Estimation and analysis of effective size in age- and stage-structured populations

Marco Andrello

UNIVERSITÉ DE GRENOBLE

THÈSE

Pour obtenir le grade de
DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE
SPECIALITE BIOLOGIE VEGETALE
Arrêté ministériel : 7 août 2006

Présentée et soutenue publiquement par
Marco ANDRELLO
le 3 décembre 2010

Estimation et analyse de la taille efficace de populations structurées en classes d'âge ou en stades

Thèse dirigée par Irène TILL-BOTTRAUD et codirigée par Oscar E. GAGGIOTTI

JURY

Bruno COLAS Maître de Conférence, Université Paris VI	Rapporteur
Oscar E. GAGGIOTTI Professeur, Université de Grenoble I	Codirecteur de thèse
Amaury LAMBERT Professeur, Université Paris VI	Examinateur
Florence NICOLÈ Maître de Conférence, Université de Saint-Étienne	Examinatrice
Nicolas PERRIN Professeur, Université de Lausanne	Rapporteur
Irène TILL-BOTTRAUD Directrice de recherche, LECA Grenoble	Directrice de thèse

Thèse préparée au sein du Laboratoire d'Ecologie Alpine (LECA), UMR-CNRS 5553, 2233 rue de la Piscine, 38041 Grenoble Cedex 9, France dans « l'Ecole Doctorale Chimie et Sciences du Vivant»

UNIVERSITÉ DE GRENOBLE

THÈSE

Pour obtenir le grade de
DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE
SPECIALITE BIOLOGIE VEGETALE
Arrêté ministériel : 7 août 2006

Présentée et soutenue publiquement par
Marco ANDRELLO
le 3 décembre 2010

Estimation and analysis of effective size in age- and stage-structured populations

Thèse dirigée par Irène TILL-BOTTRAUD et codirigée par Oscar E. GAGGIOTTI

Abstract

Effective population size N_e is a central parameter in evolutionary and conservation biology and can be estimated with demographic- and genetic-based methods. The objective of this thesis was to analyze the effects of life-history on N_e in species with overlapping generations using demographic models. One demographic and two genetic estimators were used to derive N_e in the endangered perennial plant *Eryngium alpinum*. The three methods gave considerably different estimates. The differences could be attributed to the difficulty in obtaining al the necessary data needed in the demographic model, but also to the reduced precision of genetic estimators when dealing with small genetic sample sizes. Demographic models nonetheless permitted identifying the effect of life-history on N_e through elasticity and sensitivity analyses. Numerical elasticity was employed to study the effect of stage-specific vital rates on N_e in *E. alpinum* and the endangered perennial plant *Dracocephalum austriacum*. The derived elasticity patterns were often, but not always, similar in populations with comparable demography. One demographic model was then analysed in detail to derive analytically an expression for the sensitivity of N_e/N to age-specific vital rates. This expression was used to study N_e/N in three species differing in their survivorship curves (humans, sparrows and barnacles). In this way, the differences in the sensitivity patterns among the three species could be attributed to specific components of the life-cycle. This thesis demonstrates that demographic models are powerful methods to understand the effects of the life-history on N_e and could be useful tools to complement genetic estimators when sufficient ecological and demographic data are available. The next step will be to generalize the observed elasticity and sensitivity patterns using a comparative approach on a large number of populations, which will be possible thanks to the quick accumulation of large amounts of demographic and genetic data on a great number of species.

Keywords: population genetics; demography; genetic drift; sensitivity analysis

Résumé

La taille efficace des populations, N_e, est un paramètre central en biologie de la conservation et en biologie évolutive et peut être estimée avec des méthodes démographiques et génétiques. L'objectif de cette thèse a été l'analyse des effets de l'histoire de vie sur N_e dans des espèces à générations chevauchantes en utilisant des modèles démographiques. Un modèle démographique et deux estimateurs génétiques ont été utilisés pour dériver N_e dans des population d' *Eryngium alpinum*, une plante pérenne et menacée. Les trois méthodes ont donné des estimations considérablement différentes. Les différences ont pu être attribuées à la difficulté d'obtenir toutes les données nécessaires au modèle démographique, mais aussi à la faible précision des estimateurs génétiques quand ils sont appliqués à des jeux de données limités. Les modèles démographiques ont cependant permis l'identification des effets de l'histoire de vie sur N_e à travers des analyses de sensibilité et d'élasticité. L'élasticité numérique a été utilisée pour étudier les effets sur N_e de taux vitaux stade-spécifiques dans *E. alpinum* et dans *Dracocephalum austriacum*, une plante alpine pérenne et menacée. Les patrons d'élasticité dérivés étaient souvent, mais pas toujours, similaires dans des populations à démographie comparable. Ensuite, un modèle démographique a été analysé en détail pour dériver analytiquement une expression pour la sensibilité de N_e/N aux taux vitaux âge-spécifiques. Cette expression a été utilisée pour étudier N_e/N dans trois espèces qui diffèrent dans leur patron de courbe de survie (humains, bruants et balanes). Les différences des patrons de sensibilité entre ces trois espèces ont pu être ainsi attribuées à des composantes spécifiques du cycle de vie. Cette thèse montre que les modèles démographiques sont des méthodes utiles pour comprendre les effets de l'histoire de vie sur N_e et qu'ils pourraient être des outils puissants pour complémenter les estimateurs génétiques quand des données écologiques et démographiques sont disponibles en quantité suffisante. L'étape suivante sera la généralisation des patrons de sensibilité et d'élasticité observés en utilisant une approche comparative sur un grande nombre de populations,

ce qui sera possible grâce à la rapide accumulation de grandes quantités des données démographiques et génétiques sur un grand nombre d'espèces.

Mots-clés: génétique des populations; démographie; derive génétique; analyse de sensibilité

Table of contents

Preface

Effective population size N_e is a central parameter in evolutionary and conservation biology because it measures the strength of genetic drift. Several methods exist to estimate N_e in natural populations and can be subdivided in two classes. Demographic estimators predict N_e on the basis of ecological data such as survival and fecundity rates. Genetic methods make use of signals of genetic drift contained in molecular markers. The estimation of N_e with demographic methods is particularly challenging in species with overlapping generations that exhibit age- or stage-structure. In these populations, N_e is affected by the pattern of age- or stage-specific vital rates. Studying the effect of vital rates on N_e can thus help understand the role of a species' life-history on genetic drift.

Genetic drift is an evolutionary force that has profound implication in conservation biology. The main objective of species conservation biology is to ensure long-term population persistence by minimizing extinction risk. Among the processes reducing population viability, genetic drift can play a major role leading to inbreeding depression, loss of genetic variability and accumulation of slightly deleterious mutations. It is thus of primary importance to have reliable estimators of N_e to be applied to populations of endangered and rare species.

The general objective of this thesis is to develop the utilisation of demographic models to get reliable estimates of N_e and to assess how N_e is affected by the life-history of the species. Chapter I is an introduction to the concept of effective population size and to the available methods for its estimation, in particular demographic- and genetic-based estimators. The role of effective population size in conservation biology is also presented. In Chapter II, an empirical dataset comprising demographic and genetic data is used to estimate the effective population size using three different estimators in an endangered alpine perennial plant (*Eryngium alpinum*). The specific objective of Chapter II is to assess whether estimates of effective population size obtained through demographic models are reliable and consistent with estimates obtained from genetic markers. In

Chapter III, elasticity analysis is used to study the effects of stage-specific vital rates on N_e calculated from demographic data in two plant species (*Dracocephalum austriacum* and *E. alpinum*). In Chapter IV, a model for calculating N_e/N in age-structured populations is used to derive an analytical expression for the sensitivity of N_e/N to age-specific vital rates. The derived expression is then used to study the sensitivity of N_e/N using published data on three species (humans, the white-crowned sparrow *Zonotrichia leucophrys nuttalli* and the barnacle *Balanus glandula*). Chapter V is a general discussion on the results of the thesis and a presentation of future perspectives on the topic of effective size in stage-structured populations.

The thesis is complemented by one appendix and two articles in preparation. The Appendix is the study of the demography and reproductive system in *Arabis alpina*, an alpine perennial plant that has been and will be the object of numerous evolutionary and molecular studies conducted at the Laboratoire d'Ecologie Alpine (LECA) and in other institutions. Article A contains some of the results presented in Chapter II on the elasticity analysis of N_e in *D. austriacum*. Article B is a study of population dynamics in *E. alpinum* under the effects of extreme climatic events and different management strategies. Note however that the analyses presented in Article B are still in progress and the version of the manuscript reported there will be likely subject to major changes in the months following the publication of this thesis.

Acknowledgements

I am grateful to many people for supporting me in these three years of doctoral studies. First of all, I would like to thank all the people that took part in the committee that evaluated this work: Irène Till-Bottraud, Oscar Gaggiotti, Nicolas Perrin, Florence Nicolè; Bruno Colas and Amaury Lambert, even if they have not been able to assist to my defense; the members of previous steering committees: Stefano Leonardi and Isabelle Olivieri.

I would like to thank both my advisors, Irène Till-Bottraud and Oscar Gaggiotti, for accepting me as a student, supporting my application to the doctoral school and helping me along during these years. They have always taken into consideration the doubts, questions and requests that I have been constantly addressing to them. I also thank them for being able to provide funding when that was not available to me.

Irène has always been supporting and helpful even in the most difficult moments of my thesis. The door of her office was always open for discussions. She has helped me in the office, introducing me to the other members of LECA and assisting me with many administrative tasks, and in the field, when we started the demographic study of *A. alpina*. I enjoyed discussing with her during our long field days, climbing down the mountains under the moonlight or driving back after a long day of work. Irène has had the merit of establishing a good relationship of collaboration since the beginning and this has contributed enormously to the good development of my work and my stay in Grenoble.

Oscar has been an exemplar advisor in finding the good balance between supervision and independence. I have much appreciated his experience in science, his solid knowledge of the discipline, his guidance in writing and his ability to foresee the outcome of my research project even when my ideas were still obscure. He always found time to read my works, contributing to their improvements by giving constructive comments and positive advices. I always enjoy discussing with him about science and population genetics, and I thank him for giving me the opportunity to take part to his classes as a teaching assistant.

I must thank Florence Nicolè for sharing with me the demographic data on *D. austriacum*. I used these data to develop the analyses on N_e presented in Chapter III. Florence has also been very scrupulous and insightful during the analysis and the writing of the manuscript presented in the Appendix.

The dataset of *E. alpinum* is the outcome of the hard work of numerous people. Among them, three people have been particularly important for what I have done: Myriam Gaudeul (MNHN in Paris), Jean Philippe Bizoux (Université de Liège, Belgium) and Irène Till-Bottraud. Myriam had done her doctoral dissertation on the conservation genetics of *E. alpinum* and has been able to provide me with the molecular data needed for the analysis of Chapter II. Jean Philippe was a visiting researcher in LECA in the spring 2009 to work on the demographic dataset, whose large size was discouraging for me in the beginning. I must thank Jean Philippe for his positive attitude and help, which has allowed us to start the demographic analysis and establish a nice collaboration that is still going on. The manuscript presented in the Appendix is the outcome of our joint effort. Finally, the merit for such a valuable and large dataset goes undoubtedly to Irène, who has started this study almost fifteen years ago and has carried it on with constancy year after year.

The *Arabis alpina* demography project was started as part of a larger project involving molecular analysis and local adaptation. I am grateful to numerous people for sharing very valuable information for the beginning of the demographic study. Stéphanie Manel, Bénédicte Poncet and Pierre Taberlet provided me their list of sites from which I began searching for populations in the Fall 2007. Serge Aubert and Rolland Douzet also gave me very precious advice for finding the plants and I have much appreciated their opinion whenever we discussed about the biology and ecology of *Arabis*. The field work and data handling was conducted in collaboration with numerous people: Irène Till-Bottraud, Bénédicte Poncet, Cilia Grubinstein, Stefan Willhoit, Blaise Tymen, Elsa Jullien, Jérémy Camazola, Lucas Héméry and Anne-Lise Bartalucci. I had very enriching discussion with Joerg Wunder (Max Planck Institute for Plant Breeding Research, Koln, Germany), who is currently carrying out a common garden experiment and a demographic study on *A. alpina*. Logistic support from the Station Alpine Joseph Fourier (SAJF) has been instrumental for the field study of *A. alpina*. The great physical effort and the responsibility burden of field work have been relieved by the company of many of my fellow students and colleagues that came to the SAJF during the summer for their research: Cécile, Florence Baptist, Florian, Marie-Pascale, Marco Moretti, Pascal Salze, Rolland, Sébastien I., Sébastien Lavergne, Serge Aubert, Stephane Bec, Wilfried Thuiller and many others.

I am grateful to Wilfried Thuiller and Stéphanie Manel for their statistical advices and to all the people that participated to our statistical workshops: Cécile, Claire Trichon, Cristina, Isabelle, Katja, Laure Gallien, Laure Zupan, Sebastien Lavergne, Tamara and many students and people that popped in every now and then. The LECA is a dynamic and enthralling place to do science. I enjoyed very much the occasional but enriching conversations with many of my colleagues, I have already thanked some of them above, but here I would like to add Eric Bazin, Philippe Choler, Jean-Cristophe Clement, Michel Herzog, Sébastien Lavergne and Didier Piau. My staying at LECA was made better by many friendly people and I would like to thank Alice, Angélique, Bastien Llorca, Bénédicte, Carole, Christian, Claire M., Claire R., Delphine G., Florence Baptist, Florence Sagnimorte, Flore, François Pompanon, Guillaume L., Guillaume T., Gwenaëlle C., Irene Del Cerro Márquez, Joëlle P., Kim Pla, Lucie Z., Margot, Marie Vandewalle, Mickaël, Morana, Olivier Lontin, Ozgur, Rodolphe, Rolland, Sean, Serge Aubert and Toooni Safner.

In the fall of 2009 and the spring of 2010, I had the opportunity to visit the University of Virginia. I would like to thank Jessie Abbate for inviting me there and all the people from the Labs, in particular Andrea Berardi, Daniel Sloan, Doug Taylor, Janis Antonovics and Peter Fields. I must

also thank Janis for inviting me to join him in his field trip to Alps in the summer of 2010 to study the dynamics of infectious diseases in *Dianthus pavonius*.

Even during my dissertation here in Grenoble, I have kept contacts with my former colleagues in Italy and abroad. The work we have performed together constitutes a significant part of my scientific education. I would like to thank Alain Crivelli (Centre de Recherche Tour du Valat, Arles); Gregory Maes (University of Leuven, Belgium); Paco Melià (Politecnico di Milano, Italy); Andrea Piotti, Daniele Bevacqua, Stefano Leonardi, Paolo Piovani and Simone Vincenzi (Università di Parma, Italy). A special thank to Giulio De Leo (Università di Parma) for always being supportive and encouraging, and for his great enthusiasm for science.

Graduate students have also a life outside the laboratory, which contributes to their health and the good outcome of their doctoral dissertation. Life here in Grenoble was great, thanks to numerous people, places and activities. I would like to mention the people of Café Bayard, Café des Arts, Créarc, Petit Théatre and all the friends that were with me into the mountains, around a table or in a bar and that shared with me magic moments playing music, singing or acting in a theatre. Thanks to Andy B., Greg A., Kiran V.; Miguel P-d-B.; Anne-Laure D., Aurélie D., Damiano F., Lisa E., Morgan M-L., Romano G., Viviane S. and Yasmin P; Bénédicte P., Cloé P., Francesco d-B., Jessie A., Marie V., Marta C. and Paolo L. Finally, a big grazie to my friends in Italy: Bengi, Federico D. and Marta S., and to my parents.

Préface

La taille efficace des populations, N_e, est un paramètre important en biologie évolutive et en biologie de la conservation car elle mesure l'intensité de la dérive génétique. Il y a plusieurs méthodes pour estimer N_e dans les populations naturelles : elles peuvent être subdivisées en deux classes. Les estimateurs démographiques prédisent N_e sur la base des données écologiques telles que les taux de survie et de fécondité. Les méthodes génétiques utilisent les signes de dérive génétique contenus dans les marqueurs moléculaires. L'estimation de N_e avec les méthodes démographiques est particulièrement difficile pour des espèces avec générations chevauchantes qui présentent une structure en classes d'âge ou en stades de croissance. Dans des populations de ce type, N_e est affectée par le patron de taux vitaux variables selon les classes d'âge ou les stades de croissance. L'étude de l'effet des taux vitaux sur N_e peut aider à comprendre le rôle de l'histoire de vie d'une espèce sur la dérive génétique.

La dérive génétique est une force évolutive qui a des implications profondes en biologie de la conservation. L'objectif principal de la biologie de conservation est d'assurer la persistance des populations à long terme en minimisant leur risque d'extinction. Parmi les processus capables de réduire la viabilité des populations, la dérive génétique joue en rôle déterminant en amenant à la dépression de consanguinité, la perte de variabilité génétique et l'accumulation de mutations délétères. Il est donc primordial d'avoir des estimateurs fiables pour N_e, qui puissent être utilisés pour étudier les populations d'espèces rares ou menacées.

L'objectif général de cette thèse est de développer l'utilisation des modèles démographiques pour obtenir des estimations fiables de N_e et l'évaluation des effets de l'histoire de vie d'une espèce sur N_e. Le Chapitre I est une introduction au concept de taille efficace et aux méthodes disponibles pour son estimation, en particulier les méthodes démographiques et génétiques. L'importance de la taille efficace des populations en biologie de la conservation est aussi présenté. Dans le Chapitre II, un

jeu de données empirique contenant des données démographiques et génétiques est utilisé pour estimer la taille efficace des populations en utilisant trois différents estimateurs pour une plante alpine pérenne menacée (*Eryngium alpinum*). L'objectif spécifique du Chapitre II est d'évaluer si les estimations de taille efficace obtenues avec les modèles démographiques sont fiables et cohérentes avec les estimations issues des marqueurs génétiques. Dans le Chapitre III, l'analyse d'élasticité est utilisée pour étudier les effets des taux vitaux sur N_e, calculée à partir de données démographiques, dans deux espèces de plantes en structure en stades (*Dracocephalum austriacum* et *E. alpinum*). Dans le Chapitre IV, un modèle pour calculer N_e/N pour des populations avec structure d'âge est utilisé pour dériver une expression analytique pour la sensibilité de N_e/N aux taux vitaux. L'expression obtenue est en suite utilisée pour étudier la sensibilité de N_e/N en utilisant des données publiées sur trois espèces (les humains, le bruant à couronne blanche *Zonotrichia leucophrys nuttalli* et le balane *Balanus glandula*). Le Chapitre V est une discussion générale des résultats de la thèse et une présentation des perspectives futures sur le thème de la taille efficace des populations à structure en stade.

La thèse est complétée par une Appendice et deux articles en préparation. L'Appendice est l'étude de la démographie et du système reproducteur d'*Arabis alpina*, une plante alpine pérenne qui a été et sera l'objet de nombreuses études moléculaires et de biologie évolutive exécutées au Laboratoire d'Ecologie Alpine (LECA) et dans d'autres institutions.. L'Article A contient certains résultats présentés dans le Chapitre II sur l'analyse d'élasticité de N_e dans *D. austriacum*. L'Article B est l'étude de la dynamique des populations dans *E. alpinum* sous les effets d'événements climatiques extrêmes et de pratiques de gestion différentes. Il est à noter que les analyses présentées dans l'Article B sont encore en cours donc ce manuscrit subira vraisemblablement des changements majeurs dans les mois qui suivront la publication de cette thèse.

Chapter I:

Effective population size: estimation and usefulness in population biology

Effective population size

Effective population size N_e is a central parameter in population, conservation and evolutionary biology. The concept was introduced by Sewall Wright in 1938 to quantify the strength of genetic drift. The most rigorous and general definition of N_e is the size of an ideal population that would undergo the same amount of genetic drift per generation as the population under consideration. The ideal population may be defined in various ways (e.g. Caballero 1994 p. 666), but almost always it corresponds to the Wright-Fisher population: a group of diploid, hermaphroditic, self-compatible organisms, of finite size N, with no fluctuation in size from generation to generation; random mating; complete genetic isolation (no exchange with any other population); discrete generations; all individuals contributing the same average number of gametes to the next generation (no natural selection); the sampling variation in the number of gametes contributed to the next generation given by a Poisson probability distribution.

Because genetic drift acts on numerous processes that can be of interest to the researcher, five types of effective size are usually considered:

1. The inbreeding effective size, used to describe the average increase of identity by descent ($\overline{F}$) in a population via genetic drift (Crow and Kimura 1970);

2. The variance effective size, which gives the variance in gene frequency change across generations that is induced by genetic drift (Crow and Kimura 1970);

3. The eigenvalue effective size, used to describe the rate of decrease of heterozygosity in a population via genetic drift, calculated as $N_e = (1 - \lambda)^{-1}$, where λ is the largest nonunit eigenvalue of the Markov matrix describing the temporal change in the number of alleles through generations (Ewens 2004);

4. The mutation effective size, corresponding to the size of an ideal population having the same stationary probability of gene identity-in-state as the population under consideration (Maruyama and Kimura 1980)

5. The coalescent effective size, which gives the asymptotic rate of coalescence of a pair of genes drawn at random from the population (Hudson 1991; Sjödin et al. 2005; Wakeley and Sargsyan 2009).

In general, the different types of effective size are different, but in some circumstances their values can be the same. In particular, variance and inbreeding N_e asymptote to a common value, equal to the eigenvalue N_e, with increasing generations (Yonezawa et al. 2004). In other words, one can define a population model and study how different genetic features change in time. In the first phases, variance in gene frequencies and inbreeding will proceed at different rates, so the corresponding effective sizes will be different (Crow and Kimura 1970). Once the system reaches an asymptotic state, these two quantities will change at the same rate, and the process can be correctly described by the eigenvalue effective size. To describe genetic drift in the initial phases, one should define an instantaneous effective size and specify which genetic feature is under consideration (Yonezawa et al. 2004). In this thesis, the term "effective size" will be used to indicate the variance effective size unless otherwise specified.

There are two possible approaches to estimate N_e. The first is the so-called demographic or forward method, which makes use of demographic data and information of mating system and population history to predict analytically the value of N_e. The second is the genetic or backward method, which makes use of the genetic information contained in a sample of molecular markers to infer the past value of N_e. The difference between forward and backward method is that demographic information can tell us what the value of N_e will or would be when demographic parameters take specified values, while genetic data can only tell us what the value of N_e has been in the period preceding the sampling. Another difference is that demographic-based estimates of N_e take into account only the demographic and life-history processes included in the model and not the ones that are left out; conversely, genetic-based estimates are the product of all the relevant demographical and molecular phenomena that influenced in the population.

Genetic-based estimators of N_e

Genetic methods make use of several genetic features to infer the strength of genetic drift and thus N_e. Good reviews of genetic methods have been provided by Leberg (2005), Wang (2005), Palstra and Ruzzante (2008) and Luikart et al. (2010). The various estimators can be used to infer the various types of effective sizes (variance, inbreeding, coalescent) and also classified in three categories according to the time frame of reference: the contemporary (recent) time frame, which includes the past one-to-few generations; the historical time frame, which includes the past tens-to-thousands of generations; and the ancient time frame, which includes thousands or millions of

generations in the past (Wang 2005). Here I present only the estimators available for the contemporary time frame, which has the largest relevance in conservation biology and can be compared with demographic estimates. Genetic estimators can be further subdivided in estimators requiring only one genetic sample and methods based on two on more samples. Table I.1, taken from Luikart et al. (2010), illustrates the various characteristics of the estimators.

One-sample estimators

The most widely used and well evaluated single-sample estimator of contemporary N_e is the linkage disequilibrium (LD) method (Hill 1981). The primary advantage of this moment estimator is that it requires only one sample. The principle of the LD method is that genetic drift generates nonrandom associations among alleles at different loci (linkage disequilibrium) as N_e decreases (Hill 1981). This makes it possible to use LD to estimate N_e. The provided N_e value is contemporary but it could contain information on the effective size from one or a few generations in the past, because LD can require several generations to decay (Waples and Do 2010). The method has been recently corrected for a bias for non-overlapping generations (Waples 2006), and the bias and precision are being quantified for a wide range of population and sampling scenarios including different number of loci, sample sizes and locus polymorphism (Waples and Do 2008, 2010).

The use of LD to estimate N_e in populations with overlapping generations is still under study. The original method was developed for a population with discrete generations (Hill 1981). When this method is applied to a single cohort of individuals, the resulting estimate should be interpreted as the effective number of breeding individuals N_B rather than N_e. The behavior of the estimator is unclear when multiple cohorts are sampled; Luikart et al. (2010) state that the resulting estimate will reflect something between N_B and N_e.

The LD estimator assumes an isolated equilibrium population with a constant N_e. Immigration could lead to underestimation of N_e from the LD methods, because the LD generated by migration is falsely regarded as that produced by drift. Vitalis and Couvet (2001) proposed an estimator that can disentangle migration from drift as sources of LD and thus can estimate both simultaneously.

Other single-sample estimators include the heterozygote excess method (Pudovkin et al. 1996; Luikart and Cornuet 1999; Balloux 2004), the coancestry method of Nomura (2008), the sibship method (Wang 2009), an Approximate Bayesian Computation method based on multiple summary

Table I.1. Genetic estimators of contemporary N_e, their strengths, weaknesses, key assumptions, and performance in natural populations. From Luikart et al. (2010)

N_e estimator	Strengths	Key assumptions	Comments	Software and references
One sample				
Linkage disequilibrium (LD)	Uses any 10–20 unlinked loci, and 30–50 individuals	LD signal arises only from genetic drift	Potentially strongly biased by substructure, admixture, age structure, and small samples Not clear if purely an inbreeding N_e estimator	*LDNe*; Waples and Do (2008), Waples and Do (2010)
Approximate Bayesian method using LD plus 7 other summary statistics	Uses more information than the LD method Allows prior on Ne	LD signal arises only from genetic drift	Same as above but less biased and more precise in theory Limited to microsatellites Computes Ne over several generations in the past	*ONeSAMP*; Tallmon et al. (2008)
Heterozygote excess	Estimates N_B from single sample if N_B is very small	Signal only from different allele frequencies in male and female breeders	Confidence intervals often include infinity if N_e >20 unless sample >200 progeny and 80 independent alleles	*Nb_HetEx*, Zhandanova and Pudovkin (2008), Balloux (2004)
Identity disequilibrium at 1 and 2 loci	Estimates N_e and migration rate jointly	LD signal is from genetic drift and migration	Assumes population equilibrium and known parameters for the mating system	Vitalis and Couvet (2001)
Molecular coancestry (i.e. allele sharing among sampled individuals)	Estimates N_B from single sample if N_B is very small	Nonsib pairs needed as reference for co-ancestry among individuals	Precision is poor like the heterozygote excess method The coancestry is computed from alleles identical by descent and alike in state	Nomura (2008)
Sib identification	Applies to non-random mating populations, codominant and dominant loci	Sibs and relatedness are reliably identified No/low immigration	Based on identification of full and half sibs Also yields information on number of parents, sex ratio, and variance in family size CI's too narrow with few loci	*Colony2*, Wang (2009)
Rarefaction of alleles	Estimates of N_B; precision similar to the temporal method	Progeny are produced from few adults in a large H–W equilibrium population	Rarefaction of alleles in juveniles with respect to adults by simulating production of progeny cohorts from few adults	Hedgecock et al. (2007)

Table I.1. continued

N_e estimator	Strengths	Key assumptions	Comments	Software and references
Two samples				
Heterozygosity decline	Computation is simple Much theory behind heterozygosity	Decrease in heterozygosity is caused only by small inbreeding N_e	Heterozygosity has an high variance and thus N_e estimation has low power Linear regression between H-loss and generations between samples	Harris and Allendorf (1989), Hauser et al. (2002), Miller and Waits (2003)
Temporal F-statistic moments method	Computationally rapid	Allele frequency change is only from drift No selection or migration	Bias and precision well quantified; an unbiased estimator exists for small sample sizes and skewed allele frequencies	*Ne-estimator*, Peel et al. (2004) *TempoFs* (Jorde and Ryman 2007) uses unbiased estimator;
Pseudo-ML (maximum likelihood) temporal method	Computationally rapid Allows for migration.	Allele frequency change arises only from drift (and migration if also estimating m)	Extensive simulation evaluations have been conducted Joint estimation of N_e and migration rate (m) for continent-island metapopulation model	*MLNE*, Wang (2001), Wang and Whitlock (2003)
ML and MCMC temporal method	Useful on multi-allelic loci	Allele frequency changes only from drift	Computationally slow; Wright-Fisher model	*MCLEEPS*, Anderson et a. (2000)
Coalescent Bayesian temporal method	Allows prior on N_e, which can improve precision	Same as just above One coalescent event per generation	Coalescent models are computationally faster than Wright-Fisher models (as in MCLEEPS)	*TM3*, Berthier et al. (2002) *CoNe*, Anderson (2005)
Three samples				
Coalescent Bayesian	Allows prior on N_e	Same as just above	TMVP is like TM3 but allows for 3 temporal samples	*TMVP*, Beaumont 2003

statistics (*ONeSAMP*, Tallmon et al. 2008), and a rarefaction method (Hedgecock et al. 2007) (Table I.1). The heterozygote excess method has poor precision and is seldom useful unless N_e is less than about 30 (Zhdanova and Pudovkin 2008). According to Luikart et al. (2010), *ONeSAMP* has the greatest potential to provide improved accuracy and precision because it uses multiple summary statistics and thus more information from the data. However, it has not been thoroughly evaluated and is currently limited to use with microsatellite loci.

Two-sample estimators

The most widely used and well evaluated estimators of N_e are those based on two samples and temporal change in allele frequencies. In general, the allele frequency of a population changes over time owing to mutation, selection, migration and genetic drift. When the first three forces are excluded, the observed change in allele frequency comes solely from genetic drift and can thus be used to infer N_e. The so-called temporal methods for estimating N_e is based on this logic, and was first proposed by Krimbas and Tsakas (1971). A review and refinements of the approach are provided by Waples (1989) and extensions to overlapping generations can be found in Jorde and Ryman (1995, 2007) and Waples and Yokota (2007).

The temporal method requires population samples taken from two or more time points to estimate the temporal change in gene frequencies. The original method to estimate the variance in gene frequencies was moment-based (Nei and Tajima 1981). Williamson and Slatkin (1999) presented a maximum-likelihood approach that produced less biased and more precise estimates of N_e than those obtained with the moment estimator, but required considerable computing time and was not suitable for highly polymorphic markers. Wang (2001) proposed a method for multiallelic markers, which is computationally very efficient and applies to any number of alleles per locus. Wang and Whitlock (2003) extended previous moment and maximum-likelihood methods to allow the joint estimation of N_e and migration rate (m) using genetic samples taken from different populations and times. Other probabilistic methods, based on coalescent theory, were proposed by Berthier et al. (2002), Beaumont (2003) and Anderson (2005); Tallmon et al. (2004) derived an estimator based on approximate Bayesian computation. Other N_e estimators are listed in Table I.1.

Demographic-based estimators of N_e

The simplest applications of the demographic method to calculate N_e when some feature of the population departs from the ideal conditions were developed by Wright (1938). He derived expressions for N_e for unbalanced sex-ratio and fluctuations in population size. Many of the successive investigations addressed different population features and provided formulae for N_e in various cases (reviewed by Crow and Kimura 1970; and Caballero 1994). Most of the model developments were however limited to populations with discrete, non-overlapping generations. Only later researchers began to tackle the problem of determining N_e when generations overlap. Table I.2 summarizes the models, their respective strength and their key assumptions.

Felsenstein (1971) provided equations for inbreeding and variance effective numbers in age-structured haploid and diploid species; his formulation assumes constant population size, stable age-structure, random mating and Poisson variation in family size, but an expression for variance- N_e in growing populations was also provided in the case of constant rate of population increase. Johnson (1977) and Emigh and Pollak (1979) were able to reproduce the same expression through a different mathematical derivation, and also provided an expression for N_e in populations with overlapping generations and separate sexes. Their derivation assumed constant population size, stable age-structure, random mating and Poisson variation in family size. Crow and Kimura (1972) provided a simplified expression for variance effective size in age-structured populations assuming random mating and allowing for non-equilibrium population size and an approximation for the departure from the stable age distribution.

The first formulation including variation in reproductive success was given by Hill (1972, 1979), whose simpler and more general result was expressed in terms of variances and covariances of lifetime family sizes. His result was limited to variance-effective size in age-structured populations with a constant number of newborns per time unit, but allowed for differential viabilities and fertilities in individuals of the same age-class as well as correlations of fertilities of individuals at successive ages. Hill (1972) introduced the very important concept of annual effective size N_a, defined as the size of an ideal population with a generation interval of one year that undergoes the same amount of genetic drift observed per year in the current population. N_a allows for comparison between populations with different generation times. Pollak (1979, 1990) reproduced Hill's expressions using different derivations and extended them to sex-linked loci.

Table I.2. Demographic estimators of N_e, their strengths and key assumptions.

N_e model	Strength	Key assumptions	Comments
Felsenstein (1971) Johnson (1977) Emigh and Pollak (1979)	Accommodates any age-specific life-table	Stable population size; constant vital rates; random mating Poisson variance in reproductive success	Asymptotic inbreeding and variance N_e; variance N_e also when population growths at a constant rate
Hill (1972, 1979) Pollak (1979, 1990)	Variance in reproductive success; separate sexes	Stable population size; constant vital rates; random mating	Asymptotic variance N_e Difficult to derive parameter values (life-time variance in reproductive success)
Nunney (1991, 1993, 1999)	Variance in fecundity, separate sexes Unequal life-span between sexes and unbalanced sex ratio Variation in reproductive success measurable from annual data	Stable population size, age-invariant mean fecundity Restricted to Type I and II survivorship curves	Asymptotic variance N_e Extended to various mating systems (Nunney 1993) and geographical structure (Nunney 1999)
Orive (1993)	Exact method (no approximation) Stage-structure Clonal propagation Formulated as a matrix population model	Stable population size; constant vital rates; random mating; Poisson variance in reproductive success	Asymptotic coalescent and inbreeding N_e
Yonezawa (1997) Yonezawa et al. (2000, 2004)	Stage-structure Clonal propagation Formulated as a matrix population model Accounts for growing populations, annual variation in reproductive success Includes deviation from Hardy-Weinberg proportions Variation in reproductive success measurable from annual data	Random mating	Asymptotic variance N_e. Extended to transient N_e in Yonezawa et al. (2004)
Rousset (1999)	Individuals classified in arbitrary classes (sex, stage) Geographical structure No assumptions on mating systems or family size	Constant population size; stable class-structure	Only local asymptotic inbreeding N_e
Engen et al. (2005)	Demographic and environmental stochasticity Separate sexes	Age-structured	Transient variance N_e; difficult to extend to asymptotic N_e. Environmental stochasticity has no effect on N_e

It is worth recalling the study of Choy and Weir (1978), who derived exact recursion equations for inbreeding coefficients in populations with overlapping generations. They emphasized that, in early generations, the inbreeding coefficient will not increase at the rate predicted by inbreeding N_e, which only gives the asymptotic rate of increase. The distinction between asymptotic and transient N_e was also discussed by Yonezawa et al. (2004) in the context of organisms that propagate clonally.

Nunney (1991, 1993) investigated the effects of fecundity, sex-ratio and age-structure on variance effective population size. His model assumes a population with a stable age structure, a constant number of adults per generation and variation in fecundity among adults around a constant mean (no increase in fecundity with age), but explicitly considered the effects of various mating systems other than the random union of gametes. Nunney (1991) rearranged Hill's (1972, 1979) expression in terms of means and variances in fecundity and death rate per breeding season: these estimates are easier to obtain than the variances and covariances of lifetime family sizes appearing in Hill's original formulation.

Orive (1993) developed a method for determining coalescent effective size in organisms with complex life histories, such as those with overlapping generations and both sexual and clonal reproduction. Her model allowed for classification of individuals according to life-stage rather than age, which makes it particularly suitable for organisms in which age is a poor predictor of fitness (most plants). The model assumes constant population size, random mating, Poisson variation in family sizes and stable stage (or age) structure. Under these assumptions, the coalescent effective size is equivalent to the inbreeding effective size. Gaggiotti and Vetter (1999) used a modified version of Orive's (1993) original model to consider variation in family size and periodical fluctuations in recruitment rates in two pelagic fish species. Laporte and Charleswoth (2002) used a coalescent approach to derive N_e in populations structured by age or sex; the authors also analyzed the effect of population subdivision.

Yonezawa (1997) formulated the variance effective size for partly asexually reproducing species and considered overlapping generations in the form of age or stage structure. The model can accommodate departures from random mating and from Poisson variance in family size in the case of a population with constant size. Yonezawa et al. (2000) extended the original model in order to account for varying population size. Both studies referred implicitly to asymptotic effective sizes, but an extension for instantaneous (transient) variance and inbreeding N_e was provided by

Yonezawa et al. (2004): in this paper, a full derivation of instantaneous N_e is given in the case of discrete generations and some suggestions are given to extend it to populations with overlapping generations.

Rousset (1999) calculated mutation and inbreeding effective sizes in spatially structured populations with general within-subpopulation class structure, thus including age or stage structure. His model assumes constant population size and attainment of the stable stage structure, while it does not make explicit assumptions on mating systems and distribution of family sizes. The kind of effective size developed in this approach refers to the local subpopulation.

There were also investigations of effective size in species with peculiar life-histories. Waples (1990a,b, 2002a) and Waples et al. (2010) investigated the effect of fluctuations in population number on long- and short-term variance effective size in Pacific salmon *Oncorhynchus spp.* (semelparous and exhibiting variation in annual productivity). Vitalis et al. (2004) calculated the coalescent effective size of monocarpic plant populations and annual plant populations with seed dormancy (the latter case can be thought of as a sort of age-structured model). Their model allowed for selfing and assumed Poisson variation in family size and stable age structure. Nunney (2002) considered the interaction of fluctuating population size and seed bank dynamics on N_e in annual plants.

Finally, Engen et al. (2005) formulated expressions for variance effective size in age structured populations affected by demographic and environmental stochasticity. The effective size was expressed as a function of generation time and demographic variance in vital rates (between-individual variance in age specific survival and fecundity rates). Interestingly, N_e turned out to be independent from the environmental variance in vital rates. Engen et al. (2005) provided formulae for haploids, monoecious diploids and species with separate sexes; the model can also account for growing populations subject to density dependence.

Sensitivity and elasticity analyses: life-history effects on N_e

Sensitivity and elasticity

Population dynamics in species with overlapping generations is traditionally approached by matrix population models (Caswell 2001). Matrix population models are a mathematical description of

population dynamics that is now very common among population biologists. They are useful representations of population with overlapping generations, where individuals can be classified by their age or their biological stage. In matrix population models, it is possible to represent explicitly the different biological stages that comprise an organism's life-cycle. In this way, it is possible to find the effect of distinct life stages on various parameters of population dynamics, such as the population growth rate λ.

An important step in the analysis of stage-structured populations is the investigation of how stage-specific vital rates (survival and fecundity) affect λ. This allows for an identification of the life-stage transitions that are most important for population dynamics. In species of conservation concern, these life-stages can then be the object of dedicated management actions aimed at maintaining population stability (Mills et al. 1999). The identification of the most critical vital rates can be carried out through sensitivity or elasticity analysis of the population growth rate λ. The sensitivity of λ to a given vital rate x is the change in λ caused by an infinitesimal increase in x (Caswell 1978, 1982; de Kroon et al. 1986):

$$\text{sensitivity of } \lambda \text{ to } x = \frac{\partial \lambda}{\partial x}$$

Elasticity, or proportional sensitivity, is the proportional change in λ caused by a proportional increase in x:

$$\text{elasticity of } \lambda \text{ to } x = \frac{\partial \lambda}{\partial x}\frac{x}{\lambda}$$

A positive sensitivity or elasticity indicates that a positive change in x will produce an increase in λ, or that a negative change in x will produce a reduction of λ. A negative sensitivity or elasticity means that increasing x will produce a negative change in λ, or that decreasing x will lead to larger λ.

Elasticity analysis is now applied as a standard technique in almost all demographic studies of stage-structured populations. In more recent times, the theory of elasticity analysis has been extended to stochastic and nonlinear matrix models (Tuljapurkar et al. 2003; Horvitz et al. 2005; Caswell 2008).

The effect of life-history on N_e

Sensitivity and elasticity analyses are not restricted to population dynamics models. They can be applied to any model in which a given parameter can be expressed as a function of other parameters. In this thesis, elasticity analysis is used to study how changes in vital rates affect effective population size. In species with overlapping generations, N_e is affected by the specific pattern of variation in vital rates (survival and fecundity) with life-stage. Determining the contribution of specific vital rates to N_e is important to know, for instance, whether the survival at a particular life-stage is critical to determine the range of values that N_e can take; or whether fecundity is more influential than survival. Stage-specific vital rates have received little attention in comparison to other factors affecting N_e (fluctuations in population size, variation in reproductive success, etc.), even if the majority of published models accounting for overlapping generations are explicitly framed in terms of stage- or age-specific survival and fecundity rates. For instance, the models of Felsenstein (1971) and Nunney (1991) explicitly addressed the effect of age structure on N_e. However, the approach used in these studies did not make use of elasticity analysis.

The only example of elasticity analysis of effective population size is found in Campbell and Husband (2005). These authors used elasticity analysis to study the impact of clonal propagation on genetic diversity of the perennial plant *Hymenoxis herbacea*. They found that clonal growth could either reduce or increase N_e, depending on the relative strength of variation in clonal recruitment between genets with respect to average rates of clonal growth in the population. They also evidenced that juvenile survival was the most influential vital rate on genetic drift, because the elasticity of N_e to juvenile survival was the largest one. However, it is not possible to conclude that these are general patterns because their analysis comprised only two populations.

The elasticity analysis of effective population size can be used in conjunction with the traditional elasticity analysis of population growth rate in the context of population viability analysis. As discussed in the next section, population persistence depends on the interaction of demographic and genetic factors. λ is traditionally chosen as a measure of population viability because it incorporates the effects of all life-cycle components and is easy to estimate even from minimal demographic data (Heppell et al. 2000; Caswell 2001). An elasticity analysis of N_e would allow for the identification of the vital rates that contribute the most to genetic drift and constitutes a complement to traditional elasticity analyses that are focussed on λ only. Depending on the context, one may not want to modify a vital rate that decreases effective population size, because this would entail an increased

risk of extinction by genetic stochasticity (see next section). The elasticity analysis of N_e can therefore be a useful tool in evaluating the effects of management actions on genetic variability of endangered and captive species.

Annual effective size N_a and the role of generation time

In species with overlapping generations, N_e measures the rate of genetic drift in units of generations. If the species is able to persist for several years, it might be useful to express the rate of drift in units of years rather than generations. Hill (1979) introduced the concept of annual effective size N_a, defined as the size of an ideal population experiencing the same amount of genetic drift per year as the population under consideration. In addition, Allendorf et al. (2008) argued that the generation time L of a species is a measure that should be used together with N_e. When a certain change in the life-history decreases N_e but increases L, the rate of loss of neutral genetic variation in units of time might in fact be smaller. This is a consequence of the fact that N_e is defined in units of generations and reveals the need of considering also N_a and L as parameters to describe adaptation and viability. For this reason, the elasticity analyses presented in Chapter III are also extended to generation time L and annual effective size N_a.

Effective size and population viability

Effective population size plays a key role in conservation biology. The objective of conservation biology is saving species from extinction. A species goes extinct when its last population disappears. Similarly, a population goes extinct when its last individual dies. A population may be more or less viable if it is capable of persisting in time and not going extinct. Different factors and mechanisms are involved in putting a population in danger of extinction. Among these, population size is perhaps the strongest correlate of viability, since small populations face greater risk of extinction than larger ones. This observation has led to the development of the important concept of minimum viable population size, defined as "some threshold for the number of individuals, or some multivariate set of thresholds and limits, that will insure (at some acceptable level of risk) that a population will persist in a viable state for a given interval of time" (Gilpin and Soulé 1986). This definition says clearly that viability depends on interaction of different factors ("multivariate set of thresholds"). Studying population viability requires therefore an analysis of all the different types of mechanisms through which a population can become extinct.

Shaffer (1981) classified the processes driving population extinction into deterministic and stochastic factors. Deterministic extinction occurs when something essential to the population is removed, or when something lethal is introduced. Deforestation, glaciations and other forms of habitat loss encompassing the entire habitat of the species are clear examples of deterministic factors. Indeed, habitat destruction is universally recognized as the prime cause of endangerment for most species. Other deterministic threats are the introduction of exotic species, pollution, climate change and overexploitation.

Extinctions from stochastic factors are those that result from normal, random changes or environmental disturbance. Shaffer (1981) further subdivided stochastic factors into four types: demographic, environmental, catastrophic and genetic. Demographic stochasticity is the random variation in the number of birth, number of deaths, and sex ratio in a population that results from the fates of individuals being independent outcomes of probabilistic events of reproduction, mortality, and sex determination (Goodman 1987; Menges 1990). It is generally thought that demographic stochasticity influences the fate of very small populations (less than 50 individuals) and that it can cause extinction only when populations fall below 10-20 individuals (Goodman 1987). However, the synergistic effects of variation in different demographic parameters (e.g. sex ratio and juvenile survival) can depress growth rate and lead to extinction populations of larger sizes (Lacy 2000).

Environmental stochasticity is the variation in demographic rates that results from fluctuations in the environment. Environmental disturbance acts on all individuals in an equivalent way. Some examples of perturbations causing variation in birth and death rates are disease, sporadic predation, irregular food availability and variable weather. Catastrophes are extreme forms of environmental stochasticity, such as fires, droughts, floods, which may occur at random intervals and lead to local population extinction.

The major mechanisms of population extinction that are under the influence of genetic stochasticity are inbreeding depression, loss of genetic variability, accumulation of slightly deleterious mutations, outbreeding depression and genetic swamping (Gaggiotti and Hanski 2004). The last two of these mechanisms become important only in the case of locally adapted populations connected by migration. Conversely, the first three mechanisms are of concern in every situation and are related to effective population size, as will be detailed in the following.

Inbreeding depression

Inbreeding depression is the reduction in fitness experienced by inbred individuals. An individual can be inbred when its parents are related (e.g. mating between brother and sister or between father and daughter). In small populations, the chance that the parents will be related is higher than in large populations, because the possibility for mating is limited. So it can happens that small populations that mate randomly exhibit high levels of inbreeding because sooner or later the population will be composed of individuals that are more or less related. This can become particularly evident in small captive populations, as those living in zoos. For instance, Templeton (2006; pp. 49-55) reports high levels of inbreeding in a small captive population of Speke's gazelle (*Gazella spekei*), in spite of a breeding program designed to avoid inbreeding: the observed high levels of inbreeding were due to the small size of the founder group (one male and three females).

The level of inbreeding is usually quantified by the inbreeding coefficient. There are two different types of inbreeding coefficient (Templeton 2006 pp. 48−63). The first is defined as the probability that two homologous allele copies drawn at random from an individual's genome are identical by descent: this is the relevant coefficient in the context inbreeding depression, which will be denoted by F. The second is defined as the departure from Hardy-Weinberg genotype proportions in a population and is denoted by α. Templeton (2006) warned not to mistake one for the other: F refers to a single individual, is bounded between zero and one and is mathematically a probability; α refers to the whole population, can take values comprised between –1 and 1 and is not a probability. The mean inbreeding coefficient of a population, $\overline{F}$, is the average of the Fs of all the individuals in the population. $\overline{F}$ and α are not equal and express different quantities. $\overline{F}$ is of interest in models relating demography to genetics because it can be related to the effective population size.

The average inbreeding coefficient of a population, $\overline{F}$, is expected to increase because of chance mating events involving related individuals. The increase in $\overline{F}$ will occur even in captive populations managed to avoid mating between close relatives (Templeton 2006). In an ideal population, gametes that unite to form a zygote can be thought of as a random sample of the parental gene pool. The gene pool can be defined here as the (nearly) infinite set of gametes produced by individuals in the preceding generation. The probability $\overline{F}$ that two alleles uniting to form an individual zygote are identical copies of the same parental allele is thus $1/2N$, where N is the number of parents. If the population is not ideal, such a probability can be replaced by $1/2N_e$,

where N_e is the inbreeding effective population size. A recurrence equation can be defined for the mean inbreeding coefficient in generation t as:

$$\overline{F}_t = \frac{1}{2N_e} + \left(1 - \frac{1}{2N_e}\right)\overline{F}_{t-1} \qquad \text{(I.1)}$$

The right-hand side of equation I.1 is made up of two terms, the first expressing the probability that two uniting alleles are copies of the same parental allele in the previous generation and the second expressing the probability that two uniting gametes are copies of two different alleles in the previous generation, times the probability that these alleles were themselves identical by descent. The mean inbreeding coefficient is therefore expected to increase if migration or mutation does not introduce new genes into the population and the rate of increase is controlled by the effective population size.

High levels of inbreeding can lead to inbreeding depression, defined as the decrease of fitness observed in inbred vs. non-inbred individuals (Charlesworth and Willis 2009). Two genetic mechanisms can lead to inbreeding depression: the expression of recessive deleterious alleles and the reduction in the expression of heterozygote advantage (heterosis). Of these two mechanisms, expression of recessive deleterious alleles is thought to be the most common cause of inbreeding depression. It has been demonstrated that inbreeding can reduce birth rates and fitness in a variety of organisms, including plants. Inbreeding depression can also lead to population extinction, by reducing individual survival and fertility rates under the threshold level needed for population persistence (Newman and Pilson 1997; Saccheri et al. 1998).

Loss of genetic variation

Populations can persist through environmental change only if they bear enough genetic variation to adapt to novel habitat conditions. Genetic variation is usually measured by the heterozygosity of neutral markers H or by the additive genetic variance. In the absence of immigration, mutation and selection, both quantities decline at a rate proportional to the variance effective size (Lande and Barrowclough 1987):

$$H(t+1) = H(t)\left(1 - \frac{1}{2N_e}\right) \qquad \text{(I.2)}$$

Although the theory predicts that loss of genetic variance can result in reduced adaptation potential, it has been very difficult to find evidence of this in natural populations. Most of the difficulty lies in the time-scale of this process, which is thought to affect extinction in the long-term and is therefore hard to document empirically. There is one case nonetheless where reduction of genetic variability can represent an imminent extinction threat, and it is the loss of alleles at loci involved in disease resistance in animal species (Gaggiotti and Hanski 2004).

Some plants have genetic system of self-incompatibility to prevent mating among close relatives and the risk of inbreeding depression. Self-incompatibility is controlled by one or more loci that have usually many alleles. Although these alleles are under positive frequency-dependent selection, they may be lost by random genetic drift in small populations. When genetic diversity at the self-incompatibility loci is reduced, mate availability is limited and the population may fail to reproduce. It has been observed that reproductive failures can last for several years (De Mauro 1993). A population can avoid extinction only if new self-incompatibility alleles are introduced by migration or mutation.

Numerous researchers addressed the question of how large a population should be to avoid the loss of alleles by genetic drifts. Franklin (1980) and Soulé (1980) proposed that $N_e = 500$ is sufficient for long-term maintenance of genetic variation in quantitative characters. This number was derived by assuming a balance between mutation and random genetic drift and by using experimental measures of mutation rate in *Drosophila*. Later experiments demonstrated that a large fraction of mutations are highly detrimental and the Franklin-Soulé number had to be increased to $N_e = 5000$ (Lande 1995). However, this figure should not be regarded as a magic number sufficient to ensure the viability of all species, because of differences among characters and among species in mutation rates and selective pressures.

Accumulation of slightly deleterious mutations

Under more or less constant environmental conditions, mutations hitting the genome are largely deleterious, because populations tend to be well adapted to the environmental conditions that they experience (Gaggiotti and Hanski 2004). In large populations, deleterious mutations will be efficiently removed and their frequency will be regulated by the balance of mutation and natural selection. However, when the population is too small or when the fitness effect is only slightly deleterious, introduced mutations behave effectively as neutral alleles and their fate is determined

only by random genetic drift. More precisely, natural selection becomes ineffective in removing slightly deleterious mutations when the strength of selection is weaker than that of genetic drift, which is measured by the variance effective size (Wright 1931), i.e.:

$$s < \frac{1}{2N_e} \qquad \text{(I.3)}$$

Such mutations are much harder to remove and tend to persist for long periods of time; the chance fixation of some of the deleterious alleles is therefore increased and results in the reduction of population mean fitness. This process can eventually lead to population extinction (Muller's ratchet; Muller 1964). The process of accumulation of slightly deleterious mutation is also known under the name of "mutational meltdown", because in the final phase of the process there is a positive feedback between reduction in population size and fixation of deleterious alleles that accelerates population decline. As can be seen from equation I.3, the effective size is a key parameter determining when the system will behave as effectively neutral.

Unfortunately, there is a paucity of empirical evidence for or against the mutational meltdown. At present, the impact of mutation accumulation on extinction risk appears to be less important than that of the other genetic factors and to take very many generations (Frankham 2005). There are also a number of controversial issues that need to be resolved (Gaggiotti and Hanski 2004). There is no agreement about the value of two critical parameters used in models of mutational meltdown, the per-genome mutation rates and the average fitness cost per mutation. Furthermore, most models disregarded the effect of beneficial and back mutations. When beneficial mutations are taken into account, only very small populations would face the risk of extinction due to accumulation of slightly deleterious mutants. The role of mutational meltdown as a process of population extinction remains thus still unclear.

Extinction vortices

Gilpin and Soulé (1986) and Gilpin (1987) have organized the different mechanisms of population extinction into a heuristic model of viability. When populations suffer environmental shocks, several positive feedback loops can arise among different mechanisms of extinction, therefore Gilpin and Soulé coined the expression extinction vortex. They identified and described four types of vortices, not completely independent of one another.

The R vortex: a chance reduction in population size N might increase the variance in population growth rate, Var(r), which in turn reduces population size, and so on; demographic stochasticity and random fluctuations in population size are the drivers of this process. The D vortex: a reduced N and an increased Var(r) may lead to fragmentation of a once continuous population into a number of smaller isolated populations; fragmentation might in turn increase demographic stochasticity and reduce reproductive success through density dependent processes (e.g. Allee effect). The F vortex: demographic stochasticity and spatial fragmentation can lead to effective population sizes vastly lower than the census population size N; as N_e decreases, the individuals become more and more homozygous and start suffering from inbreeding depression. The A vortex: loss of alleles due to genetic drift caused by a low N_e will make a population less able to respond adaptively to environmental change and thus leave the species more vulnerable to extinction.

The theory of extinction vortices has been implemented into the simulation package VORTEX (Lacy et al. 2005), a useful tool for evaluating the risk of extinction when sufficient knowledge is available on the relationship between genetic diversity and fitness in the species of consideration.

Chapter II:

Computation of N_e from demographic data

Introduction

The many methods proposed to estimate effective population size fall into two broad approaches. Demographic approaches use equation of N_e predicted from causal demographic parameters such as the number of breeders and the means and variances in their progeny number (Caballero 1994). In contrast to demographic methods, the genetic approach uses information about the genetic variation observed at a number of marker loci to estimate N_e (Schwartz et al. 1999; Wang 2005). The objective of this chapter is to evaluate the reliability of demographic methods to estimate N_e. In order to do so, demographic based estimates of N_e are compared to estimates obtained from molecular markers. The analysis is based on demographic and genetic data from four populations of *Eryngium alpinum*, a perennial plant that is declining over its entire distribution area and is protected throughout Europe (European Habitat Directive; Wyse Jackson and Akeroyd 1994).

Different genetic estimators of N_e can be used on the basis of the available number of genetic samples and type of molecular markers. When only one sample of microsatellite data is available, the two methods that can be employed are the linkage disequilibrium (LD) method (Hill 1981; Waples 2006) and the Bayesian *ONeSAMP* method (Tallmon et al. 2008). The LD method is based on the expectation that LD will increase due to genetic drift generating non-random associations among unlinked loci more substantially in small compared to large populations. The *ONeSAMP* method, based on approximate Bayesian computation, allows estimating N_e from a set of multiple measures (number of alleles, allele length, heterozygosity, Wright's F_{IS}, multilocus homozygosity and allele correlation at different loci). The methods used here are the demographic model of Yonezawa (1997; and Yonezawa et al. 2000), the linkage-disequilibrium method *LDNe* (Waples and Do 2008; and the Bayesian method *ONeSAMP* (Tallmon et al. 2008).

Materials and methods

Species and study site

Eryngium alpinum (Apiaceae) is a perennial rare endemic species (Figure II.1). Its distribution area extends over the Alps (France, Italy, Switzerland, Austria, Croatia and, possibly, up to Romania and Slovakia; between 1300-2500 m (Cherel and Lavagne 1982). In the Alps, this is the altitude range of the forest, however, the species is present in open sunny but relatively humid habitats. It is

Figure II.1. *Eryngium alpinum*. Photograph by Irène Till-Bottraud

therefore limited to hayfields or avalanche corridors. The species is protected by the Bern convention, the European habitat directive of Natura 2000 (Wyse Jackson and Akeroyd 1994), the French red list of protected species and considered as vulnerable by UICN (Gillot and Garraud 1995). The threat is mainly due to human activities such as cutting for commercial use and land use change (change from late hay harvest to spring grazing, or abandonment leading to land closure by forest). The taproot persists over the winter whereas aerial stems and leaves disappear each year. Flowering occurs from mid July to mid August. Flowering individuals produce 1–20 stems (mean ≈ 3), each of them measure 50 cm to 1 m high and bear one terminal inflorescence and 0–4 axillary inflorescences. Each inflorescence produces 150–350 small white flowers. *E. alpinum* is mainly pollinated by opportunistic insects such as bees and bumblebees (Gaudeul and Till-Bottraud 2004). The seed set of the terminal inflorescence is around 65% but the axillary inflorescences produce much fewer seeds because of resource limitation (Gaudeul and Till-Bottraud 2004). Mature fruits (schizocarpous diachenes) fall near the mother plant at the end of august but secondary dispersal is possible by attachment of dry sepals to animal fur (Gaudeul and Till-Bottraud 2004). Seeds germinate in the spring.

Four populations [Deslioures (DES), Bernards (BER), Boujurian (BOU) and Pralognan (PRA)] were studied (Figure II.2). The Pralognan population (Pralognan la Vanoise, Savoie, France) is located in the “aire d’adhésion” of Vanoise National Park and the other three populations are located in the “Fournel – les Bans” Natura 2000 site in the “aire d’adhésion” of Ecrins National Park (L’Argentière la Bessée, Hautes Alpes, France).

Life cycle and data collection

The life cycle of *Eryngium alpinum* was subdivided into four biological stages (Figure II.3). Seedlings (1) are new individuals that appeared following germination. Juveniles (2) are individuals

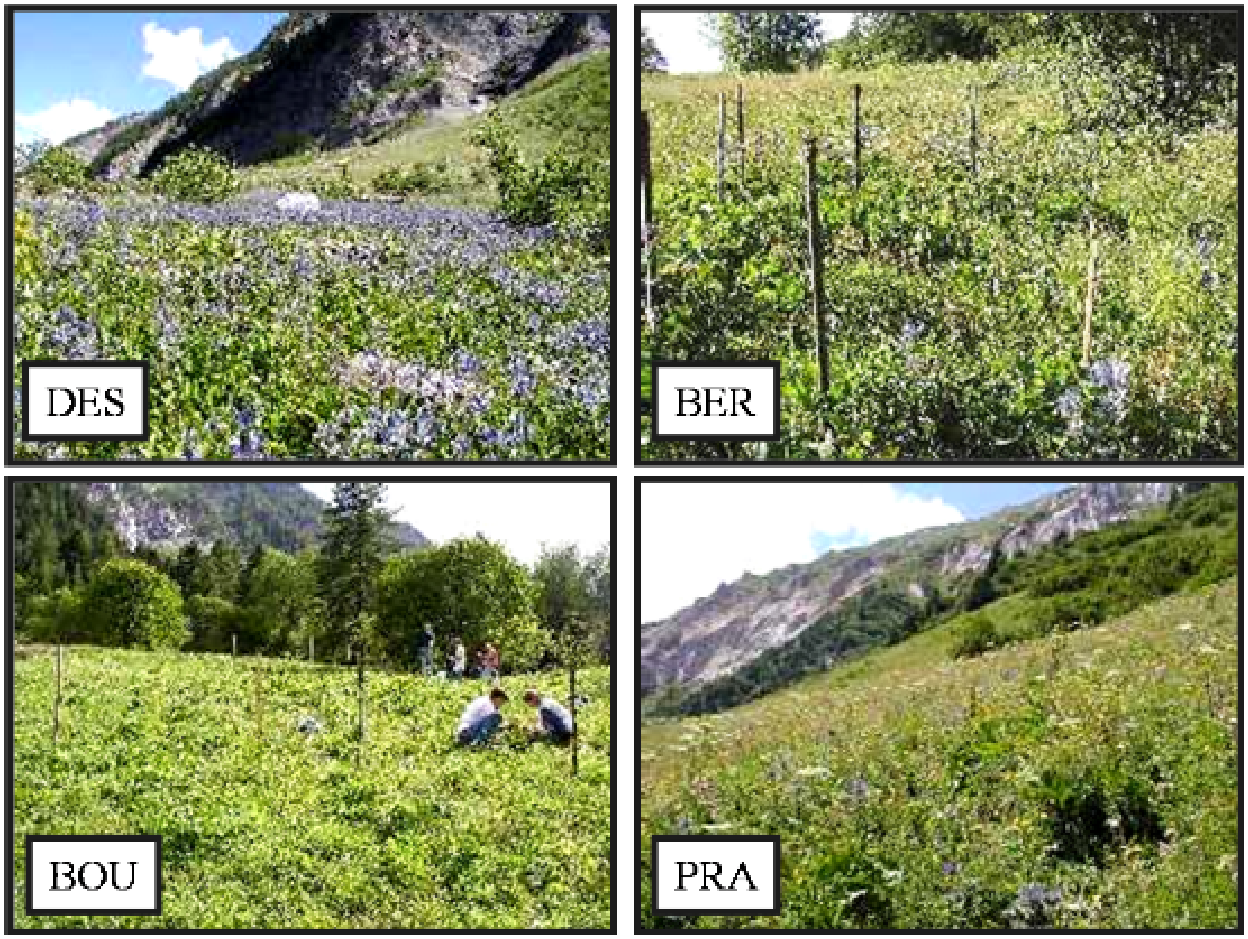


Figure II.2. The four populations of *Eryngium alpinum* analyzed in this Chapter. Desliores (DES), Les Bernards (BER) and Boujurian (BOU) are in the Ecrins National Park; Pralognan (PRA) is in the Vanoise National Park. Photographs by Irène Till-Bottraud

that are older than one year and have not yet flowered during their life. Reproductive adults (3) are plants bearing one or more inflorescences. Vegetative adults (4) are plants that do not bear inflorescences and have already flowered during their life. Seedlings become juveniles in one year, while juveniles and adults can remain in the same stage for more than one year or make a transition to another stage.

Three permanent plots were placed in each population in year 2000. In Pralognan, the population occupies an area subject to different management regimes (annual mowing, biennial mowing, grazing, unmanaged) and three permanent plots for each management regimes were placed, for a total of twelve plots. Data were pooled across plots within populations. The plants were individually tagged and their presence/absence and their biological stage were scored each year of survey from 2000 to 2008. Samples of at least 20 terminal inflorescences per population were collected and the number of viable seeds was counted and inflorescence length measured. Axial inflorescences were

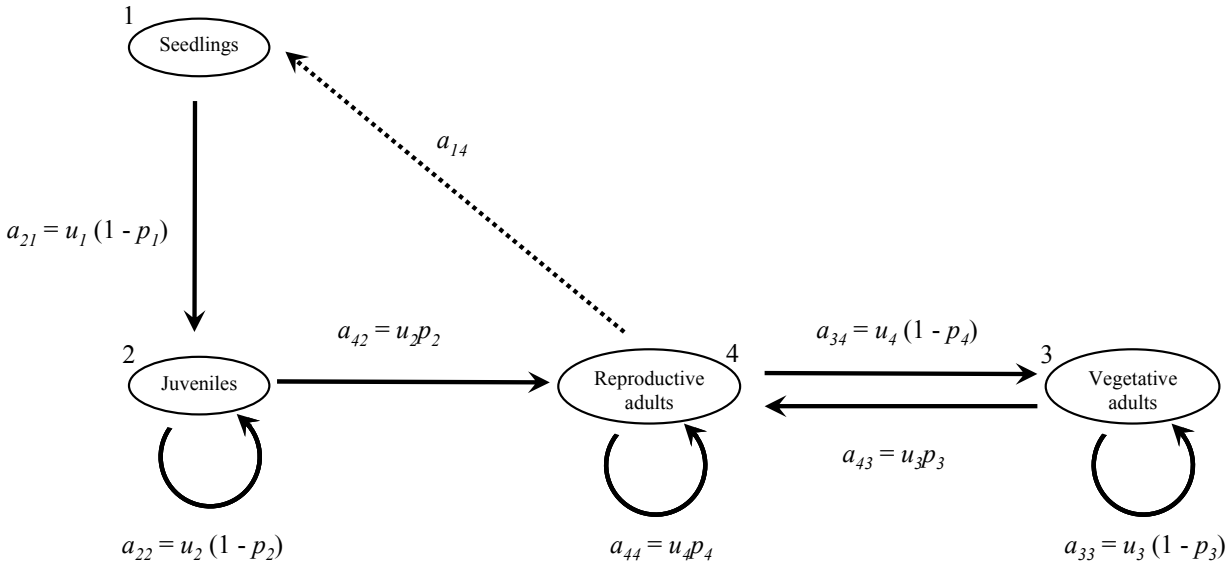


Figure II.3. Life cycle graph of *Eryngium alpinum*, representing transitions of individuals between stages (solid lines) and reproduction (dotted lines). The upper-vital rates (a_{ij}) correspond to the transitions between stages, the lower-vital rates correspond to survival (u_i) and flowering (p_i) probabilities.

disregarded because they contribute very little to seed set (Gaudeul and Till-Bottraud 2004). Seeds were considered viable when the endosperm could be felt inside the seed coat. Using these data, we fitted a linear relationship between inflorescence size h and number of viable seeds n_s ($n_s = -177 + 113h$; $t = 18.18$, $p < 0.0001$). Population and year effects were statistically tested and were not significant. The terminal inflorescences of all flowering individuals were measured each year. These data were used in connection with the inflorescence size-viable seeds relationship to estimate the mean μ_b and variance σ_b^2 of potential fecundity.

Matrix model

The life-cycle is described by a stage-classified, pre-breeding census (July), birth-pulse matrix model (Caswell, 2001). The elements of the matrix, a_{ij}, give the annual transition rates from stage j to stage i. Stage-specific survival rates u_j's (Table II.1) were obtained by summing the transition rates leaving the same stage j ($u_j = \sum_i a_{ij}$). Population growth rate λ, stable stage distribution and reproductive value were calculated by taking the leading eigenvalue and the corresponding right and left eigenvectors of the transition matrix (Caswell 2001). Generation time L was defined as the

Table II.1: Demographic parameters of the four populations of *Eryngium alpinum*. a_{ij}, transition rate (see Figure II.3); u_i, survival rate of plants belonging to stage i; p_i, flowering rate of plants belonging to stage i; μ_b and σ_b^2, mean and variance in potential fecundity; μ_k and σ_k^2, mean and variance in realized fecundity.

		Deslioures (DES)	Bernards (BER)	Boujurian (BOU)	Pralognan (PRA)
Matrix elements	a_{21}	0.06	0.01	0.48	0.05
	a_{22}	0.73	0.70	0.64	0.73
	a_{33}	0.53	0.63	0.76	0.74
	a_{34}	0.64	0.57	0.79	0.62
	a_{42}	0.10	0.10	0.11	0.05
	a_{43}	0.41	0.33	0.11	0.21
	a_{44}	0.33	0.41	0.17	0.34
Survival rates	u_1	0.06	0.01	0.48	0.05
	u_2	0.83	0.80	0.75	0.78
	u_3	0.94	0.96	0.87	0.95
	u_4	0.97	0.98	0.96	0.96
	$\overline{u}$	0.18	0.24	0.82	0.28
	$\overline{u}^2$	0.12	0.22	0.68	0.22
Flowering rates	p_2	0.12	0.12	0.14	0.07
	p_3	0.44	0.34	0.12	0.22
	p_4	0.34	0.42	0.17	0.35
Fecundity	μ_b	243	170	101	232
	σ_b^2	9839	6533	3664	8487
	$a_{14} = \mu_k$	44.05	9.32	0.81	15.91
	σ_k^2	359.38	28.44	1.04	54.71
	σ_b^2 / μ_b	40.49	38.43	36.28	36.58
	σ_k^2 / μ_k	8.16	3.05	1.28	3.44
Initial census size	N_0	2,464,580	199,715	55,459	300,346
Population growth rate	λ	1.20	0.98	0.91	1.00
Generation time	L	6.6	73.7	29.1	24.0

mean age of parents at the stable stage distribution and it was estimated according to Cochran and Ellner (1992, as reported by Caswell 2001: eq 5.13, 5.40, 5.41, 5.76 and 5.77).

Demographic estimate of N_e

Demographic estimates of N_e were derived using a stage-structured model developed for populations changing in size (Yonezawa 1997; Yonezawa et al. 2004):

$$N_e = \frac{2N_0}{V}\frac{\lambda^2(\lambda-1)}{1-(1/\lambda)^L} \qquad \text{(II.1).}$$

where N_0 is the initial census population size, λ is the population growth rate, L is the generation time and V is the variance of gene frequency change across a generation.

Parameter estimation

Census size

Initial census size N_0 was estimated by multiplying population density D by the surface area occupied by the population. Three rectangular plots were placed in each population in year 2000 and D is the ratio obtained from the average number of plants present in the plots to plot surface area. The area occupied by each population was derived by inspection of satellite images (Google Maps France, retrieved on May 17th, 2010 from http://maps.google.fr) on which population edges were identified by eye. Initial census size N_0 was used to compute N_e according to Equation II.1. To allow for comparison with N_e, census size was defined as the harmonic mean N_h of population sizes N_t during one generation,

$$N_h = \frac{L}{\sum_{t=1}^{L}\frac{1}{N_t}} = \frac{L}{\frac{1}{\lambda N_0}\sum_{t=0}^{L-1}\frac{1}{\lambda^t}} = \frac{(\lambda-1)N_0 L}{1-(1/\lambda)^L} \qquad \text{(II.2)}$$

The number of breeders was estimated as the number of reproductive adults at the stable stage distribution, $N_B = w_4 N_h$, where w_4 is the proportion of reproductive adults (the fourth element of the stable stage distribution vector).

Potential and realized fecundity

Heywood (1986) introduced two distinct quantities to describe fecundity: potential fecundity, which is measurable from the number of seeds per individual and usually dependent on plant size; and realized fecundity, i.e. number of seedlings per individual. Mean μ_b and variance σ_b^2 of potential fecundity were estimated from seed number (which was derived from inflorescence length through a linear relationship as explained in "Data collection"). Mean realized fecundity ($\mu_k = a_{14}$) was calculated as the number of seedlings in year x over number of flowering individuals in the year x-1, considering equilibrium between seed immigration and emigration in plots and no seed bank. In fact, Gaudeul (2002) studied seed germinations and suggested that seed could persist in the soil for several seasons, but it is difficult to parameterize seed bank dynamics (seed survival and germination rates) with the data available. For this reason, seed bank was disregarded, but I address the implications of this assumption in the discussion.

The variance in realized fecundity σ_k^2 was calculated by modifying Heywood's (1986) original equation A3, which applies to a population with discrete generations, by substituting the number of adults $N_{t\text{-}1}$ for the number of breeders N_B:

$$\sigma_k^2 = \frac{(\mu_b - \mu_k)}{\mu_b - \frac{1}{N_B}}\mu_k\left[1 - \frac{\left(1+\frac{\sigma_b^2}{\mu_b^2}\right)}{N_B}\right] + \mu_k^2\frac{\sigma_b^2}{\mu_b^2} \qquad \text{(II.3)}$$

This expression allows calculating variance in reproductive success from measures of fecundity. Its derivation can be found in Heywood (1986) and assumes that recruitment of seedlings from the gene pool is random. The method disregards potential variation in male fecundity.

Variance of gene frequency change

The variance V of gene frequency change over a generation was defined as (Yonezawa 1997):

$$V = 2(1+\alpha)\left(\bar{u} - \bar{u}^2\right) + (1-\bar{u})S \qquad \text{(II.4)}$$

where α is the population deviation from Hardy-Weinberg proportions (corresponding to Wright's F_{IS}); $\bar{u}$ is the annual survival rate (u_i averaged over life stages, $\bar{u} = \sum_i w_i u_i$); $\bar{u}^2$ is the average of u_i^2 ($\bar{u}^2 = \sum_i w_i u_i^2$; Table II.1); $S = (1 - \alpha) + (1 + \alpha)(\sigma_k^2/\mu_k)$, where μ_k and σ_k^2 are the mean and variance in fecundity among flowering individuals (Equation II.3); S is employed for mathematical simplicity and does not represent any quantity of genetic or demographic implications. F_{IS} was estimated by Gaudeul and Till-Bottraud (2008) on the same set of microsatellites that are used to estimate N_e; estimates did not significantly deviate from zero in any populations.

Elasticity analysis

An elasticity analysis was carried out in order to quantify the relative impact of parameter values on demographic N_e estimates. The parameters considered were survival and fecundity rates, initial census size N_0 and variance in fecundity σ_k^2. Elasticities were estimated numerically by perturbing each parameter of interest x by a small quantity proportional to its value (0.01%; Campbell and Husband 2005):

$$E_{\mathrm{Ne}}(x) = \frac{N_e^{+} - N_e^{-}}{0.0002 N_e} \qquad \text{(II.5)}$$

where N_e^+ and N_e^- are the values of N_e when x is increased or decreased by 0.01%.

Genetic estimates of N_e

The populations were sampled and scored for eight microsatellites loci (described in Gaudeul et al. 2002; Gaudeul and Till-Bottraud 2008). One locus in Pralognan had to be discarded because it was monomorphic. 24 individuals were sampled in each population, but some of them had to be discarded because some markers could not be scored (final sample sizes were 21 in DES, 24 in BER, 23 in BOU and 23 in PRA). Linkage-disequilibrium (LD) based estimates of N_e were obtained through the method of Waples (2006) as implemented in the software *LDNe* (Waples and Do 2008). As rare alleles could result in biased N_e, we calculated three estimates of N_e by excluding allele frequencies of less than P_{crit} = 0.05, 0.02 or 0.01. Confidence intervals (CI) of the estimates were derived with the jackknife method (Waples and Do 2008). Bayesian estimates were produced

by the on-line program *ONeSAMP* (Tallmon et al. 2008), with prior distribution of N_e values ranging from 2 to 10000.

Results

Stage-specific survival rates were similar across populations, seedlings exhibiting the smallest rates and reproductive adults the largest (Table II.1). BOU had smaller survival rates than the other populations, except for seedlings. The standardized variance in realized fecundity σ_k^2/μ_k was smaller than the standardized variance in potential fecundity σ_b^2/μ_b and larger than Poisson variance ($\sigma_k^2/\mu_k = 1$). Population sizes ranged from N_0 = 55,459 (BOU) to 2,464,580 (DES). The harmonic mean census size followed the same pattern (Table II.2), ranging from N_h = 9,868 (BOU) to 4,597,646 (DES). Population growth rates λ show that DES was increasing, BER was slightly decreasing, BOU was decreasing and PRA was stable. Generation time was much larger in BER (L = 73.7) relative to the other populations.

Table II.2: Harmonic census size, number of breeders and effective population size, estimated with the demographic method, the LD method *LDNe* with three thresholds for the exclusion of rare alleles, and the Bayesian *ONeSAMP* method. Confidence intervals are given in parentheses. Negative estimates of N_e are found when the observed linkage disequilibrium can be explained entirely by sampling error without invoking any genetic drift; they corresponds to an infinite N_e (Waples and Do 2008).

		Deslioures	Bernards	Boujurian	Pralognan
N_h		4,597,646	88,300	9,868	284,819
N_B		105,774	7,064	1,111	12,836
N_e	Demographic	262,968	734	822	7,094
	LDNe, P_{crit} = 0.05	20 (4 – Inf)	1,269 (11 – Inf)	-34 (25 – Inf)	71 (7 – Inf)
	LDNe, P_{crit} = 0.02	32 (7 – Inf)	70 (10 – Inf)	-54 (32 – Inf)	213 (15 – Inf)
	LDNe, P_{crit} = 0.01	32 (7 – Inf)	70 (10 – Inf)	-54 (32 – Inf)	213 (15 – Inf)
	ONeSAMP	35 (17 – 109)	27 (14 – 73)	43 (25 – 144)	29 (14 – 82)

Demographic estimates of N_e ranged over nearly three orders of magnitude (Table II.2): BER and BOU had the lowest N_e, DES had the largest value, PRA was intermediate. The large variation in N_e partially reflected the variation in census population size. Effective to census size ratios were small (N_e/N_h = 0.057 in DES, 0.008 in BER, 0.083 in BOU and 0.025 in PRA). The small N_e/N_h observed

in BER was due to the large generation time of this population. Effective to adult N_e/N_B size ratio was larger than 2 in DES and smaller in the other populations.

Table II.3: Elasticity of N_e to model parameters

	Deslioures	Bernards	Boujurian	Pralognan
u_1	1.01	2.41	3.86	1.56
u_2	2.18	8.22	12.41	5.38
u_3	0.58	20.54	3.86	4.09
u_4	0.80	14.98	3.69	2.82
p_2	0.72	1.59	2.43	1.18
p_3	0.19	1.38	1.89	0.65
p_4	0.20	1.10	0.72	0.49
a_{14}	0.07	1.69	3.48	0.78
σ_k^2	-0.88	-0.74	-0.34	-0.75
N_0	0.50	0.50	0.50	0.50

The elasticity of variance in fecundity σ_k^2 was negative, meaning that increasing σ_k^2 leads to smaller effective size, as expected from population genetics theory (Table II.3). The elasticities of initial census size N_0 were positive and similar in magnitude to those of σ_k^2. The elasticities of survival and flowering rates were similar or larger to those of σ_k^2. BER and BOU had some elasticities of N_e to survival rates that were one order of magnitude larger than the others.

The LD method provided estimates of N_e ranging over two orders of magnitudes (Table II.2). The relative rank of populations was different from that obtained through the demographic method; in particular, DES showed the smallest N_e. LDN_e provided negative estimates of N_e for BOU; negative estimates are produced when the true N_e is large and sample size is small. In this case, the observed linkage disequilibrium can be explained entirely by sampling error without invoking any genetic drift (Waples and Do 2008); the biological interpretation is $N_e = \infty$. The LD method provided lower bounds of CIs for all populations but was not able to estimate the upper bound. There was no clear trend for the effect of varying the threshold P_{crit} for excluding low frequency alleles on the estimation of N_e.

The estimates of N_e from the *ONeSAMP* estimator were much less variable than those obtained with either the demographic or the LD method (Table II.2). Median values for N_e ranged between 27 and 43 and the CI extended from 14 to 144. The CIs were largely overlapping, suggesting that the populations had similar N_e.

Discussion

The objective of this chapter was to compare different N_e estimators in order to see if they gave consistent results. In particular, demographic-based estimators have been contrasted to marker-based methods. The main result of this chapter is that there are great discrepancies among the different methods. The differences can be ascribed to: (i) different periods of reference for the demographic- and genetic-based estimates; (ii) violation of the assumptions of demographic methods and low precision in model parameters; (iii) low precision of the genetic-based estimates due to small sample sizes; and (iv) uncertainty about the performances of genetic estimators in relation to sample size and locus polymorphism. Each of these issues is discussed in detail in the following.

Period of reference

The first aspect one should consider when working with demographic-based and genetic -based methods is that these approaches do not necessarily estimate the same quantity (Nunney and Elam 1994). Demographic approaches use equations based on causal parameters to predict N_e on the basis of current census size and provide a value of N_e representative of future population state. Conversely, genetic estimates are inferred in a retrospective manner and represent realized rather than potential measures of N_e. The two approaches give consistent estimates if they refer to the same period. The genetic samples of *E. alpinum* used for this analysis were collected in 1998, so they give information on effective population size prior that date. Demographic parameters were calculated from demographic surveys taking place between 2001-2008. Genetic and demographic estimates therefore refer to different periods.

It can be expected that the larger the temporal variation in demographic and life-history parameters, the more the two quantities will differ. An additional source of difference is the occurrence of punctual historical events (e.g. bottlenecks) or fluctuations in population size, which are capable of influencing N_e for several generations: these factors may escape the demographic survey but still leave a signature in gene frequency and affect genetic-based N_e estimates (Waples 2005). The Desliores population was probably smaller in the 1990s compared to the survey period, according to the large population growth rate and field observations; this can in part explain why N_e obtained with the genetic methods was so small compared to that obtained with the demographic method and

to the census size in this population. However, there is no reason to believe that large variation of population size or vital rates occurred in the other populations.

The equivalence of genetic-based N_e values for DES, BER and BOU indicates that these sites may be part of the same population. The sites are located in the same alpine valley (Fournel) and show very weak genetic differences (Gaudeul and Till-Bottraud 2008). Global genetic differentiation was significant but low (F_{ST} = 0.013) and pairwise differentiation was significant only between BER and DES (F_{ST} = 0.018), which are located at the farthest edges of the valley. Moreover, two Bayesian clustering algorithm (*STRUCTURE*, Falush et al. 2003; and *BAPS*, Corander et al. 2003) revealed that the valley consists of a single genetic group (Gaudeul and Till-Bottraud 2008). This pattern of weak differentiation can be explained by gene flow between sites, which is plausible given the potential for long-distance pollen and seed dispersal. Alternatively, the sites may have been part of an old single population that underwent recent fragmentation. Fragmentation is thought to have begun with the radical change of land uses in the 1950s, but given the long generation time and life-span of the plant, the elapsed time was not sufficient to give rise to a significant genetic structure between sites. In addition, estimates of N_e based on linkage disequilibrium contain information on the effective size from one or a few generations in the past, because LD can require several generations to decay (Waples 2005; Waples and Do 2010). *ONeSAMP* is based mainly on LD and it is thus possible that the estimated N_e reflects the status of the population before the beginning of fragmentation. The estimates obtained in the three sites would thus represent the effective size of the ancestral population.

Assumptions and precision of the demographic estimator

Calculation of N_e through demographic methods relies upon a number of assumptions and on parameters that need to be estimated from field or genetic data. It is important to assess how violations of the model assumptions and uncertainty in parameter estimates affect the estimated value of N_e. Here I discuss the assumptions made in the model of Yonezawa (1997) and Yonezawa et al. (2000).

Stable stage distribution and constant rate of increase

The first two assumptions are that the population is at the stable stage distribution and growing at a constant rate λ. If these assumptions are not met, then the predicted λ and the vector of stable stage

distribution **w** will be different form the observed values over the study period. The observed stage distributions were compared to the stable stage distribution vectors in each population and each year. In none of the comparisons the observed distribution matched the predicted one (χ^2 comprised between 8.6 and 1845, d.f. = 3, $p < 0.05$). In addition, the assumption of stable stage distribution is necessary to calculate generation time L with the method of Cochran and Ellner (1992).

Variance in gene frequency change

The third assumption is that variance in gene frequency change V is the same over years. The variance is affected by numerous parameters (α, $\bar{u}$, $\bar{u}^2$, μ_k, σ_k^2 and N_B; Equation II.4). $\bar{u}$ and $\bar{u}^2$ are average survival rates, so they may change in presence of environmental stochasticity. Annual variation in survival rates were evidenced in the *E. alpinum* dataset (Annex III). μ_k and σ_k^2 change according to temporal variation in potential fecundity (number of seeds or inflorescence length). The populations of *E. alpinum* exhibited annual variation in inflorescence length and, thus, in seed set (Article B). In turn, variation in survival and fecundity rates generates variation in the number of breeders N_B. In presence of variation in vital rates, a more useful measure to estimate N_e is the harmonic mean of V over year (Yonezawa et al. 2000).

Departure of genotype frequencies from Hardy-Weinberg proportions

The fourth assumption is that α, the departure of genotype frequencies from Hardy-Weinberg proportions, is the same across all life-stages and constant over time. This assumption could not be checked with our data: only one genetic sample was available and individuals were not classified by life-stage. These individuals were mostly adults, so the estimated α is likely to reflect deviation from Hardy-Weinberg proportion mainly in this stage.

Random recruitment of seedlings from the gene pool

Equation II.3 is valid only when recruitment of seedlings from the gene pool is random. However, numerous factors acting during seed dispersal and seedling establishment can add further variation to realized fecundity, such as predation (Nathan and Casagrandi 2004), differential germination rates and microsite condition (Harper 1977). In addition, in sessile organisms like plants, a large portion of seeds is dispersed near the mother plant, so that spatial variation in environmental conditions could lead to differential individual success in reproduction, seed germination and

seedling survival. The method used here therefore omits several sources of variation contributing to reproductive success and probably underestimate the variance in reproductive success: the final result is thus an overestimation of N_e (see Nunney 1995). Waples (2002b) showed that, when vital rates in the first life-stages are family-correlated (individuals sharing the same parents have similar rates), the N_e/N ratio can be strongly reduced.

In order to relax the assumption of random recruitment, variation in reproductive success has to be estimated directly using the number of recruited seedlings per mother and father plant. This can be done, in theory, using molecular markers and the statistical techniques of parentage analysis. Variation in reproductive success can be converted to the probabilities that two offspring taken at random from a population are sibs sharing the same parent (Wang 2009). Then sibship assignment methods can be used to reconstruct the variance in offspring number among parents. This approach will also allow for a quantification of male variance in fecundity, which was not considered here.

There still remain practical problems such as the availability of adequate molecular markers and the risk that sampling damages seedlings because of their small size. In addition, the estimation should be replicated in time to reveal how variation in reproductive success among parents can change from one reproductive season to another. Variation in realized fecundity is an important parameter, because N_e depends linearly on it (Equation II.1), but only few studies address this issue in plant populations (see for instance Grivet et al. 2009).

Seed bank

In situ germination experiments conducted by Gaudeul (2002) suggest that seeds can persist in the soil and germinate a few years after they are produced. However, the data available from the study of Gaudeul (2002) were not sufficient to characterize adequately seed bank dynamics. Disregarding the seed bank could have introduced a downward bias in the estimation of N_e. Nunney (2002) and Vitalis et al. (2004) show with theoretical models that the effective size of a population of annual plants without a seed bank is smaller than that of a population with a seed bank. However, these models did not consider iteroparity and stage structure and there is reason to believe that the behavior of a monocarpic species may be different from that of a stage-structured species. Indeed, in the analyses presented in the next chapter, the estimation of N_e was performed after accounting for seed bank dynamics and resulted in a reduction rather than an increase in N_e, thus contradicting the expectations of theoretical models. The effect of seed dormancy on N_e will be elucidated only

when data on survival and germination rates of seeds will be available. Obtaining estimates of these parameters was the initial objective of the germination study of Gaudeul (2002), but then it was difficult to distinguish seedlings from small juveniles; as a result, the number of seedlings counted may well comprise a mixture of newborns and older individuals, making estimation of seed vital rates impossible.

Precision in parameter values

Even if all the model assumptions were met, the estimation of N_e requires demographic (survival rates, fecundity rates, variance in fecundity, census size) and genetic (the departure of genotype frequencies from Hardy-Weinberg proportions) parameters to be precisely and accurately estimated. The departure of genotype frequencies from Hardy-Weinberg proportions must be estimated from genetic markers and depends thus on the precision in the estimation of genotype frequencies and the accuracy of genotyping techniques. The other parameters are calculated from field surveys and are thus affected by sampling error. The elasticity analysis showed that all the considered parameters can be equally important in estimating N_e. This confirms the need of obtaining precise values for all the parameter appearing in the model.

Vital rates. Correct estimation of survival rate is essential in order to derive N_e under the model adopted here. Matrix elements and vital rates are estimated from demographic field studies that are conducted on a limited number of individuals. Even if the precision of vital rate estimates can be improved by adopting particular sampling procedures (Münzbergová and Ehrlén 2005), it is not possible to completely remove sampling error. In some populations, the elasticities to survival rates of adult or juvenile plants were particularly large. In some way this is fortunate, because it is easier to identify and study adults and juveniles than seedlings. One way to address the effect of sampling on estimates of N_e is bootstrapping (Efron and Tibshirani 1993). This statistical technique is used to quantify the effect of sampling error on population growth rate (Kalisz and McPeek 1992; Levin et al. 1996; Chapter III) and could be used in models for N_e estimation.

Fecundity. The estimation of potential and realized fecundity suffers from the same type of sampling error as vital rate estimation. In addition, the estimation of realized fecundity requires seedlings to be assigned to parental plants.

Census size. In the present study, it was difficult to estimate census population size N_0. The surface of the four populations is so large that a complete census of individuals was unfeasible. Census

population size had to be computed from estimates of population surface areas and measures of density in the permanent plots. Errors in estimation of surface areas introduce uncertainty in the N_e estimates but should not introduce any bias. Conversely, the density of plants in the permanent plots may not be representative of the actual population density in the entire population, because the plots were placed in areas of relative high density to increase sample size for the demographic survey. This could have introduced an upward bias in the estimation of N_0, N_h and N_e. The ranking of populations is nonetheless representative of reality, with DES being the largest and BOU the smallest.

Generation time. The four populations exhibited large variability in generation time (Table II.1). One might be tempted to attribute these differences to the different management practices adopted in the populations: BER is mowed, DES and BOU are grazed and PRA has a mix of mowing, grazing and absence of management (see also Article B for a description of management practices in *E. alpinum*). However, there are two reasons to believe that the estimated L values may not be fully reliable. First, the method used for the estimation (Cochran and Ellner 1992) makes the assumption of stable stage distribution, which was violated in all the populations analyzed. Secondly, the demographic study was carried out for a time that is too short when compared to the estimated values of L. Values such as 24 years (in PRA) or 73 years (in BRE) may be suspect when estimated from a dataset comprising only eight years of demographic study. These potential biases in L lead directly to uncertainty and lack of accuracy in the estimates of N_e.

Low precision of *LDNe*

Estimates based on the LD method had low precision and in no case upper bounds for confidence intervals could be calculated. Waples and Do (2010) performed a simulation study to understand how estimates of N_e based on the LD method are affected by sample size, number of loci, locus polymorphism and inclusion of rare alleles in the estimation. When only a limited number of individuals and loci are available, the LD method performs poorly and is not able to provide upper confidence intervals for N_e; if microsatellite polymorphism is low, the precision of the estimates is further reduced. Waples and Do (2010) showed that N_e estimates were sufficiently precise (coefficient of variation < 1) when the product of number of sampled individuals n, number of loci L and number of alleles per locus K was around $n \cdot L \cdot K \approx 1000$. Our dataset contained few loci (L = 7) and individuals (n = 24), and the loci had low polymorphism (K = 4), which gives $n \cdot L \cdot K$ = 722.

In addition, estimates of N_e become less precise when the true effective size is large (Waples and Do 2010): on the basis of the estimates from the demographic method, the effective size of the four populations can potentially be very large, and this would further reduce the performance of the LD method in providing precise values. Finally, Waples and Do (2010) noticed that using loci with many rare alleles introduce a slight upward bias in N_e and introduced the threshold P_{crit} to see how this factor could influence the estimation. We could not observe significant differences or any particular trend between the N_e estimates obtained under three P_{crit} thresholds. The reason for this is likely the low overall precision of the estimates. It seems therefore that our sample was not adequate to obtain a precise value for N_e through the LD method.

Performances of *ONeSAMP*

The Bayesian *ONeSAMP* method provided estimates for all populations with relatively narrow confidence intervals (CI). However, the ranking of populations provided by this method did not agree with that obtained with the demographic method. The demographic method suggested that DES was the population with the largest effective size, while BOU had the smallest one and BER and PRA lay in-between. Conversely, *ONeSAMP* provided estimates with CIs largely overlapping so that the four populations ranked equally. As discussed above, there is a number of factors capable of affecting the estimates of N_e obtained from the demographic method. However, it is not clear whether the disagreements are due only to the uncertainty of demographic estimates or whether there are additional factors capable of affecting the Bayesian estimates. *ONeSAMP* has never been subject to evaluation and it is thus not known whether sample size and locus polymorphism are issues capable of affecting estimation precision and accuracy. In a study comparing the performances of different genetic N_e estimators, Beebee (2009) found that *ONeSAMP* was generally very precise. In addition, estimates from *ONeSAMP* correlated well to the sibship assignment method of Wang (2009), which we could not employ because it requires samples drawn from single cohorts. Saarinen et al. (2009) found that *ONeSAMP* and *LDNe* gave consistent estimates when sample size was sufficiently large. However, in absence of an assessment for *ONeSAMP*, it is difficult to conclude which method gives the most accurate estimation.

It seems also necessary to assess how the two genetic methods perform when applied to species with overlapping generations. If the genetic samples are taken from a single cohort (by sampling seedlings for instance), the estimated values will reflect the effective number of breeders rather than

N_e (Waples 2005; Luikart et al. 2010). In the sample is taken randomly across all individuals, the effective size will be affected by correlations of individual reproductive success in successive breeding seasons (Jorde and Ryman 1995). Whether genetic estimates based on the LD and *ONeSAMP* method are affected by this issue in iteroparous species is still not clear and requires further examination. This could be carried out by a simulation study similar to that performed for *LDNe* by Waples and Do (2010).

Published comparisons

Comparison of genetic- and demographic-based estimates has not been carried out very often. To my knowledge, there are only few studies in which this has been done explicitly: Begon et al. (1980) in *Drosophila suboscura*; Husband and Barrett (1992) in the annual plant *Eichhornia paniculata*; Turner et al. (2002) in the red drum *Sciaenops ocellatus*; and Kaeuffer et al. (2004) in the domestic cat *Felis catus*.

Begon et al. (1980) studied the variance effective size of one population of *Drosophila suboscura* using three estimators: the moment-based temporal method of Krimbas and Tsakas (1971) on nine allozyme loci; a method based on lethal allelism; and one demographic method. Lethal allelism is the presence of lethal alleles at different loci in the genome and information on the frequency of lethal alleles can be used to estimate N_e. The rationale is the following: two individuals from different populations have a very low probability of carrying the same lethal allele, because these alleles arise independently by mutation. Within a small populations however, the probability of finding two individuals carrying the same lethal is higher because of inbreeding. Comparison of allelism rates between and within populations will provide information to estimate N_e. The demographic method used by Begon et al. (1980) considered the effects of unequal adult sex-ratio, variation in census size and variance in reproductive success, but ignored the mating system. Due to incomplete availability of demographic data, the authors were able to estimate N_e only within an order of magnitude of precision, which was nonetheless in good agreement with the result of the lethal allelism method. The moment-based temporal method gave much lower estimates. The authors argued that the correct N_e was that derived from the lethal allelism and the demographic methods, while the temporal estimate was downwardly biased by a putative violation of the assumption of allozyme neutrality.

Husband and Barrett (1992) used the temporal method with a set of allozymes to estimate N_e in several populations of the annual plant *Eichhornia paniculata.* They also used a combination of demographic equations that took into account the effects of variation in population size, variance in reproductive success, inbreeding and assortative mating. The ecological estimates of Husband and Barrett (1992) were consistently higher than their genetic counterparts. Variance in reproductive success was the most influential parameter, and also the most difficult to measure. Nunney (1995) pointed out some methodological errors in the study of Husband and Barrett (1992), namely the estimation of census size, gene frequencies and variance in reproductive success and the way in which inbreeding and variance reproductive success were incorporated in the ecological estimate. When these errors are corrected, genetic- and demographic-based estimates of N_e turned out to be quite consistent.

Turner et al. (2002) used the temporal method to estimate variance N_e in the red drum *Sciaenops ocellatus* and found that it is several orders of magnitude smaller than adult census size. Demographic data were not sufficient to provide an independent estimate of N_e, because variance in reproductive success among adults and between spatial locations was difficult to obtain. The authors used therefore the single population model of Nunney and Elam (1994) and the geographically structured model of Nunney (1999) to assess the effect of different demographic factors on N_e and to calculate how large variance in reproductive success needed to be for the demographic and the genetic estimate to converge to the same value. The largest effect on N_e was due to differences in productivity among demes and variance in reproductive success among female adults. Using the model without spatial structure, the calculated variance in reproductive success turned out to be unrealistically large for the estimates to converge. The authors concluded that variance in productivity among populations must be an important factor to estimate the extremely low N_e/N ratio in red drum.

Kaeuffer et al. (2004) estimated variance N_e in two domestic cat populations (*Felis catus*) through one demographic method (Nunney and Elam 1994), the moment-based temporal method of Waples (1989) and the maximum-likelihood temporal method of Berthier et al. (2002). The model of Nunney and Elam (1994) can be used to estimate N_e in age-structured populations with separate sexes by taking into account variation in reproductive success among parents, unbalanced sex-ratio and differences in life span time between sexes; it assumes constant population size and age-independent survival rates. N_e estimates from the moment-based method and the demographic

method were overall in good agreement. The maximum-likelihood method gave estimates that were far larger than the other two methods.

These four examples demonstrate that genetic and demographic based estimators can show inconsistencies in the results. The reasons can be numerous but can be reduced to three types: violations of assumptions of genetic methods, methodological mistakes in parameter estimation and lack of the necessary ecological data to calibrate the demographic models. The comparison of demographic- and genetic-based estimators is therefore a research topic that deserves further work. There is a need for increasing number of data-based comparisons as well as theoretical analyses and computer simulations that can point out the potential sources of discrepancy between the two approaches.

Conclusions

The results presented in this chapter clearly show that there is still disagreement between different estimators of effective population size. Some disagreements could be attributed to the low statistical power of the LD method when the genetic samples comprise few loci with low polymorphism and few individuals. This problem can be easily overcome by increasing sampling effort. The differences between the three methods may also be due the lack of appropriate knowledge for all the relevant parameters appearing in the demographic model. In particular, one needs reliable estimations of survival rates, variance in fecundity and census size. The need for a number of parameters that are not always readily measurable may discourage to use demographic methods and induce researchers to rely exclusively on genetic methods. Still, demographic methods can be useful to investigate the relative role of factors contributing to N_e and provide insights into the mechanistic processes that control genetic drift, as will be shown in the next chapter.

Chapter III:

Elasticity of N_e to stage-specific vital rates: numerical approach

Introduction

As illustrated in the previous chapter, demographic models can be used to calculate effective population size from demographic data. It was also illustrated how the value calculated in this way is not necessarily indicative of the "true" or realized effective size, because of violation of the model assumptions and because of the uncertainty in parameter estimates. As a consequence, demographic estimates of N_e may be considerably different from other estimates based on genetic data. However, despite the inconsistency between demographic-based and genetic-based estimates, demographic models are still a useful approach to study the effects of stage-specific vital rates on N_e.

The general goal of this chapter is studying the effects of stage-specific vital rates on N_e using numerical elasticity analysis. The data used are those from the four populations of *E. alpinum* (presented in Chapter II) and from seven populations of another endangered plant, *Dracocephalum austriacum*, (Fig. III.1; Nicolè 2005). N_e is estimated using the demographic model of Yonezawa et al. (2000), which was also described in Chapter II.

The first objective of this chapter was to find generalities in the elasticity patterns of N_e; in particular the patterns were compared between decreasing, stable and increasing populations. The second objective was to compare elasticity patterns of N_e with those of λ. A similarity in the elasticity patterns would reveal that the most important vital rates for genetic drift are also the most influential on population growth. The third objective was to study the elasticity pattern of annual effective size N_a and generation time L. These parameters are useful to measure genetic drift in populations with overlapping generations, because N_a expresses the rate of genetic drift per year, rather than per generation, and is helpful in comparing populations with different generation times. The results are discussed in relation to other published studies and in the context of population viability analysis and conservation.

Some of the results presented in this chapter are the subject of an article in preparation for publication (Article A).

Materials and methods

This section describes the biology of *Dracocephalum austriacum*, the study sites and the methods used for data collection and parameter estimation. The corresponding descriptions for *Eryngium alpinum* can be found in Chapter II.

Species and study sites

The Austrian dragonhead, *D. austriacum* (Figure III.1), is a long-lived perennial plant whose distributional area spans from the Caucasus to the Pyrenees, with scarce populations in Western Europe (Olivier et al. 1995). *D. austriacum* is present in arid, fully exposed habitats where competition with other plants is low. In France, populations are located between 1250 and 2000m of altitude on xeric, stony grasslands or heaths, preferentially on calcareous superficial soils (Lauber and Wagner 1998). The plant is a herb or a dwarf shrub of 20 to 50 cm high, with several erect or ascending hairy stems. Flowers are grouped in more or less dense spikes composed of 1 to 5 verticillasters and are insect-pollinated (large blue-violet corolla from 3.5 to 5 cm length). The fruits (tetrakens) contain seeds that are gravity dispersed, reducing dispersal potential to short distances; the species does not spread clonally.

a)

b)

Figure III.1. a) *Dracocephalum austriacum*. Photograph by Florence Nicolè. b) Typical habitat for *D. austriacum* populations; photograph by Irène Till-Bottraud

This rare species is listed as vulnerable by the International Union for Conservation of Nature (IUCN) and is under national, European and international legislations (Red List of French Endangered Flora, Habitats Directive, Bern Convention). Threats to this attractive species include pillaging and trampling, when populations are near footpaths or close to pastoral activities. Locally, plant competition with shrubs and trees can also increase vulnerability (Danton and Baffray 1995).

Only 15 populations are known in France, from Savoie to the Alpes Maritimes. 7 of the 15 French populations of the Austrian dragonhead reported in the French Alps were selected for their accessibility (Table III.1): Bessans (BE), Champcella (CH), Escoyeres (ES), L'Argentière (LA), Reynier (RE), StCristophe (ST) and Valsenestre (VA). These populations vary widely in population size, density, genetic diversity, vegetation characteristics, and soil composition (Nicolè 2005; Bonin et al. 2007; Nicolè et al. submitted). 135 Km separate the northernmost population BE, which experiences a mountain climate, and the southernmost population RE, which experiences a Mediterranean climate.

Data collection

Survival and fecundity rates of the plants were estimated from individual-based field surveys. Three to four permanent plots were installed in the seven populations in late spring 1999, except in Bessans, where the survey started in 2000. Every plant in the plots was identified by a number and its life stage was recorded. The position of each plant was mapped to facilitate finding in subsequent years. From 1999 to 2006, census was performed in June. Each plant was checked for its presence and life stage; new recruits (seedlings) were checked carefully and added to the census. The observed numbers of plant per stage per year is reported in Article A. The estimation of potential fecundity (number of seeds per plant) was performed outside the permanent plots to avoid modification of recruitment. The mean μ_b and variance σ^2_b in number of seeds produced by flowering plants was averaged over two representative samples of 20 individuals, collected in each population in 1999 and 2002. In June 1999, an exhaustive count of plants was performed over each study area to provide an estimate of total population size (N_0; Table III.3).

Table III.1 Geographical, demographic and genetic parameters of the seven populations of *Dracocephalum austriacum*. u_i, survival rate of plants belonging to stage *i*; p_i, flowering rate of plants belonging to stage *i*; μ_b and σ_b^2, mean and variance in potential fecundity; μ_k and σ_k^2, mean and variance in realized fecundity; α, deviation of population genotype frequencies from Hardy-Weinberg proportions. This table can be compared to Table II.1, which reports analogous parameters for *Eryngium alpinum*.

		Bessans (BE)	Champcella (CH)	Escoyères (ES)	L'Argentière (LA)	Reynier (RE)	StChristophe (ST)	Valsenestre (VA)
Location		7.04 E, 45.28 N	6.52E, 44.72 N	6.71 E, 44.76 N	6.47 E, 44.78 N	6.18 E, 44.32 N	6.23 E, 44.93 N	6.09 E, 44.87 N
Altitude (m)		2000	1700	1690	1550	1275	1800	1600
Survival rates	u_1	0.46	0.58	0.68	0.56	0.37	0.55	0.35
	u_2	0.94	0.86	0.70	0.71	0.55	0.78	0.79
	u_3	1.00	0.92	0.92	0.88	0.94	0.88	0.85
	u_4	1.00	0.97	0.99	0.96	0.95	0.93	0.85
	$\bar{u}$	0.94	0.89	0.93	0.82	0.64	0.84	0.76
	$\bar{u}^2$	0.91	0.81	0.87	0.69	0.48	0.72	0.61
Flowering rates	p_1	0.15	0.03	0.19	0.00	0.01	0.04	0.00
	p_2	0.17	0.13	0.37	0.12	0.16	0.17	0.21
	p_3	0.49	0.53	0.52	0.59	0.89	0.37	0.36
	p_4	0.77	0.71	0.77	0.81	0.77	0.64	0.43
Fecundity	μ_b	13.40	35.60	22.92	18.20	6.86	13.10	22.61
	σ_b^2	275.97	1286.20	390.97	448.40	123.11	198.05	399.72
	μ_k	0.18	0.20	0.14	0.39	1.12	0.28	0.55
	σ_k^2	0.22	0.30	0.15	0.59	4.22	0.35	0.76
	σ_b^2/μ_b	20.60	36.12	17.06	24.64	17.94	15.12	17.68
	σ_k^2/μ_k	1.25	1.24	1.09	1.51	3.76	1.28	1.39
	α	0.8614	0.9332	0.9190	0.8438	0.8631	0.8718	0.6779

Parameter estimation

Mean μ_b and variance σ_b^2 of potential fecundity was estimated by counting the number of seeds per individual as explained in "Data collection". Mean realized fecundity μ_k was estimated on the

pooled dataset as the number of seedlings found in years 2000-2006 divided by the number of reproductive adults in 1999-2005. The variance in realized fecundity σ_k^2 was calculated by modifying Heywood's (1986) original equation as illustrated in Chapter II (equation II.3).

The inbreeding coefficient F_{IS} was derived from a set of 87 AFLP markers (see Bonin et al. 2007 for details of the molecular techniques used to generate the data set) using ABC4F (Foll et al. 2008). ABC4F is an approximate Bayesian computation method allowing the estimation of population-based F_{IS} from dominant markers (Figure III.3). For the present analysis, F_{IS} was considered equivalent to α (the deviation of genotype frequencies from Hardy-Weinberg proportions, Table III.1), even if this is not strictly true. α is a population-level parameter and can take positive or negative values, depending on the excess of heterozygotes observed in the population. F_{IS} is defined as the probability of sampling an individual inbred for a particular locus and cannot take negative values (Foll et al. 2008, p. 928). Nonetheless, the pattern of elasticity is not affected by the actual value of α (see the section "Robustness to model assumptions" in the Results and the Discussion), so this inconsistency had no serious effect on the results.

ABC4F corrects for the effect of hidden and fixed loci on the estimation of F_{IS}. Hidden loci are the ones that cannot be observed because all individuals in the sample are homozygous for the recessive genotype and thus the band is not present. The bias parameter for hidden loci in ABC4F was set to 1: this means that the estimation procedure takes into account that the loci with less than *one* individual exhibiting the dominant phenotype (i.e. no individual) are not present in the sample. Fixed loci are the ones for which all individuals exhibit the dominant phenotype (the band), but they can be either homozygous for the dominant allele or heterozygous. Fixed loci are sometimes removed from the dataset to increase the level of polymorphism. All loci were retained in this analysis, so the bias parameter for hidden loci in ABC4F was set to 0. The "number of iterations" was set to 10000 and the "acceptance rate" to 0.001. Thus, the total number of iterations was 10,000 / 0.001 = 10,000,000 and the final average F_{IS} was estimated using 10,000 points.

Matrix model

The life cycle of *D. austriacum* was structured into four demographically distinct stages (Fig. III.2), assuming a pre-breeding census. Seedlings, the first stage, are small plants with less than 5 stems and shorter than 10 cm. They represent the new emerging plants of the year (all plants move to the juvenile stage the second year after emergence). Juveniles have also less than 5 stems but are taller

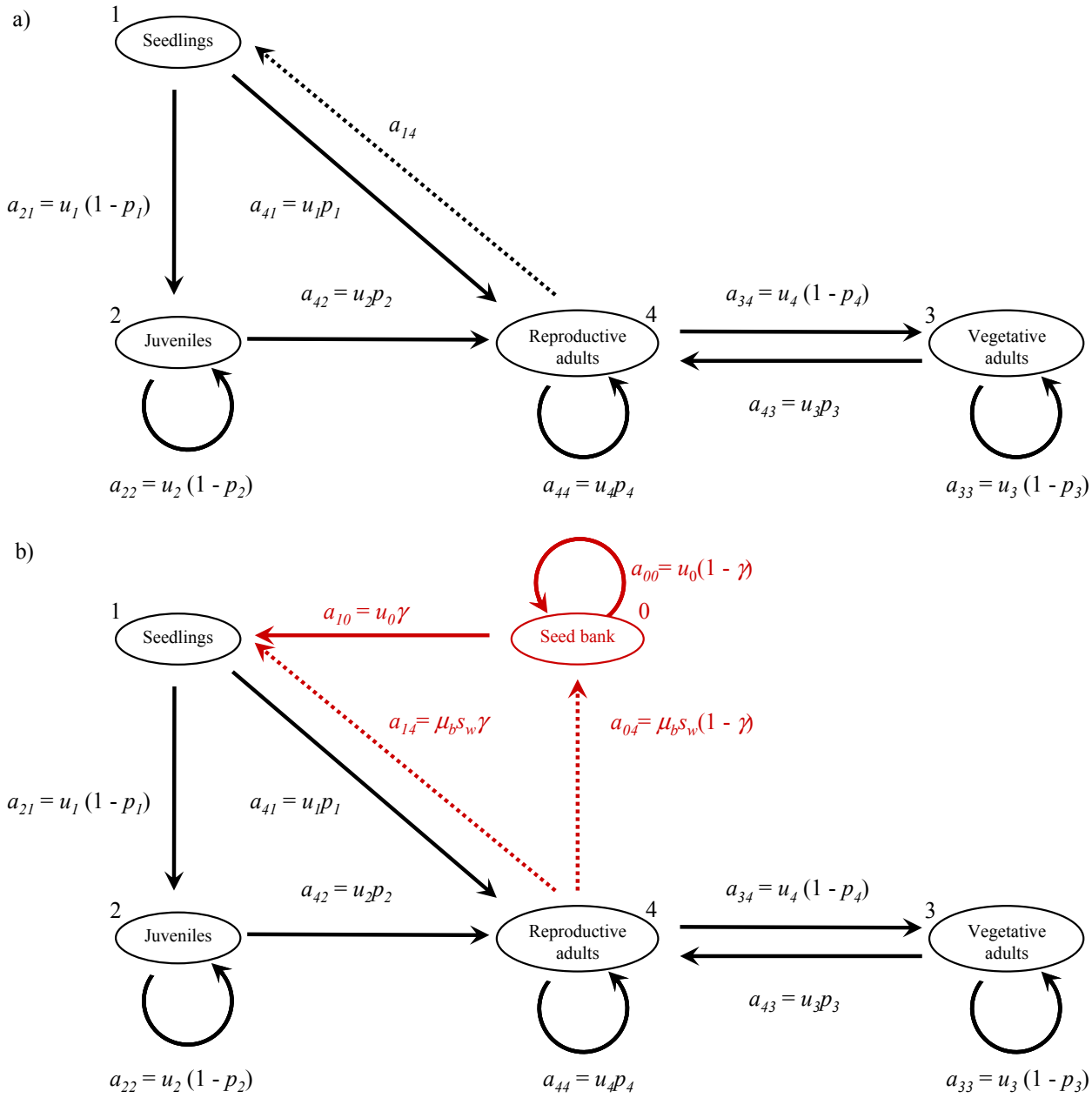


Figure III.2. a) Life cycle graph of *Dracocephalum austriacum*, representing transitions of individuals between stages (solid lines) and reproduction (dotted lines). The upper-vital rates (a_{ij}) correspond to the transitions between stages, the lower-vital rates correspond to survival (u_i) and flowering (p_i) probabilities. The life cycle of *E. alpinum* (Figure II.3 in Chapter II) is similar, the only difference being the absence of the seedling-to-reproductive-adult transition, *a*41. b) The modified life cycle graph including seed bank dynamics (in red), used to study the robustness of the elasticity pattern to model assumptions.

than 10 centimeters. A plant stays in the juvenile stage until it flowers. Adults were divided in two classes, vegetative and reproductive. Because seed dormancy was difficult to characterize, we chose to disregard seed bank dynamics, but I explored the effect of this assumption on the results by using an alternative model including a seed bank (see section "Robustness to model assumptions").

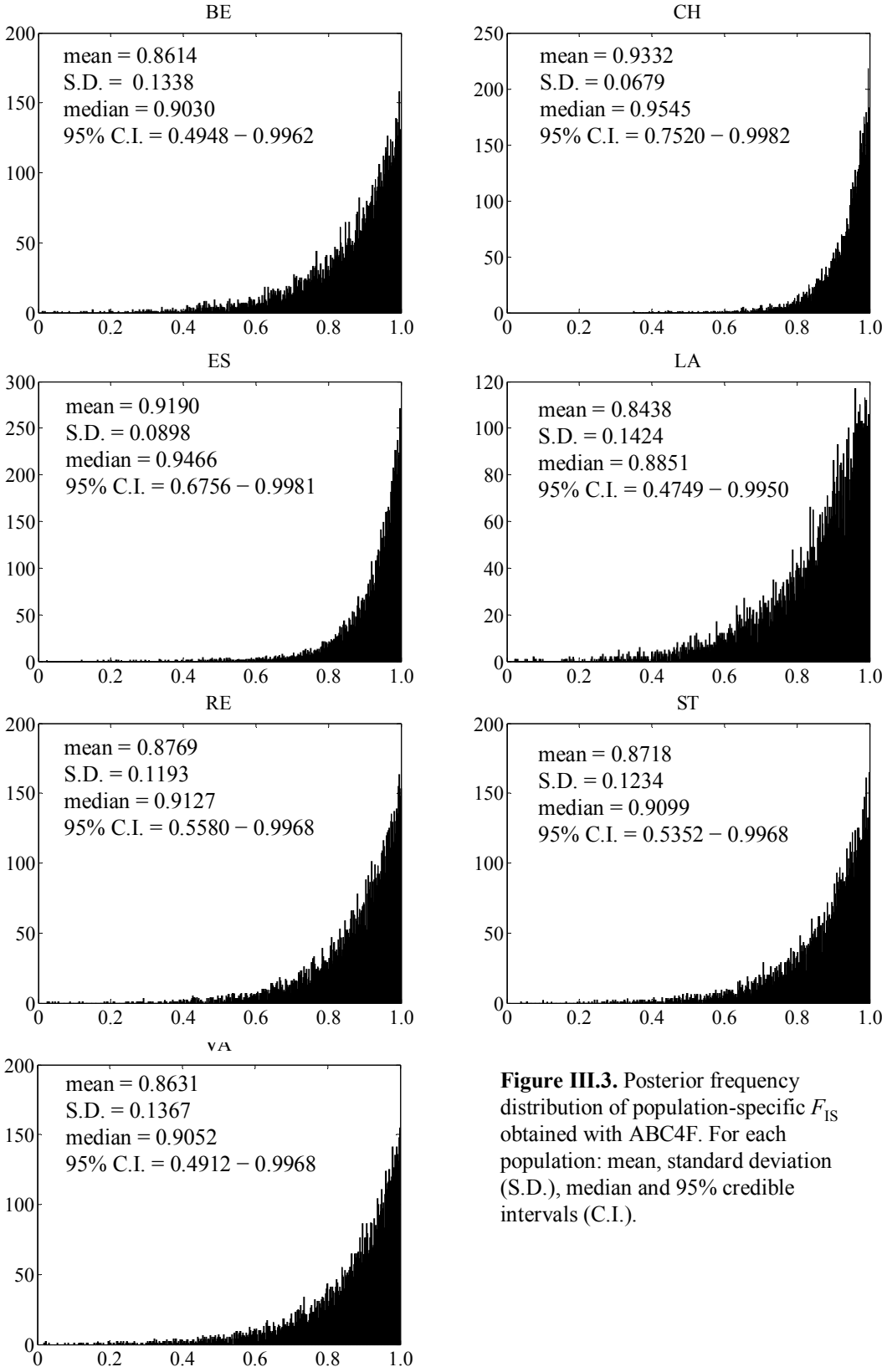


Figure III.3. Posterior frequency distribution of population-specific F_{IS} obtained with ABC4F. For each population: mean, standard deviation (S.D.), median and 95% credible intervals (C.I.).

Transition rates a_{ij} were averaged over the entire period of survey by pooling together the annual observations in the different plots in each site (Caswell 2001 p. 134-135) and then arranged in a transition matrix (Table III.2).

Table III.2: Transition matrices, stable stage distribution and reproductive value of the seven populations of *Dracocephalum austriacum*.

						Stable-stage distribution	Reproductive value
		S	J	V	R		
Bessans (BE)	S	0	0	0	0.18	0.09	1
	J	0.39	0.79	0	0	0.14	2.05
	V	0	0	0.51	0.23	0.24	3.05
	R	0.07	0.16	0.49	0.77	0.53	3.28
Champcella (CH)	S	0	0	0	0.24	0.11	1
	J	0.56	0.75	0	0	0.24	1.66
	V	0	0	0.43	0.28	0.22	3.18
	R	0.02	0.11	0.49	0.69	0.44	3.71
Escoyeres (ES)	S	0	0	0	0.14	0.08	1
	J	0.55	0.44	0	0	0.08	1.20
	V	0	0	0.44	0.23	0.24	2.23
	R	0.13	0.26	0.48	0.76	0.60	2.61
L'Argentière (LA)	S	0	0	0	0.39	0.17	1
	J	0.56	0.63	0	0	0.27	1.76
	V	0	0	0.36	0.18	0.13	6.00
	R	0.00	0.09	0.52	0.78	0.43	7.20
Reynier (RE)	S	0	0	0	1.12	0.36	1
	J	0.36	0.46	0	0	0.24	2.56
	V	0	0	0.11	0.21	0.08	14.96
	R	0.01	0.09	0.83	0.73	0.32	16.12
StCristophe (ST)	S	0	0	0	0.28	0.11	1
	J	0.53	0.65	0	0	0.20	1.64
	V	0	0	0.55	0.33	0.32	2.93
	R	0.02	0.14	0.33	0.59	0.38	3.55
Valsenestre (VA)	S	0	0	0	0.55	0.16	1
	J	0.35	0.62	0	0	0.20	2.56
	V	0	0	0.54	0.49	0.36	3.58
	R	0.00	0.17	0.30	0.37	0.27	4.25

Elasticity analysis

Effective population size was estimated using the model of Yonezawa et al. (2000) according to the methods described in Chapter II. The annual effective size N_a was calculated as the product of N_e times the generation time L. The elasticity analysis was applied to effective population size N_e,

population growth rate λ, generation time L and annual effective population size N_a. The elasticities of these parameters were calculated in relation to matrix elements a_{ij} and low-level vital rates (stage-specific survival rates u_j and flowering rates p_j). Elasticities were estimated numerically by perturbing the matrix elements (or the vital rates) by a small quantity proportional to their values (0.01%; Campbell and Husband 2005):

$$E_y(x) = \frac{y^+ - y^-}{0.0002y} \qquad \text{(III.1)}$$

where y is one of λ, N_e, L and N_a; x is a matrix element or a vital rate; y^+ and y^- are the values of y when x is increased or decreased by 0.01%. I computed pairwise correlations across populations among the E_λ, E_L, E_{Ne} and E_{Na} relative to the same vital rate (u_1, u_2, u_3 and u_4). Positive correlations imply that the vital rate in question tends to make proportional contributions to the two parameters; for instance, a positive correlation between $E_\lambda(u_3)$ and $E_{Ne}(u_3)$ indicates that if vegetative adult survival rate makes a large (or small) contribution to λ, the contribution to N_e will be large (or small) also. Negative correlations imply that large positive contributions to one parameter are associated with small or negative contributions to the other parameter, and *vice versa*. Non-significant correlations among E_{Ne} and E_λ indicate that the contributions of a given vital rate to N_e are not related to those of the same vital rate to λ. The correlations were estimated for each species separately (4 populations for *E. alpinum* and 7 populations for *D. austriacum*) and on the two species together (pooled dataset: 11 populations)

Robustness to model assumptions

The model is based on a number of assumptions and is calibrated using parameters whose estimated values could be different from the actual one. In particular, random recruitment from the seed pool (equation II.3) cannot be fully substantiated by the data obtained, thus σ_k^2 may be an incorrect measure of variation in realized fecundity. To assess whether the actual value of σ_k^2 affected the pattern of parameter elasticity, the elasticity analyses were repeated by setting σ_k^2 to half the value calculated from equation II.3. Similarly, α was estimated from a limited set of dominant molecular markers: to check the robustness of the results to the estimate of α, the elasticity analyses were repeated after halving its value (for *D. austriacum*) or setting it to $\alpha = 0.50$ (for *E. alpinum*).

Finally, I supposed that seed bank dynamics in *D. austriacum* could be disregarded because it was difficult to characterize seed dormancy and quantify germination rates. *In situ* sowing experiment resulted in very low seedling emergence (Nicolè 2005): as a consequence, it was not possible to measure germination rate with precision. In addition, *in situ* experiments make it difficult to separate germination from seed predation and mortality. However, germination rates were higher in greenhouse experiments following seed scarification (Vinciguerra 2002): this suggests that seeds can persist in the soil until the occurrence of favourable conditions for germination. To assess whether the presence of a seed bank could alter the observed elasticity pattern in *D. austriacum*, I developed an alternative version of the matrix model accounting for seed dormancy (Figure III.2) and calibrated it using arbitrary parameter values. Elasticity analyses were repeated using this modified version of the model.

Seed bank model

The modified version of the model includes an additional life-stage 0, representing dormant seeds (Figure III.2). Newly produced seeds survive their first winter in proportion s_w and then germinate at rate γ. The transitions from reproductive adults to seed bank and seedlings are

$$a_{04} = \mu_b \cdot s_w \cdot (1 - \gamma) \qquad \text{(III.2)}$$

$$a_{14} = \mu_b \cdot s_w \cdot \gamma \qquad \text{(III.3)}$$

where μ_b is mean potential fecundity (seed set; Table III.1). Seed survival rate u_0 and germination rate γ also define stasis within the seed bank

$$a_{00} = u_0(1 - \gamma) \qquad \text{(III.4)}$$

and emergence of seedlings

$$a_{10} = u_0 \gamma \qquad \text{(III.5)}$$

Germination rate is the same irrespective of seed age; in particular, newly produced seeds have the same probability of germination as seeds that have spent one or more years in the bank. The number of newly recruited seedlings n_1 is found by adding germination from the seed bank and production of new seedlings by flowering adults:

$$n_{1,t} = a_{10} \cdot n_{0,t-1} + a_{14} \cdot n_{4,t-1} = u_0 \cdot \gamma \cdot n_{0,t-1} + \mu_b \cdot s_w \cdot \gamma \cdot n_{4,t-1} \quad \text{(III.6)}$$

In order to obtain a single s_w value, the variables $n_{0,t-1}$ and $n_{4,t-1}$ were cumulated over years and I refer to them simply as n_0 and n_4, so that equation III.6 can be written as:

$$n_1 = u_0 \cdot \gamma \cdot n_0 + \mu_b \cdot s_w \cdot \gamma \cdot n_4 \quad \text{(III.7)}$$

The first term on the right hand side of equation III.7 represents emergence of seeds from the bank; the second term represents production of new seedlings by reproductive adults. From this relationship, seed winter survival rate s_w can be expressed as

$$s_w = \frac{n_1 - u_0 \cdot \gamma \cdot n_0}{\mu_b \cdot \gamma \cdot n_4} \quad \text{(III.8)}$$

It is evident from equation III.8 that, in order for s_w to be nonnegative, n_0 must satisfy the condition $n_0 < n_1 / (u_0 \cdot \gamma)$: that is, the number of seeds in the bank that germinate, $u_0 \cdot \gamma \cdot n_0$, must not exceed the number of newly recruited seedlings n_1; otherwise, s_w would be forced to take negative values in order to satisfy equation III.7. With this condition specified, the other parameters can be set to arbitrary values. By setting arbitrarily $\gamma = 0.6$, $u_0 = 0.2$ and $n_0 = 0.5 \cdot n_1 / (u_0 \cdot \gamma)$, I derived the new vital rates and matrix elements reported in Table III.4.

Results

Dracocephalum austriacum

Effective size and demographic rates

The effective population size N_e was relatively small in all populations, varying between 24 and 172 (Table III.3). N_e was always lower than the harmonic census size (mean $N_e : N_h = 0.13$) and the number of breeders N_B (mean $N_e : N_B = 0.28$). RE showed the smallest $N_e : N_h$ and $N_e : N_B$ ratios.

The largest survival rates were observed for reproductive adult plants and ranged from 0.85 (VA) to 1.00 (BE; Table III.1 and III.2). No deaths were observed among adult individuals in BE during the seven years of census. RE had the smallest survival rates, the smallest seed set μ_b, the largest

Table III.3: Population size, effective population size, generation time and population growth rate of the seven populations of *Dracocephalum austriacum*. Generation time and effective population size are also given for the modified model considering seed bank dynamics. 90% confidence intervals (CI) for λ were derived by bootstrapping the original dataset.

	BE	CH	ES	LA	RE	ST	VA
N_0	200	980	775	1650	1120	600	1400
λ	1.037 (1.027 − 1.061)	0.998 (0.979 − 1.016)	1.008 (0.998 − 1.028)	0.986 (0.965 − 1.007)	1.003 (0.982 − 1.029)	0.944 (0.917 − 0.967)	0.905 (0.876 − 0.933)
L	30	25	27	25	19	23	18
N_h	338	958	864	1371	1158	277	472
N_B	180	419	516	594	373	104	128
N_e	96	128	172	105	38	24	35
N_a	2899	3253	4672	2652	730	574	631
$N_e : N_h$	0.28	0.13	0.20	0.08	0.03	0.09	0.07
$N_e : N_B$	0.53	0.31	0.33	0.18	0.10	0.23	0.27
L (seed bank)	52	42	45	42	34	45	35
N_e (seed bank)	58	48	77	37	15	3	3

Table III.4: vital rates and matrix elements of the seed bank model for *D. austriacum*. s_w, winter survival rate of newly produced seeds; a_{04}, transition rate from reproductive adults to seed bank; a_{14}, transition rate from reproductive adults to seedlings.

	s_w	a_{04}	a_{14}
BE	0.011	0.058	0.088
CH	0.006	0.079	0.118
ES	0.005	0.049	0.073
LA	0.018	0.133	0.200
RE	0.130	0.358	0.536
ST	0.015	0.078	0.117
VA	0.017	0.152	0.228

fecundity rate μ_k and the largest variance in fecundity σ_k^2. The standardized variances in recruitment σ_k^2 / μ_k were close to unity, except in RE. The standardized variance in potential fecundity σ_b^2 / μ_b was always one order of magnitude larger than σ_k^2 / μ_k. One population was increasing (BE), two were decreasing (ST and VA) and the other four were stable. The two decreasing populations showed also low survival rates. The smallest survival rates were however

observed in RE, where fecundity was remarkably large and allowed λ to be close to unity. As a result of large survival rates, generation time *L* was remarkably large in BE.

Elasticities

The elasticity pattern of N_e presented some differences between populations (Figure III.4). In increasing and in stable populations, the largest contribution to N_e was made by transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and by adult survival rates (u_3 and u_4). In the two decreasing populations, juvenile transitions and juvenile survival rates made the largest contributions (VA) or made contributions similar to adults (ST). Survival rates were associated with larger elasticities than flowering rates and fecundity. The pattern of elasticity of λ was similar across all the seven populations. The largest elasticities were always associated with transitions among adults and with adult survival rates.

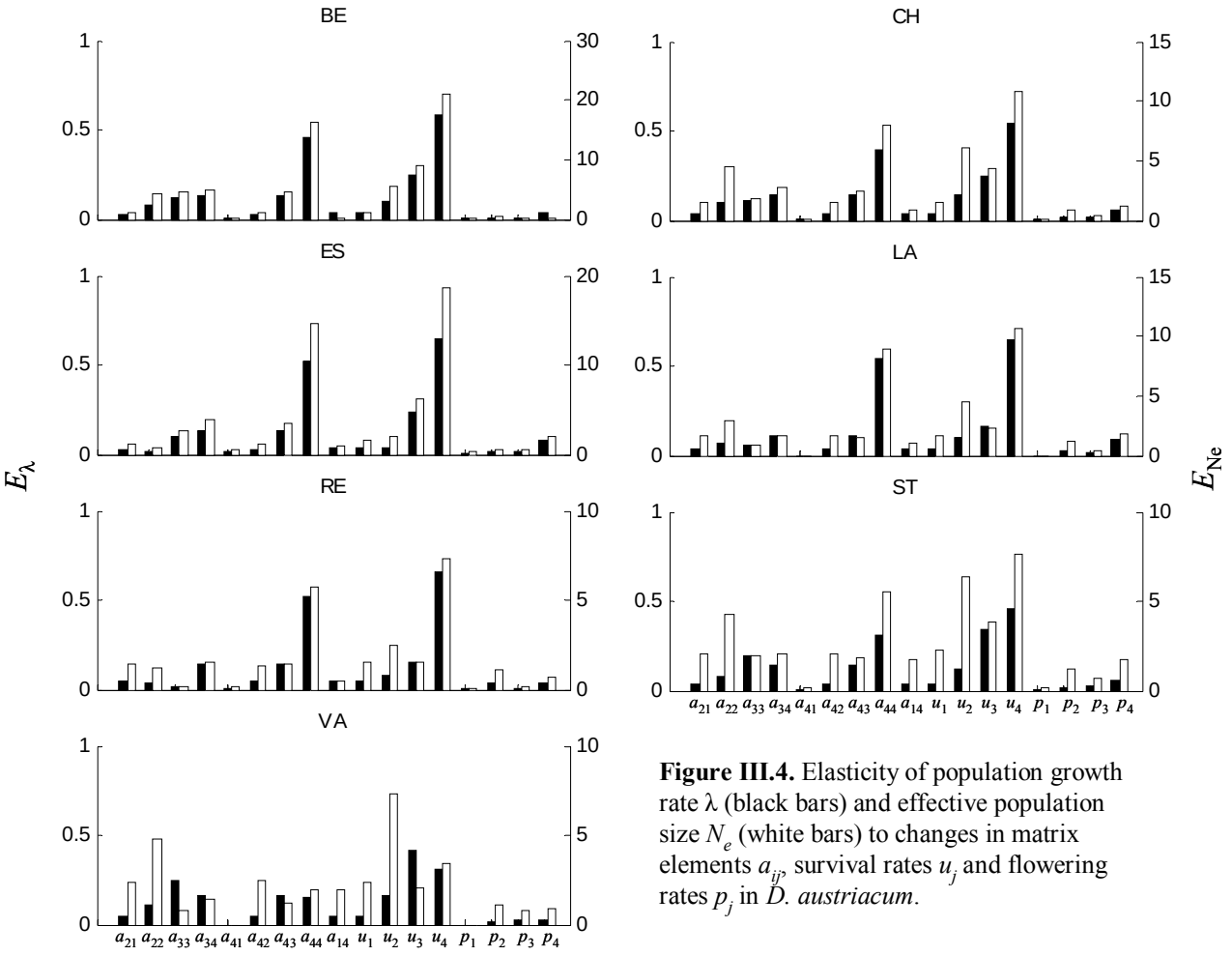


Figure III.4. Elasticity of population growth rate λ (black bars) and effective population size N_e (white bars) to changes in matrix elements a_{ij}, survival rates u_j and flowering rates p_j in *D. austriacum*.

Generation time L exhibited positive and negative elasticities (Figure III.5). Positive elasticities were limited to transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and adult survival rates (u_3 and u_4); these vital rates are the same that were associated with large elasticities of N_e and λ. The other transitions were associated with negative elasticities, meaning that increasing those transitions would shorten the generation time. Survival rates contributed more than flowering rates and fecundity. The elasticity pattern of N_a was similar to that of N_e, as in increasing and stable populations there were large elasticities associated with adult rates while in the two decreasing populations the elasticities associated to juvenile and adults had roughly the same magnitude. In the two decreasing populations, the elasticity of N_a to fecundity was larger than in the other populations.

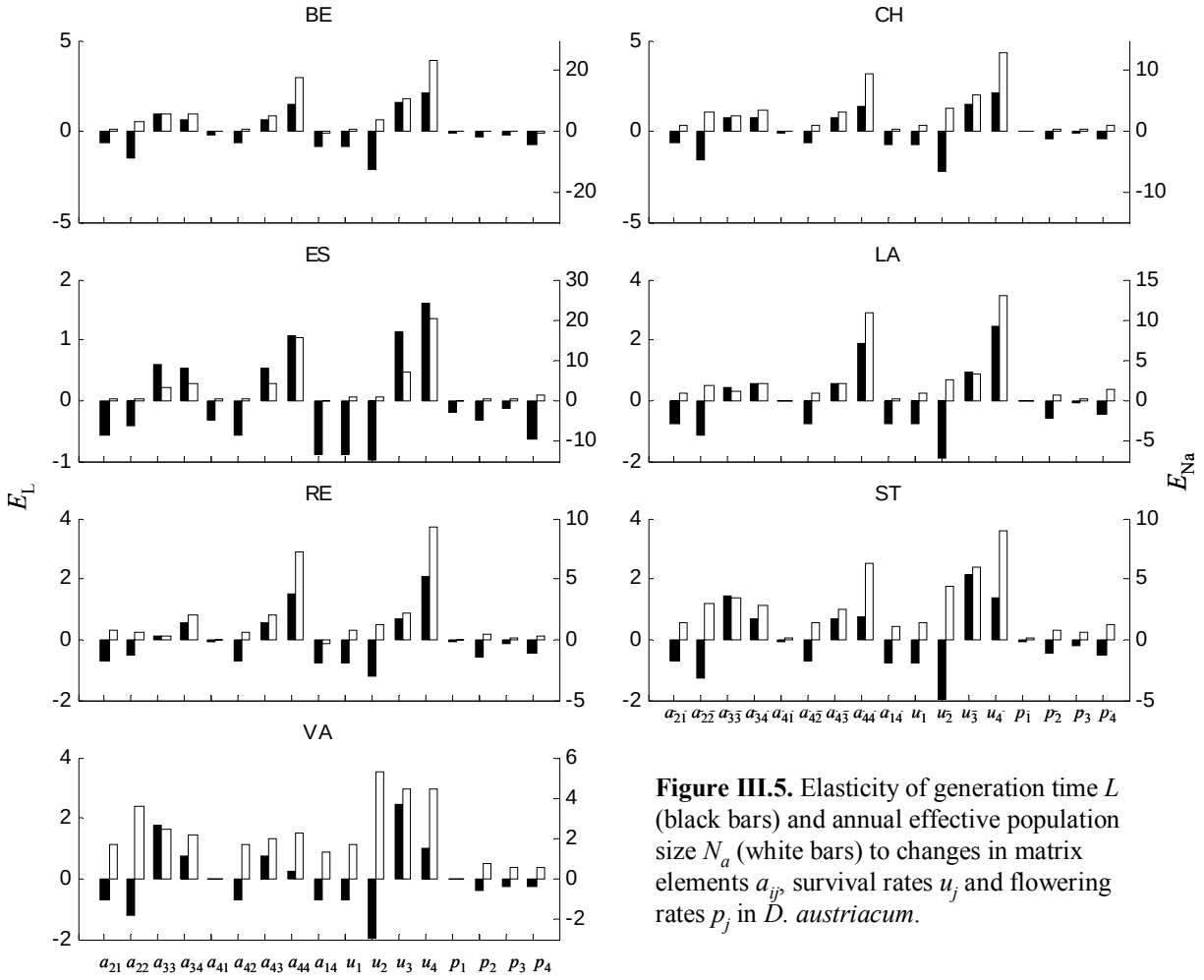


Figure III.5. Elasticity of generation time L (black bars) and annual effective population size N_a (white bars) to changes in matrix elements a_{ij}, survival rates u_j and flowering rates p_j in *D. austriacum*.

There was a positive correlation among the elasticities of N_e and λ, but only for the elasticities to juvenile survival rate u_2 (Table III.5). Significant positive correlation between E_{Ne} and E_{Na} were observed for seedling survival rate u_1, vegetative adult survival rate u_3 and reproductive adult

survival rate u_4. E_λ and E_{Na} were positively correlated, but only for juvenile survival rate u_2. All the other correlations were not significant statistically ($p > 0.05$).

Table III.5. Correlation matrices between the elasticities of λ, N_e, L and N_a to stage-specific survival rates: u_1, seedling survival; u_2, juvenile survival; u_3, vegetative adult survival; u_4, reproductive adult survival. Spearman rank correlation coefficients ($n = 7$ for *D. austriacum*; $n = 4$ for *E. alpinum*; $n = 11$ for the pooled dataset). Values in bold are significant at $p < 0.05$.

	D. austriacum			*E. alpinum*				Pooled dataset			
u_1											
	E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L
E_{Ne}	0.61			E_{Ne}	0.80			E_{Ne}	0.45		
E_L	0.50	0.29		E_L	-0.80	-1.00		E_L	-0.08	**-0.67**	
E_{Na}	0.64	**0.96**	0.50	E_{Na}	-0.80	-1.00	1.00	E_{Na}	-0.15	0.13	0.38
u_2											
	E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L
E_{Ne}	**0.93**			E_{Ne}	0.80			E_{Ne}	0.25		
E_L	-0.64	-0.68		E_L	-0.80	-1.00		E_L	-0.13	**-0.92**	
E_{Na}	**0.93**	1.00	-0.68	E_{Na}	-0.80	-1.00	1.00	E_{Na}	0.23	0.09	0.02
u_3											
	E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L
E_{Ne}	0.21			E_{Ne}	0.80			E_{Ne}	0.37		
E_L	1.00	0.21		E_L	0.40	0.20		E_L	**0.95**	0.35	
E_{Na}	0.46	**0.93**	0.46	E_{Na}	0.80	0.40	0.80	E_{Na}	**0.75**	**0.74**	**0.79**
u_4	E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L		E_λ	E_{Ne}	E_L
E_{Ne}	0.21			E_{Ne}	1.00			E_{Ne}	**0.79**		
E_L	0.57	0.50		E_L	0.40	0.40		E_L	-0.07	-0.17	
E_{Na}	0.50	**0.93**	0.61	E_{Na}	0.40	0.40	1.00	E_{Na}	**0.72**	**0.82**	0.22

Eryngium alpinum

Effective size, population growth rate and demographic rates of the four populations of *E. alpinum* are described in Chapter II.

In DES, PRA and BOU, the largest elasticities of N_e were associated with juvenile survival rate u_2 and stasis within the juvenile stage a_{22} (Figure III.6). DES was increasing (λ = 1.20; Table II.1), PRA was stable (λ = 1.00) and BOU was decreasing (λ = 0.91). In BER, which was practically stable in size (λ = 0.98; Table II.1), the largest elasticities were associated with vegetative adult survival rate u_3 and stasis within the vegetative adult stage a_{33}.

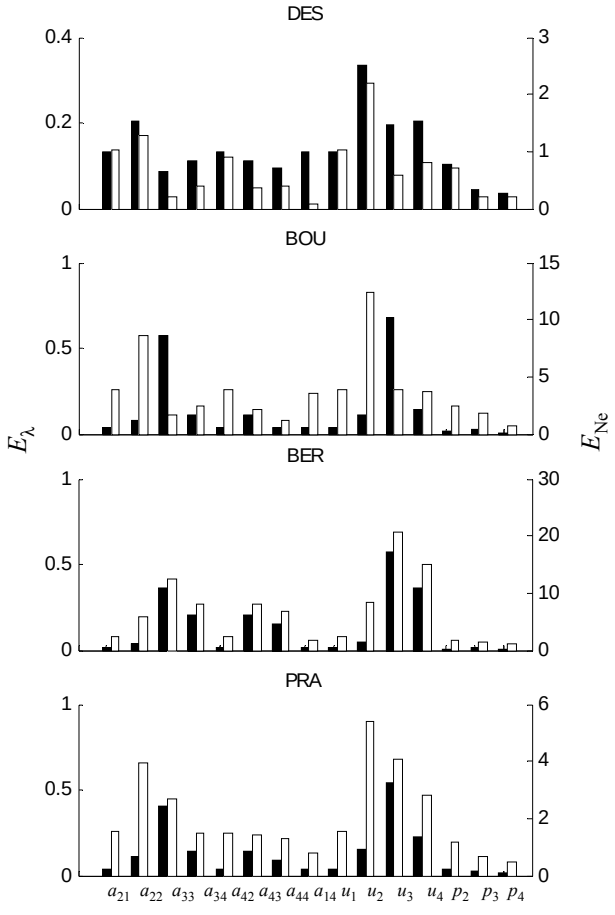


Figure III.6. Elasticity of population growth rate λ (black bars) and effective population size N_e (white bars) to changes in matrix elements a_{ij}, survival rates u_j and flowering rates p_j in *E. alpinum*.

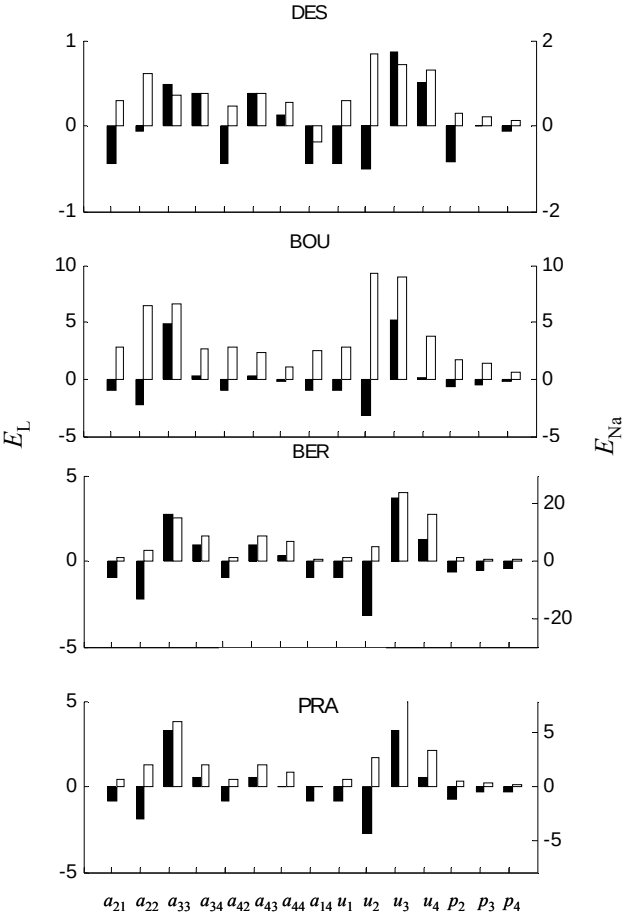


Figure III.7. Elasticity of generation time L (black bars) and annual effective population size N_a (white bars) to changes in matrix elements a_{ij}, survival rates u_j and flowering rates p_j in *E. alpinum*.

In DES, the largest elasticities of λ were associated with juvenile survival rate u_2 and stasis within the juvenile stage a_{22}. In BER, PRA and BOU, the largest elasticities of λ were associated with vegetative adult survival u_3 and stasis within the vegetative adult stage a_{33}. The patterns of elasticities of N_e and λ were similar in DES and BER, but different in PRA and BOU.

Generation time L exhibited positive and negative elasticities (Figure III.7). Positive elasticities were limited to transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and adult survival rates (u_3 and u_4). The other transitions were associated with negative elasticities, meaning that increasing those transitions would shorten the generation time. However, there were some exceptions to this pattern: in BOU, a_{44} was associated with a small negative elasticity; in PRA, a_{44} was associated with a null elasticity.

In BER and PRA, large elasticities of N_a were associated to transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and adult survival rates (u_3 and u_4)(Figure III.7). In the other two populations (DES and BOU), large elasticities of N_a were associated with transitions among adults, adult survival rates, juvenile survival rate u_2 and stasis within the juvenile stage a_{22}. In DES, the elasticity of N_a to fecundity was negative

None of the correlation coefficients among elasticities was significant (Table III.5).

Elasticities on the two species

The correlations between elasticities were then estimated on the two species together (Table III.5). There was a significant positive correlation between E_{Ne} and E_{λ}, but only for reproductive adult survival rate u_4. E_{Ne} was negatively correlated to E_{L} for seedling and juvenile survival rates, u_1 and u_2. The elasticity of annual effective population size N_a was positively correlated to the elasticity of N_e (for u_3 and u_4) and to the elasticity of λ (for u_3 and u_4). For vegetative adult survival u_3, the elasticity of N_a was correlated to that of L.

Discussion

Elasticity of N_e in decreasing, stable and increasing populations

The first objective of this chapter was to understand whether populations with different demographic dynamics and vital rates are also different in the pattern of elasticity of N_e. In the *D. austriacum* dataset, there were two decreasing populations (ST and VA), one increasing population (BE), three stable populations (CH, ES, LA) and one stable population with large fecundity (RE). The observed difference between decreasing populations on one side and stable and increasing

populations on the other side was the elasticity of N_e to juvenile survival rates. The contribution of juvenile survival rates was the largest in declining populations but not in the others. Overall, stable and increasing populations exhibited remarkably similar elasticity patterns. In particular, the pattern of elasticity of N_e in RE (an atypical population, having the largest fecundity and the lowest seedling survival) did not differ from those in other populations. The only difference between this population and the others was the remarkably small N_e:N_h ratio, probably as a consequence of its large realized fecundity μ_k. Large fecundities lead to larger variances in realized fecundity (σ_k^2, Table III.1) and in gene frequency change V (which appears in the demographic calculation of N_e, Equations II.1 and II.4) and thus smaller N_e.

E. alpinum showed similar results. The largest elasticity of N_e in BER (stable) was associated with vegetative adult survival rate and the largest elasticity of N_e in BOU (decreasing) was associated with juvenile survival rate. However, this latter elasticity was also largest in two other populations, one increasing (DES) and one stable (PRA). Therefore large N_e elasticities to juvenile survival cannot be univocally associated with declining populations.

Elasticity of N_e and elasticity of λ

The second objective of this chapter was to compare the patterns of elasticity between N_e and λ. In *D. austriacum,* the elasticities of N_e showed remarkable similarities to the elasticities of λ. The elasticity of N_e to juvenile survival rate was correlated to that of λ; however, the correlations relative to the other stages were not significant. In *E. alpinum*, only two populations out of four (DES and BER) exhibited the same patterns of elasticity for N_e and λ and the correlations between E_{Ne} and E_λ were not significant. These non-significant correlations are almost certainly due to the low statistical power of the Spearman test when performed on only four observations. When the two species were considered jointly and the correlation coefficients were measured on the pooled dataset, there were some significant correlations that were not observed on the separate datasets. In particular, the elasticity of N_e was positively correlated to that of λ, but only for reproductive adult survival rate. These results show that there can be similarities between the elasticity patterns of N_e and λ. However, it is not clear why some populations exhibit similar patterns and some others do not.

Elasticity of L and N_a

The third objective of this chapter was to study the elasticity of generation time L and annual effective size N_a. L exhibited many negative elasticities associated to most transitions and positive elasticities limited to adult survival rates. This makes sense from the definition of L as the mean age of parents, so that increasing adult survival will lengthen the mean time that plants spend in the reproductive stage. Conversely, increasing survival in the pre-breeding stages will increase the proportion of plants entering the reproductive stage relative to the plants that are already in that stage, with a net effect of decreasing the age of breeding individuals. This explains why increasing survival rates of juvenile stages (juveniles and seedlings) shortens the generation time.

The effects of generation time are important in a conservation framework because genetic processes associated with extinction risk are often better measured on a calendar time scale rather than over generations (Allendorf et al. 2008). Loss of genetic variability in time may be relatively slow in populations with small effective size but large generation time. In such a case, reducing N_e may not be critically important from a conservation perspective: if this reduction is associated with a more important increase in generation time, the net effect will be a smaller rate of genetic diversity loss per year. In these cases, the annual effective size N_a could be a more informative parameter, because it gives the rate of drift per year. The elasticities of N_a resembled those of N_e (in *D. austriacum*) or those of L (in *E. alpinum*), but no clear general pattern emerged.

Comparison with other published studies

Two other published studies analysed the effect of stage-specific vital rates on N_e using elasticity or other forms of perturbation analysis (Yonezawa et al. 2000; Campbell and Husband 2005), but they did not compare the elasticities of N_e to those of λ. Yonezawa et al. (2000) studied how variation in vital rates would affect N_e:N_0 in the clonal plant *Fritillaria camschatcensis*. They found that decreasing transition rates among large-sized plants increased N_e:N_0. This effect was attributed to the elimination of aged plants, which reduced the variance in reproductive success: if transition rates among large plants decrease, the number of large plant will decrease also and so will the variation in reproductive success. If transition rates among large plants are increased, the net effect will be an increase in variance in reproductive success, which will lead to reduction in effective size; therefore the relationship between N_e:N_0 and transition rates of large plants is negative. Although Yonezawa et al. (2000) did not use elasticity analysis, this result means that the elasticity

of N_e:N_0 to transition rates among large plants would be negative. This effect was not found in *E. alpinum* or *D. austriacum*, for which the elasticities of N_e were all positive. These results might be due to a different life-history: in *F. camschatcensis*, sexual reproduction was never observed and new seedlings were never found during the study period; reproductive success was driven only by clonal growth. Conversely, neither *D. austriacum* nor *E. alpinum* are capable of clonal growth and reproduction is only possible through seed production.

Campbell and Husband (2005) studied the effect of mode of reproduction and vital rates on N_e in *Hymenoxys herbacea*, a plant capable of both clonal and sexual reproduction. They observed that N_e was more influenced by juvenile than adult survival rates. The opposite was true for *E. alpinum* and *D. austriacum*, which exhibited larger elasticities associated with adult survival rates. In *Hymenoxys herbacea*, juvenile survival rates were larger than adult survival rates (Campbell and Husband 2005). This may result in larger elasticities of N_e to juvenile than to adult survival rates, since elasticities are proportional to the value of the modified parameter.

E. alpinum and *D. austriacum* are more similar in their life-histories, but they exhibited differences in the elasticity patterns. There are only few apparent differences in the demographic parameters of the two species. First, larger variances in realized fecundity σ_k^2 / μ_k in *E. alpinum* (mean σ_k^2 / μ_k = 3.98) than in *D. austriacum* (mean σ_k^2 / μ_k = 1.65); however, the pattern of elasticity is invariant to modest changes in σ_k^2 / μ_k (Figure III.8). Secondly, *E. alpinum* had smaller N_e:N_h ratios than *D. austriacum* (N_e:N_h = 0.043 vs. 0.130); this is a consequence of the larger σ_k^2 / μ_k of *E. alpinum*, and should not have an effect on the elasticity pattern, for the same reason as before. Finally, *E. alpinum* exhibited larger differences among populations than *D. austriacum* in relation to generation time (L is comprised between 7−74 in *E. alpinum* and between 18−30 in *D. austriacum*) and population size (N_0 is comprised between $5.5 \cdot 10^4$ −$2.5 \cdot 10^6$ in *E. alpinum* and between 200−1650 in *D. austriacum*; and N_h is comprised between $9.9 \cdot 10^3$ −$4.6 \cdot 10^6$ in *E. alpinum* and between 277−1371 in *D. austriacum*). It is difficult to make hypothesis on whether differences in generation time can alter the elasticity pattern of N_e. Generation time is determined by all vital rates, so that the differences in elasticity patterns between the two species must be attributed to the whole pattern of vital rates, i.e. the life table or the transition matrix. This leaves open the questions of what demographic character results in a particular elasticity pattern rather than another, and whether such a character exists. The effect of census size may be studied by running the elasticity analysis using a different initial census

size N_0. However, census size may have an effect on the absolute value of elasticities, but not on their relative values.

Implications for conservation

D. austriacum and *E. alpinum* are rare plants that are the object of conservation measures. Identifying which transitions have the greatest impact on population growth is important in a conservation framework, because management actions could be designed to target those life-stages (Caswell 2001; Mills et al. 1999). However, the consequences of management should be evaluated also from a genetic point of view, because some management actions could entail a reduction in genetic diversity or a modification of the genetic composition of the population (Allendorf et al. 2008). As reducing effective population size can increase extinction risk, management should avoid modifications of vital rates that reduce the effective population size.

However, this problem seems to be of minor importance for the populations examined in this chapter. In 9 populations of out 11, the life-stage transitions contributing the most to N_e were the same that contributed the most to λ. This means that if management targets the life-stage contributing the most to population growth rate, this action will also be most effective to increase N_e. This will not be true in the other two populations, because the largest elasticity of N_e was not associated with the vital rate that contributed the most to population growth: these vital rates were associated with moderate elasticities of N_e. These elasticities were however positive, which ensures that increasing those vital rates will not result in smaller effective sizes.

Robustness to model assumptions

Some assumptions or approximations could not be fully validated by the data obtained in the present analysis: random recruitment from the seed pool (equation II.3), the true value of α and, for *D. austriacum*, the absence of a seed bank. However, the pattern of elasticity turned out to be quite robust to violations of these assumptions. A reduction in either σ_k^2 or α did not modify the relative ranking of elasticities (Figure III.8) and the inclusion of a seed bank lead to only minor changes. This means that the effects of vital rates and stage-structure on N_e are relatively independent of other features, namely recruitment success, seed dormancy and deviation of genotype frequencies from Hardy-Weinberg proportions. In order words, the results of elasticity analysis are still valid

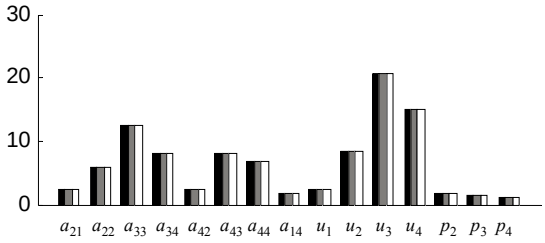


Figure III.8. Robustness of the elasticity pattern of N_e to model assumptions. The graph shows the elasticity of *Ne* in *E. alpinum* (population BER) using the original dataset (black bars); after setting the parameter $\alpha = 0.50$ (grey bars); after setting the parameter σ_k^2 to half of its original value (white bars). See the section "Robustness to model assumptions" for further details.

even if it is difficult to measure the parameters needed to describe these processes with precision. Nonetheless, the study of Yonezawa et al. (2000) on *F. camschatcensis* evidenced some elasticity patterns that were not observed in *D. austriacum* (i.e., negative elasticities) and this discrepancy may be due to a difference in life-history between the two species. This shows that it is always necessary to assess the robustness of results to model assumptions on a case-by-case basis. Future applications of elasticity analysis to other populations and species will need to take this observation into account.

It is important to note that the assumed equivalence between α and F_{IS} is not strictly true. As discussed in Material and Methods, α is the population-level deviation of genotype frequencies from Hardy-Weinberg proportions while F_{IS} is the average individual identity-by-descent probability of alleles. This distinction is of little consequence here, because the pattern of elasticity is not affected by the actual value of α. However, it is important to bear in mind this distinction when applying the model of Yonezawa et al. (2004), because α should be estimated with precision if the objective is to get a reliable estimate of N_e.

Effect of the seed bank on N_e estimation

Even if the pattern of vital rate elasticity was little affected by model assumptions, this does not mean that that N_e and N_a themselves are independent from these processes. Indeed, obtaining correct values for N_e and N_a requires a correct characterisation and estimation of many demographic parameters. This was discussed at length in Chapter II; here, I will add some considerations about the effects of a seed bank.

Dormant seeds act as a reservoir of genetic variability that buffers the population against excessive loss of gene diversity. A similar phenomenon, termed "storage effect", occurs in population with overlapping generations, where non-reproductive classes help the population maintain genetic variability (Ellner and Hairston 1996). Theoretical models confirm this intuition for monocarpic plants, in which the seed bank leads to larger N_e (Nunney 2002; Vitalis et al. 2004).

Estimates of effective population size were smaller and generation time was larger in the model including the seed bank than in the original model (Table III.3). The increase in L can be explained by a longer persistence of individuals in pre-adult stages, namely in the dormant seed stage. The reduction in N_e and in the ratio N_e/ N_B was however not expected. Theoretical models predict that N_e should increase as a result of the storage effect (Nunney 2002; Vitalis et al. 2004). However, these models were developed for monocarpic plants and may not be able to capture more complex dynamics that are typical of perennial iteroparous species. In order to understand the processes leading to changes in N_e, one should develop a model that takes into account iteroparity and stage-structure and that makes explicit the link between seed bank parameters and N_e.

Comparative approaches

The results obtained on *D. austriacum* and *E. alpinum* may be representative of a larger number of species. To explore this possibility, the analyses should be repeated on more species and the results from each species compared to the others. Comparative studies can be used to find general patterns of elasticities across different species and life-histories. Comparative studies have been carried out to analyse the elasticity patterns of λ across a wide variety of plants with different life forms and population dynamics (Silvertown et al. 1996; Franco and Silvertown 2004). Franco and Silvertown (2004) found that the elasticities of λ could be formulated in terms of elasticity to life-cycle components: survival, growth and fecundity. According to the relative magnitude of elasticities of λ to these three components, plant species can be subdivided into five categories: semelparous plants, iteroparous herbs from open habitats, iteroparous forest herbs, shrubs and trees. Each of these categories exhibits a characteristic pattern of elasticity: for instance, trees exhibit large elasticities to survival while semelparous plants present large elasticities to fecundity. A similar comparative approach, but focussed on the elasticities of N_e, could help reveal hidden patterns that were not uncovered in the two species considered here.

Silvertown et al. (1996) also identified that the elasticities of λ to a particular life-cycle component increased or decreased along a continuum that followed the rate of population growth. In *Pedicularis furbishiae* for instance, as λ increases and the population moves from a stable state to an increasing dynamics, the elasticity of λ to growth becomes progressively larger. It would be interesting to see whether similar trends can be found for N_e. The results obtained for *D. austriacum* suggest that the elasticity of N_e to juvenile survival rates becomes larger in decreasing populations.

However, this pattern emerged from only two populations and was not confirmed in *E. alpinum*. To draw more general conclusions, the analyses should be repeated on a greater number of species and populations.

Conclusions

The complexity of demographic models to calculate effective size in species with overlapping generations makes it difficult to study analytically the effects of life-history on N_e, but thanks to numerical elasticity analysis it is possible to tackle this problem empirically. It is also possible to assess whether the elasticity of N_e to a specific vital rate or life-cycle component is positively or negatively correlated to the elasticity of population growth rate; indeed, the demographic model used to calculate N_e is based on the same dataset employed by traditional matrix population models.

The results presented here show however that considerable variation in the elasticity pattern can occur also when considering species with similar life-history, such as *D. austriacum* and *E. alpinum.* Moreover, in *E. alpinum*, different populations present distinct elasticity patterns that do not correspond to variation in population growth rate, as was evidenced in *D. austriacum*. This demonstrates that the patterns of elasticity found in *D. austriacum* cannot be easily generalized. In order to find more general patterns that are common to different species, the elasticity analysis should be extended to a larger sample of plant species presenting different life-histories. This can be done using a comparative approach on a larger number of species and populations.

Chapter IV:

Sensitivity of N_e/N to age-specific vital rates: analytical derivation

Introduction

In the previous chapter, elasticity analysis was applied to empirical datasets to study the effects stage-specific vital rates on N_e. This approach was useful to study the effect of vital rates on genetic drift in a particular species, but did not provide general conclusions. An alternative way to formulate the effects of stage-specific vital rates on N_e is to derive an analytical expression for the sensitivity (or the elasticity) of N_e to stage-specific vital rates. Since the sensitivity is mathematically a derivative, it is in theory possible to find an expression for the change in N_e due to a change in a specific vital rate.

The first step is to choose a demographic model that is general enough to describe the variety of life-histories showed by natural organisms but is tractable from a mathematical point of view. The model of Felsenstein (1971) allows for variation in fecundity between ages and includes a formulation applicable to growing populations. It can thus be used to predict N_e given the life-table of the population, regardless of the particular forms of the survivorship and fecundity curves. In addition, the expression defining N_e is phrased in terms of survival rates and fecundity rates, therefore the link between effective size and these quantities is explicit. Finally, that of Felsenstein (1971) was one of the first models to derive N_e in populations with overlapping generations and constitutes an historical contribution to the topic [two other models were published before (Kimura and Crow 1963; Nei and Imazumi 1966), but their formulation was either incorrect or vague, as discussed by Felsenstein (1971)].

In this chapter, I derived an analytical expression for the sensitivity of N_e/N to survival and fecundity rates. Mathematically, the sensitivity is the derivative of N_e/N to a single vital rate (survival or fecundity rate). In order to derive the sensitivity of N_e/N, I had to first calculate the sensitivities of lower level biological parameters: the proportion of newborns w_1, generation time L, reproductive value v_k and the parameter K, which appears in the equation defining N_e but has no strict biological meaning (Felsenstein 1971).The derived expression was however too complex to be interpreted.

I then used some empirical life-tables as case studies. Since it was too complex to understand the role of survival and fecundity rates in determining the sensitivity of N_e/N, the purpose of using empirical life-tables was to search for general pattern. I particular, the derived expression can be formulated as the sum of three terms, each containing the effect of one biological parameters on the

sensitivity of N_e/N: the proportion of newborn w_1, generation time L and the parameter K. The sensitivity of N_e/N was then decomposed into these three components, to see whether some parameters had larger effects than others. Three life-tables were chosen as example: a human, a bird and an invertebrate life table.

Definitions

Consider a population structured by age, where σ_i is the survival probability from age i to age $i + 1$, b_i is the fecundity of individuals of age i (known also as the age-specific birth rate) and n denotes the number of age-classes. The life table of such a population can be represented in a matrix form (Caswell 2001):

$$\mathbf{A} = \begin{bmatrix} b_1 & b_2 & \cdots & b_{n-1} & b_n \\ \sigma_1 & 0 & \cdots & 0 & 0 \\ 0 & \sigma_2 & \cdots & 0 & 0 \\ \vdots & \vdots & \cdots & 0 & 0 \\ 0 & 0 & \cdots & \sigma_{n-1} & 0 \end{bmatrix} \qquad \text{(IV.1)}$$

The (i,j) entries of the transitions matrix can be indicated by a_{ij}; survival rates correspond to $a_{j+1,j} = \sigma_j$ and fecundity to $a_{1j} = b_j$.

l_i is defined as the probability of survival to age i :

$$l_i = \prod_{m=1}^{i-1} \sigma_m \qquad \text{(IV.2)}$$

Population growth rate λ corresponds to the dominant eigenvalue of the transition matrix **A.** When the population reaches an asymptotic state, the age structure is stable in time. The proportion of individuals of age k is then given by

$$w_k = \frac{l_k \lambda^{n-k}}{\sum_{m=0}^{n-1} l_{n-m} \lambda^m} \qquad \text{(IV.3)}$$

Fisher (1930) defined the reproductive value of an individual as the contribution of offspring to the next generations. In age-structured populations, the reproductive value of an individual of age k is given by (Caswell, 2001, pag. 94):

$$v_k = \sum_{m=k}^{n} b_m \frac{l_m \lambda^{k-m-1}}{l_k} \qquad \text{(IV.4)}$$

The generation time L is defined as the mean age of parents at the stable age distribution (Felsenstein, 1971; Caswell, 2001, eq. 5.77):

$$L = \sum_{k=1}^{n} k l_k b_k \lambda^{-k} \qquad \text{(IV.5)}$$

Finally, the formulation derived by Felsenstein (1971) for variance effective size in a population with overlapping generations is:

$$N_e = \frac{LN_1}{1 + \sum_{k=1}^{n} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \lambda^{-k}} = \frac{Lw_1 N}{1+K} = \frac{V}{1+K} \qquad \text{(IV.6a)}$$

where $N_1 = w_1 N$ is the number of newborns entering the population each year and

$$K = \sum_{k=1}^{n} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \lambda^{-k}$$

is "roughly the probability of death of an individual while it still has reproductive value" (Felsenstein 1971). However, this parameter is not strictly a probability because it can take values larger than one. The numerator of Equation IV.6a is also the total reproductive value of the population, V (Felsenstein 1971). The ratio of effective to census size N_e/N is then expressed as a function of three parameters:

$$\frac{N_e}{N} = \frac{Lw_1}{1+K} \qquad \text{(IV.6b)}$$

that is, the proportion of newborns w_1, the generation time L and the parameter K.

Derivation of sensitivities

The effect of changes in any a_{ij} on N_e can be studied by taking the derivative of N_e/N:

$$\frac{\partial \frac{N_e}{N}}{\partial a_{ij}} = \frac{\frac{\partial w_1}{\partial a_{ij}} L}{(1+K)} + \frac{w_1 \frac{\partial L}{\partial a_{ij}}}{(1+K)} - \frac{w_1 L \frac{\partial K}{\partial a_{ij}}}{(1+K)^2} \qquad \text{(IV.7)}$$

This expression contains the differentials of w_1, L and K, which are derived in the following sections. The differential of each quantity corresponds to the sensitivity to a generic vital rate a_{ij}. In order to derive the differential of K, one must first derive the differential of reproductive value v_k.

Wennergren (1994) and Caswell (1980; 2001) provide analytical methods that allow for the calculation of the sensitivities of v_k and w_k as linear functions of the entire set of eigenvectors associated to the transition matrix. Their method is very versatile and applicable to both age- and stage-classified matrices. However, the interpretation of the differential as a combination of eigenvectors is not straightforward, because the eigenvectors can often take complex magnitude. Moreover, the subdominant eigenvectors do not reflect any real biological trait. The sensitivities of of v_k and w_k , therefore, will be derived following a different approach.

In general, the differential of a given parameter can be referred to a survival rate or a fecundity rate, with different mathematical expressions in the two cases. In the following, I will derive the sensitivities to survival and fecundity rates separately.

There are some expressions that will be useful for the derivation of the various differentials. First, the sensitivity of λ to a generic entry of the transition matrix is (Caswell, 1978; 1980):

$$\frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{\sum_{m=1}^{n} v_m w_m} \qquad \text{(IV.8)}$$

where v_i and w_i are the elements of the reproductive value and the stable age structure vector, respectively. When the sensitivity of λ is evaluated relative to a survival rate ($a_{ij} = \sigma_j$, with $i = j + 1$), Equation IV.8 becomes

$$\frac{\partial \lambda}{\partial \sigma_j} = S_\lambda(\sigma_j) = \frac{v_{j+1} w_j}{\sum_{m=1}^{n} v_m w_m} \tag{IV.9}$$

and when a_{ij} represents a fecundity rate ($a_{ij} = b_j$, with $i = 1$), Equation 4.8 becomes

$$\frac{\partial \lambda}{\partial b_j} = S_\lambda(b_j) = \frac{v_1 w_j}{\sum_{m=1}^{n} v_m w_m} = \frac{w_j}{\sum_{m=1}^{n} v_m w_m} \tag{IV.10}$$

because the reproductive value vector is scaled so that $v_1 = 1$.

Similarly, the differential of l_k (the probability of a newborn to survive to age k) takes different forms depending on whether a_{ij} represents a survival or a fecundity rate. The differential of l_k to a survival rate is null when the survival rate in question is specific to any age after k:

$$\frac{\partial l_k}{\partial \sigma_j} = \begin{cases} 0 & for\ k < j+1 \\ \dfrac{l_k}{\sigma_j} & for\ k \geq j+1 \end{cases} \tag{IV.11}$$

and the differential of l_k to a fecundity rate is zero, because l_k does not depend on fecundity.

Sensitivity of reproductive value v_k

Sensitivity to survival rates

When a_{ij} represents a survival rate ($a_{ij} = \sigma_j$), the b_k's can be considered as constants and taking the differential of both sides of Equation IV.4 gives:

$$\begin{aligned} \frac{\partial v_k}{\partial \sigma_j} &= \sum_{m=k}^{n} b_m \frac{\partial}{\partial \sigma_j}\left(\frac{l_m \lambda^{k-m-1}}{l_k}\right) = \sum_{m=k}^{n} \frac{b_m}{l_k^2}\left[\frac{\partial\left(l_m \lambda^{k-m-1}\right)}{\partial \sigma_j} l_k - \left(l_m \lambda^{k-m-1}\right)\frac{\partial l_k}{\partial \sigma_j}\right] \\ &= \sum_{m=k}^{n} b_m \frac{\dfrac{\partial l_m}{\partial \sigma_j}\lambda^{k-m-1} l_k}{l_k^2} + \sum_{m=k}^{n} b_m \frac{l_m \dfrac{\partial \lambda^{k-m-1}}{\partial \sigma_j} l_k}{l_k^2} - \sum_{m=k}^{n} b_m \frac{l_m \lambda^{k-m-1}\dfrac{\partial l_k}{\partial \sigma_j}}{l_k^2} \end{aligned} \tag{IV.12}$$

The differential of v_k thus involves three terms, which are function of the differential of l_k, λ^{k-m-1} and l_m. The differential of λ^{k-m-1} is given by

$$\frac{\partial\left(\lambda^{k-m-1}\right)}{\partial\sigma_j} = (k-m-1)\lambda^{k-m-2}\frac{\partial\lambda}{\partial\sigma_j} = (k-m-1)\lambda^{k-m-2}S_\lambda(\sigma_j) \qquad \text{(IV.13)}$$

After substituting Equations IV.9 (the sensitivity of λ), IV.11 (the sensitivity of l_k) and IV.13 in Equation IV.12 and simplifying, one obtains:

$$\frac{\partial v_k}{\partial\sigma_j} = \sum_{m=j+1}^{n} b_m \frac{l_m\lambda^{k-m-1}}{\sigma_j l_k} + \sum_{m=k}^{n} b_m \frac{l_m(k-m-1)\lambda^{k-m-2}S_\lambda(\sigma_j)}{l_k} \quad \text{for } k < j+1 \qquad \text{(IV.14)}$$

and

$$\frac{\partial v_k}{\partial\sigma_j} = \sum_{m=k}^{n} b_m \frac{l_m(k-m-1)\lambda^{k-m-2}S_\lambda(\sigma_j)}{l_k} \qquad \text{for } k \geq j+1 \qquad \text{(IV.15)}$$

Sensitivity to fecundity rates

Now consider the case where a_{ij} represents a fecundity rate ($a_{1j} = b_j$). The l_m and l_k terms in Equation IV.4 can be considered as constants and the differential of reproductive value can be written as:

$$\frac{\partial v_k}{\partial b_j} = \sum_{m=k}^{n}\frac{l_m}{l_k}\left[\frac{\partial b_m}{\partial b_j}\lambda^{k-m-1} + b_m\frac{\partial\lambda^{k-m-1}}{\partial b_j}\right] = \sum_{m=k}^{n}\frac{l_m}{l_k}\frac{\partial b_m}{\partial b_j}\lambda^{k-m-1} + \sum_{m=k}^{n}\frac{l_m}{l_k}b_m\frac{\partial\lambda^{k-m-1}}{\partial b_j} \qquad \text{(IV.16)}$$

The first term in the right-hand side of expression IV.16 contains the differential of b_m. Since fecundities at different ages are supposed to be independent, their derivatives are simply:

$$\frac{\partial b_m}{\partial b_j} = \begin{cases} 1 & \text{for } m = j \\ 0 & \text{otherwise} \end{cases} \qquad \text{(IV.17)}$$

The differential of λ^{k-m-1} to a fecundity rate takes a form that is similar to Equation IV.13. After substituting and simplifying, one obtains

$$\frac{\partial v_k}{\partial b_j} = \frac{l_j}{l_k}\lambda^{k-j-1} + \sum_{m=k}^{n}\frac{l_m}{l_k}b_m(k-m-1)\lambda^{k-m-2}S_\lambda(b_j) \qquad \text{for } k < j+1 \qquad \text{(IV.18)}$$

and

$$\frac{\partial v_k}{\partial b_j} = \sum_{m=k}^{n} \frac{l_m}{l_k} b_m (k-m-1)\lambda^{k-m-2} S_\lambda(b_j) \qquad \text{for } k \geq j+1 \qquad \text{(IV.19)}$$

Sensitivity of proportion of newborns w_1

Sensitivity to survival rates

When a_{ij} represents a survival rate ($a_{ij} = \sigma_j$), the sensitivity of the proportion of individuals in stage k (w_k, Equation IV.3) can be written as:

$$\frac{\partial w_k}{\partial \sigma_j} = \frac{\partial l_k}{\partial \sigma_j} \frac{\lambda^{n-k}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} + \frac{\partial \lambda^{n-k}}{\partial \sigma_j} \frac{l_k}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\partial \left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]}{\partial \sigma_j} \frac{l_k \lambda^{n-k}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2} \qquad \text{(IV.20)}$$

The derivatives of l_k and λ^{n-k} that appear in the first two terms are found by IV.11 and IV.9. The third term in the right-hand side of Equation IV.20 requires the calculation of a differential that can be carried out as follows:

$$\frac{\partial \left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]}{\partial \sigma_j} = \sum_{m=0}^{n-1} \frac{\partial l_{n-m}\lambda^m}{\partial \sigma_j} = \sum_{m=0}^{n-1} \frac{\partial l_{n-m}}{\partial \sigma_j}\lambda^m + l_{n-m} m \lambda^{m-1} \frac{\partial \lambda}{\partial \sigma_j} \qquad \text{(IV.21)}$$

The first term on the right-hand side of expression IV.21 contains the differential of $l_{\text{n-m}}$, which is:

$$\frac{\partial l_{n-m}}{\partial \sigma_j} = \begin{cases} 0 & for \quad n-m < j+1 \\ \dfrac{l_{n-m}}{\sigma_j} & for \quad n-m \geq j+1 \end{cases} \qquad \text{(IV.22)}$$

Substituting IV.22 in IV.21 give us:

$$\sum_{m=0}^{n-1} \frac{\partial l_{n-m}\lambda^m}{\partial \sigma_j} = \sum_{m=0}^{n-j-1} \left[\frac{l_{n-m}}{\sigma_j}\lambda^m + l_{n-m} m \lambda^{m-1} \frac{\partial \lambda}{\partial \sigma_j} \right] + \sum_{m=n-j}^{n-1} l_{n-m} m \lambda^{m-1} \frac{\partial \lambda}{\partial \sigma_j} \qquad \text{(IV.23)}$$

Substituting Equations IV.9 (the sensitivity of λ), IV.11 (the sensitivity of l_k) and IV.23 in IV.20 and noting that $l_1 = 1$ by definition, the sensitivity of the proportion of newborns to a survival rate is

$$\frac{\partial w_1}{\partial \sigma_j} = \frac{(n-1)\frac{\partial \lambda}{\partial \sigma_j}\lambda^{n-2}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\left\{\sum_{m=0}^{n-j-1}\left[\frac{l_{n-m}}{\sigma_j}\lambda^m + l_{n-m}m\lambda^{m-1}\frac{\partial \lambda}{\partial \sigma_j}\right] + \sum_{m=n-j}^{n-1} l_{n-m}m\lambda^{m-1}\frac{\partial \lambda}{\partial \sigma_j}\right\}\lambda^{n-1}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2}$$

$$= \frac{(n-1)\frac{\partial \lambda}{\partial \sigma_j}\lambda^{n-2}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\left\{\sum_{m=0}^{n-j-1}\frac{l_{n-m}}{\sigma_j}\lambda^m + \frac{\partial \lambda}{\partial \sigma_j}\sum_{m=0}^{n-1} l_{n-m}m\lambda^{m-1}\right\}\lambda^{n-1}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2} \qquad \text{(IV.24)}$$

$$= \frac{(n-1)\frac{\partial \lambda}{\partial \sigma_j}\lambda^{n-2}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\left[\sum_{m=0}^{n-j-1}\frac{l_{n-m}}{\sigma_j}\lambda^m\right]\lambda^{n-1}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2} - \frac{\left[\frac{\partial \lambda}{\partial \sigma_j}\sum_{m=0}^{n-1} l_{n-m}m\lambda^{m-1}\right]\lambda^{n-1}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2}$$

Sensitivity to fecundity rates

When a_{ij} represents a fecundity rate ($a_{ij} = b_j$), the sensitivity of the proportion of individuals in stage k (w_k, Equation IV.3) can be written as:

$$\frac{\partial w_k}{\partial b_j} = \frac{\partial l_k}{\partial b_j}\frac{\lambda^{n-k}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} + \frac{\partial \lambda^{n-k}}{\partial b_j}\frac{l_k}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\partial\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]}{\partial b_j}\frac{l_k\lambda^{n-k}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2} \qquad \text{(IV.24)}$$

The first term on the right-hand side of Equation IV.24 is zero, because the sensitivity of l_k to b_j is zero (survival and fecundity rates are assumed to be independent). The second term is found from expression IV.10. The third term of expression requires the calculation of a differential that can be carried out as follows:

$$\frac{\partial\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]}{\partial b_j} = \sum_{m=0}^{n-1}\frac{\partial l_{n-m}\lambda^m}{\partial b_j} = \sum_{m=0}^{n-1}\frac{\partial l_{n-m}}{\partial b_j}\lambda^m + l_{n-m}m\lambda^{m-1}\frac{\partial \lambda}{\partial b_j} \qquad \text{(IV.25)}$$

$$= \sum_{m=0}^{n-1} l_{n-m}m\lambda^{m-1}\frac{\partial \lambda}{\partial b_j}$$

because l_k is independent of b_j. Substituting Equations IV.10 (the sensitivity of λ) and IV.25 in Equation IV.24 and noting that $l_1 = 1$ by definition, the sensitivity of the proportion of newborns to a fecundity rate is

$$\frac{\partial w_1}{\partial b_j} = \frac{(n-1)\frac{\partial \lambda}{\partial b_j}\lambda^{n-2}}{\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{\left[\sum_{m=0}^{n-1} l_{n-m} m\lambda^{m-1}\frac{\partial \lambda}{\partial b_j}\right]\lambda^{n-1}}{\left[\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right]^2} \qquad \text{(IV.26)}$$

Sensitivity of generation time *L*

Sensitivity to survival rates

When the sensitivity of generation time L (Equation IV.5) is evaluated with respect to a survival rate ($a_{ij} = \sigma_j$), the fecundity rates b_k's can be considered as constants:

$$\frac{\partial L}{\partial \sigma_j} = \sum_{k=1}^{n} k\frac{\partial l_k}{\partial \sigma_j} b_k \lambda^{-k} + \sum_{k=1}^{n} k l_k b_k (-k)\lambda^{-k-1}\frac{\partial \lambda}{\partial \sigma_j} \qquad \text{(IV.27)}$$

Recalling that the sensitivity of l_k to σ_j is null when $k < j + 1$ (Equation IV.11) it follows that:

$$\frac{\partial L}{\partial \sigma_j} = \sum_{k=j+1}^{n} k\frac{l_k}{\sigma_j} b_k \lambda^{-k} + \sum_{k=1}^{n} k l_k b_k (-k)\lambda^{-k-1}\frac{\partial \lambda}{\partial \sigma_j} \qquad \text{(IV.28)}$$

The first term in the right hand side of Equation IV.28 is always positive, while the second term is always negative. The sign of the sensitivity of L will therefore depend on the relative magnitude of the two terms.

Sensitivity to fecundity rates

When the sensitivity of L is evaluated with respect to a fecundity rate ($a_{ij} = b_j$), the l_k's can be considered as constants:

$$\frac{\partial L}{\partial b_j} = \sum_{k=1}^{n} k l_k \frac{\partial b_k}{\partial b_j}\lambda^{-k} + \sum_{k=1}^{n} k l_k b_k (-k)\lambda^{-k-1}\frac{\partial \lambda}{\partial b_j} \qquad \text{(IV.29)}$$

The sensitivity of b_k to b_j is null whenever $k \neq j$, otherwise is unity (Equation IV.17); thus

$$\frac{\partial L}{\partial b_j} = j l_j \lambda^{-j} + \sum_{k=1}^{n} k l_k b_k (-k) \lambda^{-k-1} \frac{\partial \lambda}{\partial b_j} \tag{IV.30}$$

The first term in the right hand side of this equation is always positive, while the second term is always negative. The sign of the sensitivity of L will therefore depend on the relative magnitude of the two terms.

Sensitivity of *K*

Sensitivity to survival rates

The sensitivity of K to a survival rate σ_j is:

$$\frac{\partial K}{\partial \sigma_j} = \frac{\partial}{\partial \sigma_j}\left(\sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \lambda^{-k} \right) = \sum_{k=1}^{n-1} \frac{\partial \left(l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \lambda^{-k} \right)}{\partial \sigma_j} \tag{IV.31}$$

After rewriting $dv^2_k = 2\, v_k \; dv_k$ and separating the summations:

$$\begin{aligned} \frac{\partial K}{\partial \sigma_j} = & \sum_{k=1}^{n-1} \frac{\partial l_k}{\partial \sigma_j} \sigma_k (1-\sigma_k) v_{k+1}^2 + \sum_{k=1}^{n-1} l_k \frac{\partial \sigma_k}{\partial \sigma_j} (1-\sigma_k) v_{k+1}^2 + \\ & + \sum_{k=1}^{n-1} l_k \sigma_k \frac{\partial (1-\sigma_k)}{\partial \sigma_j} v_{k+1}^2 + \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) 2 v_{k+1} \frac{\partial v_{k+1}}{\partial \sigma_j} + \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \frac{\partial \lambda^{-k}}{\partial \sigma_j} \end{aligned} \tag{IV.32}$$

The right hand side of equation IV.32 contains four terms, each of which can be simplified or developed as explained hereafter. The first term contains the sensitivity of l_k; the sensitivities of the l_k's are zeros for $k < j + 1$ (Equation IV.11), so the summation in the first term of IV.32 will be restricted to values of $k \geq j + 1$:

$$\sum_{k=1}^{n-1} \frac{\partial l_k}{\partial \sigma_j} \sigma_k (1-\sigma_k) v_{k+1}^2 = \sum_{k=j+1}^{n-1} \frac{l_k}{\sigma_j} \sigma_k (1-\sigma_k) v_{k+1}^2 \tag{IV.33}$$

The second and third terms in the right hand side of equation IV.32 contain the sensitivity of σ_k. Since the σ_k's are independent, it follows that:

$$\frac{\partial \sigma_k}{\partial \sigma_j} = \begin{cases} 0 & for\ k \neq j \\ 1 & for\ k = j \end{cases} \tag{IV.34}$$

The second and third terms in equation IV.32 will thus simplify to:

$$\sum_{k=1}^{n-1} l_k \frac{\partial \sigma_k}{\partial \sigma_j}(1-\sigma_k)v_{k+1}^2 = l_j(1-\sigma_j)v_{j+1}^2 \tag{IV.35}$$

and

$$\sum_{k=1}^{n-1} l_k \sigma_k \frac{\partial (1-\sigma_k)}{\partial \sigma_j} v_{k+1}^2 = -l_j \sigma_j v_{j+1}^2 \tag{IV.36}$$

The fourth term in the right-hand side of equation IV.32 contains the sensitivity of reproductive value. Putting together the results of Equations IV.9, IV.14, IV.33, IV.35 and IV.36, one obtains:

$$\begin{aligned} \frac{\partial K}{\partial \sigma_j} = & \sum_{k=j+1}^{n-1} \frac{l_k}{\sigma_j} \sigma_k (1-\sigma_k) v_{k+1}^2 \lambda^{-k} + l_j v_{j+1}^2 (1-2\sigma_j)\lambda^{-j} + \\ & + \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) 2 v_{k+1} \frac{\partial v_{k+1}}{\partial \sigma_j} \lambda^{-k} + \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 (-k) \lambda^{-k-1} \frac{\partial \lambda}{\partial \sigma_j} \end{aligned} \tag{IV.37}$$

The first term on the right-hand side of Equation IV.37 represents the contribution of the sensitivity of l_k and is always positive. The second term represents the contribution of the sensitivity of σ_k and it is positive if $\sigma_j < 0.5$, null if $\sigma_j = 0.5$ and negative if $\sigma_j > 0.5$. The third and fourth terms represent the contribution of the sensitivity of reproductive value v_k and are always negative. The overall sign of the sensitivity of K will thus depend on the relative magnitude of negative and positive terms.

Sensitivity to fecundity rates

When the sensitivity of K is evaluated with respect to a fecundity rate ($a_{ij} = b_j$), the l_k's and σ_k's can be considered as constants. The differential of K can be written as follows:

$$\frac{\partial K}{\partial b_j} = \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) 2 v_{k+1} \frac{\partial v_{k+1}}{\partial b_j} \lambda^{-k} + \sum_{k=1}^{n-1} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 \frac{\partial \lambda^{-k}}{\partial b_j} \tag{IV.38}$$

After substituting the sensitivity of v_k in equation (IV.38), one obtains:

$$\frac{\partial K}{\partial b_j} = \sum_{k=1}^{j-1} l_k \sigma_k (1-\sigma_k) 2 v_{k+1} \left[\frac{l_j}{l_{k+1}} \lambda^{k-j} + \sum_{m=k+1}^{n} \frac{l_m}{l_{k+1}} b_m (k-m) \lambda^{k-m-1} S_\lambda(b_j) \right] + \\ + \sum_{k=j}^{n-1} l_k \sigma_k (1-\sigma_k) 2 v_{k+1} \left[\sum_{m=k+1}^{n} \frac{l_m}{l_{k+1}} b_m (k-m) \lambda^{k-m-1} S_\lambda(b_j) \right] + \sum_{k=j}^{n-1} l_k \sigma_k (1-\sigma_k) v_{k+1}^2 (-k) \lambda^{-k-1} \frac{\partial \lambda}{\partial b_j} \quad \text{(IV.39)}$$

The last two terms in the right-hand side of Equation IV.39 are negative. The sign of the sensitivity of K to a fecundity rate depends on the relative magnitude of the first term *vs* the second and third term.

Putting all together

Substituting terms in Equation IV.7 for the sensitivities derived in the previous sections produces a complex expression that is not convenient to interpret. I tried simplifying the expression in Mathematica 5.1 (Wolfram 2003) but the resulting expressions are still too complex to be interpreted in terms of biological parameters. For instance, the sensitivity of N_e/N to σ_1 in a population with $n = 5$ age-classes turned out to be:

$$\frac{\partial \frac{N_e}{N}}{\partial \sigma_1} = \frac{\left(b_3\lambda^2 + \sigma_3 b_4 \lambda + \sigma_3\sigma_4 b_5\right)\lambda^4}{(1+K)\frac{\partial \lambda}{\partial \sigma_1}\sum_{m=0}^{n-1} l_{n-m}\lambda^m} - \frac{v_2\lambda^4\left(\sigma_1\sigma_3\sigma_4 + \sigma_1\sigma_3\lambda + \sigma_1\lambda^2\right)}{\left(\sum_{m=0}^{n-1} l_{n-m}\lambda^m\right)^2 (1+K)\frac{\partial \lambda}{\partial \sigma_1}} +$$

$$+ \frac{v_2\lambda^4}{(1+K)^2 \frac{\partial \lambda}{\partial \sigma_1}\sum_{m=0}^{n-1} l_{n-m}\lambda^m}\left[-2\sigma_1(1-\sigma_1)v_2\left(b_3\lambda^{-3} + \sigma_3 b_4\lambda^{-4} + \sigma_3\sigma_4 b_5\lambda^{-5}\right) - \sigma_1(1-\sigma_1)v_3^2\lambda^{-2} + \sigma_1\sigma_2 v_3^2\lambda^{-2}\right.$$

$$\left. - \sigma_1\sigma_3(1-\sigma_3)v_4^2\lambda^{-3} - \sigma_1\sigma_3\sigma_4(1-\sigma_4)b_5^2\lambda^{-6}\right] - \frac{v_2\lambda^4 \frac{\partial^2 \lambda}{\partial \sigma_1^2}}{(1+K)^2\left(\frac{\partial \lambda}{\partial \sigma_1}\right)^2 \sum_{m=0}^{n-1} l_{n-m}\lambda^m}$$

Since such an expression is difficult to interpret in biological terms, the problem of determining the effects of age-specific vital rates on N_e was tackled with a different strategy. I went back to Equation IV.7, which expresses the sensitivity of N_e/N in terms of the sensitivities of w_1, L and K. I tried to study the sensitivity of N_e/N by examining the relative value of these three terms by means of the life-tables of three organisms that served as examples.

Illustration with three life tables

Life tables

The three life tables chosen as examples are (Table IV.1): i) the human life-table reported in Felsenstein (1971); ii) the life table of the white-crowned sparrow *Zonotrichia leucophrys nuttalli* ("sparrow" in the following; Baker et al. 1981); and iii) the life table of barnacle *Balanus glandula* (Connell 1970). These species are interesting to analyse because they differ in the shape of the survivorship curve (Table IV.1). Humans have a Type I survivorship curve , in which survival rates decrease with age; sparrows have approximately a Type II survivorship curve, in which survival is roughly constant with age (actually, age-class one shows a lower survival rate than the adult classes); barnacles have a Type III survivorship curve, in which survival rates in the first age is extremely small. It has been proposed that the shape of the survivorship curve may be associated to the ratio of effective to census size N_e/N (Hedrick 2005).

In the human life-table, individuals are classified into five-year age classes, therefore age class one comprises individuals of 0 to 4 years of age, class two comprise individuals of 5 to 9 years of age, and so on. Conversely, the life-tables of sparrow and barnacles are formulated in the standard way where each age class corresponds to one year of age. In addition, the human life-table does not comprise post-reproductive classes, as these do not contribute to N_e.

Demography and N_e

Differences in survival and fecundities lead to differences in stable age distribution and reproductive value. Individuals are distributed evenly in the human life table while the first age class dominates the distribution in the sparrow and the barnacle (Table IV.1). As a result, the proportion of newborn is larger in sparrows and barnacles than in humans (Table IV.2). Individual reproductive value first increases and then decreases with age; the age-class reproductive value ($w_i \cdot v_i$), however, decreases steadily (Table IV.1). The ratio of total reproductive value to census size V/N is larger in sparrow and the barnacle than in humans (Table IV.2). Notice however that the distribution of age-class reproductive value is more even in humans than in sparrows or barnacles (Table IV.1). These differences are due to the longer life span and much larger newborn survival

Table IV.1. Life-tables of humans, the sparrow and the barnacle. l_i, probability of survival to age i; σ_i and b_i, survival and fecundity rates at age i; w_i, stable age distribution; v_i, age-specific reproductive value scaled so as the reproductive value of newborns is unity; $w_i \cdot v_i$, reproductive value of age-class i.

	Age	l_i	σ_i	b_i	w_i	v_i	$w_i \cdot v_i$
Human	1	1.000	0.979	0.000	0.132	1	0.132
	2	0.979	0.999	0.000	0.124	1.059	0.132
	3	0.978	0.997	0.021	0.120	1.100	0.132
	4	0.975	0.997	0.352	0.115	1.123	0.129
	5	0.972	0.997	0.416	0.111	0.816	0.090
	6	0.968	0.995	0.260	0.106	0.432	0.046
	7	0.964	0.993	0.138	0.102	0.189	0.019
	8	0.957	0.988	0.054	0.098	0.059	0.006
	9	0.946	0	0.007	0.093	0.007	0.001
Sparrow	1	1.000	0.167	0.000	0.764	1	0.764
	2	0.167	0.497	3.142	0.126	6.074	0.764
	3	0.083	0.578	3.333	0.062	6.076	0.375
	4	0.048	0.250	3.556	0.035	4.895	0.172
	5	0.012	0.500	3.750	0.009	5.640	0.049
	6	0.006	0	4.000	0.004	3.943	0.017
Barnacle	1	1	0.000	0	0.99987	1	1.000
	2	$6.2 \cdot 10^{-4}$	0.548	4600	$5.8 \cdot 10^{-5}$	17173	1.000
	3	$3.4 \cdot 10^{-5}$	0.588	8700	$3.0 \cdot 10^{-5}$	24954	0.748
	4	$2.0 \cdot 10^{-5}$	0.775	11600	$1.7 \cdot 10^{-5}$	30376	0.503
	5	$1.5 \cdot 10^{-5}$	0.710	12700	$1.2 \cdot 10^{-5}$	26764	0.323
	6	$1.1 \cdot 10^{-5}$	0.591	12700	$8.0 \cdot 10^{-6}$	22258	0.179
	7	$6.5 \cdot 10^{-6}$	0.308	12700	$4.5 \cdot 10^{-6}$	18613	0.083
	8	$2.0 \cdot 10^{-6}$	1.000	12700	$1.3 \cdot 10^{-6}$	23131	0.030
	9	$2.0 \cdot 10^{-6}$	0	12700	$1.2 \cdot 10^{-6}$	11928	0.014

rate in humans than in the sparrow or the barnacle, which lead to larger generation time L and generation overlap γin humans than in the other two species. The generation overlap is defined as $\gamma = \mathbf{vPw}/(\mathbf{vw})$, where **P** is a square matrix whose $(j+1,j)$ elements are the age specific survival σ_j and other elements are zeros, and **w** and **v** are the stable age distribution and the individual reproductive value vectors.

Parameter K is smaller than one in humans and larger than one in sparrows and barnacles; barnacles show a remarkably large value (K = 36112). The ratio of effective to census size N_e/N is larger in humans than in sparrows or in barnacles.

Table IV.2. w_1, proportion of newborns at the stable age distribution; L, generation time; K element of the denominator of equation IV.6 (K does not represent a well-defined biological property); N_e/N, ratio of effective to census size; $V/N = w_1 \cdot L$ total reproductive value of the population divided by census size; λ, population growth rate; γ, generation overlap.

	Human	Sparrow	Barnacle
w_1	0.132	0.764	1.000
L	5.211	2.801	3.881
K	0.029	7.336	36112.000
N_e/N	0.666	0.257	0.000107
V/N	0.686	2.141	3.880
λ	1.037	1.014	1.065
γ	0.838	0.652	0.790

Sensitivity

The study of the three life-tables is performed using a simplified expression for the sensitivity of N_e/N. Equation IV.7 can be written as:

$$\frac{\partial \frac{N_e}{N}}{\partial a_{ij}} = \frac{w_1}{(1+K)}\frac{\partial L}{\partial a_{ij}} + \frac{L}{(1+K)}\frac{\partial w_1}{\partial a_{ij}} - \frac{w_1 L}{(1+K)^2}\frac{\partial K}{\partial a_{ij}} \qquad \text{(IV.40)}$$

The terminology adopted in the following distinguishes between *sensitivities* and *contributions*. The *contribution* is the product of the *sensitivity* by a weighing factor: for instance, the contribution of L to the sensitivity of N_e/N is the product of the sensitivity of L by w_1 / (1 + K). Note that the contribution and the sensitivity of K will always be of opposite signs, because the sensitivity is multiplied by a negative factor.

The sensitivities to survival rates and fecundity rates are studied in separate sections. Each section is further subdivided in three parts and begins with a description of the pattern of sensitivity of N_e/N to age-specific vital rates; these patterns are shown in Figures IV.1 and IV.3. The second part concerns the sensitivities of the three biological parameters w_1, L and K, which are reported in Table IV.3. The third and last part of each section contains the description of the contributions of w_1, L and K to the sensitivity of N_e/N, which are illustrated in Figures IV.2 and IV.4.

Table IV.3. The sensitivities of λ, N_e/N, w_1, L and K to survival and fecundity rates in humans, the sparrow and the barnacle. * = Each of the values on this line is a multiplier for the whole column below it. For instance, the sensitivity of N_e/N to age one survival is $113141.61 \cdot 10^{-5} = 1.13$; that to age two survival is $7.96 \cdot 10^{-5} = 0.0000796$; and so on.

	Sensitivity to survival					Sensitivity to fecundity				
Age j	$\frac{\partial \lambda}{\partial \sigma_j}$	$\frac{\partial \frac{N_e}{N}}{\partial \sigma_j}$	$\frac{\partial w_1}{\partial \sigma_j}$	$\frac{\partial L}{\partial \sigma_j}$	$\frac{\partial K}{\partial \sigma_j}$	$\frac{\partial \lambda}{\partial b_j}$	$\frac{\partial \frac{N_e}{N}}{\partial b_j}$	$\frac{\partial w_1}{\partial b_j}$	$\frac{\partial L}{\partial b_j}$	$\frac{\partial K}{\partial b_j}$
Human										
1	0.20	0.56	-0.02	-0.27	-1.08	0.19	-0.06	0.09	-4.31	-0.05
2	0.20	0.66	0.00	-0.26	-1.10	0.18	0.03	0.09	-3.16	-0.01
3	0.20	0.74	0.01	-0.22	-1.10	0.17	0.14	0.08	-2.17	0.00
4	0.14	0.37	0.00	0.22	-0.55	0.17	0.24	0.08	-1.24	0.00
5	0.07	0.04	-0.02	0.38	-0.15	0.16	0.33	0.08	-0.38	0.01
6	0.03	-0.07	-0.02	0.27	-0.03	0.15	0.42	0.07	0.41	0.01
7	0.01	-0.09	-0.02	0.12	0.00	0.15	0.50	0.07	1.14	0.01
8	0.00	-0.06	-0.01	0.02	0.00	0.14	0.56	0.07	1.81	0.01
9						0.14	0.63	0.06	2.40	0.01
Sparrow										
1	2.17	0.87	-0.41	-2.13	-39.04	0.36	0.23	0.11	-2.13	-12.66
2	0.36	0.06	-0.06	0.47	-1.08	0.06	0.00	0.02	-0.19	-0.44
3	0.14	-0.01	-0.02	0.48	1.56	0.03	-0.01	0.01	-0.01	0.27
4	0.09	-0.04	-0.01	0.56	2.82	0.02	-0.01	0.01	0.04	0.34
5	0.02	-0.01	0.00	0.13	0.70	0.00	0.00	0.00	0.02	0.18
6						0.00	0.00	0.00	0.02	0.11
Barnacle										
*		$(\cdot 10^{-5})$	$(\cdot 10^{-5})$		$(\cdot 10^{3})$	$(\cdot 10^{-5})$	$(\cdot 10^{-10})$	$(\cdot 10^{-10})$	$(\cdot 10^{-6})$	
1	4425.04	113141.61	-87196.71	-11848.42	-490545.07	25767	918960	730341	-3395677	-62481
2	0.37	7.96	-2.81	-0.14	-28.06	1.50	12.77	42.53	-143.04	-1.76
3	0.23	3.19	-0.76	0.54	-5.68	0.77	-4.52	21.90	-45.51	-0.27
4	0.11	0.59	-0.25	0.56	3.20	0.43	-9.77	12.10	-9.58	0.24
5	0.07	-0.36	-0.15	0.53	6.12	0.31	-8.03	8.81	4.36	0.31
6	0.04	-0.65	-0.09	0.41	6.00	0.21	-6.17	5.87	10.45	0.30
7	0.03	-0.82	-0.06	0.38	6.25	0.11	-4.16	3.26	9.99	0.23
8	0.00	-0.15	-0.01	0.06	1.09	0.03	-2.02	0.94	4.10	0.11
						0.03	-1.58	0.88	4.99	0.10

Sensitivity to survival rates

Sensitivity of N_e/N

The sensitivity of N_e/N to survival rates is similar in all the three life-tables (Figure IV.1). The sensitivity is positive for younger ages and negative for older ages. This means that increasing survival rates of newborns (age class 1) will increase N_e/N, while increasing the survival rates of adults will generally lead to a reduction in N_e/N. The largest effect of increasing newborn survival is found in the sparrow and the barnacle, where survival rate in the first age class is a lot smaller than that in the older age classes, while in humans there is a smaller difference between the survival rate of the first age class and those of the older age classes.

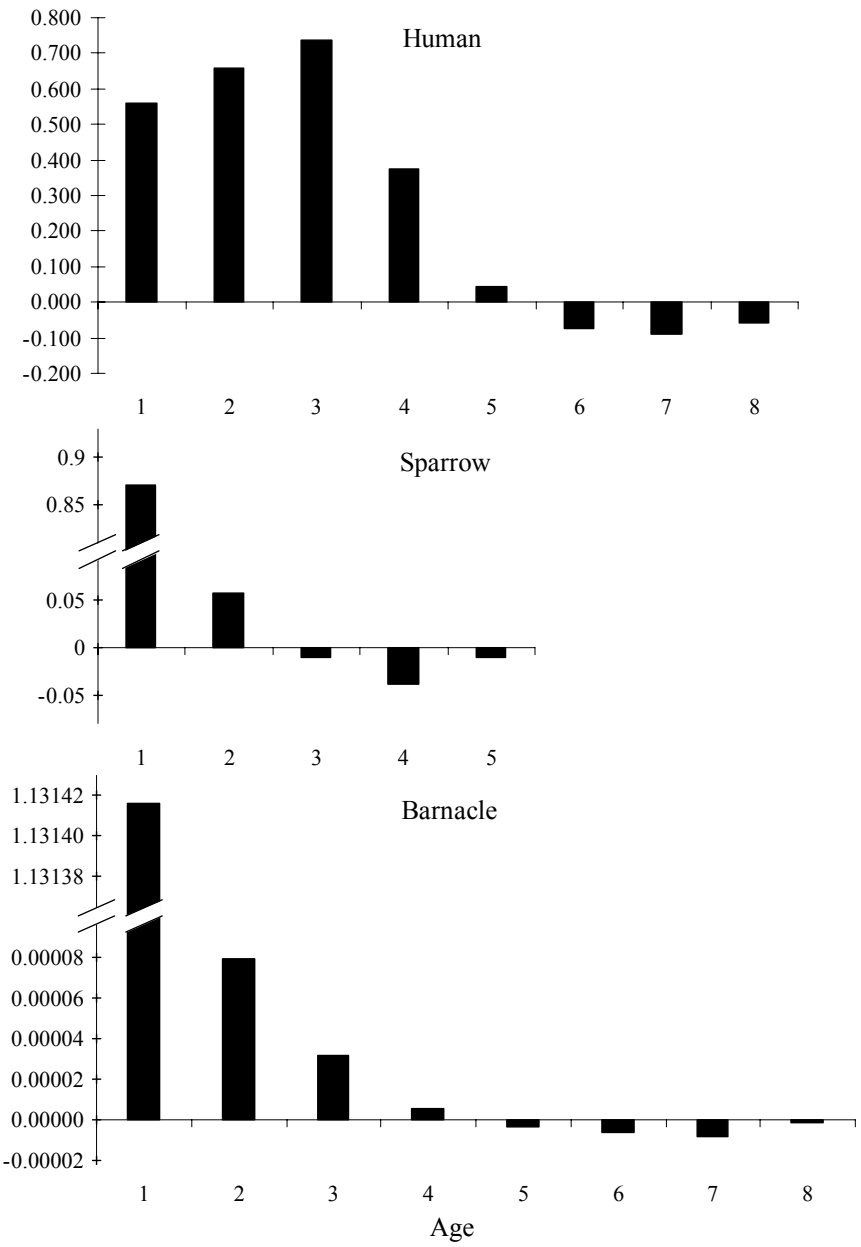


Figure IV.1. Sensitivity of N_e/N to age-specific survival rates for the three empirical datasets (humans, sparrow and barnacle).

There is a very striking difference between humans on one side and barnacles and sparrows on the other. While in humans several age classes have a comparable effect on N_e/N, in barnacles and sparrows the sensitivity to the survival of the first age class is several orders of magnitude larger than the sensitivity to the survival of other age classes. This could be related to reproductive values of different age classes: the total reproductive value of human populations is more or less

distributed across several age classes, while the opposite is true for barnacles and sparrow, i.e. the total reproductive value will be mainly due to the reproductive value of the first age class.

Sensitivities of L, K and w_1

In all the three life-tables, generation time L exhibits negative sensitivities for survival rates in the early part of the life cycle and positive sensitivities to survival rates in the late part of the life-cycle (Table IV.3). The reason for this pattern stems from the definition of L as average age of parents: increasing survival of young individuals will increase the proportion of individuals that reach the age of first reproduction, thus lowering the mean age of parents; on the contrary, increasing survival of adults will shift the mean age of parents up to older ages.

The sensitivity of K exhibits an increasing trend with age in all the three life-tables. The sensitivity of w_1 is generally negative; it increases with age in the sparrow and the barnacle, but does not exhibit a clear increasing or decreasing pattern in humans. The sensitivities of K or L are similar in magnitude, while those of w_1 are smaller.

Contributions of L, K and w_1 to the sensitivity of N_e/N

In humans, the sensitivity of N_e/N is controlled mainly by K at young ages and by L and w_1 at older ages, as revealed by the relative magnitude of their contributions (Figure IV.2). When survival at older ages is increased, generation time also increases, while the proportion of newborns decreases. The net effect is a slight reduction of N_e/N, as evidenced by the small negative sensitivities of N_e/N at ages $i = 6$, 7 and 8.

In the sparrow, the sensitivity of N_e/N is controlled by K and L. In the barnacle, the sensitivity of N_e/N is controlled mainly by K. When the survival rate of the first age class is increased, K is greatly reduced (negative sensitivity); this has a positive effect on N_e/N, because N_e is inversely proportional to K.

Here again we see a striking difference between humans on one side and barnacles and sparrows on the other. While in humans several ages contribute to the sensitivities, in barnacles and sparrows the contribution of the first age class is several orders of magnitude larger than that of other age classes.

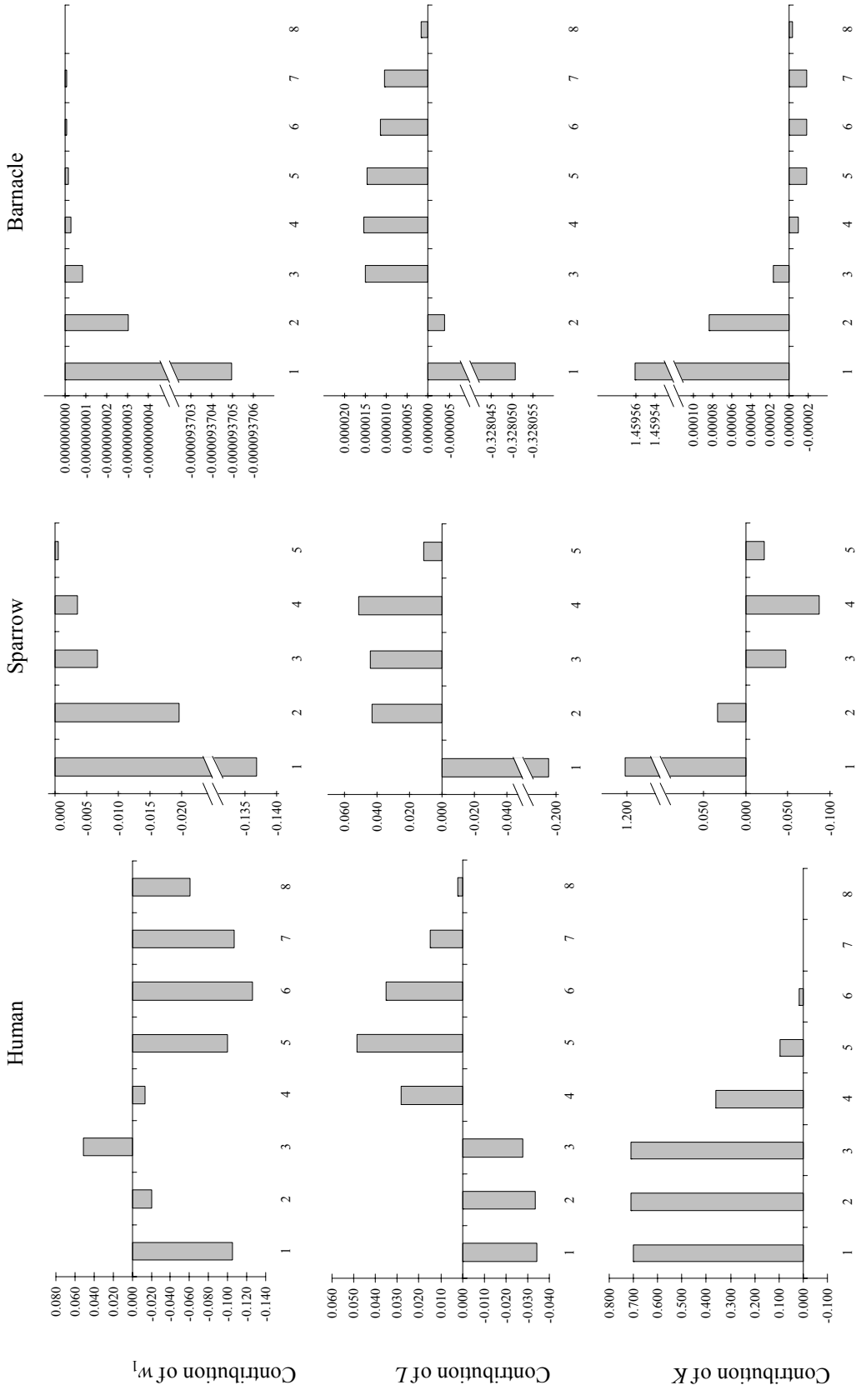


Figure IV.2. Contribution of w_1, L and K to the sensitivity of N_e/N to age-specific survival rates.

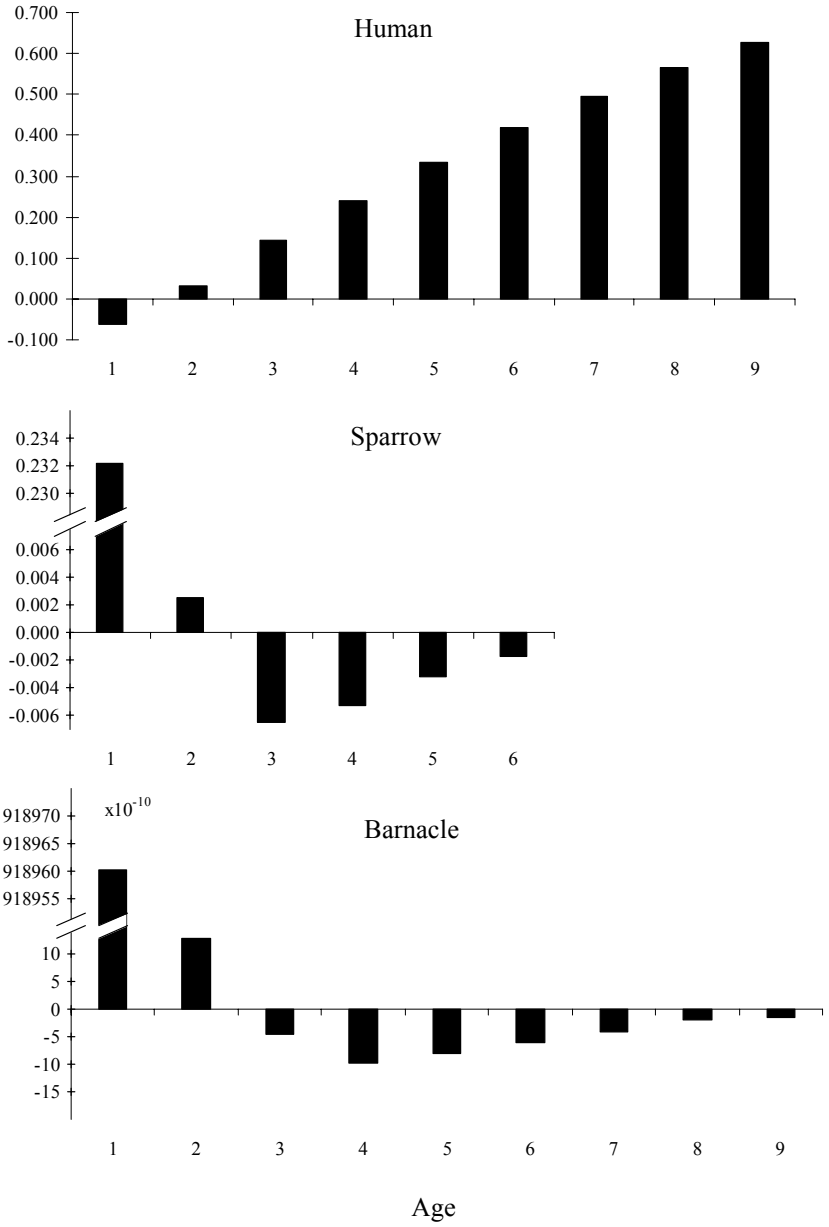


Figure IV.3. Sensitivity of N_e/N to age-specific fecundity rates.

Sensitivity to fecundity rates

Sensitivity of N_e/N

The sensitivity of N_e/N to fecundity rates exhibits different patterns depending on the life table (Figure IV.3). In the human life table, the sensitivity presents a regular increasing trend, with negative sensitivities at young ages and positive and progressively larger sensitivities at older ages. This means that the later in life fecundity is increased, the larger and more positive will be the effect on N_e/N. In the sparrow and the barnacle life table the pattern is more complex. The first age class exhibits a large, positive sensitivity, meaning that increasing fecundity of newborns will increase N_e/N. In the reproductive classes, the effects of increasing fecundity depend on the age of the class relative to the generation time. The sensitivity of N_e/N is most negative when the perturbed fecundity parameter is nearest to the mean age of parents and less negative when the change in fecundity interests age classes that are far from the generation length, i.e. younger or older reproductive age classes. The sensitivity to the first reproductive age ($i = 2$) is also positive.

Here again we observe differences between humans on one side and the two other species on the other. In the former, N_e/N is influenced by fecundity of several age classes while in the latter case, it is mainly the first age class the one that has an important effect.

Sensitivities of L, K and w_1

The sensitivity of generation time to fecundity can be positive or negative (Table IV.3). Negative sensitivities are associated with increases in fecundity in young age classes, because they lead to a decrease in the mean age of parents (recall that L is defined as a weighed sum of fecundities, Equation IV.5). When fecundity of older age classes is increased, then the mean age of parents is shifted upwards and the sensitivity of L is positive. In humans, the transition from negative to positive sensitivities occurs roughly at the age corresponding to generation time itself, i.e. between age five and six (L = 5.211). In the sparrow and the barnacle, the transition takes place somewhat later, respectively between age three and four (L = 2.801) and between age four and five (L = 3.881).

The sensitivities of the proportion of newborns w_1 exhibit a decreasing trend with age (Table IV.3). This means that the earlier in the life cycle fecundity increases, the larger the proportion of newborns in the population. The reason underlying the decreasing trend of w_1 sensitivities is that increases in fecundity at older ages are buffered by mortality: the proportion of surviving individuals l_i always decreases with age.

The sensitivities of K to fecundity increase with age (Table IV.3). In the sparrow, they reach a peak at age i = 4; in humans, they reach a plateau at age i = 5; in the barnacle, they reach a peak at age i = 5. However, the magnitude of the sensitivity (i.e. the absolute value) is largest for the first age class in all three species.

Contributions of L, K and w_1 to the sensitivity of N_e/N

In humans, the pattern is controlled mainly by L and w_1. The sensitivity of N_e/N follows that of L; the contributions of w_1 mitigate the negative contributions of L at younger ages and enhance the positive contribution of L at older ages. The contributions of K are two orders of magnitude smaller than those of w_1 and L.

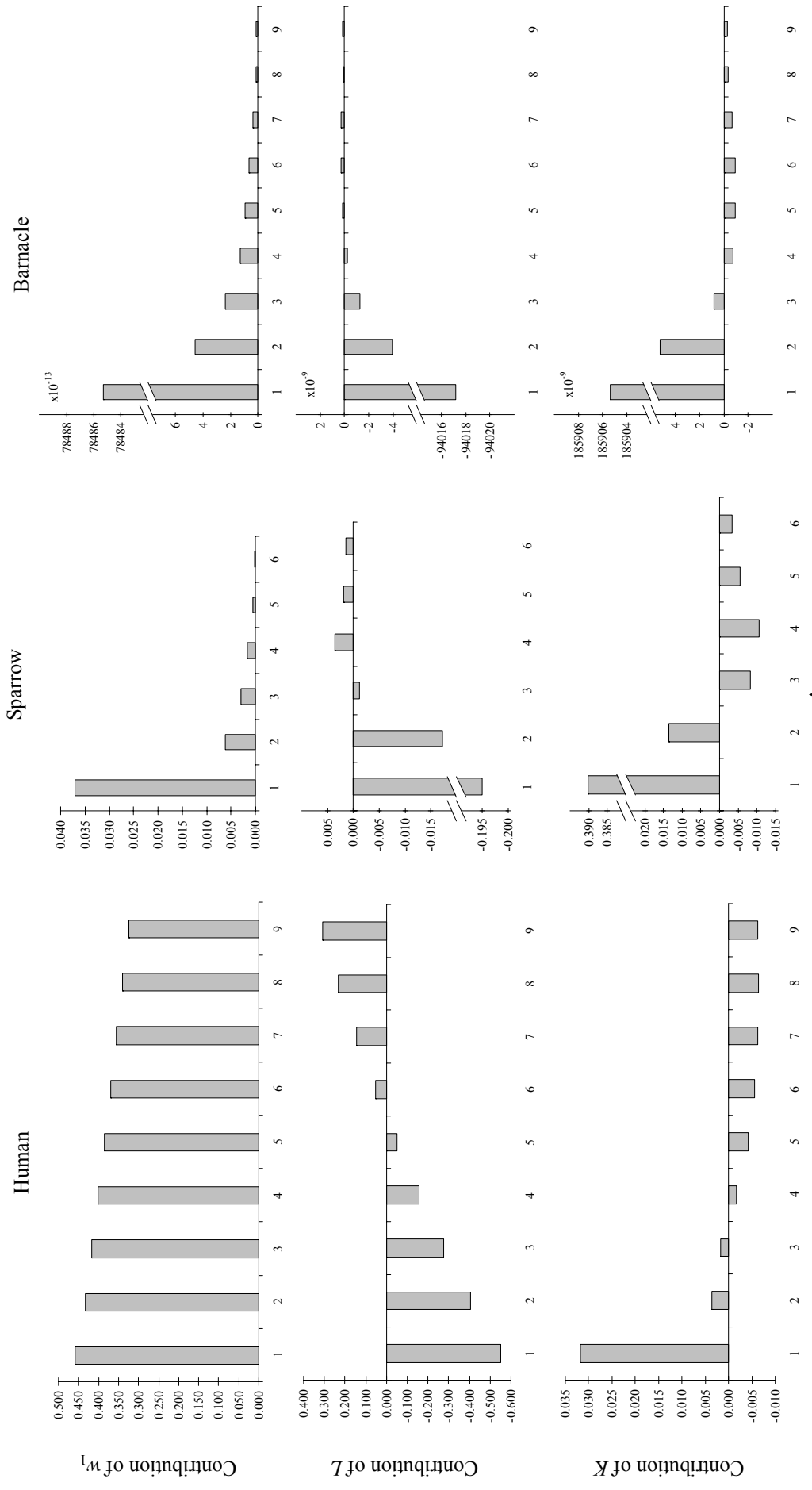


Figure IV.4. Contribution of w_1, L and K to the sensitivity of N_e/N to age-specific fecundity rates.

In the sparrow, the sensitivities of N_e/N are controlled mainly by L and K. The pattern of sensitivities of N_e/N mirrors the contributions of K, which are counterbalanced by the contributions of L and w_1. In the barnacle, the pattern of sensitivities of N_e/N mirrors the contribution of K, mitigated by the contribution of L, while the contribution of w_1 is several orders of magnitude smaller.

Discussion

The objective of this chapter was to derive an analytical expression for the sensitivity of N_e/N to changes in age-specific survival and fecundity rates. The age-structured models of Felsenstein (1971) was taken as a base to derive the sensitivity of N_e/N as a function of survival rates, fecundity rates, reproductive values and the sensitivity of population growth rate λ. The final expression for the sensitivity of N_e/N was too complex to be interpreted in terms of lower level vital rates (survival and fecundity rates). This expression was then reformulated (Equation IV.40) to highlight three terms, representing the contribution of the sensitivities of w_1, L and K to the sensitivity of N_e/N. Equation IV.40 was used to compute the sensitivity of N_e/N in three life-tables differing in the shape of the survivorship curve. In this way, it was possible to identify which component of N_e contributes the most to its sensitivity.

General patterns in sensitivities

Four general patterns were uncovered by the analysis.

1 . The most apparent pattern is that increasing survival at younger ages leads to larger N_e/N, while increasing survival at older ages leads to a reduction of N_e/N. This pattern is controlled mainly by the parameter K. Felsenstein (1971) defined this parameter as “roughly the probability of death of an individual while it still has reproductive value”. However, the cases of sparrow (K = 7.336) and barnacle (K = 36112) demonstrate that K cannot be strictly defined as a probability, because it can take values larger than one. It is difficult to find another definition of K other than Equation IV.6b.

2 . In general, generation time L increases with increasing survival and fecundity of older age classes. As explained above, this pattern can be easily understood because generation time represents the mean age of parents.

3 . The sensitivities of N_e/N to newborn vital rates are large when these vital rates are small, as evidenced for the sparrow and the barnacle. Increasing vital rates of newborns would thus lead to a great increase in N_e/N.

4 . N_e/N exhibits larger sensitivities to survival rather than fecundity rates. The effects of changes in fecundities are particularly small in the sparrow and the barnacle. As for humans, survival has larger effects than fecundity at younger ages, while the opposite is true at older ages.

Survivorship curve and N_e/N ratio

The last point suggests that the pattern of survival can greatly affect the value of N_e/N and probably the range of variation of N_e/N ratio among species and populations may lie in differences in survival rates. It is less clear however whether the shape of the survivorship curve (Type I, II and III) is the primary determinant of N_e/N or the variation should be looked for in the overall pattern of survival. There is empirical evidence that species with Type III survivorship exhibit remarkably small N_e/N (Gaggiotti and Vetter 1999; Turner et al. 2002). However, the relative role of the survivorship curve in relation to other biological and environmental perturbations is still debated. It is likely that the extremely small N_e/N ratios observed in species with Type III survivorship are the result of the synergistic action of large variation in reproductive success (Hedgecock 1994; Hedrick 2005), habitat disturbance (Turner et al. 2006) and large migration rates across a species distributional area (Wares and Pringle 2008).

Generation overlap and N_e sensitivity

Gaggiotti and Vetter (1999) argued that the N_e of species with extensive generation overlap is better buffered from environmental fluctuations than the N_e of species with low generation overlap. The sensitivities observed in humans and sparrows were similar, although these species differ in the generation overlap (γ = 0.838 and 0.652, respectively), while barnacles, which have a generation overlap intermediate between those of humans and sparrows (γ = 0.790), show very large sensitivities for N_e/N. This seems to contradict the suggestion that large generation overlap can buffer N_e from fluctuations in vital rates. However, the sensitivity analysis was conducted on the ratio N_e/N and not on N_e itself. Because the ratio N_e/N was remarkably small in barnacles, its large

sensitivities do not necessarily imply that N_e will change extensively. This issue would be better studied using the sensitivity of N_e, or the elasticity of N_e/N.

Proportionality between N_e and reproductive value

Felsenstein (1971) wrote that N_e is proportional to the total reproductive value of a population, V. However, the values in Table IV.2 show that the proportionality is no longer valid when N_e and V are divided by total population size. Since $N_e = V/(1 + K)$ (Equation IV.6a), it is clear that the parameter K can alter the proportionality, and its effect is more evident when both N_e and V are divided by total population size.

Conclusions

Arriving at a clear understanding of the link between life history and N_e/N is challenging because of the complexity of mathematical models used to calculate N_e from demographic data. Perhaps this issue can be better tackled by starting from simpler models, for instance a population with only two age-classes (pre-reproductive and reproductive). Another possibility would be to use analytical function to express age-specific survival rates; for instance, Nunney (1991) asserts that a Type I and Type II survivorship curves can be represented by a negative binomial distribution. Another simplification would be to assume that fecundity is constant with age, as in Nunney (1991); however, this would limit the generality of the model.

Chapter V:
Discussion

Effective population size can be estimated with demographic and genetic models. The first question addressed in this work is whether demographic models can provide reliable estimates of N_e (Chapter II). The second objective was to study the effects of age- and stage-structure on N_e in species with overlapping generations using a numerical (Chapter III) and an analytical (Chapter IV) approach. The general topics addressed in this discussion chapter are: the possibility of obtaining reliable estimates of N_e through demographic models; the reliability of genetic estimators; the effect of age- and stage-specific vital rates on N_e; the implications of the analyses presented in this thesis for conservation of *E. alpinum* and *D. austriacum*; and perspectives for future research.

Reliability of demographic-based estimators

As illustrated in Chapter I, numerous demographic models exist for the estimation of N_e. They differ in the biological processes modelled and in the type of effective population size that they can provide. The estimation becomes increasingly challenging as a greater number of biological processes are included in the model (e.g. asexual reproduction, demographic stochasticity, etc), because the models become mathematically complex and their parameters may be difficult to measures. Therefore, the reliability of estimates of effective population size from demographic data depends on two factors. The first is the reliability of the model, which should take into account all the biological processes that are considerable relevant for genetic drift. As it will be discussed in the following, the development of demographic models is an area of scientific investigation that is still fecund. The second factor is the availability of ecological data allowing for a precise and accurate estimation of model parameters.

Model reliability

Nowadays, demographic models for the estimation of N_e permit describing most processes contributing to genetic drift. However, despite numerous advancements in model development, there are still cases in which we do not know how to compute an effective population size. Two illustrative examples for stage-structured populations are the inclusion of demographic stochasticity and geographical structure.

Stage structure and demographic stochasticity

There is no model to compute N_e for a stage-structured population in presence of demographic and/or environmental stochasticity. Current models allow for stage-structure without demographic stochasticity (Orive 1993 for inbreeding and coalescent N_e; Yonezawa et al. 2000 for variance N_e) or demographic stochasticity with age-structure (Engen et al. 2005, 2007 for variance N_e). In plants, however, vital rates are better characterized in terms of size or other life-stages rather than age. Stage-specific vital rates can be converted into their age-specific counterparts (Cochran and Ellner 1992; de Valpine 2009) and one could choose to apply the model of Engen et al. (2005) to the converted data in order to obtain estimates of N_e in presence of demographic stochasticity. However, the converted parameters suffer from considerable error and do not reflect the true age-specific vital rates (cfr Caswell 2001, Figure 5.2). It would be preferable to develop a stage-structured model that integrates stochasticity rather than modifying stage-specific data to fit an age-specific model. After all, if age is a poor predictor of vital rates, such a model is likely to have less proximity to reality than a stage-structured model. It remains however a powerful method for animals or other species for which survival and fecundity are age-dependent (Engen et al. 2007). In some species of plants also, age seems to be a better predictor of survival than size (Lauenroth and Adler 2008).

Stage structure and geographical structure

Spatial structure was not considered in the analyses presented in this thesis, but another area of model improvement is the computation of the global effective size of a geographically-structured population. A geographically structured population is a collection of local populations (or demes) more or less tightly connected by migration. A *local* effective size can be defined for each deme, while for the entire set of populations a *global* effective size can be defined. The global effective size is the size of an ideal population that would undergo the same amount of genetic drift as the geographically structured population under consideration. There are models that formulate the global effective size of structured populations with discrete generations (Whitlock and Barton 1997). There are also models allowing for the calculation of global N_e in presence of age-structure (Kobayashi and Yamamura 2007) and various mating systems (Nunney 1999). However, the model of Kobayashi and Yamamura (2007) assumes the same vital rates for each deme and the one of Nunney (1991) was developed for populations with discrete generations. There is currently no model that defines the global effective size of a stage-structured population. Such a model could be

developed starting from existing theory on genetic differentiation in populations with different classes of individuals (Rousset 1999, 2004).

These two examples show that there is still room for improvements in the area of model development. Good reviews on the existing models can be found in Caballero (1994) and Wang and Caballero (1997). To help people choose a model that is adequate for their study among the numerous existing ones, it would be useful to review the most recent studies on demographic N_e estimation, which are not considered in the papers by Caballero (1994) and Wang and Caballero (1997). Some of these new contributions have been presented shortly in Chapter I. The next step will be to extend this review by making it fully comprehensive of all the published studies and by indicating explicitly the strength, weaknesses and assumptions of each model. Moreover, such review would be useful to identify the critical aspects of model developments that need to be addressed in future studies.

Choosing among the numerous published models is a critical step in a study aimed to estimate N_e from demographic parameters. Ideally, the estimation should be performed with a model that describes all the biological processes that are relevant for genetic drift. However, this will not be possible in practice because the model will always be an approximation of reality ("All models are wrong, but some are useful"). The choice of an appropriate model is therefore left to the investigator and will be affected by a certain degree of subjectivity. One will choose the model containing the description for the processes that are thought to be the most relevant.

Parameter accuracy and precision

The second order of problems posed by model-based estimation of N_e concerns parameter estimation. Some parameters are difficult to measure and all parameters are affected by uncertainty. In Chapter II it was evidenced how difficult it was to estimate census size and variation in reproductive success. In some situations, census size may be hard to obtain, for instance in populations that are very large or when it is difficult to observe individuals directly. For this reason, numerous methods have been developed to estimate N from genetic data (Luikart et al. 2010). Obviously, it can be discussed whether it is better to obtain an estimate of N from genetic data and use it to compute N_e with a demographic method or to estimate N_e directly from genetic data.

Variation in reproductive success was estimated from seed counts, but under the strong assumption that seedlings are randomly recruited from the gene pool. When an adequate number of suitable molecular markers is available, variation in reproductive success might be derived through parentage analysis. There are numerous published studies that use parentage analysis to infer pattern of seed and pollen dispersal in plant populations (e.g. Sezen et al. 2009; Hampe et al. 2010). The focus of most studies seems to be the analysis of spatial genetic structure of seedlings or male reproductive success through pollen dispersal. Little attention is paid to variation in reproductive success among parents. Variation in reproductive success is as important as survival rates for calculating N_e (elasticity analysis in Chapter II). Future works on N_e estimation should pay particular attention to the quantification of variation in reproductive success.

All parameters are affected by uncertainty from sampling error, even parameters that are easy to measure. For instance, survival and fecundity rates can be estimated pretty easily from demographic studies, but their values are derived from finite samples. In models, these vital rates are considered as fixed numbers and not as unknown variables that need to be estimated, making difficult to assess the effect of sampling error on the final N_e estimate. In matrix population models, where the objective is the calculation of population growth rate λ, this issue has been tackled in two ways. The first is the use of elasticity and sensitivity analysis as tools to assess the impact of parameter uncertainty on the model results. Alternatively, computer-intensive resampling techniques (such as bootstrapping) have been used to provide confidence intervals for λ as a measure of uncertainty. These techniques can also be used in models for the estimation of N_e. A third and more elegant way would be to formulate these models in terms of the means and variances of parameter values, so that the calculated N_e would no longer be just a point estimate but would have a mean and a variance. This would allow the comparison of model-based N_e with the estimates coming from molecular methods, which usually provide a mean value and a measure of variability (confidence intervals, such as in *LDNe*; or posterior credible intervals, such as in *ONeSAMP*).

Computation of coalescent N_e from demographic data

A separate issue that deserves consideration is the estimation of coalescent N_e from demographic data. I worked with coalescent models in the first period of my thesis but then I decided not to include those results in the present manuscript. This decision was motivated by the belief that demographic-based estimates of coalescent N_e must be interpreted with great care. When estimating coalescent N_e, there are problems of model reliability and parameter estimation, like for the other

types of N_e. For instance, the demographic estimator of N_e developed by Orive (1993) assumes constant vital rates and constant population size and is not suitable in cases of population growth, population decline and fluctuations in vital rates. The assumption of constant vital rates is often violated in empirical datasets: populations often exhibit positive or negative rates of increase. It is however possible to modify Orive's original model in order to account for vital rate variation and population fluctuations: for instance, Gaggiotti and Vetter (1999) modified this model in order to take into account cyclic fluctuation in recruitment rates. In this case, however, it is problematic to define an appropriate measure of generation time and thus effective population size remains undefined (indeed, N_e gives the strength of genetic drift per generation, hence it is necessary to define generation time).

Besides considerations on model assumptions, which apply equally to coalescent and variance N_e, there is one particular characteristic of coalescent N_e that should discourage the use of demographic models for its estimation, namely the reference to processes acting in the distant past. Coalescent N_e represents the asymptotic rate of coalescent in the past. However, demographic data are usually taken on limited periods of time and may not be representative of the dynamics of the population in the distant past. It is likely that survival and fecundity rates and the rate of population increase observed during the study period is different from that experienced in the past. To illustrate this point, take the case of a demographic dataset with constant vital rates but decreasing or increasing population size. In the case of increasing populations, the assumption of a constant rate of increase implies that, going backward in time, the population becomes increasingly smaller. The time to coalescence has therefore an upper limit corresponding to the moment when the population was constituted of a single individual. Conversely, when population growth rate is negative, the population becomes increasingly larger when going backward in time. In this case, it may be possible to calculate the expected time to coalescence between two genes, but one may question the reliability of population dynamics on longer time-scales. Is it realistic for a population to decline for hundreds or thousands generations? Such a population would have to be of *very* large size in the distant past!

In summary, coalescence times are usually very large in comparison to the duration of demographic studies. We have therefore no information on population dynamics in the past and we cannot calculate time to coalescence from demographic data.

Reliability of genetic-based estimators

The accuracy and precision of genetic-based estimators depends on a number of factors. Waples and Do (2010) have analysed the effect of sample size, number of markers and locus polymorphism on linkage-disequilibrium estimates of N_e and found that all these factors had a considerable effect. For instance, including rare alleles in the analysis will bias upwardly the estimate of N_e. The low precision of linkage-disequilibrium N_e in Chapter II was a consequence of the small sample size, the low number of molecular markers and their low polymorphism.

Another factors affecting the estimation and interpretation of N_e is sampling scheme (Nei and Tajima 1981; Waples 1989). If samples for genetic analysis are taken from adults after reproduction, they may contain some of the genes representing effective population size. This sampling scheme introduces a correlation between the frequencies of genes sampled in two different generations, because both may be derived from same initial population. If samples consist of juveniles or adults taken before reproduction and not replaced, then the sampled genes will no longer be part of the breeding population and gene frequencies sampled in different generations will be uncorrelated. The temporal method can accommodate for both sampling schemes, but the equations used for calculating N_e are different (Waples 1989). It is thus important that sampling follows a well defined scheme and that the correct version of the estimators is used. In species with overlapping generations, it is also important whether the sampling regime targets a single cohort or multiple cohorts (Waples 2005, 2010).

All molecular methods should be assessed for their precision and accuracy, for instance using computer simulation. This has been done in some cases, for the temporal method (Waples and Yokota 2007) and the linkage-disequilibrium method (Waples and Do 2010). The Bayesian estimator *ONeSAMP* still lacks a formal evaluation, which would be highly useful. Such an assessment is difficult because the software is web-based and each analysis takes several days to be completed. A relative evaluation can also be performed following a comparative approach, that is, comparing the outcomes of different methods applied to the same dataset (Beebee 2009; Saarinen et al. 2010). This approach can also be used to compare demographic- and genetic-based methods, as discussed in Chapter III.

Effect of stage-specific vital rates on effective size

Chapter III focused on the effect of stage-specific vital rates on effective population size through numerical elasticity analysis of demographic data. The two species analysed did not give consistent results. In *Dracocephalum austriacum*, it was possible to identify clear patterns of elasticities with respect to population dynamics. In population increasing or stable in size, the major contributors to N_e were adult survival rates; in declining populations, conversely, the life-stage transitions that affected N_e the most were those of juvenile plants. This pattern of contribution to N_e was not found in *Eryngium alpinum*. In this species, it was not possible to link clear patterns of elasticity to any population dynamics parameter. The elasticity analysis of N_e to stage-specific vital rates has not been used often in the literature: as discussed in Chapter III, the only two studies that addressed this question explicitly (Yonezawa et al. 2000 on *Fritillaria camschatcensis*; Campbell and Husband 2005 on *Hymenoxis herbacea*) reached conclusions that differ from the patterns observed in this thesis.

The differences between the elasticity patterns observed in these species does not allow us to generalize the results to other species. It seems that each species has a specific pattern of elasticity. Moreover, the results of *D. austriacum* and *E. alpinum* show also the existence of variation in elasticity pattern among populations of the same species. These inter-population differences indicate that species with fairly similar life-histories can differ greatly in the demographic features that affect their effective size. It seems thus that the effects of vital rates on N_e cannot be interpreted and understood in terms of the life-history of the species only. These effects depend on more subtle mechanisms, linked to the vital rates themselves, acting on lower levels of organisation (the single population and not the whole species).

Another approach used to study the effect of stage-specific vital rates on N_e was the derivation of an expression for N_e sensitivity through calculus (Chapter IV). This approach did not give useful results however, because the derived expression was too complex to allow its interpretation in biological terms. The basis for the derivation was the equation of Felsenstein (1971) and it is possible that the use of a different model may lead to a simpler derivation. Other models that can be analysed are those by Hill (1979) and Yonezawa (1997), which conserve the generality of Felsenstein's equation. The model developed by Nunney (1991) assumes constant population size and is not amenable to sensitivity analysis. The model of Johnson (1977) is a reformulation in

vectorial terms of the model of Felsenstein (1971); using it as a basis for the derivation of sensitivity may in theory conduct to an equivalent, but formally different, result.

Elasticity and sensitivity analyses have been rarely applied to N_e. This is partly due to the scarce number of studies using the demographic approach to compute N_e, despite the numerous models available to perform this task (see Chapter I). Another reason for the scarcity of studies employing elasticity on N_e is that most demographic models are based on the assumption of constant population size. This assumption is likely to be violated in most natural populations; more importantly, it becomes an obstacle when one wants to study the effects of changing vital rates on N_e, because a change in a single vital rate will produce a change in population dynamics, so that even if the original population could be considered at demographic equilibrium, the effect of the modified vital rate will displace it from the equilibrium condition.

As a result, the question of how vital rates affect N_e has been approached in mathematical models assuming constant population size. The paper by Nunney (1991), entitled "The influence of age-structure and fecundity on effective population size", is a good example of how mathematical models can be used to tackle this question. One of the main results of Nunney (1991) was that decreasing lifetime fecundity can increase N_e (when there is no variation in fecundity among individuals). This result is however dependent on the assumptions made in his model. Specifically, the model of Nunney (1991) assumed the invariability of survival and fecundity rate with age, an assumption that may be verified in some mammal and bird species but is certainly not valid for plants or fish, in which fecundity is an increasing function of size.

That no general pattern emerged from the elasticity analysis of N_e (Chapter III) and the absence of a simple expression for the sensitivity of N_e/N (Chapter IV) indicate that the way to understand the effects of stage-specific vital rates on N_e is the extension of empirical studies to larger datasets, comprising more species and populations. This could be done using published data (transition matrices), in a comparative study similar to those carried out on the elasticity of population growth rate (Silvertown et al. 1993; Franco and Silvertown 2004). Alternatively, it is possible to examine theoretical life-tables in which the vital rates defining the transition matrices can be varied arbitrarily.

The effect of generation time

Every model considering the effective size of populations with overlapping generations contains the generation time L. N_e is defined as the size of an ideal population with discrete generations that would undergo the same amount of gene frequency change per generation as the population under consideration. Since the life-table regulates not only the transitions of genes from one stage to another but also determines the average age of parents, any perturbation in the life-table will alter generation time and have downstream effects on the effective size through both ways. In age-structured populations the sensitivity of L follows a predictable pattern, with positive sensitivities at older age classes and negative sensitivities at younger age classes (Chapter IV). The sensitivity of L in stage-structured populations is inherently complex because stage-specific survival and fecundity rates must be converted into age-specific parameters (Cochran and Ellner 1992); as a result, perturbations of stage-specific vital rates can lead to counterintuitive changes in generation time (as evidenced in the pattern of negative and positive elasticity of L; Chapter III). To date, the sensitivity of L to stage-structure vital rates can be computed only through numerical perturbation.

Moreover, quantifying the amount of genetic drift on a time scale that is independent of generation time is of practical importance in conservation, where the focus often is to insure that a certain amount of genetic variability is retained over a time scale defined in years (Ryman et al. 1981; Allendorf et al. 2008). Hill (1979) introduced the concept of annual effective population size N_a, defined as the size of a population with discrete and non-overlapping generations that experiences the same amount of genetic drift *per year* as the population under consideration. Evaluating the sensitivity of N_e, N_a and L to vital rates can provide a more complete picture of the ways the different parameters of the life-table affects genetic drift (e.g. Yonezawa et al. 2000, 2004).

Population viability and genetic stochasticity in *D. austriacum* and *E. alpinum*

Among the different factors contributing to species decline and extinction risk, genetic stochasticity can increase mortality through inbreeding depression or reduce the potential for population adaptation through the loss of important adaptive genetic variation. However, the data available to date do not allow concluding that the reduced viability in *D. austriacum* and *E. alpinum* is a product of genetic stochasticity. This issue will be discussed separately for the two species.

Dracocephalum austriacum

Inbreeding depression

There is no information on inbreeding depression in the seven French populations considered for species monitoring. A study on inbreeding depression was carried out in populations in the Czech Republic and Slovakia, at the Northern edge of the distribution area: these populations show a negative correlation between mean seed production and mean inbreeding coefficient $\overline{F}$ (Dostalek et al. 2010). However, care should be taken in the interpretation of this result as inbreeding depression and generalizing it to the French populations.

First, mean seed production was also negatively correlated with population size (Dostalek et al. 2010) and the populations in Czech Republic and Slovakia are smaller than those in France; therefore, it may be possible that larger populations do not show great reduction in fertility.

Second, it is not known whether the lower performances of the small inbred populations were due to inbreeding depression or other processes associated with small population size (e.g. Allee effect via pollen limitation): the larger $\overline{F}$ observed in small populations might be the consequence rather than the cause of low reproductive success.

Third, the effects of inbreeding on other fitness components were not tested. If inbreeding has a negative impact on seed production only, its effects on population viability will depend on the elasticity of population growth rate to seed production. In the French populations, the elasticity of λ to fertility was one of the smallest, suggesting that this vital rate has a very small effect on population viability. More serious effects on population viability would originate if inbreeding were negatively correlated to adult or juvenile survival rates, because these stages contributed the most to population growth (the elasticity of λ to these parameters is the largest). In absence of studies on the relationship between inbreeding and survival rates, it is still difficult to conclude that inbreeding depression can reduce viability in *D. austriacum*. More studies are needed to test whether high inbreeding levels can lower survival, flowering or germination rates, or impact on other fitness components, even if this can be expected by the results of numerous studies on other species (Charlesworth and Charlesworth 1987)

Loss of genetic variability

The effects of genetic diversity on the ability of populations to respond to novel environmental conditions are traditionally difficult to document (Lande and Barrowclough 1987). The theory predicts that genetic diversity should be proportional to the long-term effective population size at equilibrium between mutation and genetic drift (Lande and Barrowclough 1987). Estimates of genetic diversity at neutral loci show that within-population genetic diversity in *D. austriacum* is high (Bonin et al. 2007). These estimates should not be correlated to N_e estimated through the demographic model or the short-term genetic estimators, because genetic diversity is the product of long-term processes: neutral genetic diversity should be correlated to estimates of long-term effective sizes, which are not available for *D. austriacum*.

Moreover, when genetic diversity is estimated on a subset of loci that exhibit signatures of natural selection, "adaptive" genetic variation turns out to be higher in small (BE) or decreasing (CH and VA) populations than in stable large populations (Bonin et al. 2007).

In absence of mutation, neutral genetic variation declines at a rate proportional to the (short-term) effective population size (Lande and Barrowclough 1987). Effective population size in the seven French sites was low (N_e = 24–172; Table III.3). If N_e remains at these low levels, the populations are expected to lose genetic variation at a rate of 0.3%–2.1% per generation (equation I.2). However, these estimates of N_e obtained through a demographic method may be very different from estimates obtained through genetic methods (Chapter I). It is therefore difficult to infer the actual strength of genetic drift in absence of more accurate estimates of N_e. On the other hand, there are several non-genetic factors whose contribution to population viability in *D. austriacum* has been clearly demonstrated. Threats to this species include pillaging, trampling and damage by grazing cattle. Global warming and its interaction with abiotic conditions such as topographic features can also reduce population viability (Nicolè et al. submitted).

More genetic studies are thus needed to assess the actual risk of inbreeding depression and loss of neutral and adaptive genetic variability. In the meantime, conservation actions should focus on ecological and anthropogenic causes of extinction, whose impact on plant mortality (Danton and Baffray 1995) and population dynamics (Nicolè et al. submitted) has been clearly documented.

Eryngium alpinum

Inbreeding depression

In *E. alpinum*, there is evidence of inbreeding depression at the earliest life stage, as seeds produced by selfing had lower germination rates than outcrossed seeds (Gaudeul and Till-Bottraud 2003). However, selfing rate in natural populations was relatively low. Only one small population (located in Malaval, 10 individuals) showed reduced outcrossing rates and large inbreeding coefficients and could therefore suffer from inbreeding depression. The consequence of reduced seed germination should however be evaluated using population dynamics modelling. If seed germination is not a critical transition in the life cycle, then inbreeding depression expressed at this stage may have little consequences for population persistence. It is difficult to know whether inbreeding depression can be expressed at older life stages.

Loss of genetic variability

Within-population genetic diversity is relatively high, but smaller populations exhibit lower levels of genetic diversity than larger ones (Gaudeul et al. 2000, 2004). It has not been tested whether genetic diversity is correlated to fitness components. The rate of loss of genetic variation due to genetic drift can be evaluated using the short-term effective size (Lande and Barrowclough 1987) In Chapter II, N_e was estimated with two genetic methods and one demographic method. Given the considerable disagreement among these three estimates, it is difficult to discuss the implications of N_e for population viability. The *ONeSAMP* estimates were the lowest (N_e = 27–43; Annex I) and, if true, would indicate that the populations lose genetic diversity at a rate of 1.2%–1.9% per generation (Equation I.2).

Other factors

In addition to inbreeding depression acting on seed germination rates, several other causes could explain the observed decline of the populations of *E. alpinum* in France. The Boujurian population has been decreasing in size over the study period, most likely as a result of overgrazing; the increasing dynamics in Deslioures is probably the result of favourable recruitment conditions and low vegetation density. Other potential causes of reduced viability include: picking for commercial use (dried bouquets); and disappearance of late hay harvest, in August, which used to limit land

closure (Gaudeul et al. 2000). Demographic stochasticity does not seem to be a problem, at least in the near future. Counts of the number of individuals have never been carried out in the French populations of *E. alpinum*. A reason for this is that some populations are so large that a rigorous census would be very time consuming. I estimated population size from aerial photographs and local density measures, obtaining very large figures for all populations ($N = 10^3$–$4 \cdot 10^6$), largely above the threshold considered relevant for genetic stochasticity (in the order of 100s individuals; Goodman 1987). However, some populations are becoming fragmented (Les Bernards) and this may constitute a risk in the future. Ongoing studies are evaluating the role of agricultural practices on species persistence (Appendix II; and Gregor Kozlowski, University of Fribourg, Switzerland, personal communication).

In summary, very small populations have large selfing rates and large inbreeding coefficients and could suffer from inbreeding depression; in addition, genetic diversity is lower in smaller populations. However, it is difficult to predict the relative effect of genetic stochasticity relative to other potential mechanisms of population extinction, such as agricultural practices or environmental stochasticity. Future investigations should aim at integrating the vast amount of demographic and genetic data now available for *E. alpinum* and produce an assessment of all the potential causes of species endangerment.

Elasticity analysis of N_e and conservation strategies

One objective of conservation programs for endangered species is to find the life-stages that contribute critically to population viability. These life-stages can then be targeted by specific management actions (Mills et al. 1999). Usually, the targeted life-stage transitions are those contributing the most to population growth rate. This is usually done by performing an elasticity or sensitivity analysis of λ. When the elasticity analysis is extended to N_e, it is possible to identify the vital rates that contribute the most to genetic drift. This has a potential impact on viability in small populations, which can experience high levels of genetic stochasticity and be at risk of extinction by genetic factors. The elasticities of N_e are thus informative on the life-stages that are critical for genetic stochasticity and can be used to plan management actions to decrease extinction risk.

If there are vital rates associated with negative elasticities of N_e, there will be a problem because increasing the values of those vital rates would decrease the effective size. An objective in species conservation is to maintain the effective size of populations at a sufficient high level to minimize

extinction risk by genetic factors. Modifying the vital rates associated with negative elasticities would therefore go in the opposite direction and is a type of management action that should generally be avoided. However, the decision about which life-stages should be the target of management actions will depend on what factor is the most important source of danger for the population. If a population is very small and at risk of extinction by demographic stochasticity, one may want to increase population size and population growth rate even if this can decrease the effective size. The same would be true for populations not showing clear signs of genetic impoverishment or where low genetic diversity has no effects on fitness. The problem can be nonetheless ignored for *D. austriacum* and *E. alpinum*, because the elasticities of N_e had the same signs as those of λ.

Future perspectives

The work conducted in this thesis have raised new questions and evidenced the need for further investigations, for instance:

1. *Incorporating sampling errors in demographic estimators.* Demographic-based estimation of N_e requires measurements of numerous ecological parameters whose values are affected by sampling error. However, the mathematical expressions to calculate N_e from demographic data consider these parameters as known quantities. These expressions should be reformulated in order to account for sampling error in demographic parameters. Ideally, demographic estimators should be able to provide average N_e values and confidence intervals based on the uncertainty of ecological measurements.

2. *Comparing demographic- and genetic-based estimates.* Very few studies have investigated the consistency of demographic- and genetic-based estimates of N_e. One difficulty surrounding this issue is finding model systems with enough demographic and genetic information to estimate N_e using the two approaches. An alternative is using simulation studies with individual-based stage-structured models (e.g. *rmetasim*; Strand 2002).

3. *Linkage disequilibrium N_e estimators in iteroparous species.* The relationship between N_e and linkage disequilibrium (LD) in iteroparous species is still unclear. The original method to estimate N_e from LD was proposed for populations with discrete and non-overlapping generations (Hill 1981). Extensions have been proposed for semelparous populations with

overlapping generations, such as salmon (Waples 2005). In iteroparous populations, sampling only one cohort will provide an estimate of the effective number of breeders rather than N_e. It is still unclear how the estimators perform when multiple cohorts are sampled.

4. *Using N_e to estimate demographic parameters.* Demographic estimators are based on equations that express N_e as a function of demographic parameters. If the equations are rearranged, they can be used to estimate demographic variables when N_e is known. For instance, genetic-based estimates of N_e can be used to infer population decline (Antao et al. 2010) or census size when the N_e/N ratio is known (Nunney and Elam 1994). In plant populations, it might be possible to estimate variation in reproductive success with the model of Yonezawa et al. (2000), provided that survival and fecundity rates are known. In all cases, N_e needs to be estimated accurately and precisely and uncertainties surrounding its estimation (confidence intervals) should be taken into account in the final estimation of the ecological variables of interest. As discussed in point 1 above, the estimation should be conducted a rigorous statistical framework.

5. *Studying variation in reproductive success.* Variation in reproductive success (σ_k^2, variation in number of seedlings per adult plant) is a key parameter for estimating N_e. However, few estimates of variation in reproductive success are available for plant populations, apart from estimates of seed production. However, seed production represents variation in female fecundity but does not give information on male variation, except in completely self-fertilizing species. More importantly, seed production need not be related to reproductive success measured as production of seedlings or even older life stages. Empirical studies on model system are needed to gain basic information on this phenomenon, such as: what is the expected range of values for σ_k^2? Does σ_k^2 exhibit extensive variation over years? What are the demographic variables determining σ_k^2 (variation in seed production, pollen production, pollination efficiency, germination rates, seedling survival)? These questions can be addressed using parentage analysis and individual based demographic studies.

6. *Global N_e in populations with spatial and stage structure.* Demographic estimators of N_e can be further extended in order to describe realistic situations. A formulation of N_e for geographically structured population with stage-structure or with deme-variation in age- or stage- structure is still lacking. The model of Rousset (2004) can be used as a starting point.

7. *N_e in stage-structured populations with demographic stochasticity.* Current models allow for stage-structure without demographic stochasticity (Orive 1993 for inbreeding and coalescent N_e; Yonezawa et al. 2000 for variance N_e) or demographic stochasticity with age-structure (Engen et al. 2005 for variance N_e). A model including stage-structure and demographic stochasticity would be useful to study numerous organisms, such as plants, where vital rates are expressed better as a function of stage rather than age.

Effectiveness of selection when life-history evolves

In addition, the analysis of the effect of life-history on variance effective size suggests that interesting dynamics can emerge when life-history traits are under selection. In order to discuss this effect, it is better to recall two concepts. The first is that population growth rate λ is equal to mean population fitness (Crow and Kimura 1970). The second is the concept of selection differential, defined as the derivative of mean population fitness (λ) to a given life-history trait; the selection differential indicates how fitness changes in response to a change in that trait. Positive selection differentials mean that increasing the trait value will increase fitness, while negative selection differentials mean that increasing the trait value will reduce fitness. As a consequence, heritable traits having positive selection differentials are expected to increase their mean value. For instance, if the selection differential to adult survival rate is positive and adult survival is heritable, then adult survival is expected to increase. This is strictly true only if there is no covariation between different fitness traits (e.g. negative covariance between survival and fecundity); in cases of covariation between life-history traits, selection can indeed have counter-intuitive outcomes (Charlesworth 1980). Since λ is equivalent to mean population fitness, the selection differential to a given vital rate is equivalent to the sensitivity of λ to that vital rate. The sensitivities can therefore be used to predict the outcome of evolution (Lande 1982).

In addition to excluding covariation, the above line of reasoning also assumes that genetic drift is disregarded. However, when genetic drift is strong, the effectiveness of selection is reduced (see equation I.3, also known as Wright's "rule of thumb" for the effectiveness of selection). For now, let us assume that covariation between fitness traits can be excluded and examine the interplay between drift and selection on the evolution of a single life-history trait. The strength of genetic drift can be measured by N_e. The elasticity or sensitivity of N_e to vital rates describes the effect of life-history on N_e. If selection changes the components of life-history (by increasing survival rates

for instance), the elasticity of N_e indicates whether N_e will increase or decrease in response to this change.

In *D. austriacum* and *E. alpinum*, the elasticities of N_e were always positive, but there are cases where N_e can exhibit negative elasticities, for instance in *Fritillaria camschatcensis* (Yonezawa et al. 2000). If a certain life-history trait is associated with negative elasticity of N_e and positive elasticity of λ, then the outcome of selection on that trait will entail a reduction of effective population size. This can have a negative feedback on selection of other slightly advantageous alleles, because when the effective size is reduced, genetic drift is stronger and the effectiveness of selection is reduced. If, on the contrary, the elasticity of N_e to the trait affected by selection is positive, then the net effect of selection will be to increase the effective size and thus decrease the strength of genetic drift. This will have a positive feedback on selection, which will become more and more effective. This mechanism can help very small populations in the process of adaptation.

It would be interesting to check whether the above hypothesis is true, but in order to do so the relevant processes need to be formulated in mathematical terms. The processes controlling the evolution of life-history traits are of course more complex than the simple description given here. First, one should consider genetic covariances between life-history traits. Secondly, the feedbacks described here are expected to be strong only in populations with very small effective sizes or on genes which are only slightly advantageous (recall Wright's "rule of thumb", equation I.3). Finally, it should not be forgotten that effective population size describes genetic drift for neutral genes, but genes affected by selection may experience different levels of genetic drift than those predicted by N_e. Moreover, genes that are unaffected directly by selection can still be influenced by selection on unlinked traits. In this case, the effective population size is reduced (Caballero 1994, section 5.2).

Analytical treatments of effective size under selection are available for population with discrete generations (Robertson 1961 and Wray et al. 1990, in Caballero 1994). There is currently no analytical investigation on the effect of selection on N_e in populations with overlapping generations and/or stage structure. On the other hand, such a task seems mathematically very challenging. One difficulty is that effective population size will continuously change as the life-history traits of the population evolve under the effect of selection. Therefore the effective population size will be different at each generation or even at each time step. In the face of these analytical difficulties, a pilot study using computer simulation could probably shed light on the most important parameters controlling the process.

Conclusions

The object of this thesis was to assess demographic estimators of effective population size and use them to study the effects of age- and stage-structure in populations with overlapping generations. The application of demographic and genetic estimators to empirical data showed that there can be great differences between the two approaches. Demographic models need further theoretical developments in order to describe all the relevant mechanisms acting on genetic drift and demographic parameters need to be estimated with accuracy and precision. On the other hand, genetic estimators still need to be assessed using empirical and simulated datasets.

Nonetheless, demographic models are a powerful tool to study the effect of life-history on N_e. This issue was addressed both analytically and numerically. The derived analytical expression for the sensitivity of N_e/N was too complex to be interpreted in terms of simple demographic parameters (survival and fecundity rates). Conversely, numerical elasticity analysis was successful in revealing patterns of elasticities over several populations. The next step will be to generalize the observed patterns using a comparative approach on a large number of populations, which will be possible thanks to the quick accumulation of large amounts of demographic and genetic data on a great number of species.

Conclusions

L'objectif de cette thèse était d'évaluer les estimateurs démographiques de la taille efficace des populations et leur utilisation pour l'étude de populations avec structure en classes d'âge ou en stades et avec générations chevauchantes. L'application des estimateurs démographiques et génétiques à des données empiriques a montré qu'il peut y avoir des grandes différences entre les deux approches. Les modèles démographiques nécessitent des développements théoriques ultérieurs afin de pouvoir décrire tous les processus pertinents qui influencent la dérive génétique, et, les paramètres démographiques doivent être estimés avec précision et sans biais. D'autre part, les estimateurs génétiques nécessitent toujours d'être évalués en utilisant des données empiriques et simulées.

Cependant, les modèles démographiques sont un outil puissant pour étudier les effets de l'histoire de vie sur N_e. Ce thème a été approché par voie analytique aussi bien que par voie numérique. L'expression pour la sensibilité de N_e/N qui a été dérivée était trop complexe pour être interprétée en terme de paramètres démographiques simples (taux de survie et de fécondité). Au contraire, l'analyse numérique d'élasticité a réussi à dévoiler des patrons d'élasticité sur plusieurs populations. L'étape suivante du travail sera de généraliser les patrons observés en utilisant une approche comparative sur un grand nombre de populations, ce qui serait possible grâce à la rapide accumulation de grandes quantités de données démographiques et génétiques sur un grand nombre d'espèces.

References

Allendorf, F. W., P. R. England, G. Luikart, P. A. Ritchie and N. Ryman. 2008. Genetic effects of harvest on wild animal populations. *Trends in Ecology and Evolution* 23: 327-337.

Anderson, E. C. 2005. An efficient Monte Carlo method for estimating N_e from temporally spaced samples using a coalescent-based likelihood. *Genetics* 170: 955-967.

Anderson, E. C., E. G. Williamson and E. A. Thompson. 2000. Monte Carlo evaluation of the likelihood for N_e from temporally spaced samples. *Genetics* 156: 2109-2118.

Baker, M. C., L. R. Mewaldt and R. M. Stewart. 1981. Demography of White-Crowned Sparrows (*Zonotrichia leucophrys nuttalli*). *Ecology* 62: 636-644.

Balloux, F. 2004. Heterozygote excess in small populations and the heterozygote-excess effective population size. *Evolution* 58: 1891-900.

Beaumont, M. A. 2003. Estimation of population growth or decline in genetically monitored populations. *Genetics* 164: 1139-60.

Beaumont, M. A., W. Zhang and D. J. Balding. 2002. Approximate Bayesian computation in population genetics. *Genetics* 162: 2025-35.

Beebee, T. J. 2009. A comparison of single-sample effective size estimators using empirical toad (*Bufo calamita*) population data: genetic compensation and population size-genetic diversity correlations. *Mol Ecol* 18: 4790-7.

Begon, M., C. B. Krimbas and M. Loukas. 1980. The genetics of *Drosophila suboscura* populations. XV. Effective size of a natural population estimated by three independent methods. *Heredity* 45: 335-350.

Berthier, P., M. A. Beaumont, J. M. Cornuet and G. Luikart. 2002. Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. *Genetics* 160: 741-51.

Bonin, A., F. Nicole, F. Pompanon, C. Miaud and P. Taberlet. 2007. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. *Conserv Biol* 21: 697-708.

Caballero, A. 1994. Developments in the prediction of effective population size. *Heredity* 73: 657-679.

Campbell, L. G. and B. C. Husband. 2005. Impact of clonal growth on effective population size in *Hymenoxys herbacea* (Asteraceae). *Heredity* 94: 526-32.

Caswell, H. 1978. A general formula for the sensitivity of population growth rate to changes in life history parameters. *Theoretical Population Biology* 14: 215-230.

Caswell, H. 1980. On the Equivalence of Maximizing Reproductive Value and Maximizing Fitness. *Ecology* 61: 19-24.

Caswell, H. 1982. Optimal Life Histories and the Maximization of Reproductive Value - a General Theorem for Complex Life-Cycles. *Ecology* 63: 1218-1222.

Caswell, H. 2001. *Matrix population models: construction, analysis, and interpretation*. Sinauer Associates, Sunderland, Massachusetts.

Caswell, H. 2008. Perturbation analysis of nonlinear matrix population models. *Demographic Research* 18: 59-115.

Charlesworth, B. 1980. *Evolution in age-structured populations*. Cambridge University Press, Cambridge, United Kingdom.

Charlesworth, D. and B. Charlesworth. 1987. Inbreeding Depression and Its Evolutionary Consequences. *Annual Review of Ecology and Systematics* 18: 237-268.

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. *Nature Reviews Genetics* 10: 783-796.

Cherel, O. and A. Lavagne. 1982. Aire de répartition, phénologie, biologie, reproduction d'*Eryngium alpinum*, "la Reine des Alpes", dans la vallée du Fournel. Propositions de mesures de protection de l'espèce. *Travaux Scientifiques du Parc National des Ecrins* 2: 53-92.

Choy, S. C. and B. S. Weir. 1978. Exact Inbreeding Coefficients in Populations with Overlapping Generations. *Genetics* 89: 591-614.

Cochran, M. E. and S. Ellner. 1992. Simple Methods for Calculating Age-Based Life-History Parameters for Stage-Structured Populations. *Ecological Monographs* 62: 345-364.

Connell, J. H. 1970. A Predator-Prey System in Marine Intertidal Region .1. *Balanus glandula* and Several Predatory Species of Thais. *Ecological Monographs* 40: 49-and.

Corander, J., P. Waldmann and M. J. Sillanpaa. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* 163: 367-374.

Crow, J. F. and M. Kimura. 1970. *An introduction to population genetics theory*. Harper and Row, New York.

Danton, P. and M. Baffray. 1995. *Inventaire des plantes protégées en France*. Nathan, Paris, France.

de Valpine, P. 2009. Stochastic development in biologically structured population models. *Ecology* 90: 2889-2901.

de Kroon, H., A. Plaisier, J. Vangroenendael and H. Caswell. 1986. Elasticity - the Relative Contribution of Demographic Parameters to Population-Growth Rate. *Ecology* 67: 1427-1431.

De Mauro, M. M. 1993. Relationship of Breeding System to Rarity in the Lakeside Daisy (*Hymenoxys acaulis var. glabra*). *Conservation Biology* 7: 542-550.

Dostalek, T., Z. Munzbergova and I. Plackova. 2010. Genetic diversity and its effect on fitness in an endangered plant species, *Dracocephalum austriacum* L. *Conservation Genetics* 11: 773-783.

Efron, B. and R. Tibshirani. 1993. *An introduction to the bootstrap*. Chapman and Hall, New York.

Ellner, S. and N. G. Hairston. 1994. Role of Overlapping Generations in Maintaining Genetic-Variation in a Fluctuating Environment. *American Naturalist* 143: 403-417.

Emigh, T. H. and E. Pollak. 1979. Fixation Probabilities and Effective Population Numbers in Diploid Populations with Overlapping Generations. *Theoretical Population Biology* 15: 86-107.

Engen, S., R. Lande and B. E. Saether. 2005. Effective size of a fluctuating age-structured population. *Genetics* 170: 941-954.

Engen, S., T. H. Ringsby, B. E. Saether, R. Lande, H. Jensen, M. Lillegard and H. Ellegren. 2007. Effective size of fluctuating populations with two sexes and overlapping generations. *Evolution* 61: 1873-1885.

Ewens, W. J. 2004. *Mathematical population genetics*. Springer, New York.

Falush, D., M. Stephens and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

Felsenstein, J. 1971. Inbreeding and Variance Effective Numbers in Populations with Overlapping Generations. *Genetics* 68: 581-597.

Fisher, R. A. 1930. *The genetical theory of natural selection*. The Clarendon press, Oxford,.

Foll, M., M. A. Beaumont and O. Gaggiotti. 2008. An approximate Bayesian computation approach to overcome biases that arise when using amplified fragment length polymorphism markers to study population structure. *Genetics* 179: 927-939.

Franco, M. and J. Silvertown. 2004. Comparative demography of plants based upon elasticities of vital rates. *Ecology* 85: 531-538.

Frankham, R. 2005. Genetics and extinction. *Biological Conservation* 126: 131-140.

Franklin, I. R. 1980. Evolutionary changes in small populations. *In*: M. E. Soulé and B. A. Wilcox (eds.) *Conservation Biology: an evolutionary-ecological perspective*. Sinauer Associates,

Sunderland, Massachusetts.

Gaggiotti, O. E. and I. Hanski. 2004. Mechanisms of population extinction. *In*: I. Hanski and O. E. Gaggiotti (eds.) *Ecology, genetics and evolution of metapopulations*. Elsevier Academic Press, Amsterdam, The Netherlands.

Gaggiotti, O. E., F. Jones, W. M. Lee, W. Amos, J. Harwood and R. A. Nichols. 2002. Patterns of colonization in a metapopulation of grey seals. *Nature* 416: 424-427.

Gaggiotti, O. E. and R. D. Vetter. 1999. Effect of life history strategy, environmental variability, and overexploitation on the genetic diversity of pelagic fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 1376-1388.

Gaudeul, M., Y. Naciri-Graven, P. Gauthier and F. Pompanon. 2002. Isolation and characterization of microsatellites in a perennial Apiaceae, *Eryngium alpinum* L. *Molecular Ecology Notes* 2: 107-109.

Gaudeul, M., P. Taberlet and I. Till-Bottraud. 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L.. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 9: 1625-1637.

Gaudeul, M. and I. Till-Bottraud. 2003. Low selfing in a mass-flowering, endangered perennial, *Eryngium alpinum* L. (Apiaceae). *American Journal of Botany* 90: 716-723.

Gaudeul, M. and I. Till-Bottraud. 2004. Reproductive ecology of the endangered alpine species *Eryngium alpinum* L. (Apiaceae): Phenology, gene dispersal and reproductive success. *Annals of Botany* 93: 711-721.

Gaudeul, M. and I. Till-Bottraud. 2008. Genetic structure of the endangered perennial plant *Eryngium alpinum* (Apiaceae) in an alpine valley. *Biological Journal of the Linnean Society* 93: 667-677.

Gaudeul, M., I. Till-Bottraud, F. Barjon and S. Manel. 2004. Genetic diversity and differentiation in *Eryngium alpinum* L. (Apiaceae): comparison of AFLP and microsatellite markers. *Heredity* 92: 508-518.

Gillot, P. and L. Garraud. 1995. *Eryngium alpinum* (L.). *Livre Rouge de la Flore Menacée*.

Museum National d'Histoire Naturelle, Conservatoire Botanique National de Porquerolles, Ministère de l'Environnement, Paris, France.

Gilpin, M. E. 1987. Spatial structure and population vulnerability. *In*: M. E. Soulé (ed.) *Viable populations for conservation*. Cambridge University Press, Cambridge, United Kingdom.

Gilpin, M. E. and M. E. Soulé. 1986. Minimum viable populations: processes of species extinction. *In*: M. E. Soulé (ed.) *Conservation biology: the science of scarcity and diversity*. Sinauer Associates, Sunderland, Massachusetts.

Goodman, D. 1987. The demography of chance extinction. *In*: M. E. Soulé (ed.) *Viable populations for conservation*. Cambridge University Press, Cambridge, United Kingdom.

Grivet, D., J. J. Robledo-Arnuncio, P. E. Smouse and V. L. Sork. 2009. Relative contribution of contemporary pollen and seed dispersal to the effective parental size of seedling population of California valley oak (*Quercus lobata*, Nee). *Molecular Ecology* 18: 3967-3979.

Hampe, A., L. El Masri and R. J. Petit. 2010. Origin of spatial genetic structure in an expanding oak population. *Molecular Ecology* 19: 459-471.

Harper, J. L. 1977. *Population biology of plants*. Academic Press, London.

Harris, R. B. and F. W. Allendorf. 1989. Genetically effective population size of large mammals — an assessment of estimators. . *Conservation Biology* 3: 181-191.

Hauser, L., G. J. Adcock, P. J. Smith, J. H. B. Ramirez and G. R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America* 99: 11742–11747.

Hedgecock, D. 1994. Temporal and spatial genetic-structure of marine animal populations in the California current. *California Cooperative Oceanic Fisheries Investigations Reports* 35: 73–81.

Hedgecock, D., S. Launey, A. I. Pudovkin, Y. Naciri, S. Lapegue and F. Bonhomme. 2007. Small effective number of parents (N_b) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. *Marine Biology* 150: 1173-1182.

Hedrick, P. 2005. Large variance in reproductive success and the N_e / N ratio. *Evolution* 59: 1596-1599.

Heppell, S. S., D. T. Crouse and L. B. Crowder. 2000. Using matrix models to focus research and management efforts in conservation. *In*: S. Ferson and M. Burgman (eds.) *Quantitative methods for conservation biology*. Springer-Verlag, New York.

Heywood, J. S. 1986. The Effect of Plant Size Variation on Genetic Drift in Populations of Annuals. *American Naturalist* 127: 851-861.

Hill, W. G. 1972. Effective Size of Populations with Overlapping Generations. *Theoretical Population Biology* 3: 278-and.

Hill, W. G. 1979. Note on Effective Population-Size with Overlapping Generations. *Genetics* 92: 317-322.

Hill, W. G. 1981. Estimation of Effective Population-Size from Data on Linkage Disequilibrium. *Genetical Research* 38: 209-216.

Horvitz, C. C., S. Tuljapurkar and J. B. Pascarella. 2005. Plant-animal interactions in random environments: Habitat-stage elasticity, seed predators, and hurricanes. *Ecology* 86: 3312-3322.

Hudson, R. R. 1991. Gene genealogies and the coalescent process. *In*: D. Futuyama and J. Antonovics (eds.) *Oxford Surveys in Evolutionary Biology*. Oxford University Press, Oxford, United Kingdom.

Husband, B. C. and S. C. H. Barrett. 1992. Effective Population-Size and Genetic Drift in Tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 46: 1875-1890.

Johnson, D. L. 1977. Inbreeding in Populations with Overlapping Generations. *Genetics* 87: 581-591.

Jorde, P. E. and N. Ryman. 1995. Temporal Allele Frequency Change and Estimation of Effective Size in Populations with Overlapping Generations. *Genetics* 139: 1077-1090.

Jorde, P. E. and N. Ryman. 2007. Unbiased estimator for genetic drift and effective population size.

Genetics 177: 927-935.

Kaeuffer, R., D. Pontier, S. Devillard and N. Perrin. 2004. Effective size of two feral domestic cat populations (*Felis catus* L.): effect of the mating system. *Molecular Ecology* 13: 483-490.

Kalisz, S. and M. A. Mcpeek. 1992. Demography of an Age-Structured Annual - Resampled Projection Matrices, Elasticity Analyses, and Seed Bank Effects. *Ecology* 73: 1082-1093.

Kimura, M. and J. F. Crow. 1963. The measurement of effective population number. *Evolution* 17: 279-288.

Kobayashi, Y. and N. Yamamura. 2007. How to compute the effective size of spatiotemporally structured populations using separation of time scales. *Theoretical Population Biology* 71: 174-181.

Krimbas, C. B. and S. Tsakas. 1971. Genetics of *Dacus oleae* .5. Changes of Esterase Polymorphism in a Natural Population Following Insecticide Control-Selection or Drift. *Evolution* 25: 454-and.

Lacy, R. C. 2000. Considering threats to the viability of small populations using individual-based models. *Ecological Bulletins* 48: 39-51.

Lacy, R. C., M. Borbat and J. P. Pollak. 2005. VORTEX: A Stochastic Simulation of the Extinction Process. 9.50 ed. Brookfield, Illinois: Chicago Zoological Society.

Lande, R. 1982. A Quantitative Genetic Theory of Life-History Evolution. *Ecology* 63: 607-615.

Lande, R. 1995. Mutation and Conservation. *Conservation Biology* 9: 782-791.

Lande, R. and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. *In*: M. E. Soulé (ed.) *Viable populations for conservation*. Cambridge University Press, Cambridge, United Kingdom.

Laporte, V. and B. Charlesworth. 2002. Effective population size and population subdivision in demographically structured populations. *Genetics* 162: 501-519.

Lauber, K. and G. Wagner. 1998. *Flora Helvetica*. Verlag Paul Haupt, Bern, Switzerland.

Lauenroth, W. K. and P. B. Adler. 2008. Demography of perennial grassland plants: survival, life expectancy and life span. *Journal of Ecology* 96: 1023-1032.

Leberg, P. 2005. Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management* 69: 1385–1399.

Levin, L., H. Caswell, T. Bridges, C. DiBacco, D. Cabrera and G. Plaia. 1996. Demographic responses of estuarine polychaetes to pollutants: Life table response experiments. *Ecological Applications* 6: 1295-1313.

Luikart, G. and J. M. Cornuet. 1999. Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* 151: 1211-1216.

Luikart, G., N. Ryman, D. A. Tallmon, M. K. Schwartz and F. W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics* 11: 355-373.

Maruyama, T. and M. Kimura. 1980. Genetic-Variability and Effective Population-Size When Local Extinction and Recolonization of Sub-Populations Are Frequent. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* 77: 6710-6714.

Menges, E. S. 1990. Population Viability Analysis for an Endangered Plant. *Conservation Biology* 4: 52-62.

Miller, C. R. and L. P. Waits. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proceedings of the National Academy of Sciences of the United States of America* 100: 4334–4339.

Mills, L. S., D. F. Doak and M. J. Wisdom. 1999. Reliability of conservation actions based on elasticity analysis of matrix models. *Conservation Biology* 13: 815-829.

Muller, H. J. 1964. The relation of recombination to mutational advance. *Mutat Res* 106: 2–9.

Munzbergova, Z. and J. Ehrlen. 2005. How best to collect demographic data for population viability analysis models. *Journal of Applied Ecology* 42: 1115-1120.

Nathan, R. and R. Casagrandi. 2004. A simple mechanistic model of seed dispersal, predation and plant establishment: Janzen-Connell and beyond. *Journal of Ecology* 92: 733-746.

Nei, M. and Y. Imazumi. 1966. Genetic structure of human populations. II: Differentiation of blood group gene frequencies among isolated populations. *Heredity* 21: 183-190, 344.

Nei, M. and F. Tajima. 1981. Genetic drift and estimation of effective population size. *Genetics* 98: 625–640.

Newman, D. and D. Pilson. 1997. Increased probability of extinction due to decreased genetic effective population size: Experimental populations of *Clarkia pulchella*. *Evolution* 51: 354-362.

Nicolè, F. 2005. *Biologie de la conservation appliquée aux plantes menacées des Alpes*. PhD Thesis, Université Joseph Fourier, Grenoble, France.

Nomura, T. 2008. Estimation of effective number of breeders from molecular coancestry of single cohort sample. *Evolutionary Applications* 1: 462-474.

Nunney, L. 1991. The Influence of Age Structure and Fecundity on Effective Population-Size. *Proceedings of the Royal Society of London Series B-Biological Sciences* 246: 71-76.

Nunney, L. 1993. The Influence of Mating System and Overlapping Generations on Effective Population-Size. *Evolution* 47: 1329-1341.

Nunney, L. 1995. Measuring the Ratio of Effective Population-Size to Adult Numbers Using Genetic and Ecological Data. *Evolution* 49: 389-392.

Nunney, L. 1999. The effective size of a hierarchically structured population. *Evolution* 53: 1-10.

Nunney, L. 2002. The effective size of annual plant populations: The interaction of a seed bank with fluctuating population size in maintaining genetic variation. *American Naturalist* 160: 195-204.

Nunney, L. and D. R. Elam. 1994. Estimating the Effective Population-Size of Conserved Populations. *Conservation Biology* 8: 175-184.

Olivier, L., J. P. Galland and H. Maurin. 1995. *Livre Rouge de la flore menacée de France. Tome I:*

Espèces prioritaires. Collection Patrimoines Naturels, Paris, France.

Orive, M. E. 1993. Effective population size in organisms with complex life-histories. *Theoretical Population Biology* 44: 316–340.

Palstra, F. P. and D. E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology* 17: 3428-3447.

Peel, D., J. R. Ovenden and S. L. Peel. 2004. Neestimator: software for estimating effective population size. Version 12 ed.: Queensland Government, Department of Primary Industries and Fisheries, Brisbane, Australia.

Pollak, E. 1979. Effective Population Numbers and Mean Times to Extinction in Monoecious Populations with Overlapping Generations. *Mathematical Biosciences* 46: 87-106.

Pollak, E. 1990. The Effective Population-Size of an Age-Structured Population with a Sex-Linked Locus. *Mathematical Biosciences* 101: 121-130.

Pudovkin, A. I., D. V. Zaykin and D. Hedgecock. 1996. On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics* 144: 383-387.

Robertson, A. 1961. Inbreeding in artificial selection programmes. *Genetical Research* 2: 189-194.

Rousset, F. 1999. Genetic differentiation in populations with different classes of individuals. *Theoretical Population Biology* 55: 297-308.

Rousset, F. 2003. Effective size in simple metapopulation models. *Heredity* 91: 107-111.

Rousset, F. 2004. *Genetic structure and selection in subdivided populations*. Princeton University Press, Princeton, New Jersey.

Ryman, N., R. Baccus, C. Reuterwall and M. H. Smith. 1981. Effective Population-Size, Generation Interval, and Potential Loss of Genetic-Variability in Game Species under Different Hunting Regimes. *Oikos* 36: 257-266.

Saarinen, E. V., J. D. Austin and J. C. Daniels. 2010. Genetic estimates of contemporary effective population size in an endangered butterfly indicate a possible role for genetic compensation.

Evolutionary Applications 3: 28-39.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392: 491-494.

Schwartz, M. K., D. A. Tallmon and G. Luikart. 1999. Using genetics to estimate the size of wild populations: many methods, much potential, uncertain utility. *Animal Conservation* 2: 321-323.

Sezen, U. U., R. L. Chazdon and K. E. Holsinger. 2009. Proximity is not a proxy for parentage in an animal-dispersed Neotropical canopy palm. *Proceedings of the Royal Society B-Biological Sciences* 276: 2037-2044.

Shaffer, M. L. 1981. Minimum Population Sizes for Species Conservation. *Bioscience* 31: 131-134.

Silvertown, J., M. Franco and E. Menges. 1996. Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. *Conservation Biology* 10: 591-597.

Silvertown, J., M. Franco, I. Pisanty and A. Mendoza. 1993. Comparative Plant Demography - Relative Importance of Life-Cycle Components to the Finite Rate of Increase in Woody and Herbaceous Perennials. *Journal of Ecology* 81: 465-476.

Sjodin, P., I. Kaj, S. Krone, M. Lascoux and M. Nordborg. 2005. On the meaning and existence of an effective population size. *Genetics* 169: 1061-1070.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. *In*: M. E. Soulé and B. A. Wilcox (eds.) *Conservation Biology: an evolutionary-ecological perspective*. Sinauer Associates, Sunderland, Massachusetts.

Strand, A. E. 2002. METASIM 1.0: an individual-based environment for simulating population genetics of complex population dynamics. *Molecular Ecology Notes* 2: 373-376.

Tallmon, D. A., A. Koyuk, G. Luikart and M. A. Beaumont. 2008. ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. *Molecular Ecology Resources* 8: 299-301.

Tallmon, D. A., G. Luikart and M. A. Beaumont. 2004. Comparative evaluation of a new effective

population size estimator based on approximate Bayesian computation. *Genetics* 167: 977-988.

Templeton, A. R. 2006. *Population genetics and microevolutionary theory*. Wiley-Liss, Hoboken, N.J.

Tuljapurkar, S., C. C. Horvitz and J. B. Pascarella. 2003. The many growth rates and elasticities of populations in random environments. *American Naturalist* 162: 489-502.

Turner, T. F., M. J. Osborne, G. R. Moyer, M. A. Benavides and D. Alo. 2006. Life history and environmental variation interact to determine effective population to census size ratio. *Proceedings of the Royal Society B-Biological Sciences* 273: 3065-3073.

Turner, T. F., J. P. Wares and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics* 162: 1329-1339.

Vinciguerra L. (2002). Programme de conservation sur *Dracocephalum austriacum*. Rapport interne du Conservatoire Botanique National Alpin, Gap, France.

Vitalis, R. and D. Couvet. 2001. Estimation of effective population size and migration rate from one- and two-locus identity measures. *Genetics* 157: 911-925.

Vitalis, R., S. Glemin and I. Olivieri. 2004. When genes go to sleep: The population genetic consequences of seed dormancy and monocarpic perenniality. *American Naturalist* 163: 295-311.

Wakeley, J. and O. Sargsyan. 2009. Extensions of the Coalescent Effective Population Size. *Genetics* 181: 341-345.

Wang, J. L. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research* 78: 243-257.

Wang, J. L. 2005. Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B-Biological Sciences* 360: 1395-1409.

Wang, J. L. 2009. A new method for estimating effective population sizes from a single sample of

multilocus genotypes. *Molecular Ecology* 18: 2148-2164.

Wang, J. L. and A. Caballero. 1999. Developments in predicting the effective size of subdivided populations. *Heredity* 82: 212-226.

Wang, J. L. and M. C. Whitlock. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163: 429-446.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121: 379-91.

Waples, R. S. 1990a. Conservation Genetics of Pacific Salmon .2. Effective Population-Size and the Rate of Loss of Genetic-Variability. *Journal of Heredity* 81: 267-276.

Waples, R. S. 1990b. Conservation Genetics of Pacific Salmon .3. Estimating Effective Population-Size. *Journal of Heredity* 81: 277-289.

Waples, R. S. 2002a. Effective size of fluctuating salmon populations. *Genetics* 161: 783-91.

Waples, R. S. 2002b. Evaluating the effect of stage-specific survivorship on the N_e/N ratio. *Molecular Ecology* 11: 1029-1037.

Waples, R. S. 2005. Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Mol Ecol* 14: 3335-52.

Waples, R. S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics* 7: 167-184.

Waples, R. S. 2010. Spatial-temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. *Molecular Ecology Resources* 10: 785-796.

Waples, R. S. and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* 8: 753-756.

Waples, R. S. and C. Do. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3: 244-262.

Waples, R. S., D. W. Jensen and M. McClure. 2010. Eco-evolutionary dynamics: fluctuations in population growth rate reduce effective population size in chinook salmon. *Ecology* 91: 902-14.

Waples, R. S. and M. Yokota. 2007. Temporal estimates of effective population size in species with overlapping generations. *Genetics* 175: 219-233.

Wares, J. P. and J. M. Pringle. 2008. Drift by drift: effective population size is limited by advection. *Bmc Evolutionary Biology* 8: -.

Wennergren, U., P. Weinerfelt and G. Forsling. 1994. Comparative Sensitivity Analysis of Stable Stage Structures and Reproductive Values. *Bulletin of Mathematical Biology* 56: 945-957.

Whitlock, M. C. and N. H. Barton. 1997. The effective size of a subdivided population. *Genetics* 146: 427-441.

Williamson, E. G. and M. Slatkin. 1999. Using maximum likelihood to estimate population size from temporal changes in allele frequencies. *Genetics* 152: 755-761.

Wolfram Research, I. 2004. Mathematica. 5.1 ed. Wolfram Research, Inc, Champaign, Illinois.

Wray, N. R., J. A. Woolliams and R. Thompson. 1990. Methods for Predicting Rates of Inbreeding in Selected Populations. *Theoretical and Applied Genetics* 80: 503-512.

Wright, S. 1931. Evolution in Mendelian Populations. *Genetics* 16: 97-159.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. *Science* 87: 430-431.

Wyse-Jackson, P. S. and J. R. Akeroyd. 1994. *Guidelines to be followed in the design of plant conservation or recovery plans*. Council of Europe (Nature and environment, 68), Strasbourg, France.

Yonezawa, K. 1997. Effective population size of plant species propagating with a mixed sexual and asexual reproduction system. *Genetical Research* 70: 251-258.

Yonezawa, K., T. Ishii and T. Nagamine. 2004. The effective size of mixed sexually and asexually reproducing populations. *Genetics* 166: 1529-1539.

Yonezawa, K., E. Kinoshita, Y. Watano and H. Zentoh. 2000. Formulation and estimation of the effective size of stage-structured populations in *Fritillaria camtschatcensis*, a perennial herb with a complex life history. *Evolution* 54: 2007-2013.

Zhdanova, O. L. and A. I. Pudovkin. 2008. Nb_HetEx: A Program to Estimate the Effective Number of Breeders. *Journal of Heredity* 99: 694-695.

Appendix:

Demography and reproductive system in *Arabis alpina*

Introduction

Arabis alpina (L. Brassicaceae) is a perennial plant inhabiting alpine regions of Europe, Africa and Middle-East. Recently, this plant is receiving increasing attention for the possibility to use it as a new model species. Numerous studies have been conducted or are currently under development at LECA on the phylogeography (Assefa et al. 2007), local adaptation (Manel et al. 2010; Poncet et al. 2010; Poncet 2010), stress resistance (Michel Herzog, study in progress) and sequencing (Stéphane Lobreaux, Christelle Melodelima, study in progress) of *A. alpina*. The purpose of my work was to complement these studies with ecological data from natural populations.

My project focused on demography and reproductive biology. The purpose of the demographic study was to measure vital rates (survival and germination rates, seed production) in natural conditions and to determine the environmental factors responsible for their variation in time and space. Along with data on the reproductive system, these studies can be used to develop mathematical models for the estimation of important demographic and evolutionary parameters, such as the population dynamics modes to estimate population growth rates (Appendix II) or models for the estimation of effective population size (Chapter I).

a)

b)

Figure A.1. a) Rosette of *A. alpina* during the winter; b) plant bearing fruits (siliques)

Demography was studied using individual-based field studies. To this purpose, we chose eight natural sites where the species was sufficiently abundant (i.e., at least fifty plants). These sites were also used to sample seeds for fecundity estimation and for a germination experiment. The sites were characterized by collecting local data on climate (temperature) and soil (nitrogen and carbon content). Climatic data were collected on site by means of data loggers that measured temperature every two hours. A pilot experiment was conducted to study the reproductive strategy of the species. The purpose of the experiment was to assess self-compatibility and the possibility of selfing. Collection and translocation of

adult plants from the field to the common garden in LECA failed, as plants may have been damaged when collected and may have suffered from the different climatic conditions found at low altitudes. For this reason, we decided to perform the reproductive strategy experiment *in situ*

The *A. alpina* project started at the beginning of my PhD and is still in progress, because demographic studies need to be performed for several years in order to embrace enough temporal variability in vital rates. Some pilot experiments (reproductive system, germination) were needed to assess the feasibility of more thorough investigations in the future. The whole budget of my project allowed us to conduct field observation (travelling, accommodation, purchasing of field materials) but not to perform genetic analysis, even if tissue samples have been collected for future analysis. Here I report the first results obtained on demography (survival and fecundity rates, seed production) and reproductive system.

Materials and methods

Species and study sites

Arabis alpina is a perennial plant distributed in the arctic-alpine regions of Europe, Middle East and Africa. Growing at 500–3200m altitude, this plant prefers moist, open, low-competition rocky habitats, reproducing sexually or asexually by stoloniferous growth. The plant forms rosettes that persist during the winter (Figure A.1a). Each reproductive stem can produce up to 30 flowers, which are pollinated by insects or by the wind and selfing is probably possible (Titz 1971). The fruit is a silique (Figure A.1b).

Sites were chosen so as to encompass the widest altitudinal gradient of the species (Table A.1). Vercors and Chartreuse are calcareous ranges, while sites in Lautaret-Galibier are located on schist bedrock (Figure A.2). All sites had a number of plants comprised between 50 and 500 individuals with moderate density. Sites with very dense *A. alpina* cover were not included because identification of individual plants would have been difficult; sites where plant distribution was too sparse were disregarded as well. Sites have also been chosen for their accessibility and protection from human disturbance. As a consequence, although the sites comprise the widest possible altitudinal gradient of the species, they may not reflect the complete range of variation of *A. alpina* habitats.

In each site, the demographic study was conducted using permanent plots, whose number, shape and size were dictated by the density and distribution of plants (Table A.1). In BRU, CHA and VIL, the permanent plots were installed in October-November 2007. In the other sites, the permanent plots were installed in June-July 2008. Plots were placed on similar portion of each site in order to minimize within-site variability in environmental and demographic characteristics.

Table AI.1: Description of the study sites. k, number of demographic plots

Site		*k*	Elevation	Exposure	N	E	Remarks
Vercors							
Gorges du Bruyant	BRU	2	1000	S	45.15	5.60	Screes; soil very unsteady; forest
Combe de Chaulange	CHA	3	1500	NW	45.06	5.59	Screes with some shrubs
Villard de Lans	VIL	3	2000	SW	45.01	5.57	Screes; no tree/shrub cover
Chartreuse							
Grande Sure	SUR	3	1600	NW	45.33	5.72	Coniferous forest (exploited)
Chamechaude	CHM	1	2000	W	45.28	5.78	In an opening of the rock
Lautaret-Galibier							
Lautaret	LAU	3	2000	N	45.02	6.38	River side with shrubs
Galibier	GAL	3	2600	N	45.05	6.40	Under cliff; no shrub/tree
Pic Blanc du Galiber	PIC	4	3000	E	45.05	6.38	Screes; almost bare

Environmental data

Air temperature and humidity were measured with iButton® DS1923 data loggers (Figure A.3a; www.maxim-ic.com). One logger per plot was installed 5-10cm above soil surface on a wooden stake set in the ground. Loggers were sheltered from direct sunlight with a wooden or plexiglas 15-by-15cm plate (Figure A.3b). Temperature was recorded every two hours from the date of installation in Autumn 2008 to the date of demographic census in Summer 2009. The dates of installation and census varied among sites.

Figure A.4 reports a profile of temperature data retrieved from the logger. The retrieved environmental variables were the duration of snow cover; the number and fraction of frost days; minimal, mean and maximal temperature after snow melt (hereafter, “temperature”); minimal, mean and maximal temperature in the summer (hereafter, “summer temperature”). The presence of snow

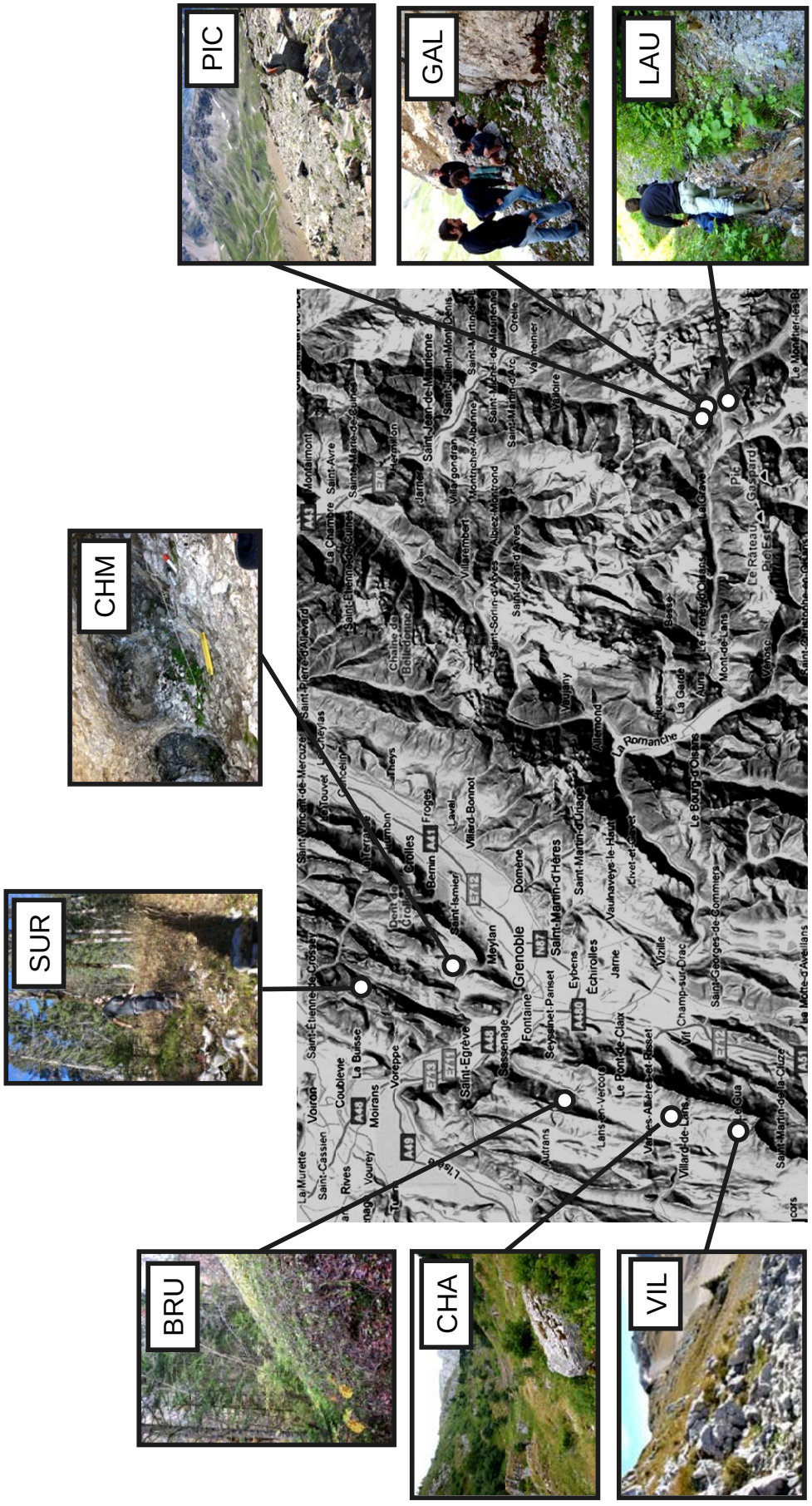
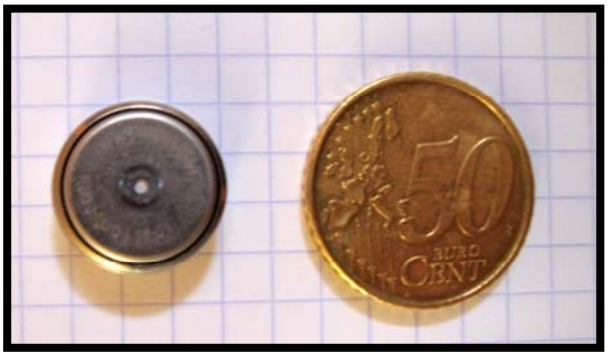


Figure A.2. Location of the eight study sites.

a)



b)

Figure A.3. a) Temperature data logger (the coin is for size comparison); b) the device used to shelter data loggers from direct sunlight.

cover corresponded to the time when temperature and humidity exhibited constant values. Frost days were defined as those days in which temperature fell below 0°C. The fraction of frost days was counted on the period preceding and following snow cover. Minimal, mean and maximal temperatures were defined as the average minimal, mean and maximal daily temperature over the 32 days following the end of snow cover. This period corresponds therefore to the same phenological state in all sites. Summer minimal, mean and maximal temperatures were calculated as the average minimal, mean and maximal daily temperature from June 20th 2009 to July 15th 2009. Summer temperatures are thus calculated on the same calendar time period in all sites but correspond to different phenological states for plants in different sites: as a matter of fact, on the same date, the measures corresponds to the onset of vegetative growth in high altitude sites (e.g. PIC), flowering in mid-altitude sites (e.g. VIL and LAU) and fruit maturation in low altitude sites (e.g. BRU).

Three soil samples per plot were collected in Autumn 2008 in all sites. Samples were let to dry for at least two days, then sieved (2mm mesh). Soil acidity (pH) was measured in water with a pH meter; it was not possible to measure the pH in BRU, CHA and SUR because the collected samples did not contain enough soil to conduct one measure per sample. We decided not to pool individual samples together and to collect larger samples in the future. Total nitrogen and carbon content was measured with a FlashEA 1112 analyzer (www.thermoscientific.com) by Bruno Couchaud (équipe TDE, LECA) in May 2009. Two to five µg of finely ground soil was used for nitrogen and carbon determination.

Differences between sites in environmental variables [fraction of frost days; minimal, mean and maximal temperatures; summer minimal, mean and maximal temperatures; soil pH; soil nitrogen

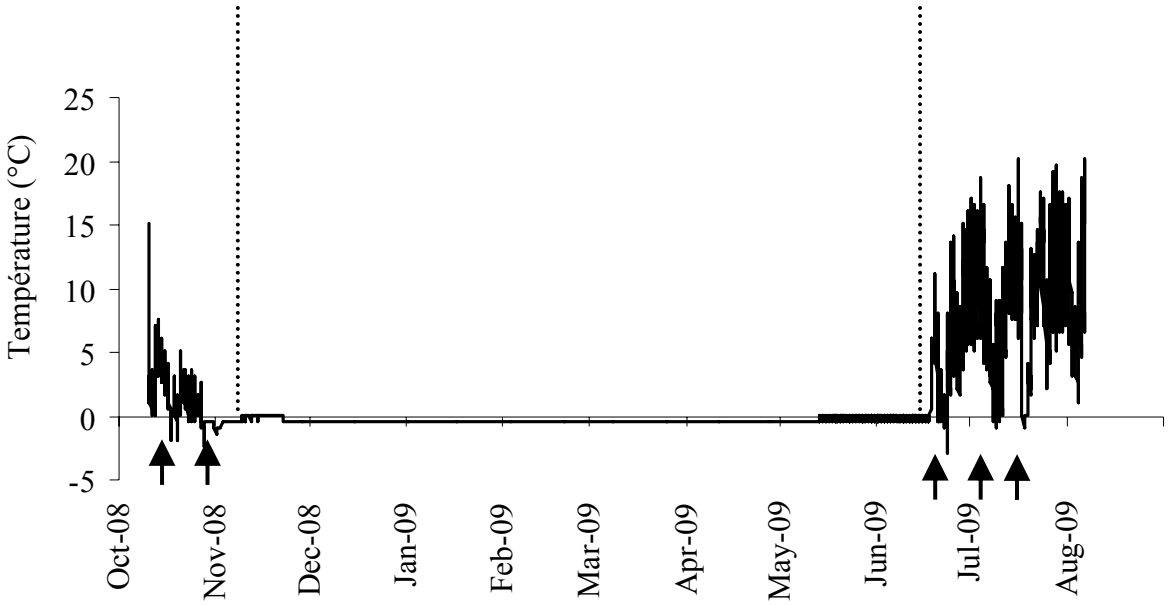


Figure A.4. Temperature recording for the GAL-2 site. Dotted vertical lines delimit the beginning and the end of snow cover; small arrows indicates frost events.

content (%N); soil carbon to nitrogen ratio (C/N ratio)] were tested using non-parametric ANOVA (Kruskal-Wallis test).

Survival rates

At the beginning of the study, each plant in the plot was marked with a metal tag (Figure A.5) nailed to the ground and the position of all plants was recorded by measuring the distance from two plot vertices to the base of the plant. These data were used to construct a map of the plot (see an example in Figure A.6) by transforming distance measures into x-y coordinates through standard trigonometric relationships. When the soil surface was too rough and in presence of big rocks, the reconstruction of plant position from field measures was not sufficiently precise to accurately construct the map. In such cases, a photograph of the plant along with its tag was taken. The practice of taking photographs was adopted as a standard for all new plants from the 2009 season onward. The joint use of metal tags, maps and photographs assure a correct identification of individual plants. Seedlings cannot be individually marked because

Figure A.5. A metal tag used to mark an individual.

they are too small, therefore only their number and approximate position were recorded.

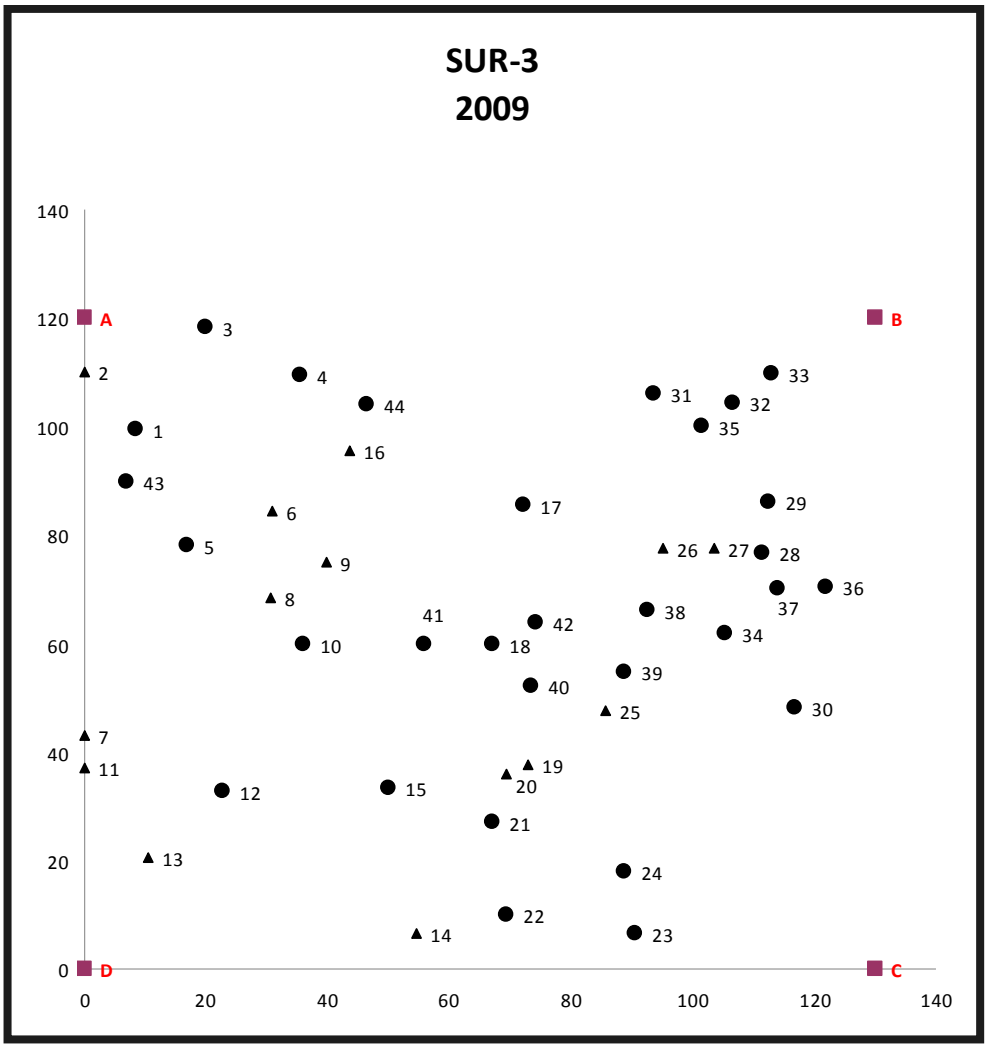


Figure A.6. Map of a demographic plot showing the position of the plants.

Each year, we counted the number of vegetative stems, reproductive stems and fruits/flowers per flowering stem. The field observations were used to construct individual life-histories. Survival rate were calculated for each site as the number of plant present in 2008 that survived until 2009 divided by the number of plants present in 2008.

The effect of site on the number of vegetative and reproductive stems per plant was tested using non-parametric ANOVA (Kruskal-Wallis test). The effect of site on survival rates was tested with a χ^2 test. The effect of environmental variables (soil nitrogen percentage, the C/N ratio, the fraction of frost days, minimal, mean and maximal temperatures, and the number of vegetative stems) on survival rate was analysed through generalized linear regression with a binomial error distribution and a logit link. Variable selection was performed through forward stepwise regression based on the

Akaike Information Criterion (AIC). All statistical analyses were performed with R (R Development Core Team 2009).

Seed production

A sample of twenty plants was collected from each of the VIL, LAU and GAL sites at the time of fruit maturation. Plants were stored individually in cellophane bags and let dry for one/two weeks. The number of seeds per plant was counted and, when it was possible, the number of seeds per fruit (some fruits opened naturally and released seeds into the cellophane bags, so this measure was not available for all fruits). All the seeds of a single plant were weighed on a precision balance to obtain the average seed mass per plant. Differences in seed number per fruit were tested with a non-parametric ANOVA (Kruskal-Wallis test). Differences in seed mass were tested with parametric ANOVA (*F* test).

Germination rates

A pilot experiment was conducted to estimate the rate of *in-situ* seed germination. The experiment was conducted in the sites VIL and GAL, both located at 2000m. Twenty mother plants per site were sampled in July 2008. For each site, seeds from all plants were mixed together. Sowing was performed in four experimental plots located close to the demographic permanent plots. Each plots was divided in two adjacent 20 x 20 cm sections (Figure A.7): the left section was sowed with 200 seeds; the right section was kept as a control. Sowing was performed in October 2008 and seedlings were scored in 2009. The difference in germination rate between the two sites was analysed with Wilcoxon rank sum test.

Figure A.7. The quadrat used for the germination experiment. The left section is sowed with a known number of seeds and the right section is kept as a control.

Reproductive system

The experiment was conducted in July 2008 near the GAL site. We selected 20 plants that had at least three stems. The first stem was isolated from pollinators with a nylon mesh before anthesis (Figure A.8). When the buds opened, they were manually fertilized with pollen coming from the same plant. This treatment allowed us to assess whether the plant is self-compatible. The second stem was bagged as the first, the only difference being the absence of manual pollination. This treatment served to evaluate the possibility for spontaneous self-fertilization. The third stem was left without mesh and acted as a control.

The experiment started on July 7th 2008 when plants were selected and bagged. Three additional visits were made during the experiment. In a first visit (July 16th), opened flowers in the first treatment were manually pollinated. In the second visit (July 24th), some nylon bags were removed to allow complete fruit maturation. In the third and last visit (August 7th), stems bearing fruits were collected. For logistic reason, fruits were collected before complete maturation; fruits were stored in 70° alcohol to fix the seeds to the placenta for subsequent counting. Fruits were observed by microscope and the number of seeds was recorded. Aborted and malformed seeds were not included in the counts. The effect of pollination treatment on seed set was analyzed using ANOVA. Pairwise differences in seed set between treatments were assessed with Tukey's HSD method with the *multcomp* package in R.

Figure A.8. plants used in the reproductive system experiment with nylon mesh to prevent cross-pollination.

Results

Environmental data

Climatic data were available for only 18 of the 22 data loggers initially installed (the logger in the SUR-2 was lost during the winter; CHA-1 returned aberrant data; LAU-1 had missing data; PIC-2 was discarded, because the device had been placed in direct sunlight). Climatic data are reported in Table A.2

Table AI.2. Climatic data and survival rates in the eight study sites. Frost days are indicated in fraction and total number (in parentheses)

Site	Plot	Snow cover			Frost days	Temperature			Summer temperature			Survival
		Starting date	End date	Duration		Min	Mean	Max	Min	Mean	Max	
BRU	1	18/11/2008	31/03/2009	133 days	0.007 (2)	1.79	5.27	10.39	9.35	14.19	23.79	0.79 (33/42)
	2	22/11/2008	01/04/2009	130	0 (0)	2.11	5.26	9.06	9.56	14.21	21.42	
CHA	1	17/11/2008	14/04/2009	148	na	na	na	na	na	na	na	0.72 (58/80)
	2	12/11/2008	26/03/2009	134	0.041 (11)	1.62	5.10	9.69	9.35	16.60	26.27	
	3	13/11/2008	07/05/2009	175	0.029 (8)	4.09	12.58	13.06	6.67	14.87	25.40	
VIL	1	11/11/2008	02/05/2009	172	0.029 (8)	0.02	9.32	15.19	5.85	12.16	22.11	0.65 (53/81)
	2	11/11/2008	11/05/2009	181	0.021 (6)	5.41	9.72	18.73	5.90	12.15	21.01	
	3	11/11/2008	07/05/2009	177	0.021 (6)	3.71	8.46	15.55	6.11	11.90	20.74	
SUR	1	12/11/2008	06/05/2009	175	0 (0)	-0.17	10.81	24.40	9.02	14.07	23.24	0.55 (53/97)
	2	na	na	na	na	na	na	na	na	na	na	
	3	12/11/2008	20/04/2009	159	0 (0)	-0.79	6.87	13.88	9.52	13.11	19.46	
CHM	1	07/11/2008	30/04/2009	174	0.013 (4)	6.10	5.50	19.02	7.32	8.42	9.91	0.70 (28/40)
LAU	1	04/11/2008	11/06/2009	219	0.015 (4)	na	na	na	na	na	na	0.70 (63/90)
	2	07/11/2008	12/06/2009	217	0.01 (3)	2.27	10.05	11.97	6.33	10.70	16.62	
	3	28/10/2008	29/05/2009	213	0 (0)	3.57	7.11	17.09	5.93	9.68	14.29	
GAL	1	17/10/2008	01/06/2009	227	0.036 (11)	4.75	4.86	6.85	3.13	6.49	10.08	0.74 (82/111)
	2	27/10/2008	18/06/2009	234	0.043 (13)	2.05	7.12	7.11	3.00	7.44	12.33	
	3	27/10/2008	15/06/2009	231	0.05 (15)	2.90	8.36	10.88	2.90	8.66	16.56	
PIC	1	28/10/2008	17/05/2009	201	0.124 (38)	6.24	5.50	16.89	0.95	8.80	19.13	0.70 (35/50)
	2	28/10/2008	19/06/2009	234	na	na	na	na	na	na	na	
	3	29/10/2008	06/05/2009	189	0.196 (60)	1.42	4.52	13.79	-0.17	9.21	20.01	
	4	28/10/2008	18/05/2009	202	0.114 (35)	-0.21	6.78	17.91	0.97	10.77	25.65	

There were significant differences between sites in the duration of snow cover (χ^2 = 17.73, d.f. = 7, p = 0.013), which ranged from 130 in BRU to 234 in PIC (mean duration: 187 days). The fraction of frost days did not differ significantly between sites (χ^2 = 13.54, d.f. = 7, p = 0.059), but PIC exhibit a somewhat larger values respect to the other sites. Temperatures did not differ significantly between sites (χ^2 = 7.03, d.f. = 7, p = 0.42 for minimal temperature; χ^2 = 7.07, d.f. = 7, p = 0.42 for mean temperature; χ^2 = 13.33, d.f. = 7, p = 0.06 for maximal temperature). The averages over all sites are 2.9°C, 7.4° and 13.8°C for minimal, mean and maximal temperature, respectively. Conversely, minimal and mean summer temperatures differed between sites (χ^2 = 16.14, d.f. = 7, p = 0.024 and χ^2 = 16.39, d.f. = 7, p = 0.022, respectively); maximal summer temperatures did not show significant differences between sites (χ^2 = 13.78, d.f. = 7, p = 0.055).

There was a significant site effect for carbon percentage (χ^2 = 60.59, d.f. = 7, p < 0.001), nitrogen percentage (χ^2 = 59.72, d.f. = 7, p < 0.001) and the C/N ratio (χ^2 = 60.59, d.f. = 7, p < 0.001) (Figure 9). The largest nitrogen percentages were found in BRU (2.68%), CHA (2.96%) and SUR (2.27%). The largest C/N was observed in LAU, where it also showed considerable inter-plot variability (from 22.27 to 66.03), while in the other sites the average C/N was 14.53. Soil acidity (pH) differed significantly between sites (χ^2 = 10.22, d.f. = 4, p < 0.037) and was comprised between 6.30 (in VIL) and 7.93 (in LAU) (Figure 10).

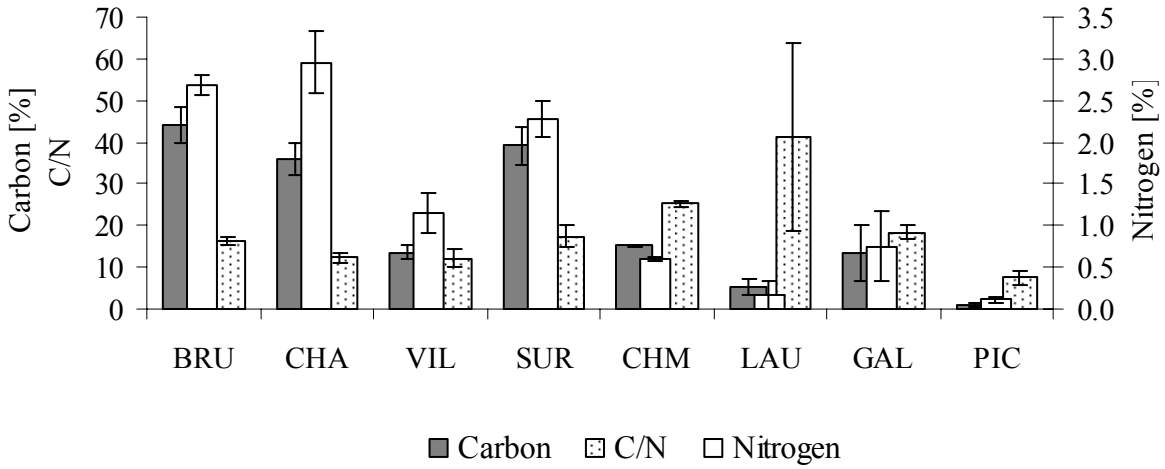


Figure A.9. Differences in soil carbon and nitrogen percentages between sites.

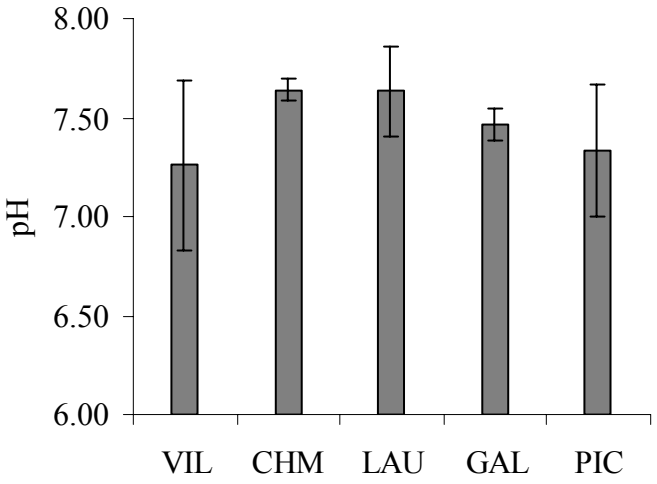


Figure A.10. Differences in pH between sites

Demography and plant survival

There was a significant site effect on the number of vegetative stems per plant (χ^2 = 129.05, d.f. = 7, $p < 0.001$) and flowering stems per plant (χ^2 = 88.10, d.f. = 7, $p < 0.001$). Survival rates did not differ significantly among sites (χ^2 = 13.24, d.f. = 7, $p = 0.067$). The model selected by stepwise forward regression included the number of vegetative stems and the fraction of frost days as explanatory variables of survival (AIC = 647.8), but only the number of vegetative stems had a significant positive effect on survival (Table A.3).

Table A.3: Generalized linear regression model on survival rates. S.E., standard error of the estimate

Factors	Estimate	S. E.	z	p
Intercept	0.61	0.14	4.25	<0.001
Fraction of frost days	-3.92	2.47	1.58	0.112
Number of vegetative stems	0.15	0.05	3.03	0.002

Seed production

The number of seeds per plant (mean ± standard deviation) was 24.82 ± 5.43 in GAL, 25.16 ± 6.97 in LAU and 25.33 ± 4.33 in VIL and did not differ between sites (χ^2 = 0.031, d.f. = 2, $p = 0.98$). Seed mass (mean ± standard deviation) was 0.039 ± 0.024 mg in GAL, 0.123 ± 0.034 mg in LAU and 0.157 ± 0.028 mg in VIL. Mean seed mass was significantly different among sites (F = 73.78,

d.f. = 2, 44, $p < 0.001$); there were significant differences in seed mass between GAL and LAU ($t = 7.71$, $p < 0.001$), GAL and VIL ($t = 12.02$, $p < 0.001$), and LAU and VIL ($t = 3.32$, $p = 0.005$).

In situ germination experiment

Seed germination rate (mean ± standard deviation) was 0.121 ± 0.109 in LAU and 0.137 ± 0.034 in VIL and did not differ between sites (W = 5, $p = 0.49$). There were no seedlings in the control plot, except for one plot in VIL. In this plot, one adult *A. alpina* plant was present at the time of sowing (Autumn 2008) and was eradicated; however, some seeds may have already been present in the soil and gave rise to the observed seedlings.

Reproductive system

Some plants or stems were broken and some bags went lost. As a result, only six of the originally twenty plants had data for all the three treatments. Two to seven fruits per stem were available for statistical analysis. Seed number per plant (mean ± standard deviation) was 27.11 ± 13.10 in the control, 19.45 ± 13.42 in the manual self-pollination treatment and 14.15 ± 11.81 in the natural self-pollination treatment and differed significantly between treatments ($F = 8.02$, d.f. = 2, 88, $p < 0.001$). There was a significant difference in seed set between the control and natural self-pollination plants ($t = 3.94$, $p < 0.001$); the difference between control and manual self-pollination was barely significant ($t = 2.38$, $p = 0.051$); the difference between manual and natural self pollination was clearly not significant ($t = 1.54$, $p = 0.276$). The number of seeds in the control was not significantly different from the number of seeds in natural conditions ($t = 0.84$, d.f. = 42, $p = 0.40$; GAL, LAU and VIL pooled together; see ‘Fecundity’ above).

Discussion

Survival rates

There was no effect of temperatures or frost occurrence on plant survival rates. This result could be due to low statistical power, because little among-site variation was observed in survival and temperatures and the analysis was conducted using only one year of data. Alternatively, the observed lack of effect could reflect a real biological pattern (local adaptation). However, survival

rates are not the only components of fitness, which is also a function of germination, growth, fecundity and dispersal rates. Additional analyses are thus needed to test whether these fitness components are affected with environmental variables.

Moreover, the choice of which environmental variables to include in the set of potential explanatory factors for survival is fundamental. Temperatures were evaluated on the first 32 days after snow melt. Therefore, all sites were expected to exhibit similar climatic conditions and, accordingly, there was no statistically significant difference in temperature between sites. Conversely, when temperatures are compared on the same calendar period (summer temperatures), significant differences emerged. The question is: which is the time period of interest that could potentially explain variation in survival rates or other demographic and biological parameters? In my opinion, environmental variables should be measured relatively to the same life-cycle phase (e.g. germination, vegetative growth, buds formation, flower opening, fruit maturation, seed dispersal, winter dormancy, etc.), because their effect on survival may depend on the state of the plants. For instance, frost damage may differ between seedlings and plants completing seed maturation. I chose to consider the time immediately following snow melt because it was the easiest to identify using the recorded temperature data without the need of visiting the sites. The choice of another time period may not have lead to the same results.

Finally, it should not be forgotten that the range of environmental variation included in our study is likely not representative of the overall natural variation experienced by *A. alpina*. For logistic reasons, only the sites where plant abundance and density was sufficiently large were included in the study. Small, isolated and sparse populations were often found in the field but were not included in the analysis. These populations may experience more extreme environmental conditions. One way to assess whether the chosen sites are representative of average climatic and environmental conditions is to compare them with geographical occurrence data in connection with environmental variables from geographic information system (GIS) projections (Zimmermann and Kienast 1999).

The use of data loggers to monitor temperature and humidity was very satisfying. The device gives local measurements that can be compared with data from nearby meteorological stations or with GIS projections (Zimmermann and Kienast 1999). Climate stations and GIS projections do not provide local measures, yet they are the only available dataset so far to infer relationship between environmental and biological variables. For instance, GIS projections were used to infer

correlations between gene frequencies and environmental variables in *A. alpina* (Poncet et al. 2010). It would be interesting to test whether local climatic variables give the same correlations.

Seed production

The differences in seed mass between GAL and the other two sites were likely the result of different sampling time. Seeds were collected earlier in GAL, when fruits had not attained maturation: this can explain the low seed mass in this site.

The number of seeds per fruit was not different between sites. This means that counting the number of fruits per plant can give information on total seed set. Such data could be potentially very useful in demographic models, but they can be time consuming to obtain. A faster but less precise estimate of seed set of individual plants could be obtained by counting the number of reproductive stems. Eventually, the effect of obtaining precise parameter values on the result of a demographic model should be checked with sensitivity or elasticity analyses. Some other components of reproductive success (e.g. germination rates) may be more important to determine population growth rates and other relevant demographic parameters. In such a case, seed production may be measured with lesser precision without significantly affecting demographic projections.

Germination rates

Germination rates were similar between the two sites, but the low number of replicates used may have reduced statistical power considerably. There was however considerable within-site variation in germination rates, which likely reflects micro-site conditions. It is not known whether seeds can persist in the soil in a dormant state for several years. The occurrence of *A. alpina* in disturbed habitats (road sides, screes, river banks) suggests that seed dormancy is likely. A larger experiment, involving more replicates and scoring of seedlings over several years, may help clarifying this issue and obtaining more precise estimates of germination rates. Moreover, sowing experiments using translocated seeds may be used to test for local adaptation at the phenotypic level. The main obstacle to these studies is the availability of suitable sites for sowing, because populations are often found on steep, rocky, unstable soils and sowing in these conditions may result in great seed losses. Yet, most populations are found in this type of habitat, which therefore seems the most favourable for growth and survival. Experiment might be conducted in a common garden, but this would not permit estimating *in-situ* germination rates.

Reproductive system

The pollination experiment showed that plants are self-compatible and can produce seeds through natural selfing. However, there was a significant reduction of seed set between naturally selfed plants and plants kept in natural conditions (control). This effect may be due to the lack of pollen vectors in the natural pollination treatment. If lack of pollinators can reduce seed set, we expect a lower number of seeds in naturally vs. manually self-pollinated plants. The results were confounding because natural self pollination resulted in smaller seed production than manual self pollination, but the difference was not statistically significant. However, the sample size was small ($n = 6$) and disentangling this issue requires extending the experiment to larger samples.

An alternative explanation for the reduction in seed production in self pollination is inbreeding depression acting on embryos shortly after fertilization. Alternatively, we cannot exclude the possibility that reduction in seed set in bagged plants was due to an experimental artifact. The nylon mesh could prevent efficient air exchange or weigh down the plant stem (Figure 8), leading to suboptimal reproductive capacity (Gaudeul and Till-Bottraud 2003). Nonetheless, one purpose of this pilot study was to assess the feasibility of *in-situ* pollination experiments for *A. alpina*, and the obtained results are encouraging.

We were not able to measure seed mass or germination rate for seeds produced in the pollination experiment (fruits were collected before dehiscence for logistic reasons). Inbreeding depression can be expressed at different stages of the life-cycle, therefore it would be interesting to test whether seeds from selfed plants have lower mass and/or germination rates.

The use of molecular markers could be very useful for studying the reproductive system. Paternity analysis can be used to determine what fraction of seeds in natural conditions are generated through selfing. It would be interesting to test whether selfing rates change with altitude or other environmental variables, as selfing may be evolutionary advantageous where pollinators are scarce or at the edges of species distributions. Also, the breeding system may be at the origin of the observed differences in inbreeding levels in populations of *A. alpina* in the Alps and the Appennines: the mean F_{IS} estimated for allozyme loci is 0.553 in the Alps and in 0.076 the Appennines (Ansell et al. 2008). One hypothesis for the difference in F_{IS} is that plants are self-compatible in the Alps and self-incompatible in the Appennines (Ansell et al. 2008). *In-situ* pollination experiments and paternity analysis may thus be useful to test this hypothesis.

References

Ansell, S.W., M. Grundmann, S. J. Russell, H. Schneider and J. C. Vogel. 2008. Genetic discontinuity, breeding-system change and population history of *Arabis alpina* in the Italian Peninsula and adjacent Alps. *Molecular Ecology* 17: 2245–2257.

Assefa, A., D. Ehrich, P. Taberlet, S. Nemomissa, C. Brochmann. 2007. Pleistocene colonization of afro-alpine "sky islands" by the arctic-alpine Arabis alpina. Heredity 99: 133.

Gaudeul, M. and I. Till-Bottraud. 2003. Low selfing in a mass-flowering, endangered perennial, *Eryngium alpinum* L. (Apiaceae). *American Journal of Botany* 90: 716-723.

Manel, S., B.N. Poncet, P. Legendre, F. Gugerli, and R. Holderegger. 2010. Common factors drive genetic variation of adaptive relevance at different spatial scales. *Molecular Ecology* (In press).

Poncet, B. N. 2010. *Modèles de distributions d'allèles pour la détection de la variabilité génétique adaptative chez une espèce non modèle, Arabis alpina*. PhD thesis, Université Joseph Fourier, Grenoble, France.

Poncet, B. N., D. Herrmann, F. Gugerli, P. Taberlet, R. Holderegger, L. Gielly, D. Rioux, W. Thuiller, S. Aubert and S. Manel. 2010. Tracking genes of ecological relevance using a genome scan in two independent regional population samples of *Arabis alpina*. *Molecular Ecology* 19: 2896–2907.

R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Titz, W. 1971. Zur Evolution der Gattung Arabis im Alpenraum. Berichte der Deutschen Botanischen Gesellschaft 84: 697-704.

Wang, R. H., S. Farrona, C. Vincent, A. Joecker, H. Schoof, F. Turck, C. Alonso-Blanco, G. Coupland and M. C. Albani. 2009. *PEP1* regulates perennial flowering in *Arabis alpina*. *Nature* 459: 423-427.

Zimmermann, N. E. and F. Kienast. 1999. Predictive mapping of alpine grasslands in Switzerland: species versus community approach. *Journal of Vegetation Science* 10: 469–482.

Article A:

The effect of stage-specific vital rates on population growth and effective population size: a numerical elasticity approach

Title: The effect of stage-specific vital rates on population growth rate, effective population size and generation time in the endangered iteroparous plant *Dracocephalum austriacum*

Authors: Marco Andrello[1], Florence Nicolè[2], Irène Till-Bottraud[1] and Oscar E. Gaggiotti[1]

[1] Laboratoire d'Ecologie Alpine (LECA), CNRS UMR 5553, 38041 Grenoble Cedex 09, France

2 Laboratoire BV pam - EA 3061, Faculté des Sciences et Techniques, Université Jean Monnet, PRES "Université de Lyon", 23, rue du Docteur Michelon, F-42023 Saint-Etienne cedex 2, France

Submitted to: Conservation Biology

Corresponding author E-mail: marco.andrello@gmail.com

Keywords: matrix population models; overlapping generations; genetic drift; annual effective size; comparative demography; elasticity analysis

Running headline: effect of vital rates on N_e and λ.

Word count: 6152 words

Abstract

Evaluating the effects of the life-history of a species on the effective size N_e of its populations is one of the main problem in conservation and evolutionary biology, because N_e determines the level of genetic diversity and measures the strength of genetic drift. This problem can be addressed using demographic models, which make it possible to evaluate the effects of various demographic factors on N_e and also on other parameters of interest, such as population growth rate λ, annual effective size N_a and generation time L. Evaluating multiple parameters simultaneously is important in endangered species because some vital rates may have contrasting effects on population growth and genetic drift, for instance if they contribute positively to λ and negatively to N_e. We used elasticity analysis to study the effect of stage-specific survival and flowering rates on N_e, N_a, λ and L in seven populations of the iteroparous endangered plant *Dracocephalum austriacum*. The elasticities of N_e and N_a were similar to those of λ, indicating that the vital rates having the largest effect on λ are also those contributing the most to genetic drift, and we did not observe any contrasting effect. As expected, L exhibited negative elasticities to vital rates reducing the mean age of parents, such as the transition of juvenile individuals to the flowering stage. In general, survival rates had larger effects than flowering rates. Elasticities were similar across populations, the only difference being larger elasticities to juvenile vital rates in decreasing populations. The type of analysis presented here is an easy approach to study the simultaneous effects of demographic parameters on genetic drift and population growth in species with complex life-histories. The next step will be to generalize the observed patterns by extending the analysis to a wider array of populations and species.

Introduction

Effective population size (N_e) is a widely used parameter in population biology. It measures the rate of inbreeding and the loss of genetic variation, two important processes that can reduce population fitness and lead to extinction (Frankham 2005; Gaggiotti 2003; Lande & Barrowclough 1987). N_e also controls the relative strength of selection and gene flow relative to genetic drift (in populations with small N_e, gene flow and selection are less effective in modifying gene frequencies) and thus is important for studying genetic differentiation and adaptation in natural populations.

Effective population size is defined as the size of an ideal population experiencing the same amount of genetic drift as the population under consideration. It is possible to estimate N_e through molecular methods based on various genetic measures, for instance temporal variance of gene frequencies (Jorde & Ryman 2007) or linkage disequilibrium (Hill 1981; Waples 2006; Waples & Do 2008, 2010). Another approach to estimate N_e is based on demographic models that incorporate various processes known to affect N_e, e.g. sex-ratio variation, mating system or variation in reproductive success (reviewed by Caballero 1994 and Wang and Caballero 1997). Strictly speaking, these models cannot be considered estimation methods, because they take into account the effects of one or few factors only, but they are useful to study the relative effect of these factors on N_e.

In species with overlapping generations, N_e is affected by the pattern of age- or stage-specific survival and fecundity rates (Felsenstein 1971). Due to the complexity of the models that calculates N_e in species with overlapping generation, it is better to study the effects of age- and stage-specific vital rates using numerical sensitivity and elasticity analyses, which are widely used in population dynamics. Sensitivity is defined as the change in a parameter of interest caused by an infinitesimal change in a given vital rate. Elasticities (or proportional sensitivity) are used to express this change when the perturbation of parameters is proportional to their values. Elasticity analysis has been applied to study the effect of vital rates on population growth rate λ (Caswell 1978; Dekroon et al. 1986; Franco & Silvertown 2004). In the framework of model-based calculation of N_e, it is possible to perform an elasticity analysis based on the same demographic data that are used to define a matrix population model. When both λ and N_e are estimated from the same demographic dataset, the pattern of elasticity of N_e can be compared to that of λ. This joint elasticity analysis is powerful to explore possible antagonistic effects of single vital rates on population growth (λ, which is also

69 equivalent to mean population fitness) and genetic drift (N_e). This can have an application in
70 conservation biology, when it is important to identify critical life-stages that can be the object of
71 conservation measures for endangered species (Mills et al. 1999). Vital rates or life-stage transitions
72 exhibiting the largest elasticity to λ are often chosen as the object of management actions. Obtaining
73 the elasticity for both N_e and λ can help assessing whether acting on the vital rate exhibiting the
74 largest elasticity for λ, will also be beneficial in terms of genetic diversity and inbreeding
75 avoidance, i.e. whether it will increase N_e.

76 In species with overlapping generations, N_e measures the rate of genetic drift in units of generations.
77 However, if the species is able to persist for several years, it might be useful to express the rate of
78 drift in years rather than generations. Hill (1979) introduced the concept of annual effective size N_a,
79 defined as the size of an ideal population experiencing the same amount of genetic drift per year as
80 the population under consideration. N_a allows for comparison between species or populations with
81 different generation times L.

82 The general aim of this work was to study jointly the effect of stage-specific vital rates on N_e, N_a, λ
83 and L. We applied a numerical elasticity analysis on these parameters using a demographic dataset
84 composed of seven populations of the Austrian dragonhead, *Dracocephalum austriacum* L.
85 (Lamiaceae)(Nicolè 2005). N_e and N_a were estimated using the approach of Yonezawa et al. (2000)
86 based on the same transition matrices that are used to calculate λ. This model allows for the
87 calculation of variance N_e from demographic data in stage-structured species even when population
88 size is not stable in time ($\lambda \neq 1$). Specifically, the first objective of our work was to study whether
89 the parameters contributing the most to N_e and N_a were also the same contributing the most to
90 population growth rate. This was done by comparing the elasticities of these parameters and the
91 correlations among the elasticities. The second objective was to study the elasticities of generation
92 time L. The third and final objective was to see whether the elasticity patterns of N_e and N_a differed
93 in increasing and decreasing populations. This was motivated by the observation of Silvertown et al.
94 (1996), who evidenced a change in the elasticity pattern of λ when populations of the same species
95 were observed in conditions of population growth or population decline. A similar pattern can be
96 expected for the elasticities of N_e and N_a if the elasticities of N_e, N_a, and λ are correlated.

Methods

Species and study sites

The Austrian dragonhead, *D. austriacum,* is a long-lived perennial plant whose distributional area spans from the Caucasus to the Pyrenees, with scarce populations in Western Europe (Dostalek et al. 2010; Olivier et al. 1995). This rare species is listed as vulnerable by the International Union for Conservation of Nature (IUCN) and is under national, European and international legislations (Red List of French Endangered Flora, Habitats Directive, Bern Convention). Threats to this attractive species include pillaging and trampling, when populations are near footpaths or close to pastoral activities. Locally, plant competition with shrubs and trees can also increase vulnerability (Danton & Baffray 1995).

Only 15 populations are known in France, from Savoie to the Alpes Maritimes. We selected 7 of the 15 French populations of the Austrian dragonhead reported in the French Alps for their accessibility (Table I): Bessans (BE), Champcella (CH), Escoyères (ES), L'Argentière (LA), Reynier (RE), StChristophe (ST) and Valsenestre (VA). These populations vary widely in population size, density, genetic diversity, vegetation characteristics, and soil composition (Bonin et al. 2007; Nicolè 2005)(Nicolè et al. unpublished manuscript).

Data collection

Survival and fecundity rates of the plants were estimated from individual-based field surveys. Three to four permanent plots were installed in the populations in late spring 1999, except in Bessans, where the survey started in 2000. Every plant in the plots was identified by a number and its life stage was recorded. The position of each plant was mapped to facilitate finding in subsequent years. From 1999 to 2006, census was performed in June. Each plant was checked for its presence and life stage; new recruits (seedlings) were checked carefully and added to the census. The estimation of potential fecundity (number of seeds per plant) was performed outside the permanent plots to avoid modification of recruitment. The mean μ_b and variance σ^2_b in number of seeds produced by flowering plants was averaged over two representative samples of 20 individuals, collected in each population in 1999 and 2002.

Matrix model

The life cycle was structured into four demographically distinct stages (Fig. 1), assuming a pre-breeding census. Seedlings, the first stage, are small plants with less than 5 stems and shorter than 10 cm. They represent the new emerging plants of the year (all plants move to the juvenile stage the second year after emergence). Juveniles have also less than 5 stems but are taller than 10 centimeters. A plant stays in the juvenile stage until it flowers. Adults were divided in two classes, vegetative and reproductive.

We chose to disregard seed bank dynamics because seed dormancy was difficult to characterize. To assess whether the assumption of absence of seed bank could alter the observed elasticity pattern, we developed an alternative version of the matrix model accounting for seed dormancy with plausible parameter values from field observations and laboratory experiments (Supporting Information). Elasticity analyses were repeated using this modified version of the model. Except in one case (the elasticities of N_a in VA), the resulting elasticities were well correlated with the ones obtained with the original model, so the main conclusions of the work were not altered by the presence of a seed bank.

Transition rates a_{ij} were averaged over the entire period of survey by pooling together the annual observations in the different plots in each site (Caswell 2001 p. 134-135) and then arranged in a transition matrix (Table II). The matrices were used to obtain stage-specific survival rates u_j's and flowering probabilities p_j's, conditional on survival (Table I), population growth rate λ, stable stage distribution and reproductive value (Caswell, 2001). The uncertainty of the estimates of λ was assessed by bootstrapping (Efron & Tibshirani 1993) the individual life-histories (10^5 iterations). Confidence intervals for λ were derived by the percentile method. Generation time L was defined as the mean age of parents at the stable stage distribution and was estimated according to Cochran & Ellner (1992).

Calculation of N_e

Demographic estimates of N_e were derived using the stage-structured model of Yonezawa et al. (2000):

151 $$N_e = \frac{2N_0}{V}\frac{\lambda^2(\lambda-1)}{1-(1/\lambda)^L} \quad (1)$$

152 where N_0 is the initial census population size (obtained from an exhaustive count of plants
153 performed in June 1999), λ is the population growth rate, L is the generation time and V is the
154 variance of gene frequency change across a generation. The annual effective size N_a was calculated
155 as the product of N_e by the generation time L. The variance V of gene frequency change over a
156 generation was defined as (Yonezawa et al. 2000):

157 $$V = 2(1+\alpha)(\bar{u} - \bar{u}^2) + (1-\bar{u})S \quad (2)$$

158 where α is the deviation of genotype frequencies from Hardy-Weinberg proportions (Table I); $\bar{u}$ is
159 the annual survival rate (u_i averaged over life stages, $\bar{u} = \sum_i w_i u_i$); $\bar{u}^2$ is the average of u_i^2
160 ($\bar{u}^2 = \sum_i w_i u_i^2$; Table I); $S = (1 - \alpha) + (1 + \alpha)(\sigma_k^2/\mu_k)$, where μ_k and σ_k^2 are the mean and variance
161 in realized fecundity (see below).

162 Mean μ_b and variance σ_b^2 of potential fecundity was estimated by counting the number of seeds per
163 individual as explained in "Data collection". Mean realized fecundity μ_k was estimated on the
164 pooled dataset per population as the number of seedlings found in years 2000-2006 divided by the
165 number of reproductive adults the year before, in 1999-2005. The variance in realized fecundity σ_k^2
166 was calculated by modifying Heywood's (1986) original equation A3, which applies to a population
167 with discrete generations, by substituting the number of adults $N_{t\text{-}1}$ for the number of breeders N_B:

168 $$\sigma_k^2 = \frac{(\mu_b - \mu_k)}{\mu_b - \frac{1}{N_B}}\mu_k\left[1 - \frac{\left(1+\frac{\sigma_b^2}{\mu_b^2}\right)}{N_B}\right] + \mu_k^2\frac{\sigma_b^2}{\mu_b^2} \quad (3)$$

169 The number of breeders is estimated as the number of reproductive adults at the stable stage
170 distribution, $N_B = w_4 N_h$, where w_4 is the proportion of flowering individuals at the stable stage
171 distribution (the fourth element of the dominant right eigenvector of the transition matrix; Table II)
172 and N_h is the harmonic mean census number over one generation assuming constant population
173 growth (λ) and an initial size N_0. Expression (3) therefore allows the calculation of variance in

reproductive success from measures of fecundities. This expression assumes random recruitment of seedlings from the seed pool and does not consider variation in paternal contributions. For these reasons, the calculated σ_k^2 is likely biased downward, which leads to an upward bias in the calculation of N_e. The implications of this bias for the results of the elasticity analysis were addressed by repeating the analysis after setting σ_k^2 to ten times its original values, but leaving mean fecundity a_{14} to its observed value. The elasticities changed in magnitude, as a result of the change in the calculated N_e, but their values were proportional and correlated to the original results. The conclusions of our work were therefore not affected by the difficulty in estimating variance in reproductive success.

The inbreeding coefficient F_{IS} was derived from a set of 87 AFLP markers (see Bonin et al. 2007 for details of the molecular techniques used to generate the data set) using ABC4F (Foll et al. 2008) (Fig. 2). ABC4F is an approximate Bayesian computation method allowing the estimation of population-based F_{IS} from dominant markers. The bias parameters for hidden and fixed loci in ABC4F was set to 1and 0, respectively. The "number of iterations" was set to 10000 and the "acceptance rate" to 0.001. Actually, this means that the total number of iterations was 10,000 / 0.001 = 10,000,000 and the final average F_{IS} was estimated using 10,000 points. For the present analysis, F_{IS} was considered equivalent to α (the deviation of genotype frequencies from Hardy-Weinberg proportions, Table III.1), even if this is not strictly true. α is a population-level parameter and can take positive or negative values, depending on the excess of heterozygotes observed in the population. F_{IS} is defined as the probability of sampling an individual inbred for a particular locus and cannot take negative values (Foll et al. 2008, p. 928). Nonetheless, we repeated the elasticity analysis after setting α to half of its original value and the pattern of elasticity remained unchanged (new elasticities proportional and correlated to the original ones), so this inconsistency had no serious effect on the results.

Elasticity analysis

We calculated the elasticity of λ, N_e, L and N_a to matrix elements a_{ij} and lower level vital rates (survival rates u_j and flowering probabilities p_j). Elasticities were estimated numerically by perturbing the matrix elements (or the vital rates) by a small quantity proportional to their values (0.01%; Heppell et al. 2000; Campbell & Husband 2005) :

$$E_y(x) = \frac{y^+ - y^-}{0.0002x} \quad (4)$$

where y is one of N_e, N_a, λ or L; x is a matrix element or a vital rate; y^+ and y^- are the values of y when x is respectively increased or decreased by 0.01%.

The elasticities were grouped into three life-cycle components according to Silvertown et al. (1996): stasis and retrogression (comprising the elasticities to the elements (a_{22}, a_{33}, a_{34}, a_{44}), growth (elements a_{21}, a_{41}, a_{42}, a_{43}), and fecundity (a_{14}). Because the absolute values of elasticities of N_e and N_a present a large variability across populations, we expressed the relative contributions of life-cycle components as the ratio of the contribution of stasis and retrogression over the contribution of growth.

Results

Effective size and demographic rates

The effective population size N_e was relatively small in all populations (Table III), varying from 24 to 172. N_e was always lower than census size (mean $N_e : N_h = 0.13$) and the number of breeders N_B (mean $N_e : N_B = 0.28$). RE showed the smallest $N_e : N_h$ and $N_e : N_B$ ratios.

The largest survival rates were observed for reproductive adult plants and ranged from 0.85 (VA) to 1.00 (BE; Table I and II). No deaths were observed among adult individuals in BE during the seven years of census. RE had the smallest survival rates, the smallest seed set μ_b, the largest fecundity rate μ_k and the largest variance in fecundity σ_k^2 (Table I).

The ratio between the variance in fecundity and the mean, also called the standardized variance in fecundity (Nunney 1991) is informative because ideal populations under the Wright-Fisher model have a ratio of 1 (Crow & Kimura 1970). When the standardized variance is larger than one, then the effective size is expected to decrease as a consequence of unequal parental contribution to the gene pool of the next generation. The standardized variances in recruitment σ_k^2 / μ_k were close to unity, except in RE. The standardized variance in potential realized fecundity σ_b^2 / μ_b was always one order of magnitude larger than σ_k^2 / μ_k.

One population was increasing (BE, $\lambda > 1$; Table III), two were decreasing (ST and VA, $\lambda < 1$) and the other four were stable (confidence intervals for λ comprising 1). The two decreasing populations showed also low survival rates. The smallest survival rates were however observed in RE, where fecundity was remarkably large and allowed λ to be close to unity. As a result of large survival rates, generation time L was remarkably large in BE.

The inbreeding coefficients F_{IS} calculated with ABC4F were very large in all populations (mean values ranging from 0.8438 in LA to 0.9332 in CH; Figure 2). The posterior distributions were skewed towards the upper theoretical limit ($F_{IS} = 1$). Changing the number of iterations or the acceptance rate resulted in similar results.

Elasticities

The elasticity pattern of N_e presented some differences between populations (Fig. 3, black bars). Two groups could be distinguished: in LA, CH, RE, ES and BE (the increasing and stable populations), the largest contribution to N_e was made by transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and by adult survival rates (u_3 and u_4). In VA and ST (the two decreasing populations), juvenile transitions and juvenile survival rates made the largest contributions (VA) or made contributions similar to adults (ST). Survival rates were associated with larger elasticities than flowering rates and fecundity, both in decreasing and in stable/increasing populations. The elasticities of N_e to flowering rates were somewhat larger in decreasing populations than in stable or increasing populations. The elasticities of stasis and retrogression were larger than the elasticities to growth, as shown by their ratios (Table IV). The ratios were larger in stable/increasing populations than in decreasing populations (linear regression between λ and the ratio: $y = -15 + 19x$, adj-$R^2 = 0.64$, $F_{1,5} = 11.6$, $p < 0.05$).

The elasticity pattern of N_a was similar to that of N_e (Fig. 3, grey bars), as in increasing and stable populations there were large elasticities associated with adult rates, while in the two decreasing populations the elasticities associated to juvenile and adult transitions had roughly the same magnitude. Similarly to what was observed for N_e, the elasticities were much larger for survival than flowering or fecundity rates. In the two decreasing populations, the elasticity of N_a to flowering rates and fecundity were larger than in the other populations. The elasticities to stasis and retrogression were larger than the elasticities to growth (Table IV). The ratios were larger in

stable/increasing populations than in decreasing populations (linear regression between λ and the ratio: $y = -18 + 22x$, adj-$R^2 = 0.75$, $F_{1,5} = 18.9$, $p < 0.01$).

The pattern of elasticity of λ (Fig 3, white bars) was similar across all the seven populations. The largest elasticities were always associated with transitions among adults and with adult survival rates. The elasticities associated with survival rates were always larger than the elasticities to flowering rates and fecundity, similarly to what was observed for N_e and N_a. The elasticities to stasis and retrogression were larger than the elasticities to growth (Table IV). The ratios were larger in stable/increasing populations than in decreasing populations, although the regression between λ and the ratio only approached significance ($y = -7 + 11x$, adj-$R^2 = 0.46$, $F_{1,5} = 6.1$, $p = 0.057$). It is noteworthy that the patterns of elasticities of N_e, N_a and λ were very similar in LA, CH, RE, ES and BE (the stable/increasing populations) while they presented some dissimilarities in VA and ST (the decreasing populations).

Contrary to N_e, N_a and λ, generation time L exhibited both positive and negative elasticities (Fig. 4). The pattern of elasticities of L was similar among all populations. Positive elasticities were limited to transitions among adults (a_{33}, a_{34}, a_{43} and a_{44}) and adult survival rates (u_3 and u_4); these vital rates are also those associated with large elasticities of N_e, N_a and λ. The other transitions were associated with negative elasticities, meaning that increasing those transitions would shorten the generation time. Similarly to what was observed for N_e, N_a and λ, the elasticities of L to survival rates were generally larger than the elasticities to flowering rates and fecundity.

The elasticities of N_e and N_a were positively correlated to those of λ (Fig. 5). All the correlation coefficients were significant, except that for the correlation between E_{Na} and E_λ in BE, which however was nearly significant ($p = 0.056$) A significant positive correlation indicates that vital rates having a large effect on λ have also a large effect on N_e and N_a. In addition, the elasticities of N_e and N_a were positively correlated among themselves (Fig. 6). Contrary to the general pattern of correlation among the elasticities of N_e, N_a and λ in all populations, the other correlation coefficients (between and E_L and E_λ, E_L and E_{Ne}, E_L and E_{Na},) were not significantly different from zero.

Discussion

We calculated effective population size with the demographic matrix approach of Yonezawa et al. (2000) and then evaluated the elasticities of N_e, N_a, λ and L to the matrix elements and to the stage-specific vital rates. Demographic models are useful approaches to study how particular processes can reduce N_e (Caballero 1994; Nunney & Elam 1994), so we could use them to address the three main questions of our work: i) do N_e, N_a and λ exhibit similar patterns of elasticities? ii) Is there a general pattern in the elasticities of L? iii) Do the elasticity patterns of N_e and N_a differ between increasing and decreasing populations? We discuss the general patterns that were uncovered by the analysis along with the potential implications of elasticity analyses of N_e and N_a for species conservation and the relationship between the results obtained here and those found in other published works.

General patterns

The answer to the first question was positive. The elasticities of N_e and N_a showed remarkable similarities to the elasticities of λ. In addition, the elasticities of λ, N_e and N_a were correlated in all populations. This pattern of correlation indicates that vital rates contributing the most to population growth are also those contributing the most to N_e and N_a and thus to genetic drift. If this pattern were confirmed on a wider array of populations and species, then one could use the elasticities of λ as proxies for the elasticities of N_e and N_a. However, in VA and ST there were some differences between the elasticities of N_e, N_a and λ (Fig. 3) and the correlation between E_λ and E_{Na} was weak in VA (Fig. 5), so before generalizing the observed patterns it would be better to consider a larger number of species with different life-histories.

The second objective of our work was to study the elasticities of generation time. The elasticity pattern of L was different from those of λ, N_e and N_a. with many negative elasticities associated to most transitions and positive elasticities limited to adult survival rates. This makes sense from the definition of L as the mean age of parents, so that increasing adult survival will lengthen the mean time that plants spend in the reproductive stage. The effects of generation time are important in a conservation framework because genetic processes associated with extinction risk are often better measured in units of years rather than generations (Allendorf et al. 2008). Loss of genetic variability in time may be relatively slow in populations with small effective size but large generation time. In

such a case, reducing N_e may not be critically important from a conservation perspective if this reduction is associated with an increase in generation time that results in an increase in N_a. However, we have evidenced that the elasticities of N_e and N_a are positively correlated in *D. austriacum*, so that a decrease in effective size will translate not only in a higher rate of genetic drift per generation but also per year.

The third objective of our study was to understand whether the pattern of elasticities of N_e and N_a differed between increasing and decreasing populations. In our dataset, there were two decreasing populations (ST and VA), one increasing population (BE), three stable populations (CH, ES, LA) and one stable population with large fecundity (RE). In the two decreasing populations, juvenile survival rates made larger contributions to N_e with respect to the other populations; apart from this difference, however, all the other populations exhibited remarkably similar elasticity patterns. The pattern of elasticity of N_e in RE (an atypical population, having the largest fecundity and the lowest seedling survival) did not differ from the other populations either.

By analyzing different populations of the perennial iteroparous herb *Pedicularis furbishiae*, Silvertown et al. (1996) observed that populations in low-density habitats exhibited increasing dynamics ($\lambda > 1$) and large elasticities of λ associated with growth rates (elements of the population transition matrix corresponding to growth to an upper life-stage, i.e. a_{ij} with $i > j$). Conversely, populations situated in dense vegetation habitats showed a tendency to decline ($\lambda < 1$) and exhibited large elasticities associated with stasis and retrogression (elements corresponding to persistence in the same life-stage or retrogression to a lower life-stage, i.e. a_{ij} with $i \leq j$). In our dataset, the elasticities of λ to flowering rates were always smaller than those to survival rates, but increasing populations did not show larger flowering rates than decreasing populations. Moreover, we observed that the ratio between the elasticities to stasis and retrogression and the elasticities to growth was larger in increasing than in decreasing populations, contrary to the pattern observed by Silvertown et al. (1996). Given these contrasting patterns and the low number of populations examined here (7) and in the study by Silvertown et al. (1996)(15), it seems premature to draw definite conclusions on this point. In addition, the number of life-stages specified in the transition matrix and the life-span of the species can increase the importance of stasis relatively to other life-cycle components (Enright et al. 1996) and comparing species with different life spans and number of life-stages may thus be misleading.

Comparison with other studies

Two other published studies analysed the effect of stage-specific vital rates on N_e using elasticity or other forms of perturbation analysis (Yonezawa et al. 2000; Campbell & Husband 2005), but they did not compare the elasticities of N_e to those of λ. Yonezawa et al. (2000) studied how variation in vital rates would affect N_e:N_0 in the clonal plant *Fritillaria camschatcensis*. They found that decreasing transition rates among large-sized plants increased N_e:N_0. Yonezawa et al. (2000) argued that if transition rates among large plants decrease, the number of large plants will also decrease and so will the variation in reproductive success. The effective size would consequently decrease. Although Yonezawa et al. (2000) did not use elasticity analysis, this result means that the elasticity of N_e:N_0 to transition rates among large plants would be negative. This effect was not found in *D. austriacum*, for which the elasticities of N_e were all positive. Apart from the possibility of clonal growth, an important difference between the two species is the existence of reproduction in different life stages in *F. camschatcensis*, so that a decrease in survival of large plants translates into a reduction in variation in reproductive success because the production of new individuals is shifted to the small size classes. Conversely, in *D. austriacum* reproduction occurs only in the flowering adult class and reproductive success is determined only by the vital rates of this class.

Campbell & Husband (2005) studied the effect of mode of reproduction and vital rates on N_e in *Hymenoxys herbacea* and observed that N_e was more influenced by juvenile than adult survival rates. The opposite was true for *D. austriacum*, which exhibited larger elasticities associated with adult survival rates. One difference between the two species is however that in *H. herbacea* juvenile survival rates were larger than adult survival rates (Campbell & Husband 2005). This may result in larger elasticities of N_e to juvenile than to adult survival rates, since elasticities are proportional to the value of the modified parameter.

The comparison among these studies and the results of our work suggests that different species can exhibit varying patterns of elasticity of N_e to vital rates. This dependence of the elasticity patterns upon the life-history features of a species was also observed for the elasticities of λ in plants (Silvertown et al. 1993, 1996; Franco & Silvertown 2004). When the analysis is extended to dozens of species and populations, as done in these studies, it is possible to find general patterns through a comparative approach. One question to be answered before performing such an exercise with N_e is whether the number of life-stages of the transition matrix and the life-span of the species can affect

the final pattern of elasticity, as this issue was important in the studies addressing the elasticities of λ (Enright et al. 1996).

Implications of elasticity analysis of N_e for conservation

D. austriacum is a rare plant submitted to conservation measures. Identifying which transitions have the greatest impact on population growth is important in a conservation framework, because management actions can be designed to target those life-stages (Mills et al. 1999; Caswell 2001). However, the consequences of management should be evaluated also from a genetic point of view, because some management actions could entail a reduction in genetic diversity or a modification of the genetic composition of the population (Allendorf et al. 2008; Allendorf & Luikart 2007; Lande & Barrowclough 1987). As reducing effective population size can increase extinction risk, management should avoid modifications of vital rates that reduce the effective population size. However, this problem seems to be of minor importance for the populations examined in this study, because both N_e and λ exhibited high and positive elasticities to the same group of parameters. Adult survival rates were associated with the largest E_λ and E_{Ne} in increasing populations, while juvenile and adult survival rates had similar contributions in the decreasing populations. Thus vital rates did not show any antagonistic effect on population growth and genetic drift.

Conclusions

We have presented a study of the effects of life-history on N_e and N_a by taking as an example an iteroparous plant, which might be representative of many other species with similar life-history. The correlations between the elasticities of N_e, N_a and λ were highly significant, indicating that vital rates having a large effect on λ are also expected to have a large effect on N_e and N_a and thus on genetic drift. This conclusion may be of high relevance in conservation studies, where the demographic parameters of a species may be altered in order to ensure population persistence, but at the same time it is important to preserve the effective size above a minimum critical value (Allendorf & Luikart 2007; Lande & Barrowclough 1987). Similarly, the relationship between effective size and population growth rate, which is equivalent to the mean population fitness, can be informative when it is important to quantify the relative strength of genetic drift and other evolutionary forces such as migration, selection or the mating system (Campbell & Husband 2005; Husband & Barrett 1992).

We are convinced, however, that the results from only seven populations are difficult to generalize to other populations or other species. With larger datasets, it will be possible to search for general patterns on the elasticities in increasing and decreasing populations analogously to what was done by Silvertown et al. (1993, 1996) and Franco & Silvertown (2004). The next step in this study will thus be to extend the comparative analysis to more populations and species that present different dynamics and life-histories.

Acknowledgments

We thank A. Vivat, L. Garraud, A. Bouffier, G. Damour, S. Ayata, F. Tellier, A. Bonin, E. Rigot, C. Grebenstein, J. Ehrlen and C. Conord for help with census and field experiments. M.A. was funded by the French Ministry of Research and F.N. by the Région Rhône-Alpes.

Supporting Information

Supporting Information S1 is available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Literature cited

Allendorf, F. W., P. R. England, G. Luikart, P. A. Ritchie, and N. Ryman. 2008. Genetic effects of harvest on wild animal populations. Trends in Ecology & Evolution **23**:327-337.

Allendorf, F. W., and G. Luikart 2007. Conservation and the genetics of populations. Blackwell, Malden, Mass. ; Oxford.

Bonin, A., F. Nicole, F. Pompanon, C. Miaud, and P. Taberlet. 2007. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv Biol **21**:697-708.

Caballero, A. 1994. Developments in the prediction of effective population size. Heredity **73**:657-679.

Campbell, L. G., and B. C. Husband. 2005. Impact of clonal growth on effective population size in Hymenoxys herbacea (Asteraceae). Heredity **94**:526-532.

Caswell, H. 1978. A general formula for the sensitivity of population growth rate to changes in life history parameters. Theoretical Population Biology **14**:215-230.

Cochran, M. E., and S. Ellner. 1992. Simple Methods for Calculating Age-Based Life-History Parameters for Stage-Structured Populations. Ecological Monographs **62**:345-364.

Crow, J. F., and M. Kimura 1970. An introduction to population genetics theory. Harper & Row, New York,.

Danton, P., and M. Baffray 1995. Inventaire des plantes protégées en France. Nathan, Paris, France.

Dekroon, H., A. Plaisier, J. Vangroenendael, and H. Caswell. 1986. Elasticity - the Relative Contribution of Demographic Parameters to Population-Growth Rate. Ecology **67**:1427-1431.

Dostalek, T., Z. Munzbergova, and I. Plackova. 2010. Genetic diversity and its effect on fitness in an endangered plant species, Dracocephalum austriacum L. Conservation Genetics **11**:773-783.

Efron, B., and R. Tibshirani 1993. An introduction to the bootstrap. Chapman & Hall, New York.

Felsenstein, J. 1971. Inbreeding and Variance Effective Numbers in Populations with Overlapping Generations. Genetics **68**:581-597.

Foll, M., M. A. Beaumont, and O. Gaggiotti. 2008. An approximate Bayesian computation approach to overcome biases that arise when using amplified fragment length polymorphism markers to study population structure. Genetics **179**:927-939.

448 Franco, M., and J. Silvertown. 2004. Comparative demography of plants based upon elasticities of
449 vital rates. Ecology **85**:531-538.

450 Frankham, R. 2005. Genetics and extinction. Biological Conservation **126**:131-140.

451 Gaggiotti, O. E. 2003. Genetic threats to population persistence. Annales Zoologici Fennici **40**:155-
452 168.

453 Hill, W. G. 1981. Estimation of Effective Population-Size from Data on Linkage Disequilibrium.
454 Genetical Research **38**:209-216.

455 Husband, B. C., and S. C. H. Barrett. 1992. Effective Population-Size and Genetic Drift in
456 Tristylous Eichhornia-Paniculata (Pontederiaceae). Evolution **46**:1875-1890.

457 Jorde, P. E., and N. Ryman. 2007. Unbiased estimator for genetic drift and effective population
458 size. Genetics **177**:927-935.

459 Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use
460 in population management in M. E. Soulé, editor. Viable populations for conservation.
461 Cambridge University Press, Cambridge, United Kingdom.

462 Mills, L. S., D. F. Doak, and M. J. Wisdom. 1999. Reliability of conservation actions based on
463 elasticity analysis of matrix models. Conservation Biology **13**:815-829.

464 Nicolè, F. 2005. Biologie de la conservation appliquée aux plantes menacées des Alpes. Université
465 Joseph Fourier, Grenoble, France.

466 Nunney, L., and D. R. Elam. 1994. Estimating the Effective Population-Size of Conserved
467 Populations. Conservation Biology **8**:175-184.

468 Olivier, L., J. P. Galland, and H. Maurin 1995. Livre Rouge de la flore menacée de France. Tome I:
469 Espèces prioritaires. Collection Patrimoines Naturels, Paris, France.

470 Wang, J. L., and A. Caballero. 1999. Developments in predicting the effective size of subdivided
471 populations. Heredity **82**:212-226.

472 Waples, R. S. 2006. A bias correction for estimates of effective population size based on linkage
473 disequilibrium at unlinked gene loci. Conservation Genetics **7**:167-184.

474 Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from
475 data on linkage disequilibrium. Molecular Ecology Resources **8**:753-756.

476 Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary N-e using
477 highly variable genetic markers: a largely untapped resource for applied conservation and
478 evolution. Evolutionary Applications **3**:244-262.

479

480

Tables

Table I. Geographical, demographic and genetic parameters of the seven populations of *D. austriacum*.

		VA	ST	LA	CH	RE	ES	BE
	Location (Longitude, latitude)	6.10 E, 44.88 N	6.23 E, 44.93 N	6.47 E, 44.78 N	6.53 E, 44.72 N	6.18 E, 44.33 N	6.72 E, 44.76 N	7.04 E, 45.29 N
	Altitude (m)	1600	1800	1550	1700	1275	1690	2000
Survival rates[1]	u_1	0.35	0.55	0.56	0.58	0.37	0.68	0.46
	u_2	0.79	0.78	0.71	0.86	0.55	0.70	0.94
	u_3	0.85	0.88	0.88	0.92	0.94	0.92	1.00
	u_4	0.85	0.93	0.96	0.97	0.95	0.99	1.00
	$\overline{u}$	0.76	0.84	0.82	0.89	0.64	0.93	0.94
	$\overline{u}^2$	0.61	0.72	0.69	0.81	0.48	0.87	0.91
Flowering rates[2]	p_1	0.00	0.04	0.00	0.03	0.01	0.19	0.15
	p_2	0.21	0.17	0.12	0.13	0.16	0.37	0.17
	p_3	0.36	0.37	0.59	0.53	0.89	0.52	0.49
	p_4	0.43	0.64	0.81	0.71	0.77	0.77	0.77
Fecundity rates[3]	μ_b	22.61	13.10	18.20	35.60	6.86	22.92	13.40

σ_b^2	399.72	198.05	448.40	1286.20	123.11	390.97	275.97
μ_k	0.55	0.28	0.39	0.20	1.12	0.14	0.18
σ_k^2	0.76	0.35	0.59	0.30	4.22	0.15	0.22
σ_b^2/μ_b	17.68	15.12	24.64	36.12	17.94	17.06	20.60
σ_k^2/μ_k	1.39	1.28	1.51	1.24	3.76	1.09	1.25
α	0.86	0.87	0.85	0.93	0.88	0.90	0.86

[4]

[1] u_i: survival rate of plants belonging to stage *i*

[2] p_i: flowering rate of plants belonging to stage i

[3] μ_b and σ_b^2 : mean and variance in potential fecundity; μ_k and σ_k^2 : mean and variance in realized fecundity

[4] α: deviation of population genotype frequencies from Hardy-Weinberg proportions.

Table II. Transition matrices, stable stage distribution and reproductive value for the seven populations of *D. austriacum*..

490

Population		Matrix				Stable stage distribution	Reproductive value
		S	J	V	R		
VA	S	0	0	0	0.55	0.16	1
	J	0.35	0.62	0	0	0.20	2.56
	V	0	0	0.54	0.49	0.36	3.58
	R	0.00	0.17	0.30	0.37	0.27	4.25
ST	S	0	0	0	0.28	0.11	1
	J	0.53	0.65	0	0	0.20	1.64
	V	0	0	0.55	0.33	0.32	2.93
	R	0.02	0.14	0.33	0.59	0.38	3.55
L	S	0	0	0	0.39	0.17	1
	J	0.56	0.63	0	0	0.27	1.76
	V	0	0	0.36	0.18	0.13	6.00
	R	0.00	0.09	0.52	0.78	0.43	7.20
CH	S	0	0	0	0.24	0.11	1
	J	0.56	0.75	0	0	0.24	1.66
	V	0	0	0.43	0.28	0.22	3.18
	R	0.02	0.11	0.49	0.69	0.44	3.71
RE	S	0	0	0	1.12	0.36	1
	J	0.36	0.46	0	0	0.24	2.56
	V	0	0	0.11	0.21	0.08	14.96
	R	0.01	0.09	0.83	0.73	0.32	16.12
ES	S	0	0	0	0.14	0.08	1
	J	0.55	0.44	0	0	0.08	1.20
	V	0	0	0.44	0.23	0.24	2.23
	R	0.13	0.26	0.48	0.76	0.60	2.61
BE	S	0	0	0	0.18	0.09	1
	J	0.39	0.79	0	0	0.14	2.05
	V	0	0	0.51	0.23	0.24	3.05
	R	0.07	0.16	0.49	0.77	0.53	3.28

S, seedlings; J, juveniles; V, vegetative adults; R, reproductive adults

Table III. Demographic parameters and effective population sizes of the seven populations of *D. austriacum*.

	VA	ST	LA	CH	RE	ES	BE
N_0	1400	600	1650	980	1120	775	200
N_h	472	277	1371	958	1158	864	338
N_B	128	104	594	419	373	516	180
N_e	35	24	105	128	38	172	96
N_a	631	574	2652	3253	730	4672	2899
N_e / N_B	0.27	0.23	0.18	0.31	0.10	0.33	0.53
N_e / N_h	0.07	0.09	0.08	0.13	0.03	0.20	0.28
λ	0.905 (0.876 − 0.933)*	0.944 (0.917 − 0.967)	0.986 (0.965 − 1.007)	0.998 (0.979 − 1.016)	1.003 (0.982 − 1.029)	1.008 (0.998 − 1.028)	1.037 (1.027 − 1.061)
L	18.2	23.5	25.3	25.4	19.3	27.2	30.3

N_0, initial census size; N_h, harmonic census size over one generation, calculated assuming constant
495 population growth rate (λ) and initial census size N_0; N_B, number of breeders; N_e, effective population size; N_a, annual effective population size; λ population growth rate; L generation time;

* 90% confidence intervals (CI) for λ were derived by bootstrapping the original dataset.

Table IV. ratio of the elasticities to retrogression and stasis over the elasticities to growth for $N_{e,}$ N_a and λ in the seven populations of *D. austriacum*.

	VA	ST	LA	CH	RE	ES	BE
N_e	1.47	2.23	2.97	3.10	2.03	3.56	4.37
N_a	1.95	2.85	4.18	3.78	3.07	4.53	5.13
λ	2.51	3.31	4.29	3.50	3.04	4.01	4.24

500

Figure legends

Figure 1: life cycle graph of *Dracocephalum austriacum*, representing transitions of individuals between stages (solid lines) and reproduction (dotted lines). The upper-level vital rates (a_{ij}) correspond to the transitions between stages, the lower-level vital rates correspond to survival (u_i) and flowering (p_i) probabilities.

Figure 2. Posterior frequency distribution of population-specific F_{IS} obtained with ABC4F. For each population: mean, standard deviation (S.D.), median and 95% credible intervals (C.I.). A 95% credible interval is defined as the set of values for which the posterior probability that F_{IS} lies there is 0.95.

Figure 3. Elasticities of effective population size N_e (black), annual effective population size N_a (grey) and population growth rate λ (white) to the matrix elements (a_{ij}) and the lower level vital rates (survival rates u_i and flowering rates p_i). The elasticities of N_e and N_a have been downscaled to be drawn on the same axis of the elasticities of λ by multiplying them by 0.03 (for ES and BE) or 0.05 (for all the other populations).

Figure 4. Elasticities of generation time L to the matrix elements (a_{ij}) and the lower level vital rates (survival rates u_i and flowering rates p_i).

Figure 5. Spearman rank correlation coefficients between the elasticities of N_e (black) and N_a (grey) and the elasticities of λ.

Figure 6. Spearman rank correlation coefficients between the elasticities of N_e and the elasticities of N_a.

Figure 1

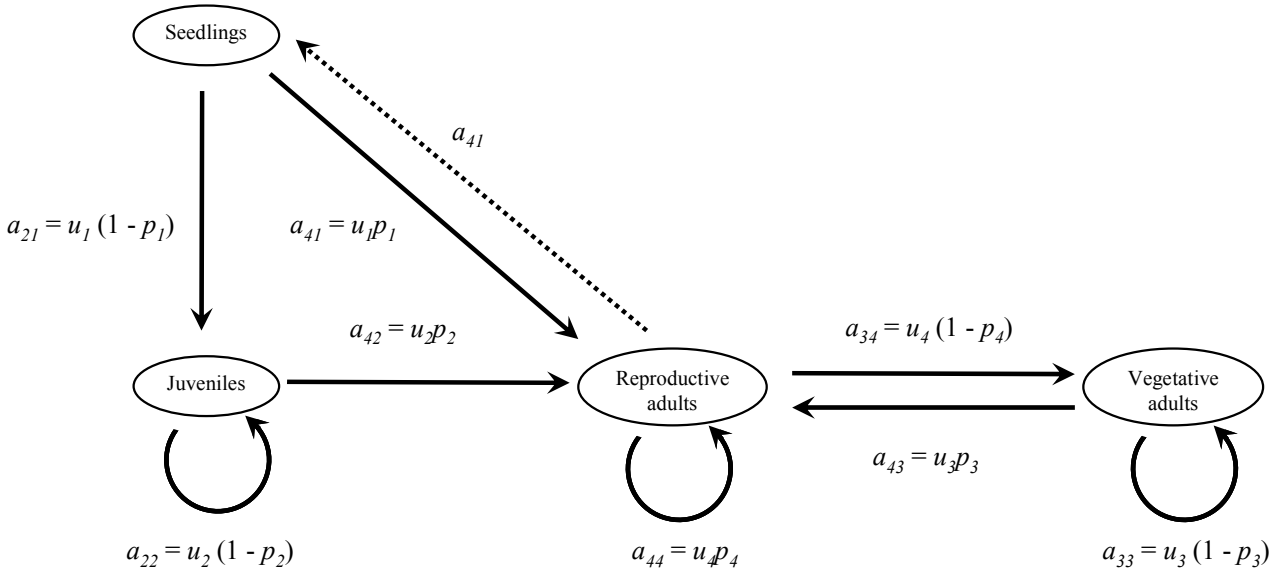
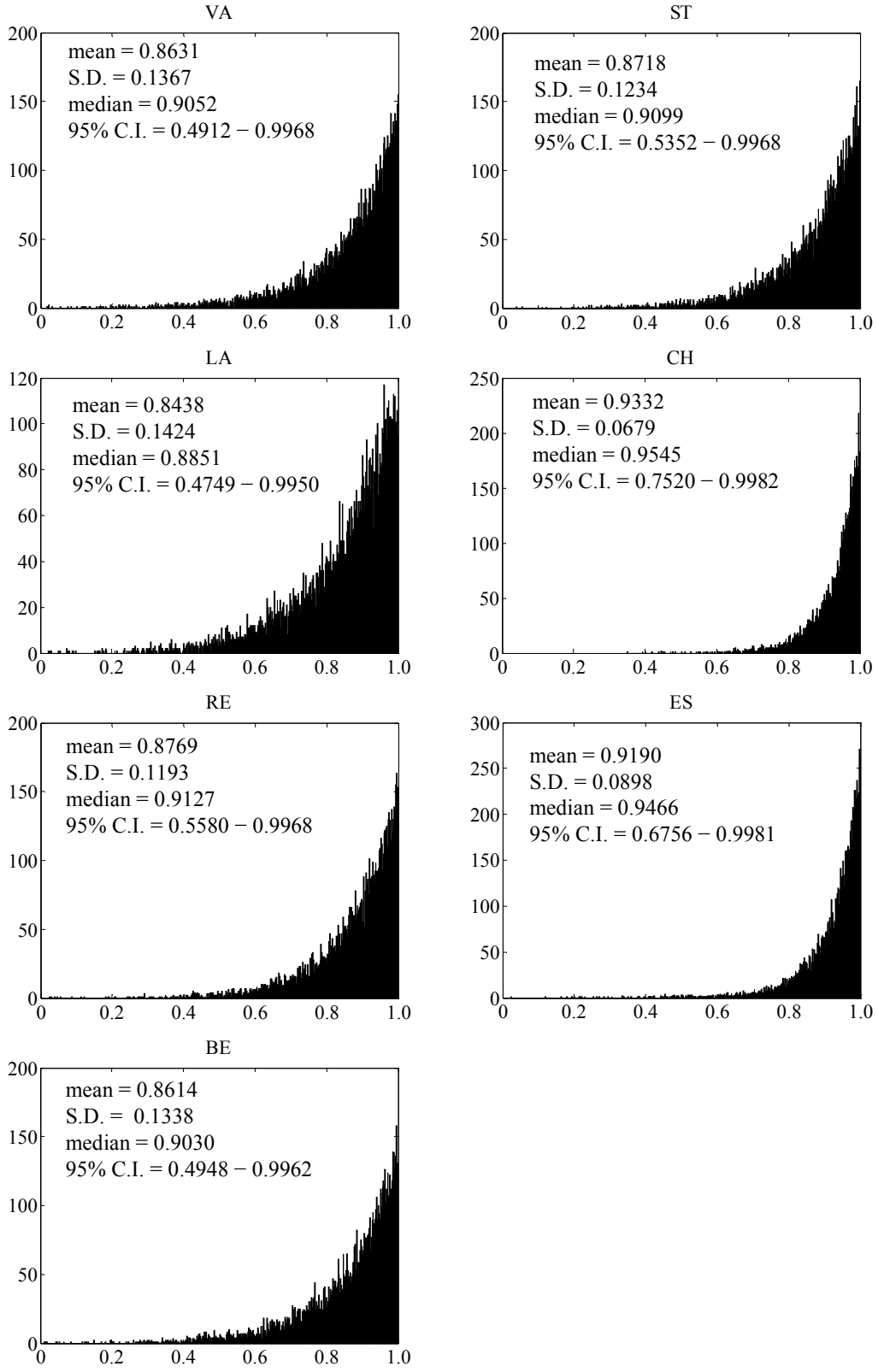

Seedlings
Juveniles
Reproductive adults
Vegetative adults
a_{41}
$a_{21} = u_1 (1 - p_1)$
$a_{41} = u_1 p_1$
$a_{42} = u_2 p_2$
$a_{34} = u_4 (1 - p_4)$
$a_{43} = u_3 p_3$
$a_{22} = u_2 (1 - p_2)$
$a_{44} = u_4 p_4$
$a_{33} = u_3 (1 - p_3)$

Figure 2


VA
mean = 0.8631
S.D. = 0.1367
median = 0.9052
95% C.I. = 0.4912 − 0.9968
ST
mean = 0.8718
S.D. = 0.1234
median = 0.9099
95% C.I. = 0.5352 − 0.9968
LA
mean = 0.8438
S.D. = 0.1424
median = 0.8851
95% C.I. = 0.4749 − 0.9950
CH
mean = 0.9332
S.D. = 0.0679
median = 0.9545
95% C.I. = 0.7520 − 0.9982
RE
mean = 0.8769
S.D. = 0.1193
median = 0.9127
95% C.I. = 0.5580 − 0.9968
ES
mean = 0.9190
S.D. = 0.0898
median = 0.9466
95% C.I. = 0.6756 − 0.9981
BE
mean = 0.8614
S.D. = 0.1338
median = 0.9030
95% C.I. = 0.4948 − 0.9962

525

Figure 3

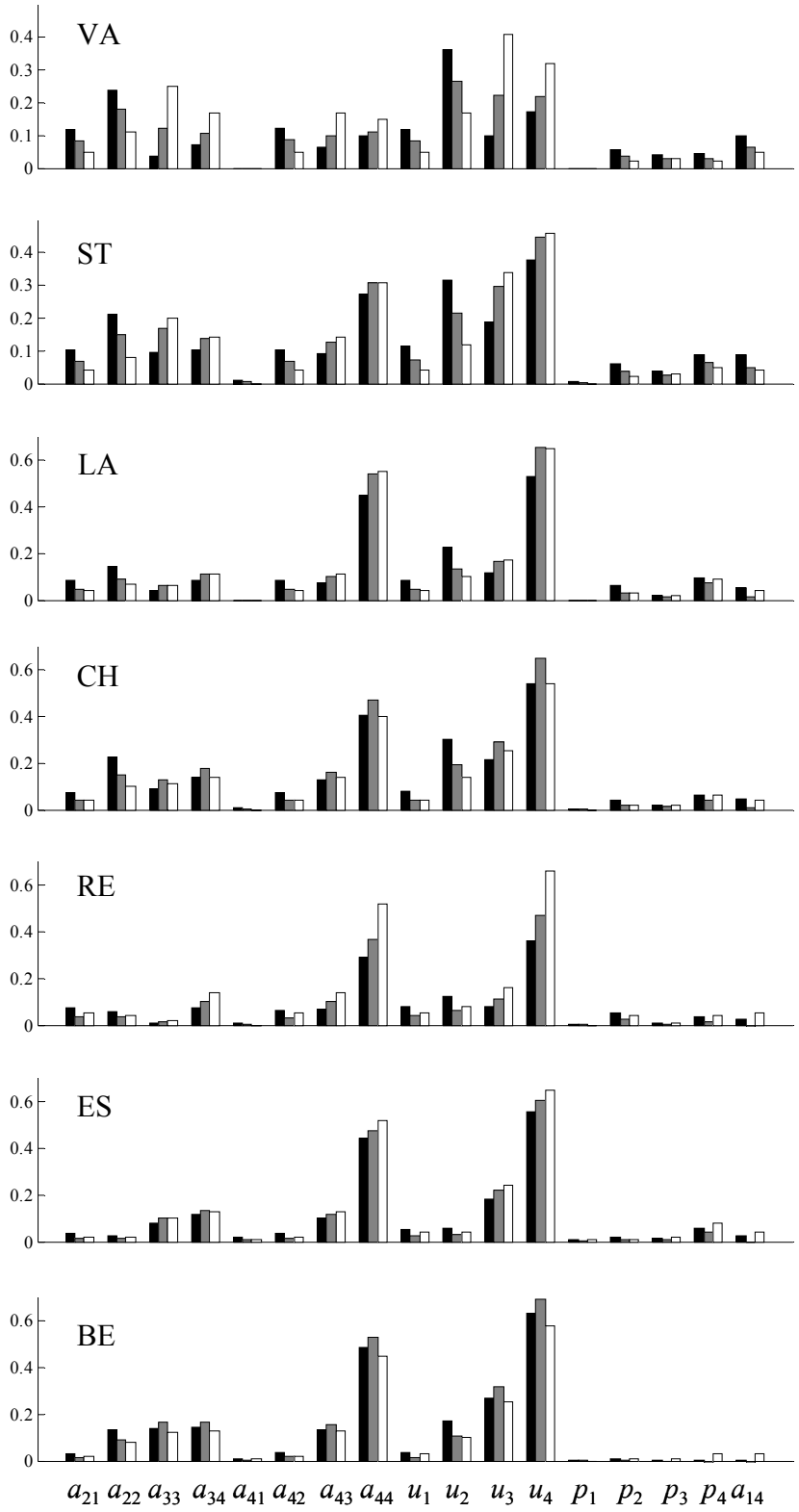
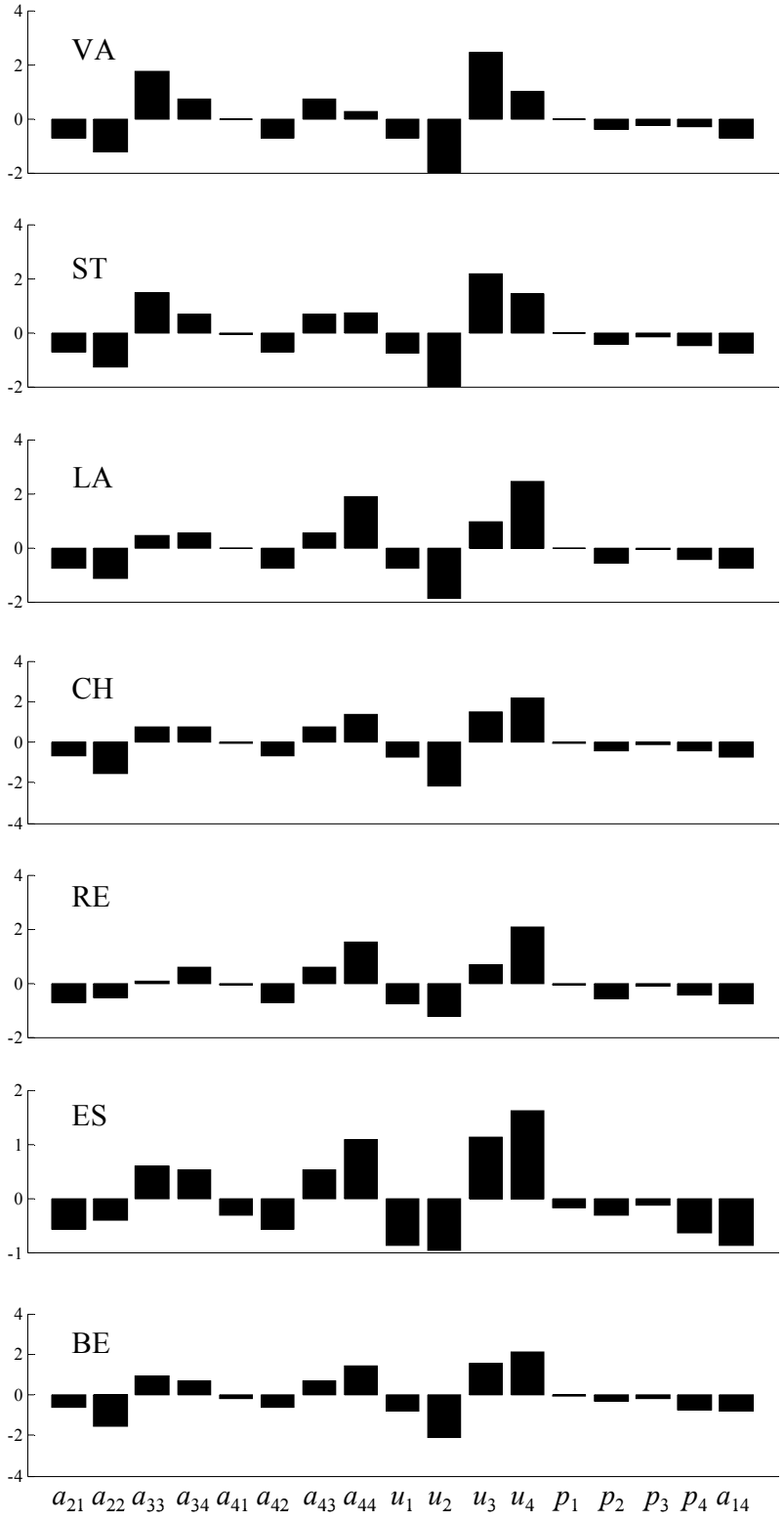

VA
ST
LA
CH
RE
ES
BE
0.4
0.3
0.2
0.1
0
0.6
a_{21} a_{22} a_{33} a_{34} a_{41} a_{42} a_{43} a_{44} u_1 u_2 u_3 u_4 p_1 p_2 p_3 p_4 a_{14}

Figure 4


VA
ST
LA
CH
RE
ES
BE
a_{21} a_{22} a_{33} a_{34} a_{41} a_{42} a_{43} a_{44} u_1 u_2 u_3 u_4 p_1 p_2 p_3 p_4 a_{14}

530 **Figure 5**

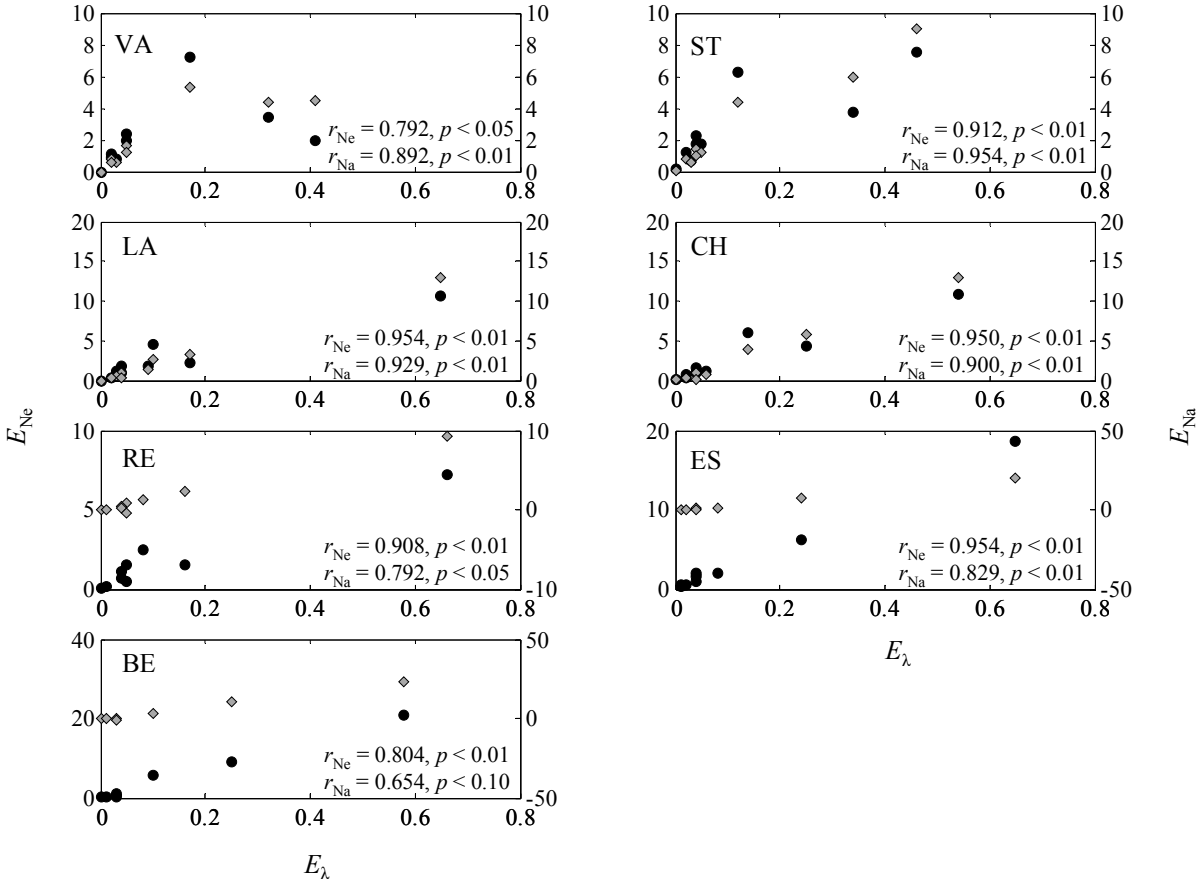
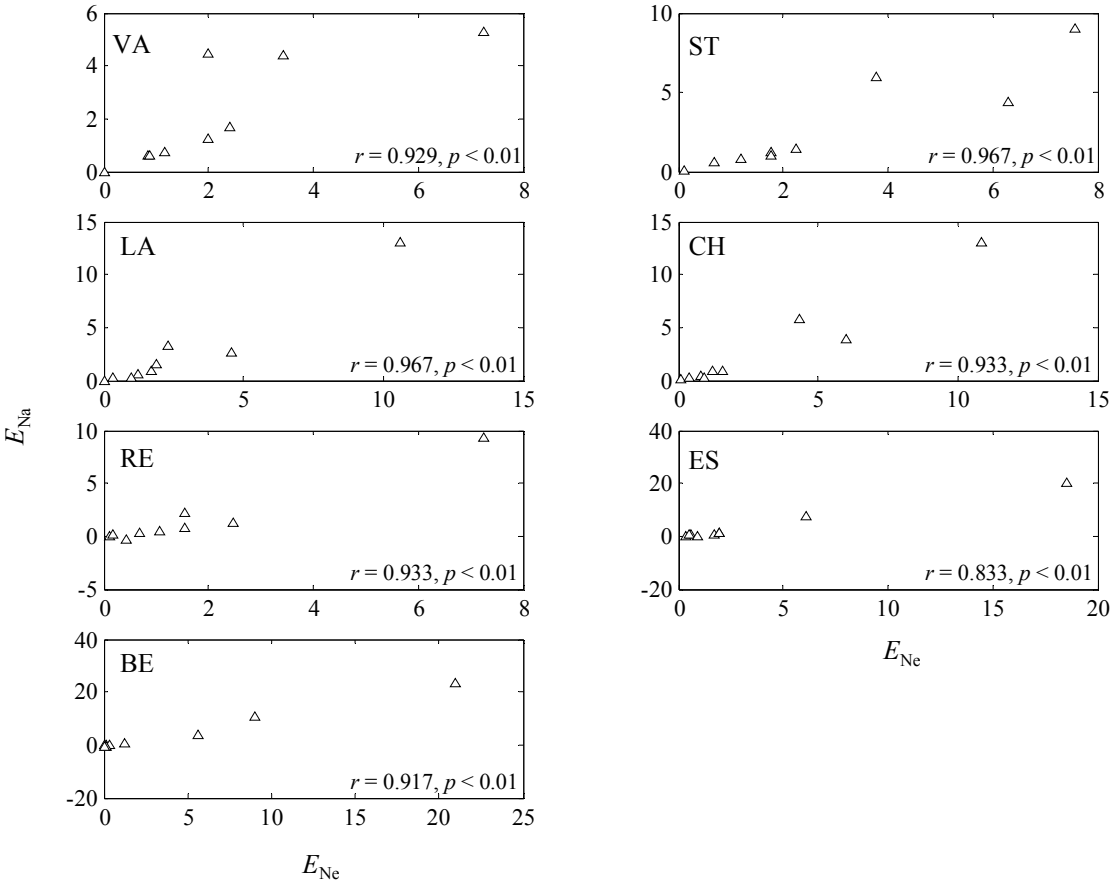

VA
r_{Ne} = 0.792, p < 0.05
r_{Na} = 0.892, p < 0.01
ST
r_{Ne} = 0.912, p < 0.01
r_{Na} = 0.954, p < 0.01
LA
r_{Ne} = 0.954, p < 0.01
r_{Na} = 0.929, p < 0.01
CH
r_{Ne} = 0.950, p < 0.01
r_{Na} = 0.900, p < 0.01
RE
r_{Ne} = 0.908, p < 0.01
r_{Na} = 0.792, p < 0.05
ES
r_{Ne} = 0.954, p < 0.01
r_{Na} = 0.829, p < 0.01
BE
r_{Ne} = 0.804, p < 0.01
r_{Na} = 0.654, p < 0.10
E_{Ne}
E_{Na}
E_λ
E_λ

Figure 6


VA
r = 0.929, p < 0.01
ST
r = 0.967, p < 0.01
LA
r = 0.967, p < 0.01
CH
r = 0.933, p < 0.01
RE
r = 0.933, p < 0.01
ES
r = 0.833, p < 0.01
BE
r = 0.917, p < 0.01
E_Na
E_Ne
E_Ne

Article B:

Demographic effects of extreme drought and management practices on an endangered alpine perennial, *Eryngium alpinum*

Title: Effect of the 2003 heatwave on the perennial *Eryngium alpinum* L. (Apiaceae) under different management regimes.

Authors: Marco ANDRELLO[1*], Jean-Philippe BIZOUX[2*], Morgane BARBET-MASSIN[3], Myriam GAUDEUL[4], Florence NICOLÈ[5] and Irène TILL-BOTTRAUD[1]

* The first two authors contributed equally to this work

Author's affiliations

[1] Université de Grenoble, Laboratoire d'Écologie Alpine, CNRS UMR 5553, F-38041 Grenoble CEDEX 09, France

[2] University of Liege, Gembloux Agro-Bio Tech, Biodiversity and Landscape Unit, Passage des Déportés, 2, B-5030 Gembloux, Belgium.

[3] Muséum National d'Histoire Naturelle, UMR 7204 MNHN-CNRS-UPMC, Centre de Recherches sur la Biologie des Populations d'Oiseaux, CP 51, 55 Rue Buffon, 75005 Paris, France

[4] Muséum National d'Histoire Naturelle, UMR CNRS 7205 'Origine, Structure et Evolution de la Biodiversité', 16 rue Buffon, CP 39, F-75005 Paris, France.

[5] Université de Lyon, F-69003, Lyon, France; Université de Saint-Etienne, F-42000, Saint-Etienne, France; Laboratoire BVpam, EA 3061; 23 rue du Dr Michelon, F-42000, Saint-Etienne, France.

Corresponding author E-mail: marco.andrello@gmail.com

In preparation for: *Journal of Ecology*.

Keywords: matrix population models; LTRE; demographic compensation; vital rates; mowing; grazing

Running title: Heatwave and management effect on *E. alpinum*

Summary

1. In the summer of 2003, Europe was impacted by a heatwave that altered ecosystem productivity and increased plant mortality in natural areas. We analyse the effect of this extreme climatic event on the demography of the protected alpine plant *Eryngium alpinum*. We also consider how population dynamics vary in relation to local ecological conditions and management regimes (mowing, grazing and unmanaged).

2. Vital rates of different life-stages (fecundity, survival and flowering rates of seedlings, juvenile, vegetative adult and reproductive adult plants) were estimated in seven sites of *E. alpinum* in the French Alps between 2001 and 2010. Spatiotemporal variation of vital rates and its impact on population dynamics (deterministic and stochastic population growth rate, λ and r) were studied using matrix population models and life table response experiments.

3. Fecundity rates did not show significant temporal variation. Reduction in survival rates was observed following the extreme 2003 summer. Flowering rates exhibited great variation between years but no effect of the 2003 heatwave was detected. λ was smaller during the heatwave and simulations showed that increasing the occurrence probability of a 2003-like event resulted in smaller r.

4. Fecundity rates were reduced in sites subject to grazing. Between-site variation in seedling and juvenile survival rates contributed considerably to variation of λ. There were few differences in vital rates and population dynamics between mowed and unmanaged sites.

5. Spatiotemporal variation in vital rates was consistent across juvenile and adult plants and for flowering and survival rates, i.e. favourable years for flowering in one life-stage were also favourable for flowering in the other stages and for survival. Conversely, fecundity and seedling survival rates exhibited opposite trends, with juvenile and adult vital rates sometimes compensating the spatiotemporal variation in λ.

6. *Synthesis.* This study demonstrates that plant vital rates can exhibit differential response to changing environmental conditions and illustrates which vital rates make the largest contribution to difference in population dynamics of *E. alpinum*. Survival rates could, on

their own, account for between-year variation, whereas fecundity and survival rates were both needed to explain between-site variation.

Introduction

Extreme climatic events can negatively affect plant population dynamics and increase extinction risk in threatened species (Maschinski *et al.* 2006; Marrero-Gomez *et al.* 2007). Increased temperature extremes and severe drought induce significant stress to plants, which can lead to increased mortality rates (Yordanov, Velikova & Tsonev 2000; McDowell *et al.* 2008; Saccone *et al.* 2009) and reduced photosynthetic rates and reproductive performances (Schulze 1986; Chaves 1991; Epron & Dreyer 1993; Yordanov, Velikova & Tsonev 2000). Europe has undergone an extremely dry and hot summer in 2003 (Meehl & Tebaldi 2004; Schär *et al.* 2004; Zaitchik *et al.* 2006), which resulted in exceptional human mortality (Le Tertre *et al.* 2006), decreased ecosystem productivity (Ciais *et al.* 2005; Reichstein *et al.* 2007), loss in crop yield (van der Velde, Wriedt & Bouraoui 2010) and increased plant mortality (Saccone *et al.* 2009). Such reductions in plant survival may compromise the persistence of vulnerable plant populations and increase their risk of extinction. A temperature anomaly such as that registered in 2003 had never been observed in the last 140 years and its return period has been estimated to several millions years (Schar *et al.* 2004). The occurrence probability of such an extreme event can nonetheless increase with global climate change as a result of the greater between-year variability in meteorological conditions and an increase of mean temperatures (Meehl & Tebaldi 2004; Schar *et al.* 2004).

Many Alpine plant species can be found in sites that were historically used as pastures for grazing by domestic animals or meadows harvested by farmers. Current management strategies in alpine ecosystems still include seasonal mowing or grazing, but with varying frequency and intensity, and absence of management is also common. These new management regimes can lead to changes in plant community structure (Stammel, Kiehl & Pfadenhauer 2003; Jantunen *et al.* 2007) and to an increase of extinction risk for the most vulnerable species (Lennartsson & Oostermeijer 2001; Brys *et al.* 2004; Jantunen *et al.* 2007; Marage, Garraud & Rameau 2008). Populations can exhibit different vital rates and demography between sites subject to different management strategies and characterized by variable ecological conditions. Such local variation can interact with large scale climatic variability and result in different sensitivities of local populations to extreme climatic events (Maschinski *et al.* 2006).

The effects of large scale climatic variability, local ecological conditions and management regimes translate into variation in vital rates at different phases of the life-cycle and affect plant fitness in a complex way. Depending on the species life-form, population dynamics is controlled mainly by survival (perennial iteroparous plants) or reproductive rates (annual species) (Silvertown *et al.* 1993; Franco & Silvertown 2004). The effects of vital rates variability on overall population growth depend on the relative sensitivity of population growth rate λ to survival, flowering and fecundity rates (Dekroon *et al.* 1986; Mills, Doak & Wisdom 1999; Caswell 2001). Vital rates exhibiting large variation in response to climatic variability may however not be important for population dynamics if they are associated with a small sensitivity of λ. On the other hand, vital rates that seem relatively unaffected by environmental variation may contribute disproportionately and unexpectedly to variation in λ if they are associated with large sensitivities. Moreover, the same environmental conditions may not affect all vital rates in the same way, and the overall effect on λ will be the outcome of sometimes opposing, compensating contributions of different vital rates (Doak & Morris 2010).

The objective of this work was to study the population dynamics of *E. alpinum,* a rare Alpine perennial plant threatened by changes in land use (Cherel & Lavagne 1982; Gillot & Garraud 1995; Gaudeul & Till-Bottraud 2003; 2004; 2008), in relation to: i) the effects of the 2003 heatwave, ii) the effects of local conditions and management regimes, and iii) the patterns of covariation of vital rates. Field data were collected between 2001 and 2010 in seven sites and survival, flowering and fecundity rates were measured for plants of different life-stages: seedlings, juveniles, vegetative adults and reproductive adults. The effect of the 2003 heatwave and local conditions on vital rates was tested using generalised linear models where year and site were used as fixed effects. Since each site was subject to a different management regime, we could not treat management explicitly as a distinct effect and we limited our statistical analysis to the site effect. Matrix population models (Caswell 2001) and life table response experiments (LTREs) (Levin *et al.* 1996; Cooch, Rockwell & Brault 2001; Lucas, Forseth & Casper 2008) were used to complete the statistical analysis of vital rates by a study of the overall consequences of the 2003 heatwave, local environmental conditions and management regimes on population dynamics. LTREs were used to weigh the observed variation in vital rates by the sensitivity of λ to those vital rates and constitute therefore the appropriate analysis to assess the impact of vital rates variation on demography and population viability.

Materials and methods

Species and study sites

Eryngium alpinum L. (Apiaceae) is a perennial, rare species. Its distribution area extends over the Alps (France, Italy, Switzerland, Austria, Croatia; between 1300-2500 m (Cherel & Lavagne 1982)). In the Alps, this is the altitudinal range of the forest. However, the species is present in open sunny but relatively humid habitats; it is therefore restricted to hayfields and avalanche corridors. The species is protected by the Bern convention, the European Habitat Directive of Natura 2000 (Wyse-Jackson & Akeroyd 1994), the French Red List of protected species and it is considered vulnerable by UICN (Gillot & Garraud 1995). The threats are mainly due to human activities such as cutting for commercial use and land use change (from late hay harvest to spring grazing, or abandonment leading to land closure by forest). Flowering occurs from mid July to mid August. Flowering individuals produce 1–20 stems (mean ≈ 3), each of them measuring 50 cm to 1 m high and bearing one terminal and 0–4 axillary inflorescences. Each inflorescence produces 150–350 small white flowers. The seed set of the terminal inflorescence is around 65% but the axillary inflorescences produce much fewer seeds because of resource limitation (Gaudeul & Till-Bottraud 2004). Mature fruits (schizocarpous diachenes) fall near the mother plant at the end of August and seeds germinate in the spring.

Two populations, Pralognan and Fournel, were studied. The Pralognan population (Pralognan la Vanoise, Savoie, France) is located in the "aire d'adhésion" of Vanoise National Park and the Fournel population (L'Argentière la Bessée, Hautes Alpes, France) is located in the "Fournel – les Bans" Natura 2000 site and the "aire d'adhésion" of Ecrins National Park. The two populations are situated at about the same altitude (1500 m) but in contrasting situations: Pralognan is in the Northern Alps on a steep East-facing slope while Fournel is in the Southern Alps on a mild North-facing slope. The climatic conditions are thus quite different, with Fournel presenting lower precipitations and greater temperature variation than Pralognan (Irène Till-Bottraud, unpublished data). Respectively three (DES, BER and BOU in Fournel) and four (PRA, PRB, PRC and PRD in Pralognan) different sites were studied. In each site, three permanents plots were established for annual census. In the two areas, the National Parks are testing various management practices to limit or even reverse the population decline observed due to agricultural abandonment. These management practices are based on local traditional and present uses of mountain prairies: sheep

grazing in autumn (DES), late mowing (after 15 August; BER), sheep grazing in spring (BOU), absence of management (PRA), late mowing every other year (PRB), late mowing (PRC), and cow grazing in spring (PRD).

Life cycle and data collection

The life cycle of *E. alpinum* was subdivided into four biological stages (Fig. 1). Seedlings (S) are individuals that appeared following germination. Juveniles (J) are individuals that are older than one year and have not yet flowered during their life. Reproductive adults (R) are plants bearing one or more inflorescences. Vegetative adults (V) are plants that do not bear inflorescences but have already flowered during their life. Seedlings become juveniles in one year, while juveniles and adults can remain in the same stage for more than one year or make a transition to another stage.

For the first two years of survey (2001 and 2002), juveniles and vegetative adults were distinguished on the basis of morphological traits (number of basal leaves and length of the longest petiole). An individual was considered juvenile when it bore less than four leaves and when the longest petiole measured less than 20 mm, otherwise it was classified as a vegetative adult. These criteria had to be slightly modified for BOU, where grazing results in enhanced vegetative growth and plants show more leaves (juveniles identified as individuals bearing less than ten leaves with the longest petiole measuring less than 20 mm).

In each permanent plot, plants were individually tagged and their presence/absence and biological stage were scored every year from 2001 to 2010, leading to a dataset including nine annual transitions. Stage-specific annual survival rates σ_i were defined as the ratio of the number of plants surviving from one census to the next one over the number of plants present in the first census. Stage-specific flowering rates ϕ_i were defined as the proportion of plants making the transition to the flowering stage the next year, conditional on survival. The annual fecundity rate f for year t was defined as the ratio of the number of seedlings emerging in year $t + 1$ over the number of reproductive adults in year t, assuming equilibrium between seed immigration and emigration among plots and no seed bank. As the number of seedlings was only recorded since 2003, seedling survival rate in the 2001 and 2002 transitions and fecundity rate in the 2001 transition were not available. No reproductive adult was observed in BOU in 2008, so survival and flowering rate could not be estimated. Since census was performed in July, the effect of the 2003 heatwave summer were associated with both the 2002 and the 2003 transitions.

Spatiotemporal variation in vital rates

We tested for spatio-temporal variation in fecundity rates, survival rates and flowering rates. Fecundity rates were analysed using a linear model with Gaussian errors. Survival rates and flowering rates were analysed with generalised linear models with binomial errors and a logit link. Site, year and the interaction between site and year were included in the models as fixed factors; the three permanent plots were considered as independent replicates of the same site-by-year cell. For each vital rate, the optimal model was selected through a backward stepwise procedure based on the Akaike Information Criterion (AIC). The AIC is commonly used to select an optimal statistical model on the basis of the likelihood of the model and the number of parameters that need to be estimated (Burnham & Anderson 2002). Models with lower AIC are characterized by higher likelihood and less parameters. We report the difference between the AIC of the optimal model and that of the second-optimal one (ΔAIC). When the year and/or the site factor was retained in the optimal model but the interaction was discarded, the differences in vital rates between the levels of each factor were tested using Tukey's post-hoc multiple comparison method. When the interaction between site and year was also retained, the comparisons between the levels of one factor were tested separately for each level of the other factor (e.g. differences between years were tested separately for each site). When the year factor was retained in the optimal model, additional planned comparisons were used to test for significant differences between transitions affected by the heatwave (2002 and 2003, hereafter also referred to as dry years) and transitions over normal years (all the others). All statistical analyses were performed in R 2.10.1 (R Development Core Team 2008) using the packages *lme4* (Pinheiro & Bates 2000) and *multcomp* (Hothorn, Bretz & Westfall 2010).

Deterministic analysis

The life-cycle was described by a birth-pulse matrix model with pre-breeding census (Caswell 2001). The elements of the **A** matrix, a_{ij}, gave the annual transition rates from stage j to stage i and were defined in terms of survival rates σ_j, flowering rates ϕ_j and fecundity rate f:

$$\mathbf{A} = \begin{pmatrix} 0 & 0 & 0 & f \\ \sigma_S & \sigma_J(1-\phi_J) & 0 & 0 \\ 0 & 0 & \sigma_V(1-\phi_V) & \sigma_R(1-\phi_R) \\ 0 & \sigma_J\phi_J & \sigma_V\phi_V & \sigma_R\phi_R \end{pmatrix}$$

Within each site, the observations from the three plots were pooled together to increase precision of the matrix element estimates. We defined a transition matrix for each site and each transition from 2001 to 2009. Since seedling survival rates were not available for 2001 and 2002 and fecundity rates were not available for 2001 (see 'Life-cycle and data collection'), their values were set equal to their averages over the other years. The transition matrix for BOU in 2008 was not estimated because of lack of data on the reproductive adult stage.

The asymptotic rate of population increase λ was estimated for each site and transition as the eigenvalue of the **A** matrix. The uncertainty in λ estimation was estimated by bootstrapping individual life-histories for each population and each year. Ninety five percent confidence intervals (CIs) were derived by the percentile methods using 2000 bootstrap replicates (Caswell 2001). We derived the sensitivities and elasticities of λ to survival, flowering and fecundity rates as indicated in Caswell (2001; pp. 218-220 and 232). Sensitivities and elasticities were calculated in relation to the overall mean matrix and to the matrices of each site. The calculation of eigenvalues, as well as the following LTRE and stochastic analyses, were carried out in Matlab (The Mathworks 2001)

Life-Table Response Experiment (LTRE)

The differences in λ between sites and years were analysed with a Life-Table Response Experiment (LTRE) (Caswell 2001; Cooch, Rockwell & Brault 2001). This method allows the estimation of site and year effects, and the decomposition of the effects into contribution of single vital rates. The LTRE thus complements the analysis of the spatiotemporal variation of vital rates because it reflects the contribution that single vital rates have on the overall population dynamics (λ). In its mathematical formulation, the LTRE weights the effect on λ of the variability in vital rates by the sensitivity of λ to those vital rates. To first-order approximation, λ can be defined in terms of site and year effects analogously to a linear model:

$$\lambda^{(l,m)} = \lambda^{(\cdot\cdot)} + \alpha^{(l)} + \beta^{(m)} + (\alpha\beta)^{(l,m)}$$

where $\lambda^{(l,m)}$ is the growth rate of site *l* in year *m*, associated to the corresponding transition matrix $\mathbf{A}^{(l,m)}$. $\lambda^{(..)}$ is the growth rate of the mean transition matrix $\mathbf{A}^{(\cdot\cdot)}$ over all sites and years. $\alpha^{(l)}$, $\beta^{(m)}$ and $(\alpha\beta)^{(l,m)}$ represent the effect of site *l*, year *m* and the interaction between site and year, respectively. In our case, there were seven levels for the site effect, corresponding to the seven sites studied, and nine levels for the year effect, corresponding to the transitions between years 2001 and 2010. The

extent of effects $\alpha^{(l)}$, $\beta^{(m)}$ and $(\alpha\beta)^{(l,m)}$ can be estimated directly to first-order approximations by the following equations, which also decompose them into the contributions of lower-level vital rates x_p (Caswell 2001):

Site effect: $$\hat{\alpha}^{(l)} = \lambda^{(l,\cdot)} - \lambda^{(\cdot\cdot)} = \sum_p \sum_{i,j} \left(x_p^{(l,\cdot)} - x_p^{(\cdot\cdot)}\right) \frac{\partial a_{ij}}{\partial x_p} \frac{\partial \lambda}{\partial a_{ij}}\bigg|_{(1/2)\left(A^{(l,\cdot)} + A^{(\cdot\cdot)}\right)}$$

Year effect: $$\hat{\beta}^{(m)} = \lambda^{(\cdot,m)} - \lambda^{(\cdot\cdot)} = \sum_p \sum_{i,j} \left(x_p^{(\cdot,m)} - x_p^{(\cdot\cdot)}\right) \frac{\partial a_{ij}}{\partial x_p} \frac{\partial \lambda}{\partial a_{ij}}\bigg|_{(1/2)\left(A^{(\cdot,m)} + A^{(\cdot\cdot)}\right)}$$

Interaction site*year effect:

$$\left(\alpha\hat{\beta}\right)^{(l,m)} = \lambda^{(l)} - \hat{\alpha}^{(l)} - \hat{\beta}^{(m)} - \lambda^{(\cdot\cdot)} = \sum_p \sum_{i,j} \left(x_p^{(l,m)} - x_p^{(\cdot\cdot)}\right) \frac{\partial a_{ij}}{\partial x_p} \frac{\partial \lambda}{\partial a_{ij}}\bigg|_{(1/2)\left(A^{(l,m)} + A^{(\cdot\cdot)}\right)} - \hat{\alpha}^{(l)} - \hat{\beta}^{(m)}$$

In these equations, $\mathbf{A}^{(l,\cdot)}$ is the mean transition matrix for site l (averaged over all years) and $\mathbf{A}^{(\cdot,m)}$ is the mean transition matrix for year m (averaged over all sites); $a_{ij}^{(l,m)}$ denotes the i, j element of the transition matrix $\mathbf{A}^{(l,m)}$. The sensitivities $\frac{\partial \lambda}{\partial a_{ij}}$ are evaluated at the mid-point between the treatment matrix ($\mathbf{A}^{(l,\cdot)}$, $\mathbf{A}^{(\cdot,m)}$ or $\mathbf{A}^{(l,m)}$) and the mean matrix $\mathbf{A}^{(\cdot\cdot)}$ (Caswell 2001; Cooch, Rockwell & Brault 2001; Lucas, Forseth & Casper 2008). x_p indicates one of the lower-level vital rates, i.e. survival rates σ_j, flowering rates φ_j or fecundity rate f.

In order to identify the vital rates contributing the most to the overall between-site and between-year differences in λ, we summed the contributions across sites and years for each vital rate. The overall contribution of a given vital rate to between-site differences was obtained by summing the absolute values of its contributions to between-site differences across sites. Similarly, the overall contribution to between-year differences was obtained by summing across years.

Stochastic projection

We used stochastic projections to study the effect of increased heatwave occurrence probabilities on population dynamics. The stochastic rate of population increase $r = \ln\lambda_{stoch}$ was calculated under different heatwave occurrence probabilities p using a numerical approach as explained in Caswell (2001). Briefly, population dynamics was simulated for a large number of years (T = 1000). Each

year t, a transition matrix was sampled from the set of matrices and the corresponding rate of population increase was calculated as $r_t = \ln N(t + 1) - \ln N(t)$. Each time a heatwave was selected to occur, the corresponding year t was assigned the 2002 matrix and the following year $t + 1$ was assigned the 2003 matrix. In years that were not affected by a heatwave, the matrix was sampled randomly from the remaining set, with equal probability for each year. Reported r values are the average of the r_t's over the T years of simulation. A 95% confidence interval for r was calculated as $1.96 \cdot (\mathrm{Var}(r_t) / T)^{0.5}$, where $\mathrm{Var}(r_t)$ is the observed variance in r_t over the T years of simulation (Caswell 2001). On the basis of climatic projections (Schar *et al.* 2004), we chose to consider values of heatwave occurrence probability p varying between 0 (no heatwave) and 0.2 (one heatwave every 5 years).

Results

Spatiotemporal variation in vital rates

Mean fecundity rate f (± S.D.) was 22 ± 36 seedlings per reproductive adult. The optimal model included the effect of site only (ΔAIC = 5.4). DES and PRC exhibited significantly higher f than the other sites (Tukey's multiple comparisons; Fig. 2).

Mean seedling survival rate σ_S (± S.D) was 0.10 ± 0.20. The optimal model included the effects of site, year and the interaction between site and year (ΔAIC = 367). We decomposed thus the model to test the year and site effects separately. In DES, σ_S was significantly smaller in dry than in normal years (planned comparison: 0.19 vs 0.01, $P < 0.001$); in PRC, σ_S was significantly larger in dry than in normal years (0.04 vs. 0.16, $P < 0.001$); in the other sites, there was no significant difference between dry and normal years. There was no general pattern for the between-site variation in σ_S. Some sites exhibited significant differences in some years but not in others, e.g. DES had a significantly larger survival rates than all the other sites in 2009 but not in 2006 (Tukey's multiple comparison; supplementary material). BOU exhibited very large σ_S in 2005, 2006 and 2007 (Fig. 3), but these rates were estimated on very small numbers of censused seedlings and were not significantly larger than in the other sites (Tukey's multiple comparison; Supporting Information). In BER, σ_S was often small: in 2004, 2008 and 2009, $\sigma_S = 0$; in 2006 σ_S was

significantly lower than in all other sites; and in 2007 significantly lower than in all other sites except PRD.

Mean juvenile survival rate σ_J (± S.D) was 0.78 ± 0.26 (Fig. 3). The optimal model included the effects of site, year and the interaction between site and year (ΔAIC = 17.69). We decomposed the model to test the year and site effects separately. σ_J was significantly smaller in dry than in normal years only in four sites out of seven: DES (0.60 vs 0.92, $P< 0.001$), PRA (0.32 vs. 0.82, $P < 0.001$), PRB (0.36 v. 0.83, $P < 0.001$) and PRC (0.35 vs. 0.87, $P < 0.001$); no significant difference between dry and normal years was observed in BER, BOU or PRD. However in PRD, the dry year 2003 exhibited a smaller σ_J than 2005, 2006 and 2008 (Tukey's multiple comparison; $P < 0.05$; Supporting Information). σ_J was markedly different between sites in the first three years of survey. In 2001, 2002 and 2003, the σ_J's are often larger in DES and BOU than in the other sites (Tukey's multiple comparison; Supporting Information). In BER, $\sigma_J = 1$ for six consecutive years (no death observed between 2003-2008). There was no other apparent general pattern in between-site differences in juvenile survival rate.

Mean vegetative adult survival rate σ_V (± S.D) was 0.93 ± 0.09 (Fig. 3). The optimal model included the year and site effects without the interaction (ΔAIC = 32.53). σ_V was significantly smaller in dry than in normal years (0.87 vs 0.94, z = -8.4, $P < 0.001$). BER and PRB exhibited the largest σ_V and BOU the smallest one.

Mean reproductive adult survival rate σ_R (± S.D) was 0.95 ± 0.13 (Fig. 3). The optimal model included the year effect only (ΔAIC = 8.64). σ_R was significantly smaller in dry than in normal years (0.87 vs 0.97, $P < 0.001$).

Mean juvenile flowering rate ϕ_J (± S.D.) was 0.09 ± 0.14 (Fig. 4). The optimal model included the year and site effects without the interaction (ΔAIC = 20.36). ϕ_J was significantly reduced in dry vs. normal years (0.063 vs 0.093, $z = - 3.25$, $P = 0.001$). However, there was strong temporal variation in ϕ_J and the dry year 2003 exhibited a value that was very similar to the normal year 2007 but smaller than the dry year 2002. Between-site differences in ϕ_J were smaller: BER exhibited the largest rate and PRC the smallest, but the two values were not significantly different (Tukey's multiple comparison).

Mean vegetative adult flowering rate ϕ_V (± S.D.) was 0.28 ± 0.21. The optimal model included the effects of site, year and the interaction between site and year (ΔAIC = 49.44). We decomposed the model to test the year and site effects separately. The difference in ϕ_V between dry and normal year was significant only in DES (0.23 vs 0.29, z = -2.360, P = 0.018) but not in the other sites. DES had a significantly larger ϕ_V than PRA, PRB, PRC and PRD in 2001, 2004, 2006 and 2009. Apart from this, ϕ_V varied among sites without any general clear pattern, and the sign and significance of between-site differences was very variable across years.

Mean reproductive adult flowering rate ϕ_R (± S.D.) was 0.35 ± 0.29. The optimal model included the effects of site, year and the interaction between site and year (ΔAIC = 114.4). We decomposed the model to test the year and site effects separately. ϕ_R did not differ significantly between dry and normal years in any site except PRA, where ϕ_R was larger in dry than in normal years (0.57 vs. 0.31, P = 0.004). Between-site differences in ϕ_R did not show any apparent general pattern, except for DES, BER and PRC, which often exhibited larger ϕ_R than the other sites in normal years.

Deterministic analysis

The asymptotic population growth rate λ was comprised between 0.726 (95% CI: 0.626 – 0.812) in PRD in 2003 and 2.070 (95% CI: 1.896 – 2.232) in DES in 2009 (Fig. 5). DES showed much larger λ than the other sites in 2005 and 2009. On the overall mean matrix, the largest sensitivity of λ was associated with seedling survival rate σ_S and the smallest with fecundity f (Table 1). This pattern was observed also in each site, except for DES and PRC (largest sensitivity associated with juvenile flowering rate ϕ_J) and for BOU (largest sensitivity associated with vegetative adult survival rate σ_V). On the overall mean matrix, as well as in each site, the largest elasticities were associated with juvenile, vegetative and reproductive adult survival rates (Table 1). The elasticities to σ_S and f were equal and similar to the elasticities to flowering rates. Elasticities represent *potential* contributions to λ; however, in retrospective analysis like LTRE, the *realized* contributions are calculated as a function of sensitivities, not elasticities. Thus variation in σ_S contributes more to λ than variation in f due to the much larger sensitivity of σ_S compared to f.

Life Table Response Experiment (LTRE)

The site and year effect sizes were comparable so differences in λ between years were similar to those between sites. There were also interactions between the site and year effects (Supporting Information).

DES showed a large positive effect ($\hat{\alpha}^{(\mathrm{DES})} = 0.263$; Fig. 6), while BOU showed a large negative effect ($\hat{\alpha}^{(\mathrm{DES})} = -0.171$); all the other sites showed negative effects. The vital rates contributing the most to differences between sites were fecundity rate f (sum of absolute values of contributions: 0.518) and seedling survival rate σ_1 (0.317). Fecundity made large contributions in DES (positive), BOU (negative) and, to a lesser extent in PRA, PRB, PRD (negative) and PRC (positive). DES was also the site exhibiting the largest fecundity rate (Fig. 2). Seedling survival made moderately large contributions in DES, BOU (positive) and PRB, PRC and PRD (negative). The positive contributions of seedling survival thus corresponded to the sites exhibiting the largest values for this vital rate (Fig. 3). The contributions of the other vital rates were of lesser importance, with few exceptions (e.g. juvenile survival rate in PRA and juvenile flowering rate in PRC).

The years 2001, 2002, 2003, 2004 and 2009 exhibited negative effects (Fig. 7), while the other years showed positive effects. The effect of 2003 was very large and negative ($\hat{\beta}^{(2003)} = -0.191$) and the effect of 2005 was very large and positive ($\hat{\beta}^{(2005)} = 0.201$). The large negative effect showed by 2003 was due to negative contributions of all vital rates: in particular, there were large negative contributions of juvenile and vegetative adult survival rate and juvenile flowering rate. The positive effect of 2005 was due mainly to positive contributions of seedling survival rate, fecundity rate, juvenile survival rate and juvenile flowering rate. Across all years, the vital rates contributing the most to the differences in λ were juvenile survival rate (0.439), seedling survival rate (0.363), juvenile flowering rate (0.285) and fecundity rate (0.185).

The contributions of flowering rates to the year effect were consistent across life-stages, i.e. when juvenile flowering rate contributed positively to one year, then vegetative and reproductive adult flowering rates contributed also positively. The contributions of survival rates were also consistent across the juvenile and adult life-stages. Moreover, there was consistency between the contribution of flowering rates and the contributions of survival rates (except in 2002 and in 2007), so years benefiting from positive contributions from flowering rates were also associated with positive contributions from survival rates.

In contrast to the consistency between flowering rates and survival rates among adults and juveniles, fecundity rate and seedling survival rate made contributions that tended to compensate each other and those of the other life-stages. In years where survival and flowering rates made positive contribution to λ (2004, 2005, 2006, 2008), fecundity and seedling survival rates made negative contributions (2005 and 2006 for fecundity and 2004 and 2008 for seedling survival). This resulted in opposite contributions: the contributions of survival and flowering rates were compensated by the contribution of fecundity (in 2005 and 2006) and seedling survival (in 2004 and 2008). In all the four years therefore fecundity and seedling survival provided opposite contributions.

Stochastic projections

In absence of heatwaves ($p = 0$), the stochastic rate of population increase r was comprised between –0.108 (95% CI: –0.117, –0.098) in BOU and 0.344 in DES (95% CI: 0.324, 0.363; Fig. 8). Increasing heatwave occurrence probability p had a positive effect only in BER. In the other sites, r decreased as p increased; the reduction in r was most pronounced in DES. The differences in r between different values of p were smaller compared to difference between sites. DES showed always the largest r and BOU the smallest, for all values of heatwave occurrence p.

Discussion

Impact of the 2003 heatwave on vital rates and population growth

Survival rates of *E. alpinum* were reduced by the heatwave of 2003 in all the surveyed sites. This extreme climatic event was associated with rainfall deficit and extreme summer heat that impaired plant productivity in all Europe through heat stress and drought stress (Ciais *et al.* 2005; Reichstein *et al.* 2007). At a smaller scale, the summer of 2003 lead to reduced survival rates in tree populations located in the same geographical area as the *E. alpinum* populations studied here (Saccone *et al.* 2009). Drought can lead to direct plant death by hydraulic failure, or reduce photosynthetic rates following stomatal closure, leading to a cascade of downstream effects that reduce plant fitness and increase mortality (McDowell *et al.* 2008). When photosynthesis is inhibited, plants must rely on resources stored in previous seasons, and individuals with smaller roots as seedlings or juveniles are more exposed to severe drought stress than larger, adult plants

(McDowell *et al.* 2008). In agreement with this prediction, we observed the largest reductions in survival rates for juvenile plants (from σ_J = 0.86 to 0.41 on average in DES, PRA, PRB and PRC), while adults were much less affected by the 2003 heatwave (from σ_V = 0.95 to 0.87 and from σ_R = 0.97 to 0.87 on average). However, seedlings did not conform to these expectations. Seedling survival rates showed a reduction in only one site out of seven (DES), while they remain stable in others or even increased in one site (PRC). Plant responses to the heatwave were therefore variable across life-stages and geographic sites. The contrasting results from DES and PRC are puzzling, because both sites have large fecundity and local conditions seem favourable to seed germination, yet seedling responses to the heatwave were opposite. One explanation to this pattern could be that DES is drier than PRC so that the effects of the 2003 drought were more severe. Overall, the reduction in survival rates observed during the 2003 heatwave lead to small population growth rates λ in this year. The negative impact on λ could be ascribed mainly to the reduced survival rates rather than to a change in the pattern of flowering or fecundity.

Fecundity rates did not show significant interannual variation and did not contribute much to the difference in λ between 2003 and the other transitions. In contrast, flowering rates exhibited strong temporal variation, but it was not possible to unequivocally attribute these changes to the effects of the 2003 heatwave. Flowering rates were consistently reduced in the 2003 transition only for juvenile plants, while vegetative adult flowering rate was reduced only in DES and reproductive adult flowering rate was not affected by the heatwave. Flowering rates exhibited nonetheless considerable year-to-year variation but the causes of these observed temporal variations remain unknown and warrant further investigation.

The effects of increasing heatwave occurrence on future population dynamics were limited. Even when the frequency of heatwave occurrence was maximal (p = 0.2, one heatwave every five years on average), some sites still presented positive population growth (e.g. DES and BER). This result is reassuring for the persistence of *E. alpinum* populations, but it cannot be taken as evidence that the species will not be affected by climate change. The dramatic increase in the frequency of heatwaves and other extreme events is only one consequence of global climate change (Meehl & Tebaldi 2004; Schär *et al.* 2004). Climate warming will also lead to modifications in species interaction, community dynamics and productivity, and ecosystem processes (Easterling *et al.* 2000; Araujo & Luoto 2007; Hagvar & Klanderud 2009; Klanderud 2010). The projections that we used may therefore not be representative of the population dynamics under climate change, because they

only take into account the effects of increased heatwave frequency and disregard potential modifications of vital rates due to other processes.

Impact of local conditions and management regimes

Overall, populations of *E. alpinum* were stable (Fig. 8 for $p = 0$), except in the DES site, which was increasing ($r = 0.344$), and in the BOU and PRD sites, which exhibited a pronounced (BOU; $r = -0.108$) or modest (PRD; $r = -0.012$) decline. We discuss the results of these three sites first and examine the factors controlling population dynamics in the other sites afterwards. DES is located in an avalanche debris cone that favours soil water retention and thus creates favourable conditions for plant growth and seedling establishment. The site is part of a protected area (Natura 2000) and is subject to regular shrub and tree cutting as well as sheep grazing in the autumn, which prevent forest regeneration. As a consequence, the vegetation is sparse, *E. alpinum* is the dominant species and exhibits a positive population growth rate. On the basis of plant density in the demographic plots and the surface occupied by *E. alpinum*, the site counts potentially tens of thousands reproductive adults (Andrello et al., unpublished manuscript). The population has large levels of genetic diversity (Gaudeul & Till-Bottraud 2008) and is the one of least risk for the species conservation.

Conversely, BOU and PRD exhibited a pattern of population decrease. This pattern is very likely explained by the impact of management regime (intense sheep grazing). Heavy grazing can impair plant survival and reduce seed set (Huhta *et al.* 2001; Lennartsson & Oostermeijer 2001; Brys *et al.* 2004) and this effect was evident for adult survival rates, which were lower in BOU than in other sites (Fig. 3). The effect of grazing on fecundity can be positive or negative: grazing can reduce fecundity rates by lowering seed set (Lennartsson & Oostermeijer 2001; Brys *et al.* 2004), but can also increase fecundity by creating favourable conditions for seedling establishment, through the prevention of litter accumulation and the creation of gaps (Lennartsson & Oostermeijer 2001). However, fecundity rates in BOU were the smallest of all sites and were the parameters contributing the most to the reduction in λ (Fig. 7). This reduction in fecundity was at least partly driven by a decrease in seed set (data not shown), but we have no data to assess the potential changes in seed germination and seedling establishment. The effects of grazing can also be seen on plant morphology, as plants usually reduce their flowering rates, allocate more resources to vegetative growth and show higher foliage productivity (Turner, Seastedt & Dyer 1993; Huhta *et al.* 2001;

Brys *et al.* 2004). In agreement with these published observations, flowering rates were lower in BOU than in the other sites and vegetative growth was greatly enhanced (the mean number of basal leaves per vegetative adult was 56 compared to 8 in the other sites). To a lesser extent, the negative effects of grazing could also be also detected by comparing the fecundity in PRD (grazed) with those in PRB and PRC (both mowed). In summary, the main factor explaining the declining population dynamics in grazed sites was the reduction of fecundity associated to lower adult survival rates. Intense grazing must therefore be discouraged as a management regime in sites where the conservation of *E. alpinum* is a priority.

The populations growing in the other sites (BER, PRA, PRB and PRC) were stable or slightly declining. These populations were left unmanaged (PRA) or subject to various regimes of mowing (see 'Species and study sites'). Absence of management can lead to limited germination possibilities due to the disappearance of suitable microsites for recruitment (Brys *et al.* 2004), decline in species diversity and increase in the abundance of tall plants (Huhta *et al.* 2001; Stammel, Kiehl & Pfadenhauer 2003; Lanta *et al.* 2009; Jacquemyn *et al.* 2011). At the altitudinal range where *E. alpinum* is found, land abandonment translates into invasion by tree species and forest regeneration, and the populations of *E. alpinum* tend to become smaller and more fragmented (Cherel & Lavagne 1982). Conversely, mowing can have positive consequences for persistence of weakly competitive species. Seed germination and seedling establishment are favoured by litter removal and harvest of competitive species (Huhta *et al.* 2001; Lennartsson & Oostermeijer 2001; Brys *et al.* 2004; Kahmen & Poschlod 2008). In spite of these expectations, there were no clear signs of better population dynamics in mowed vs. unmanaged sites, apart from PRC (mowed every year) exhibiting larger fecundity rates and PRB (mowed every second year) exhibiting larger vegetative adult survival rates than PRA (unmanaged). No reduction in seedling, juvenile or reproductive adult survival rates was observed in PRA compared to the mowed sites. The negative effects of land abandonment will perhaps emerge in the future, as tree and shrub invasion likely occurs on longer time scales than the duration of our study; some trees and shrubs were observed on the site in the last years of survey. Mowing did not have clear positive effects in BER: seedling survival rates were often very low even though fecundity rates, juvenile survival rates and adult survival rates were similar to or even larger than those observed in the other sites. The reduction in seedling survival rates seems the result of local conditions rather than management regime. This site is characterised by dry conditions and dense vegetation cover, two features that could reduce survival rates in the youngest stage.

Patterns and effects of vital rate variation

The impact of environmental variation on population dynamics depends not only on the effect of environmental (e.g. climatic) conditions on demographic rates, but also on the sensitivity of λ to those rates (Mills, Doak & Wisdom 1999; de Kroon, van Groenendael & Ehrlen 2000). Due to their pronounced temporal variation and large sensitivity of λ to these rates, juvenile and seedling survival rates were the demographic parameters contributing the most to differences in λ among years (Fig. 7; Table 1). Nevertheless, even vital rates associated with small sensitivities can contribute considerably to variation in population growth rate if they have large effects. This was the case of fecundity, which was largely contributing to differences in λ between sites, and juvenile flowering rate, which contributed to interannual differences, even if they were associated with smaller sensitivities than seedling or adult survival rates.

Flowering rates and survival rates of juveniles, vegetative and flowering adults varied consistently over years. In general, a favourable year for juvenile flowering rate was also a favourable year for flowering of the other stages (except in 2005). In addition, there was a positive covariation between juvenile flowering and survival rates (except 2002). The underlying factors driving these dynamics may be linked to large-scale climatic variability, which would affect all the sites in the same way. However, significant site*year interactions for seedling, juvenile survival rates and adult flowering rates suggest that local conditions may alter the effects of large scale pattern of climatic variation.

Some vital rates exhibited contrasting patterns of variation over years compared to other vital rates. In particular, fecundity rates and seedling survival rates did not match the pattern emerging from the other life stages. As a consequence, the contributions of these vital rates to population growth rate compensated those of other vital rates. However, the years affected by the heatwave did not exhibit compensation between survival and fecundity: in 2003, all vital rates contributed negatively to λ. Likely, environmental conditions were so harsh that a tipping point in the relationship between climatic variables and demographic rates was crossed (Doak & Morris 2010).

Conclusions

The analyses presented here uncovered different pattern of variation in vital rates in *E. alpinum* following the extreme heatwave of 2003 and the variability in local conditions and management regimes of the different sites where the species is present. A clear result of this study was that

grazing must be discouraged in sites where the conservation of *E. alpinum* is a priority because it reduces recruitment and survival rates under the minimum needed for population viability ($\lambda \geq 1$). Field experiments are needed to clarify the dynamics of recruitment through estimation of germination rates and seedling establishment rates. Mowed and unmanaged sites did not exhibit very different demographic patterns, but some differences may emerge in the future as a result of forest regeneration. The need to document such a long-term dynamics encourages us to continue the efforts of this demographic survey in the future.

The 2003 heatwave was detrimental for population growth, but an increase in occurrence rates of 2003-like events would not impair population persistence by itself. This result was derived on the basis of a long term demographic dataset, which allowed us to compare the reduction in plant vital rates following an extreme summer heatwave with the baseline temporal fluctuations observed on the whole decade. For vital rates that show pronounced variability over years, the reduction due to the 2003 heatwave was not significant (e.g. vegetative and adult flowering rates). The climatic conditions experienced by the plants in the summer 2003 were exceptional when considered from a climatic standpoint only, but flowering rates were not different from those experienced in any other year. This demonstrates the importance of considering long-term data when analysing extreme climatic events, since short-term studies may miss the intrinsic temporal variability of vital rates. In addition, the demographic effect of the heatwave were comparable to those of grazing, the only difference being that grazing exert a constant negative impact because it is performed every year, while heatwaves will hit the population with a much lower frequency. Population persistence is therefore more sensitive to differences in management regimes and loacal conditions than to the occurrence of extreme climatic events.

Literature cited

Araujo, M.B. & Luoto, M. (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography,* **16,** 743-753.

Brys, R., Jacquemyn, H., Endels, P., De Blust, G. & Hermy, M. (2004) The effects of grassland management on plant performance and demography in the perennial herb Primula veris. *Journal of Applied Ecology,* **41,** 1080-1091.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach,* 2nd edn. Springer-Verlag, New York.

Caswell, H. (2001) *Matrix population models : construction, analysis, and interpretation,* 2nd edn. Sinauer Associates, Sunderland, Massachusetts.

Chaves, M.M. (1991) Effects of Water Deficits on Carbon Assimilation. *Journal of Experimental Botany,* **42,** 1-16.

Cherel, O. & Lavagne, A. (1982) Aire de répartition, phénologie, biologie, reproduction d'*Eryngium alpinum*, "la Reine des Alpes", dans la vallée du Fournel. Propositions de mesures de protection de l'espèce. *Travaux Scientifiques du Parc National des Ecrins,* **2,** 53-92.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T. & Valentini, R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature,* **437,** 529-533.

Cooch, E., Rockwell, R.F. & Brault, S. (2001) Retrospective analysis of demographic responses to environmental change: A Lesser Snow Goose example. *Ecological Monographs,* **71,** 377-400.

de Kroon, H., van Groenendael, J. & Ehrlen, J. (2000) Elasticities: A review of methods and model limitations. *Ecology,* **81,** 607-618.

Dekroon, H., Plaisier, A., Vangroenendael, J. & Caswell, H. (1986) Elasticity - the Relative Contribution of Demographic Parameters to Population-Growth Rate. *Ecology,* **67,** 1427-1431.

Doak, D.F. & Morris, W.F. (2010) Demographic compensation and tipping points in climate-induced range shifts. *Nature,* **467,** 959-962.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. (2000) Climate extremes: Observations, modeling, and impacts. *Science,* **289,** 2068-2074.

Epron, D. & Dreyer, E. (1993) Long-Term Effects of Drought on Photosynthesis of Adult Oak Trees [Quercus-Petraea (Matt) Liebl and Quercus-Robur L] in a Natural Stand. *New Phytologist,* **125,** 381-389.

Franco, M. & Silvertown, J. (2004) Comparative demography of plants based upon elasticities of vital rates. *Ecology,* **85,** 531-538.

Gaudeul, M. & Till-Bottraud, I. (2003) Low selfing in a mass-flowering, endangered perennial, Eryngium alpinum L. (Apiaceae). *American Journal of Botany,* **90,** 716-723.

Gaudeul, M. & Till-Bottraud, I. (2004) Reproductive ecology of the endangered alpine species Eryngium alpinum L. (Apiaceae): Phenology, gene dispersal and reproductive success. *Annals of Botany,* **93,** 711-721.

Gaudeul, M. & Till-Bottraud, I. (2008) Genetic structure of the endangered perennial plant Eryngium alpinum (Apiaceae) in an alpine valley. *Biological Journal of the Linnean Society,* **93,** 667-677.

Gillot, P. & Garraud, L. (1995) Eryngium alpinum (L.). *Livre Rouge de la Flore Menacée*, pp. 185. Museum National d'Histoire Naturelle, Conservatoire Botanique National de Porquerolles, Ministère de l'Environnement, Paris, France.

Hagvar, S. & Klanderud, K. (2009) Effect of simulated environmental change on alpine soil arthropods. *Global Change Biology,* **15,** 2972-2980.

Hothorn, T., Bretz, F. & Westfall, P. (2010) multcomp: Simultaneous Inference for General Linear Hypotheses.

Huhta, A.P., Rautio, P., Tuomi, J. & Laine, K. (2001) Restorative mowing on an abandoned semi-natural meadow: Short-term and predicted long-term effects. *Journal of Vegetation Science,* **12,** 677-686.

Jacquemyn, H., Van Mechelen, C., Brys, R. & Honnay, O. (2011) Management effects on the vegetation and soil seed bank of calcareous grasslands: An 11-year experiment. *Biological Conservation,* **144,** 416-422.

Jantunen, J., Saarinen, K., Valtonen, A. & Saarnio, S. (2007) Flowering and seed production success along roads with different mowing regimes. *Applied Vegetation Science,* **10,** 285-292.

Kahmen, S. & Poschlod, P. (2008) Does germination success differ with respect to seed mass and germination season? Experimental testing of plant functional trait responses to grassland management. *Annals of Botany,* **101,** 541-548.

Klanderud, K. (2010) Species recruitment in alpine plant communities: the role of species interactions and productivity. *Journal of Ecology,* **98,** 1128-1133.

Lanta, V., Dolezal, J., Lantova, P., Kelisek, J. & Mudrak, O. (2009) Effects of pasture management and fertilizer regimes on botanical changes in species-rich mountain calcareous grassland in Central Europe. *Grass and Forage Science,* **64,** 443-453.

Le Tertre, A., Lefranc, A., Eilstein, D., Declercq, C., Medina, S., Blanchard, M., Chardon, B., Fabre, P., Filleul, L., Jusot, J.F., Pascal, L., Prouvost, H., Cassadou, S. & Ledrans, M. (2006) Impact of the 2003 heatwave on all-cause mortality in 9 French cities. *Epidemiology,* **17,** 75-79.

Lennartsson, T. & Oostermeijer, J.G.B. (2001) Demographic variation and population viability in Gentianella campestris: effects of grassland management and environmental stochasticity. *Journal of Ecology,* **89,** 451-463.

Levin, L., Caswell, H., Bridges, T., DiBacco, C., Cabrera, D. & Plaia, G. (1996) Demographic responses of estuarine polychaetes to pollutants: Life table response experiments. *Ecological Applications,* **6,** 1295-1313.

Lucas, R.W., Forseth, I.N. & Casper, B.B. (2008) Using rainout shelters to evaluate climate change effects on the demography of Cryptantha flava. *Journal of Ecology,* **96,** 514-522.

Marage, D., Garraud, L. & Rameau, J.C. (2008) The influence of management history on spatial prediction of Eryngium spinalba, an endangered endemic species. *Applied Vegetation Science,* **11,** 139-148.

Marrero-Gomez, M.V., Oostermeijer, J.G.B., Carque-Alamo, E. & Banares-Baudet, A. (2007) Population viability of the narrow endemic Helianthemum juliae (CISTACEAE) in relation to climate variability. *Biological Conservation,* **136,** 552-562.

Maschinski, J., Baggs, J.E., Quintana-Ascencio, P.E. & Menges, E.S. (2006) Using population viability analysis to predict the effects of climate change on the extinction risk of an endangered limestone endemic shrub, Arizona cliffrose. *Conservation Biology,* **20,** 218-228.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yepez, E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* **178,** 719-739.

Meehl, G.A. & Tebaldi, C. (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science,* **305,** 994-997.

Mills, L.S., Doak, D.F. & Wisdom, M.J. (1999) Reliability of conservation actions based on elasticity analysis of matrix models. *Conservation Biology,* **13,** 815-829.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag, New York.

R Development Core Team (2008) R: A language and environment for statistical computing. Vienna, Austria.

Reichstein, M., Ciais, P., Papale, D., Valentini, R., Running, S., Viovy, N., Cramer, W., Granier, A., Ogee, J., Allard, V., Aubinet, M., Bernhofer, C., Buchmann, N., Carrara, A., Grunwald,

T., Heimann, M., Heinesch, B., Knohl, A., Kutsch, W., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Pilegaard, K., Pumpanen, J., Rambal, S., Schaphoff, S., Seufert, G., Soussana, J.F., Sanz, M.J., Vesala, T. & Zhao, M. (2007) Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biology,* **13,** 634-651.

Saccone, P., Delzon, S., Pages, J.P., Brun, J.J. & Michalet, R. (2009) The role of biotic interactions in altering tree seedling responses to an extreme climatic event. *Journal of Vegetation Science,* **20,** 403-414.

Schar, C., Vidale, P.L., Luthi, D., Frei, C., Haberli, C., Liniger, M.A. & Appenzeller, C. (2004) The role of increasing temperature variability in European summer heatwaves. *Nature,* **427,** 332-336.

Schär, C., Vidale, P.L., Luthi, D., Frei, C., Haberli, C., Liniger, M.A. & Appenzeller, C. (2004) The role of increasing temperature variability in European summer heatwaves. *Nature,* **427,** 332-336.

Schulze, E.D. (1986) Carbon-Dioxide and Water-Vapor Exchange in Response to Drought in the Atmosphere and in the Soil. *Annual Review of Plant Physiology and Plant Molecular Biology,* **37,** 247-274.

Silvertown, J., Franco, M., Pisanty, I. & Mendoza, A. (1993) Comparative Plant Demography - Relative Importance of Life-Cycle Components to the Finite Rate of Increase in Woody and Herbaceous Perennials. *Journal of Ecology,* **81,** 465-476.

Stammel, B., Kiehl, K. & Pfadenhauer, J. (2003) Alternative management on fens: Response of vegetation to grazing and mowing. *Applied Vegetation Science,* **6,** 245-254.

The Mathworks, I. (2001) Matlab.

Turner, C.L., Seastedt, T.R. & Dyer, M.I. (1993) Maximization of Aboveground Grassland Production - the Role of Defoliation Frequency, Intensity, and History. *Ecological Applications,* **3,** 175-186.

van der Velde, M., Wriedt, G. & Bouraoui, F. (2010) Estimating irrigation use and effects on maize yield during the 2003 heatwave in France. *Agriculture Ecosystems & Environment,* **135,** 90-97.

Wyse-Jackson, P.S. & Akeroyd, J.R. (1994) *Guidelines to be followed in the design of plant conservation or recovery plans*. Council of Europe (Nature and environment, 68), Strasbourg, France.

Yordanov, I., Velikova, V. & Tsonev, T. (2000) Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica,* **38,** 171-186.

Zaitchik, B.F., Macalady, A.K., Bonneau, L.R. & Smith, R.B. (2006) Europe's 2003 heat wave: A satellite view of impacts and land-atmosphere feedbacks. *International Journal of Climatology,* **26,** 743-769.

680

Table 1. Sensitivities and elasticities of population growth rate λ to stage-specific vital rates (σ_i, survival rate; ϕ_i, flowering rate; f, fecundity) on the overall mean matrix.

Vital rate	σ_S	σ_J	σ_V	σ_R	ϕ_J	ϕ_V	ϕ_R	f
Sensitivity	1.12	0.37	0.38	0.24	0.84	0.15	0.10	0.00
Elasticity	0.09	0.28	0.33	0.21	0.07	0.04	0.03	0.09

Figures

Figure 1. Life-cycle of *Eryngium alpinum*. S, seedlings; J, juveniles; V, vegetative adults; R, reproductive adults; σ_i, survival rate; ϕ_i, flowering rate; f, fecundity rate.

Figure 2. Fecundity rate f (number of seedlings per reproductive adult) in the seven sites averaged over all years. Sites with different letters are significantly different at $P < 0.05$ after Tukey's multiple comparison test. Bars represent standard deviations.

Figure 3. Stage-specific survival rates σ_i for the nine years of study in the seven sites. Bars represent standard deviations.

Figure 4. Stage-specific flowering rates ϕ_i for the nine years of study in the seven sites. Bars represent standard deviations.

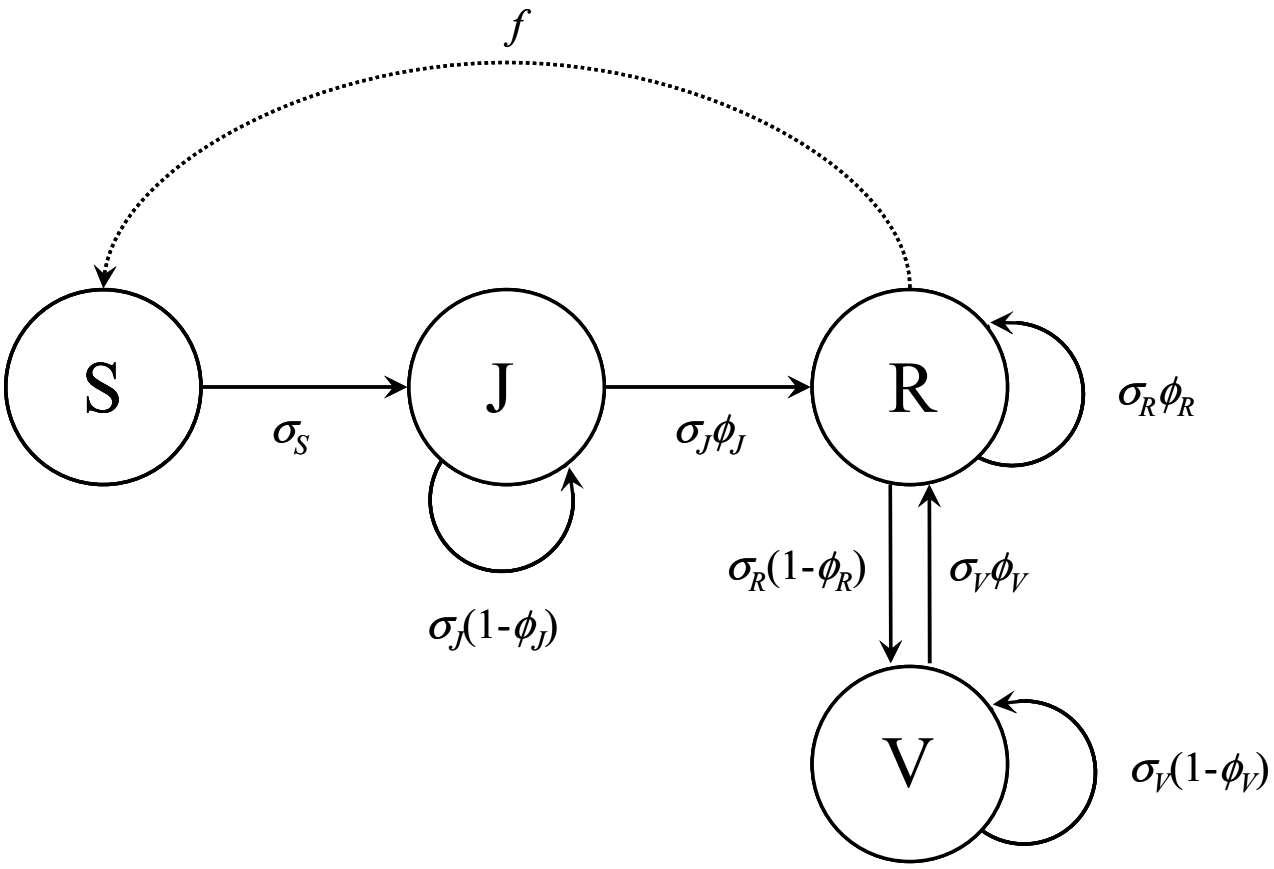
Figure 5. Asymptotic rate of population increase λ for each site and year of study. Bars represent 95% confidence intervals calculated through a bootstrap procedure.

Figure 6. Contributions of the different vital rates to differences in λ between sites. The mean λ for each site and the difference $\hat{a}$ between the site mean and the overall mean are indicated.

Figure 7. Contributions of the different vital rates to differences in λ between years. The mean λ for each year and the difference $\hat{b}$ between the annual mean and the overall mean are indicated.

Figure 8. Stochastic growth rate $r = \log\lambda$ for each site under several simulated probabilities of drought occurrence.

Figure 1


f
S
J
R
V
σ_S
$\sigma_J\phi_J$
$\sigma_R\phi_R$
$\sigma_J(1-\phi_J)$
$\sigma_R(1-\phi_R)$
$\sigma_V\phi_V$
$\sigma_V(1-\phi_V)$

705

Figure 2

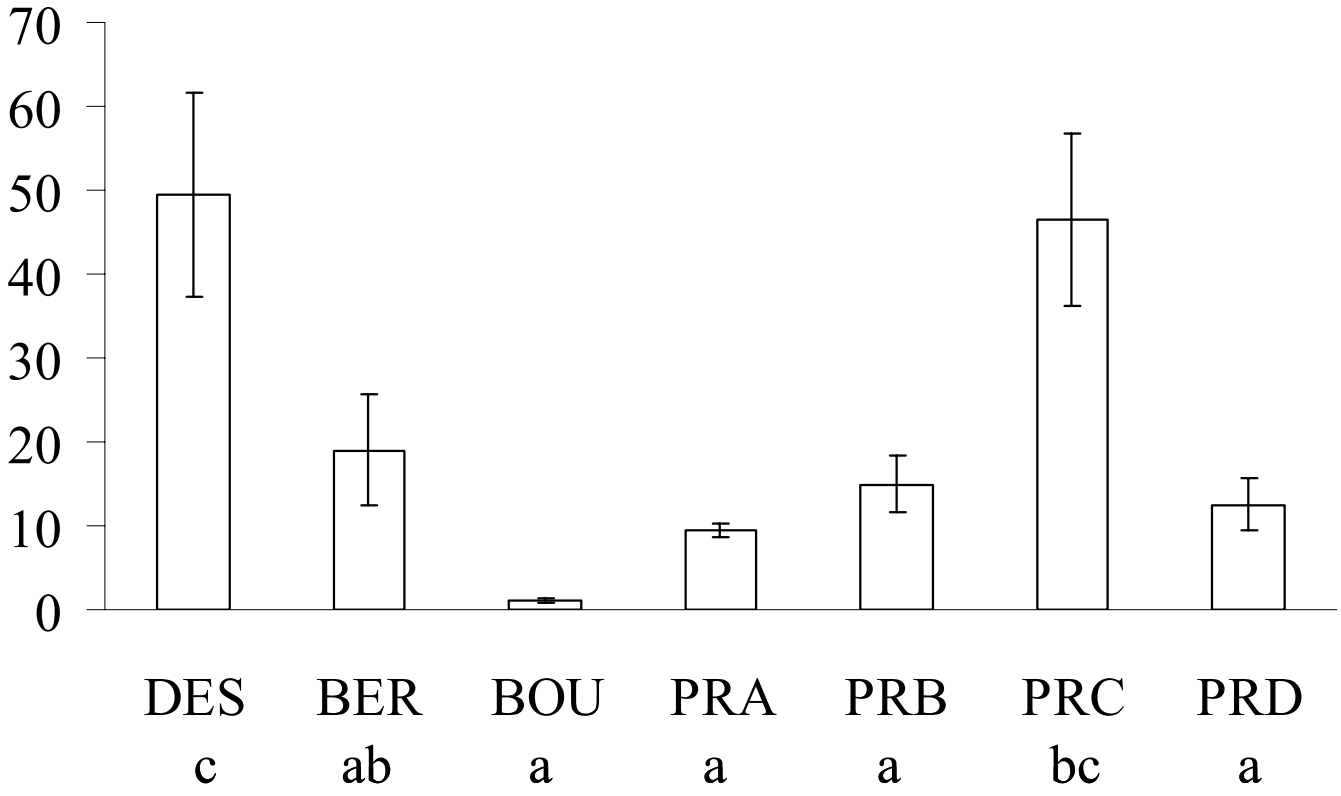
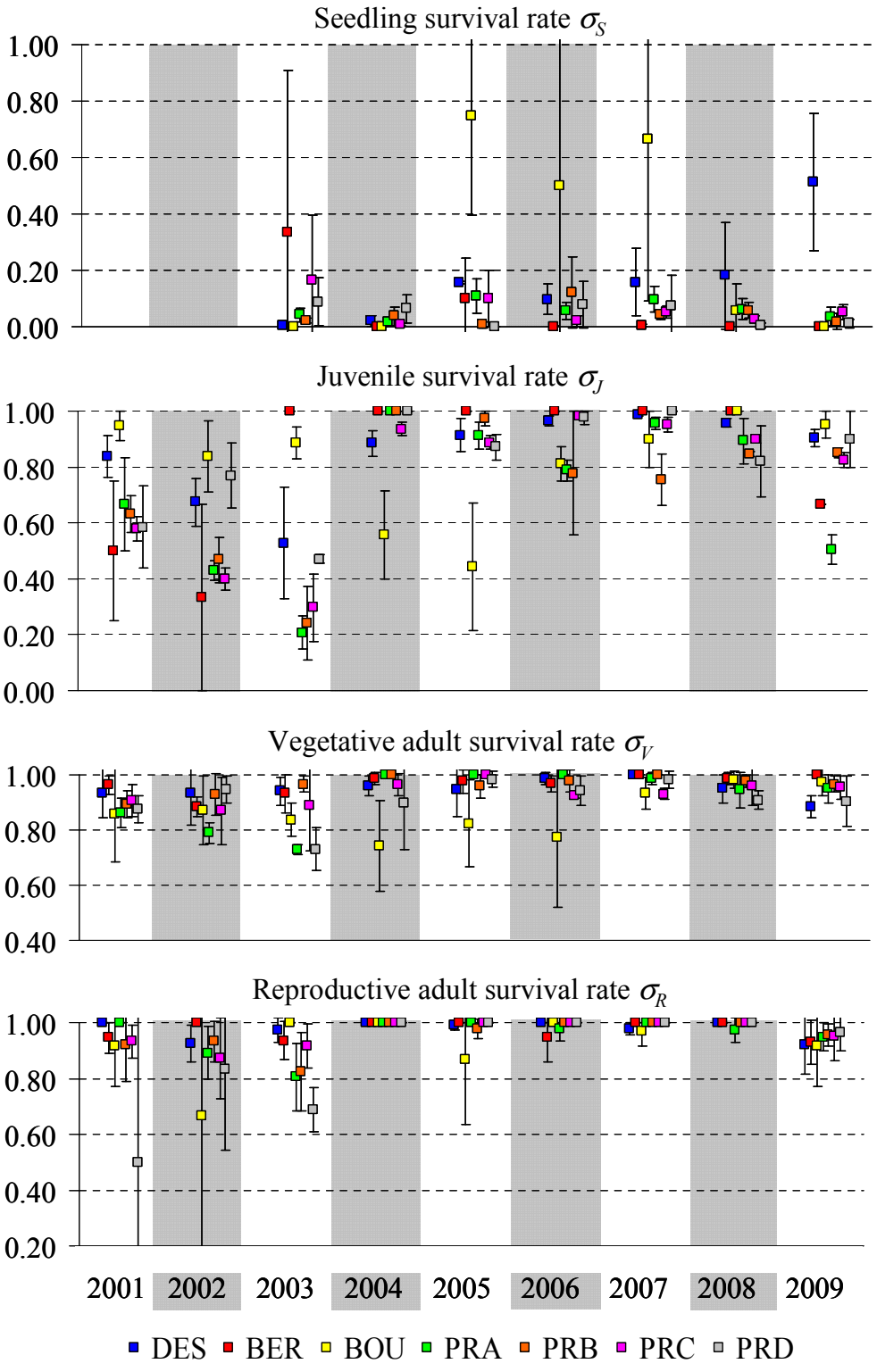


Figure 3


Seedling survival rate σ_S
1.00
0.80
0.60
0.40
0.20
0.00
Juvenile survival rate σ_J
1.00
0.80
0.60
0.40
0.20
0.00
Vegetative adult survival rate σ_V
1.00
0.80
0.60
0.40
Reproductive adult survival rate σ_R
1.00
0.80
0.60
0.40
0.20
2001
2002
2003
2004
2005
2006
2007
2008
2009
DES
BER
BOU
PRA
PRB
PRC
PRD

710

Figure 4

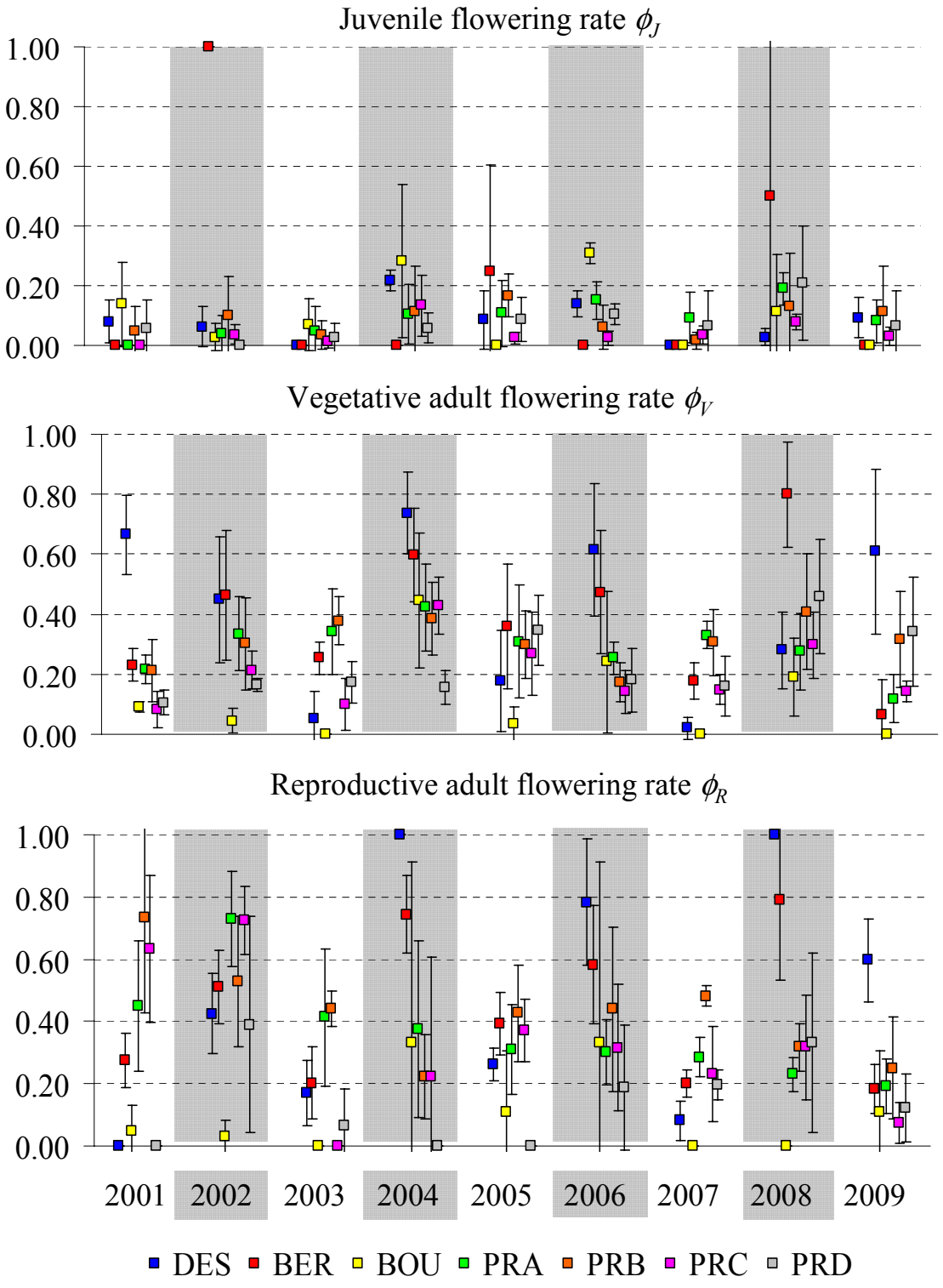

Juvenile flowering rate ϕ_J
1.00
0.80
0.60
0.40
0.20
0.00
Vegetative adult flowering rate ϕ_V
1.00
0.80
0.60
0.40
0.20
0.00
Reproductive adult flowering rate ϕ_R
1.00
0.80
0.60
0.40
0.20
0.00
2001
2002
2003
2004
2005
2006
2007
2008
2009
DES
BER
BOU
PRA
PRB
PRC
PRD

Figure 5

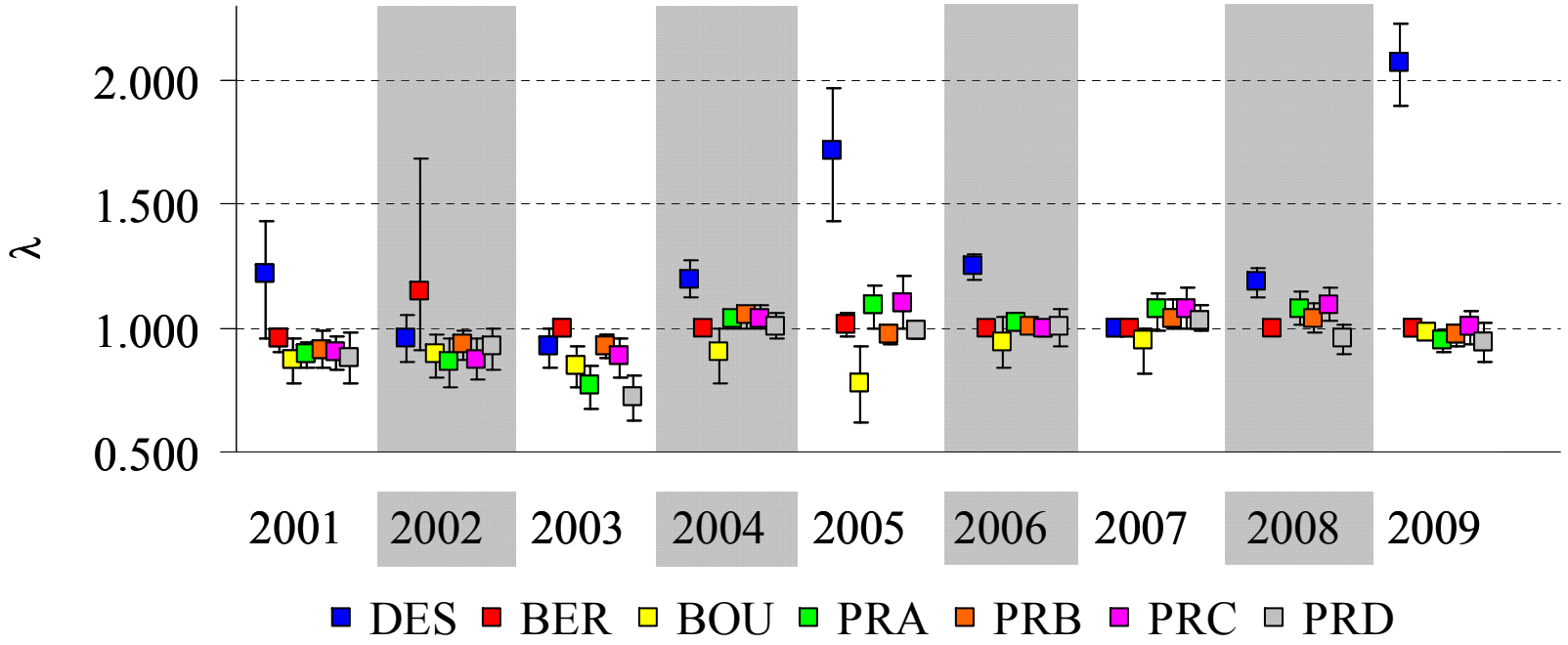

λ
2.000
1.500
1.000
0.500
2001
2002
2003
2004
2005
2006
2007
2008
2009
DES
BER
BOU
PRA
PRB
PRC
PRD

Figure 6

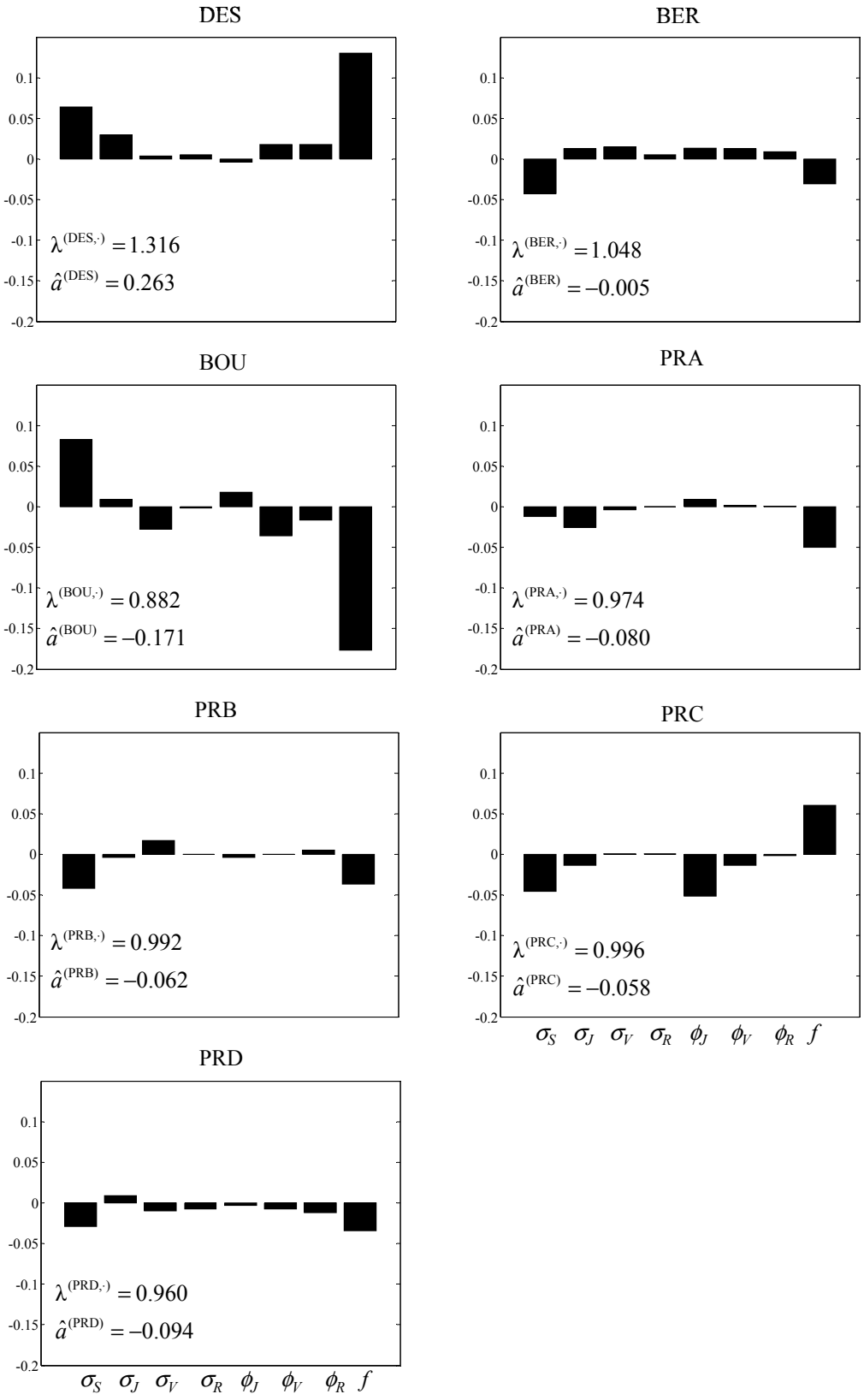

DES
$\lambda^{(DES,\cdot)} = 1.316$
$\hat{a}^{(DES)} = 0.263$
BER
$\lambda^{(BER,\cdot)} = 1.048$
$\hat{a}^{(BER)} = -0.005$
BOU
$\lambda^{(BOU,\cdot)} = 0.882$
$\hat{a}^{(BOU)} = -0.171$
PRA
$\lambda^{(PRA,\cdot)} = 0.974$
$\hat{a}^{(PRA)} = -0.080$
PRB
$\lambda^{(PRB,\cdot)} = 0.992$
$\hat{a}^{(PRB)} = -0.062$
PRC
$\lambda^{(PRC,\cdot)} = 0.996$
$\hat{a}^{(PRC)} = -0.058$
PRD
$\lambda^{(PRD,\cdot)} = 0.960$
$\hat{a}^{(PRD)} = -0.094$
σ_S σ_J σ_V σ_R ϕ_J ϕ_V ϕ_R f

Figure 7

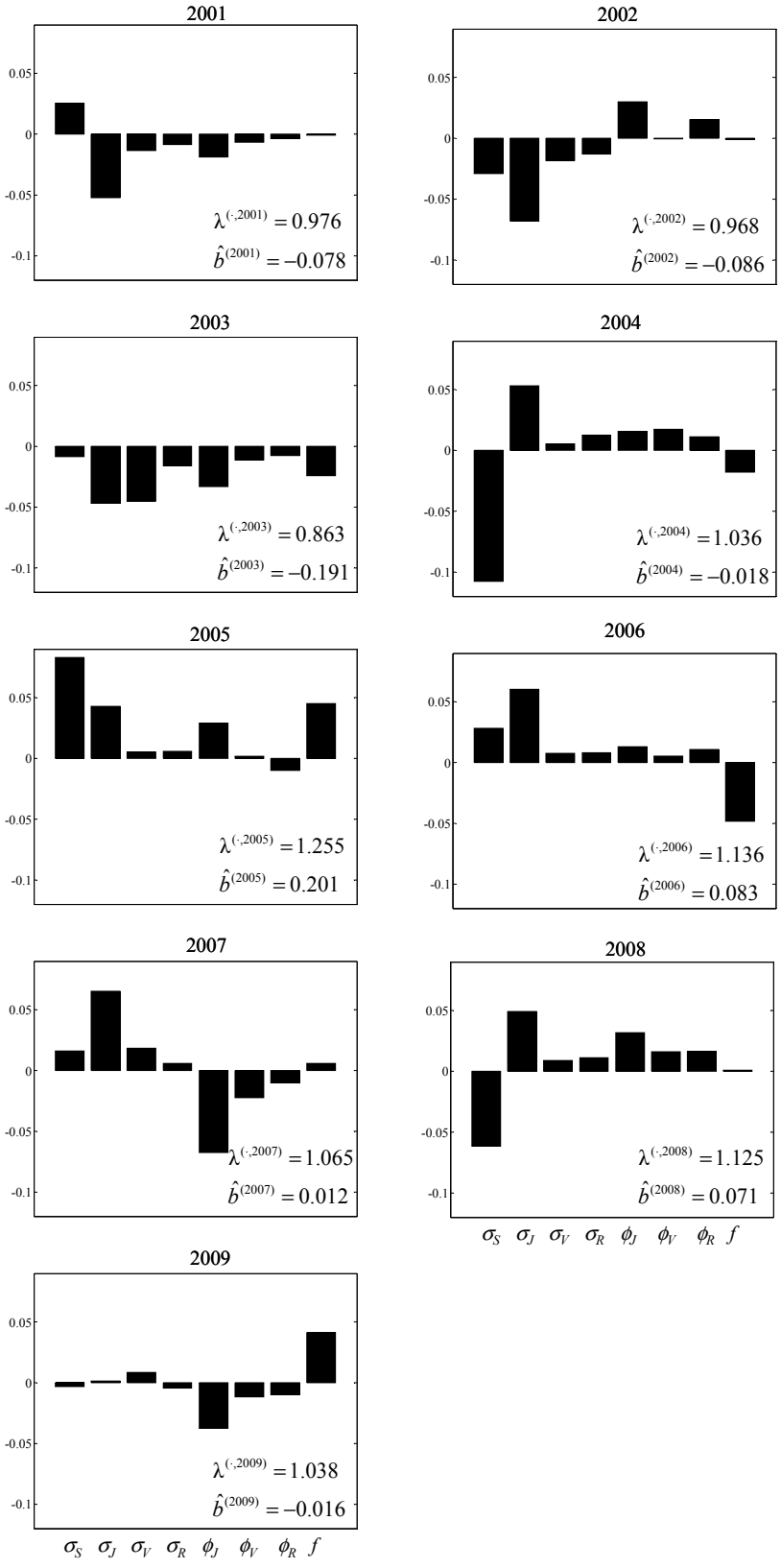
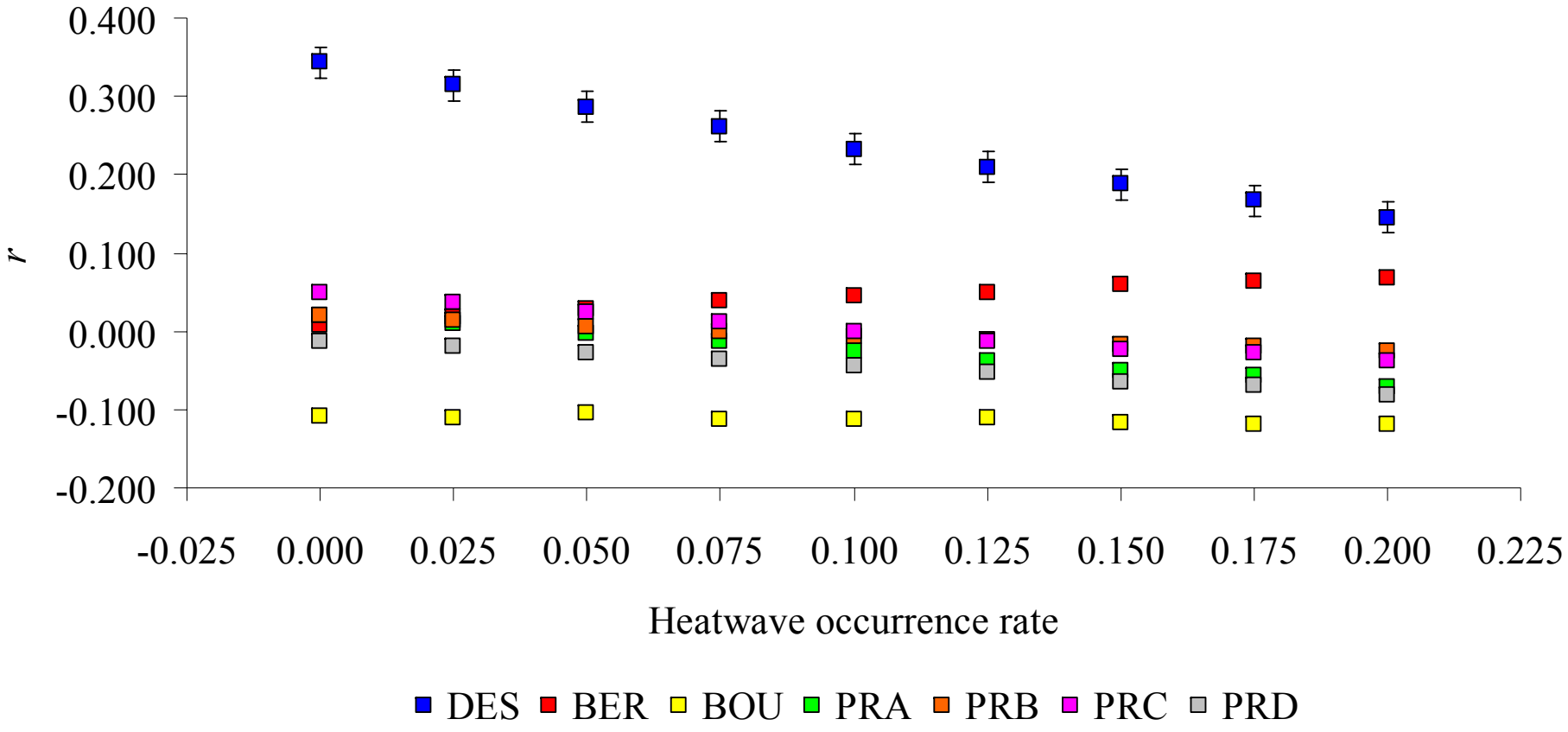

2001
$\lambda^{(\cdot,2001)} = 0.976$
$\hat{b}^{(2001)} = -0.078$
2002
$\lambda^{(\cdot,2002)} = 0.968$
$\hat{b}^{(2002)} = -0.086$
2003
$\lambda^{(\cdot,2003)} = 0.863$
$\hat{b}^{(2003)} = -0.191$
2004
$\lambda^{(\cdot,2004)} = 1.036$
$\hat{b}^{(2004)} = -0.018$
2005
$\lambda^{(\cdot,2005)} = 1.255$
$\hat{b}^{(2005)} = 0.201$
2006
$\lambda^{(\cdot,2006)} = 1.136$
$\hat{b}^{(2006)} = 0.083$
2007
$\lambda^{(\cdot,2007)} = 1.065$
$\hat{b}^{(2007)} = 0.012$
2008
$\lambda^{(\cdot,2008)} = 1.125$
$\hat{b}^{(2008)} = 0.071$
2009
$\lambda^{(\cdot,2009)} = 1.038$
$\hat{b}^{(2009)} = -0.016$
0.05
0
-0.05
-0.1
σ_S σ_J σ_V σ_R ϕ_J ϕ_V ϕ_R f

Figure 8


0.400
0.300
0.200
0.100
0.000
-0.100
-0.200
r
-0.025
0.000
0.025
0.050
0.075
0.100
0.125
0.150
0.175
0.200
0.225
Heatwave occurrence rate
DES
BER
BOU
PRA
PRB
PRC
PRD

720