high genetic variation over many generations without gene flow, if managed properly (i.e., through sod cutting at an optimal frequency) and assuming no stochastic environmental variability. This is possible because negative effects of inbreeding and drift on genetic diversity disappear after the rapid increase in population size that follows sod cutting. The more the allele frequencies in the small populations are skewed, however, the higher the probability that some alleles will be lost even under proper management. As a result, within-population genetic variation will become very low unless restored through gene flow.

In an ensemble of many small *G. pneumonanthe* populations with growth rates of ≥ 1 (as a result of optimal management through sod cutting) under absence of gene flow, we may expect the among-population component of genetic variation to be high and the within-population genetic variation to be negligible. As our results show, under sufficiently high gene flow, the situation will be reversed. With an assumption of optimal management on a regional scale, the total genetic diversity under both scenarios will remain high and approach that in a large population in Hardy-Weinberg equilibrium. The question is what scenario is optimal for managed *G. pneumonanthe* to ensure high viability and persistence and preserve evolutionary potential.

The scenario that includes gene flow would be preferred if the species does not demonstrate outbreeding depression (unlikely to occur if there is substantial gene flow), is self-incompatible, and has high inbreeding depression. The opposite would be true for a scenario in which gene flow is absent (i.e., when the species occupies different habitats, where it undergoes different selection pressures, demonstrates local adaptation, has no self-incompatibility, and experiences no or low inbreeding depression).

Because properly managed small populations are not prone to extinction, fixing different rare alleles in different populations prevents their loss by drift. Thus, maximizing between-population variability and minimizing within-population variability might be effective, depending on the likelihood of extirpation. *G. pneumonanthe* is self-compatible with a modest effect of inbreeding (Raijman et al. 1994; Oostermeijer et al. 1996).

Oostermeijer and colleagues failed to detect either local adaptation or outbreeding depression in this species (Oostermeijer et al. 1995, 1996). It is unlikely that outbreeding depression effect will be detected unless crosses occur over > 100 km (Oostermeijer et al. 2003). But outbreeding depression may occur after crosses between heathland and grassland populations because of their

very different demographic behaviors (and thus their likely adaptations; Oostermeijer et al. 1996). Establishing among-population gene flow and maximizing the within-population component of variability as a part of management should not have negative consequences as long as management focuses on gene flow within and not among the two main habitat types.

An advantage of maintaining high genetic diversity within small populations over sustaining genetically dissimilar small populations with reduced genetic diversity is the lower probability of losing some alleles from the regional gene pool. The extinction of several local populations will then have little effect on the regional genetic diversity because they are replications of the same genetically diverse gene pool over space. In contrast, extinction of several genetically differentiated local populations may result in a significant reduction of regional diversity. Although random extinction was not included in the simulation, environmental stochasticity might, in extreme events, drive some populations to extinction. Hence, preserving within-population diversity might be even more important in reality than is shown in the model.

Our model did not include the explicit mechanism of gene exchange; instead, immature individuals were swapped between populations. We have not considered a geographically explicit metapopulation structure or varied the among-population distances. Therefore, our conclusions apply to an area where populations are rather dense and evenly distributed in space, and where they experience similar environmental conditions (e.g., core populations). Managing isolated, remote (peripheral) populations might be a different issue and is not addressed here. In practical terms, improving genetic composition of small populations through gene flow can be achieved in several ways, under either long- or short-term management strategies. For the former the best option would be to reduce fragmentation by increasing landscape connectivity (wide seminatural corridors). If, for whatever reason, gene exchange by natural migration would not be possible, pollen carryover from adjacent populations or introduction of seeds or seedlings could be the two options for short-term management.

Acknowledgments

We thank B. Rider for his invaluable help in developing the model. We are also grateful to M. Burgman and three anonymous reviewers for comments that greatly improved the manuscript. Support for this study was granted to S.V. by the Israel Council for Higher Education (a "Bikura" postdoctoral fellowship).

Literature Cited

- Bohrer, G., S. Shem-Tov, E. Summer, K. Or, and D. Saltz. 2002. The effectiveness of various rabies spatial vaccination patterns in a simulated host population with clumped distribution. *Ecological Modelling* **152**:205–211.
- Burgman, M. A., and B. B. Lamont. 1992. A stochastic model for the viability of *Banksia cuneata* populations—environmental, demographic and genetic effects. *Journal of Applied Ecology* **29**:719–727.
- Chapman, S. B., R. J. Rose, and R. T. Clarke. 1989. The behavior of populations of the marsh gentian (*Gentiana pneumonanthe*)—a modeling approach. *Journal of Applied Ecology* **26**:1059–1072.
- Crone, E. E., and J. L. Gehring. 1998. Population viability of *Rorippa columbiae*: multiple models and spatial trend data. *Conservation Biology* **12**:1054–1065.
- Doubledee, R. A., E. B. Muller, and R. M. Nisbet. 2003. Bullfrogs, disturbance regimes, and the persistence of California red-legged frogs. *Journal of Wildlife Management* **67**:424–438.
- Drechsler, M., B. B. Lamont, M. A. Burgman, H. R. Akcakaya, E. T. F. Witkowski, and Y. Supriyadi. 1999. Modelling the persistence of an apparently immortal *Banksia* species after fire and land clearing. *Biological Conservation* **88**:249–259.
- Ferson, S. 1991. RAMAS/stage 1.4 manual. Applied Biomathematics, Setauket, New York.
- Freckleton, R. P., and A. R. Watkinson. 2002. Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology* **90**:419–434.
- Gross, K., J. R. Lockwood, C. C. Frost, and M. F. Morris. 1998. Modeling controlled burning and trampling reduction for conservation of *Hudsonia montana*. *Conservation Biology* **12**:1291–1301.
- Hunt, L. P. 2001. Heterogeneous grazing causes local extinction of edible perennial shrubs: a matrix analysis. *Journal of Applied Ecology* **38**:238–252.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* **241**:1455–1460.
- Lennartsson, T. and J. G. B. Oostermeijer. 2001. Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. *Journal of Ecology* **89**:451–463.
- McCarthy, M. A., H. P. Possingham, and A. M. Gill. 2001. Using stochastic dynamic programming to determine optimal fire management for *Banksia ornata*. *Journal of Applied Ecology* **38**:585–592.
- Menges, E. S. 1991. The application of minimum viable population theory to plants. Pages 45–61 in D. A. Falk and K. E. Holsinger, editors. *Genetics and conservation of rare plants*. Oxford University Press, Oxford, United Kingdom.
- Menges, E. S. 1992. Stochastic modeling of extinction in plant populations. Pages 253–275 in P. L. Fiedler and S. K. Jain, editors. *Conservation biology: the theory and practice of nature conservation, preservation and management*. Chapman and Hall, New York.
- Menges, E. S. 2000. Applications of population viability analyses in plant conservation. *Ecological Bulletins* **48**:73–84.
- Menges, E. S., and R. W. Dolan. 1998. Demographic viability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. *Journal of Ecology* **86**:63–78.
- Oostermeijer, J. G. B. 2000. Population viability analysis of the rare *Gentiana pneumonanthe*: the importance of genetics, demography and reproductive biology. Pages 313–334 in A. G. Young and G. M. Clarke, editors. *Genetics, demography and viability of fragmented populations*. Cambridge University Press, Cambridge, United Kingdom.
- Oostermeijer, J. G. B., R. G. M. Altenburg, and J. C. M. den Nijs. 1995. Effects of outcrossing distance and selfing on fitness components in the rare *Gentiana pneumonanthe* (Gentianaceae). *Acta Botanica Neerlandica* **44**:257–268.
- Oostermeijer, J. G. B., M. L. Brugman, E. R. DeBoer, and J. C. M. den Nijs. 1996. Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. *Journal of Ecology* **84**:153–166.
- Oostermeijer, J. G. B., J. C. M. den Nijs, L. E. L. Raijmann, and S. B. J. Menken. 1992. Population biology and management of the

- marsh gentian (*Gentiana pneumonanthe* L.), a rare species in the Netherlands. *Botanical Journal of the Linnean Society* **108**: 117–130.
- Oostermeijer, J. G. B., S. H. Luijten, and J. C. M. den Nijs. 2003. Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113**:389–398.
- Oostermeijer, J. G. B., R. Vantveer, and J. C. M. den Nijs. 1994. Population structure of the rare, long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management in the Netherlands. *Journal of Applied Ecology* **31**:428–438.
- Osunkoya, O. O. 2003. Two-sex population projection of the endemic and dioecious rainforest shrub, *Gardenia actinocarpa* (Rubiaceae). *Biological Conservation* **114**:39–51.
- Quintana-Ascencio, P. F., E. S. Menges, and C. W. Weekley. 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.
- Raijmann, L. L., N. C. Vanleeuwen, R. Kersten, J. G. B. Oostermeijer, J. C. M. den Nijs, and S. B. J. Menken. 1994. Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conservation Biology* **8**:1014–1026.
- Schonewald-Cox, C. M., S. M. Chambers, B. MacBryde, and W. L. Thomas. 1983. *Genetics and conservation: a reference for managing wild animal and plant populations*. Benjamin Cummings, Menlo Park, California.
- Valverde, T., and J. Silvertown. 1997. A metapopulation model for *Primula vulgaris*, a temperate forest understorey herb. *Journal of Ecology* **85**:193–210.
- Young, A. G., A. H. D. Brown, B. G. Murray, P. H. Thrall, and C. H. Miller. 2000. Genetic erosion, restricted mating and reduced viability in fragmented populations of the endangered grassland herb *Rutidosia leptorrhynchoides*. Pages 335–359 in A. G. Young, and G. M. Clarke, editors. *Genetics, demography and viability of fragmented populations*. Cambridge University Press, Cambridge, United Kingdom.

