

**Variation of life history characteristics, long-term
population dynamics and genetic differentiation of
Himantoglossum hircinum (Orchidaceae)**

—

**Implications for conservation and
general questions in plant ecology**

**Dissertation
zur Erlangung des akademischen Grades doctor rerum naturalium
(Dr. rer. nat.)**

**vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät
der Friedrich-Schiller- Universität Jena**

von Diplom-Biologin Marion Pfeifer

geboren am 18.12.1976 in Weimar

Jena, den 31.12.2004

Gutachter:

1.:

2.:

3.:

Tag der Doktorprüfung:

Tag der öffentlichen Verteidigung:

THE BLIND MEN AND THE ELEPHANT

1

It was six men of Indostan
To learning much inclined,
Who went to see the Elephant
(Though all of them were blind),
That each by observation
Might satisfy his mind.

2

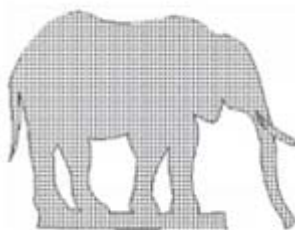
The First approached the Elephant,
And happening to fall
Against his broad and sturdy side,
At once began to bawl:
"God bless me! but the Elephant
Is very like a wall!"

3

The Second, feeling of the tusk,
Cried, "Ho! what have we here?
So very round and smooth and sharp?
To me 'tis mighty clear
This wonder of an Elephant
Is very like a spear!"

4

The Third approached the animal,
And happening to take
The squirming trunk within his hands,
Thus boldly up and spake:
"I see," quoth he, "the Elephant
Is very like a snake!"



5

The Fourth reached out an eager hand,
And felt about the knee.
"What most this wondrous beast is like
Is mighty plain," quoth he;
"'Tis clear enough the Elephant
Is very like a tree!"

6

The Fifth who chanced to touch the ear,
Said: "E'en the blindest man
Can tell what this resembles most;
Deny the fact who can,
This marvel of an Elephant
Is very like a fan!"

7

The Sixth no sooner had begun
About the beast to grope,
Than, seizing on the swinging tail
That fell within his scope,
"I see," quoth he, "the Elephant
Is very like a rope!"

8

And so these men of Indostan
Disputed loud and long,
Each in his own opinion
Exceeding stiff and strong,
Though each was partly in the right
And all were in the wrong!

John Godfrey Saxe (after an ancient Indian tale)

Contents

List of tables	iv
List of figures	iv
List of equations	v
1 Introduction	1
2 Methods	3
2.1 Study site	3
2.2 Study species	4
2.3 Data set	7
2.3.1 Long-term data set	7
2.3.2 Data recorded between October 2002 and August 2004	7
2.4 Analysis of population dynamics	7
2.4.1 Multiple linear regression analysis	7
2.4.2 Transition matrix analysis	9
2.5 AFLP - molecular genetic approach in plant ecology	10
2.5.1 Methods to analyse genetic diversity	10
2.5.2 AFLP	10
2.6 Spatial pattern analysis in plant ecology	11
3 High variability of life history characteristics and life state transitions of an orchid over 26 years	14
3.1 Abstract	14
3.2 Introduction	15
3.3 Methods	16
3.3.1 Population structure	16
3.3.2 State variables and matrix analysis	16
3.3.3 Abiotic control of population characteristics	18
3.4 Results	18
3.4.1 Population growth and structure	18
3.4.2 Survivorship curves	20
3.4.3 Life span and dormancy/flowering pattern	21
3.4.4 Transition probabilities and matrix analysis	23
3.4.5 Abiotic control of population trends	24
3.5 Discussion	25
3.5.1 Demographic analysis	25
3.5.2 Plasticity of transition probabilities and matrix analysis	27
3.5.3 Abiotic control of population trends	27

3.6 Conclusions	28
4 Climate, size, and flowering history determine flowering pattern of <i>Himantoglossum hircinum</i> (L.)	29
4.1 Abstract	29
4.2 Introduction	30
4.3 Methods	31
4.3.1 Population growth and structure	31
4.3.2 Biotic and abiotic influencing factors	32
4.4 Results	33
4.4.1 Growth and flowering pattern of the population	33
4.4.2 Flowering pattern and plant age	35
4.4.3 Flowering pattern and plant size	35
4.4.4 Variability of flowering pattern between years	36
4.4.5 Influence of weather on flowering pattern of the population	37
4.4.6 Reproductive effort	40
4.5 Discussion	40
4.5.1 Flowering pattern of the individuals and the population	40
4.5.2 Flowering pattern and plant age	41
4.5.3 Flowering pattern and plant size	42
4.5.4 Factors controlling flowering pattern	42
4.6 Conclusions	45
5 Influence of geographical isolation on genetic diversity of <i>Himantoglossum hircinum</i> (Orchidaceae)	46
5.1 Abstract	46
5.2 Introduction	47
5.3 Material and methods	48
5.3.1 Data sampling	48
5.3.2 DNA extraction and AFLP protocol	49
5.3.3 Data analysis	50
5.4 Results	51
5.4.1 Genetic diversity within populations	51
5.4.2 Analysis of gene flow and population differentiation	52
5.4.3 Genetic distances between the populations	52
5.5 Discussion	55
5.5.1 Within population genetic diversity	55
5.5.2 Population differentiation and interconnectedness	56
5.5.3 Genetic relationships between the populations	57
5.5.4 Implications for conservation	57

6 Spatial pattern and dynamics of <i>Himantoglossum hircinum</i>	59
6.1 Abstract	59
6.2 Introduction	60
6.3 Methods	61
6.3.1 Spatial autocorrelation analysis	61
6.3.2 Spatial variability of individual and population performance	64
6.3.3 Intra- and interspecific relationships: point pattern analysis	64
6.4 Results	65
6.4.1 Spatial autocorrelation analysis	65
6.4.2 Spatial variability of population characteristics	65
6.4.3 Spatial variability of individual performance	67
6.4.4 Spatial point pattern analysis	69
6.5 Discussion	72
6.5.1 Spatial spread	72
6.5.2 Spatial variability of performance	72
6.5.3 Intra- and interspecific relationships	73
7 Conclusions and directions for future research	75
7.1 Long-term studies and trends in orchid ecology	75
7.2 Variability of individual characteristics and population dynamics	76
7.3 Conservation issues	76
7.4 Directions for future research	77
Summary	79
Zusammenfassung	81
References	84
Appendices	A1-A3
Acknowledgements	

List of tables

Table 3.1. Cohort half-life values for the <i>H. hircinum</i> cohorts on the study site.	20
Table 3.2. Growth pattern of flowering and non-flowering <i>H. hircinum</i> plants.	21
Table 3.3. Life span of flowering and dormant <i>H. hircinum</i> plants.	22
Table 3.4. Mean transition probabilities between size-based life states of <i>H. hircinum</i> on the study site, calculated for two periods.	23
Table 3.5. Elasticity matrices based on transition matrices for the two periods, 1979 to 1990 and 1990 to 1999.	24
Table 4.1. Frequency of flowering of <i>H. hircinum</i> plants on the study site.	34
Table 4.2. Contribution of quarterly weather variables to the eigenvectors from the PCA.....	38
Table 4.3. Models of flowering probability from the multiple linear regression analyses.	39
Table 5.1. Genetic diversity of three <i>H. hircinum</i> populations.	51
Table 5.2. Pairwise genetic differences and gene flow between three <i>H. hircinum</i> populations....	52
Table 5.3. Pairwise genetic distances based on Nei's unbiased genetic distance (1978).	53
Table 6.1. Characterization of the spatial plots studied.	65
Table 6.2. Influence of habitat characteristics on individual performance.	68
Table 6.3. Size of reproductive structures and fruit set of <i>H. hircinum</i> plants depending on habitat structure.	68
Appendix 1. Air temperature and precipitation data of the University of Jena weather station	A1
Appendix 2. Mean number of days with soil temperature below 0°C (SFD) and mean minimum temperature (Tmin) of the University of Jena weather station	A2
Appendix 3. Number of leaves and size of reproductive structures of <i>H. hircinum</i>	A3

List of figures

Fig. 2.1. Location of the permanent monitoring site.	3
Fig. 2.2. Photography of the permanent monitoring site.	4
Fig. 2.3. European distribution of <i>H. hircinum</i> (L.) (after Meusel et al., 1965).	5
Fig. 2.4. The phases of growth of <i>Himantoglossum hircinum</i>	6
Fig. 2.5. Photographs of the sterile and flowering state of <i>H. hircinum</i>6
Fig. 2.6. Univariate second-order spatial statistics (pair-correlation function).	13
Fig. 3.1. Life-cycle graphs and projection matrix for <i>H. hircinum</i>	17
Fig. 3.2. Population turnover of <i>H. hircinum</i> between 1979 ad 1999.	19
Fig. 3.3. Size class distribution of the population between 1979 and 1999.	19
Fig. 3.4. Life span of flowering <i>H. hircinum</i> plants.	23
Fig 4.1. Population growth and number of individuals flowering each year (both on a log10-scale) 1976 and 2001 on the study site.	33
Fig. 4.2. Proportion of emerged individuals flowering and of emerged individuals with 8 - 15 leaves as well as 5 - 7 leaves.	34
Fig. 4.3. Distribution of age of flowering <i>H. hircinum</i> plants between 1979 and 2001.....	35

Fig. 4.4. Distribution of size of flowering <i>H. hircinum</i> plants between 1977 and 2001.	36
Fig. 4.5. Relationship between proportion of individuals flowering and proportion of individuals with 8 - 15 leaves between 1977 and 2001.	37
Fig. 4.6. Reproductive effort of <i>H. hircinum</i> on the study site expressed as decreased size after flowering.	41
Fig. 4.7. Scheme of potential factors influencing flowering frequency of <i>the H hircinum</i> population in each plant year.	43
Fig. 5.1. Topographical map of the area south of Jena showing the distances between the three populations sampled to study genetic diversity.	49
Fig. 5.2. UPGMA dendrogram of the populations based on Nei's (1978) genetic distance.	53
Fig. 5.3. UPGMA dendrogram based on Jaccard's coefficient of similarity for pairwise comparisons of all <i>H. hircinum</i> individuals sampled.	54
Fig. 5.4. PCoA ordination of the genetic distances between the <i>H. hircinum</i> individuals sampled.	55
Fig. 6.1. Neighbourhood relations in a spatial autocorrelation analysis in a grid.	61
Fig. 6.2. Composition of the permanent monitoring site in the nature reserve "Leutratal" and localization of the spatial plots.	63
Fig. 6.3. Trend of spatial autocorrelation between the squares regarding number of <i>H. hircinum</i> individuals between 1977 and 2001.	66
Fig. 6.4. Classified growth rates regarding number of <i>H. hircinum</i> individuals in patches within the study site.	67
Fig. 6.5. Means and standard errors of length growth rate and maximum number of leaves of <i>H. hircinum</i> plants depending on habitat structure.	69
Fig. 6.6. Univariate point pattern (Pair Correlation Function) of <i>H. hircinum</i> individuals.	70
Fig. 6.7. Bivariate point pattern (Mark Correlation Function) to test for spatial relationships between <i>H. hircinum</i> plants and individuals of shrub species.	71
Fig. 6.8. Bivariate point pattern (Mark Correlation Function) to test for spatial relationships between <i>H. hircinum</i> plants and individuals of <i>P. veris</i> and <i>P. media</i>	71

List of equations

Eq. 2.1. Linear regression equation.	8
Eq. 4.1. Exponential growth model of the <i>H. hircinum</i> population.	31
Eq. 4.2. Logistic regression model to explain size-dependent probability of flowering of <i>H. hircinum</i> on the study site.	36
Eq. 6.1. Definition of Moran's I to calculate spatial autocorrelation.	62
Eq. 6.2. Definition of Geary's C to calculate spatial autocorrelation.	62

1. Introduction

The causes of similarities in the set of physiological, morphological and life history traits of unrelated species in widely separated but physically similar habitats worldwide is a central question facing ecologists (Mooney, 1977; Cody and Mooney, 1978; Orians and Paine, 1983). We seek to understand how populations are structured and how they change from an ecological and evolutionary perspective (Silvertown and Charlesworth, 2001). Populations have several kinds of structure including an age structure (proportion of young and old individuals), a size structure (relative number of large and small individuals), a genetic structure (patchiness of gene frequency and genotypes), and a spatial structure (variation in individual performance and dynamics within a habitat). Population biology attempts to explain the origin of these different kinds of structure, to understand how they influence each other, and how they change with time (Silvertown and Charlesworth, 2001).

Pattern in plant ecology is caused by the constraints of the abiotic and biotic environment (Tilman, 1988). Plants are ideal model organisms in which to study fundamental ecological and evolutionary principles, since they cannot escape from unfavourable conditions. The questions arise whether similar patterns on the species and on the community level are influenced by a few general underlying processes or whether these patterns are unrelated, each requiring a unique explanation. It is unclear, whether there is a dominant intrinsic mechanism causing variable patterns in the life history of a plant species, or whether species mainly respond to environmental factors including varying weather conditions, presence of competing species, natural disturbances and human influence.

Long-term ecological research is one of the essential approaches for developing an ecological understanding, which is necessary for the detection of pattern, to determine which factors control and regulate patterns in space and time, and for predicting the effects of human activities on ecological processes (Likens, 1989). In plant ecology, long-term studies generally have been focussed on rare and conspicuous species, predominantly orchids. They predominantly considered some aspects of the life of the plant individuals and populations, this mostly on a small spatial scale.

Molecular methods have rarely been applied in studies of orchid conservation (Scacchi et al., 1990; Scacchi et al., 1991; Case, 1994; Arduino et al., 1996; Sun, 1996; Aargaard, 1999; Gustafsson, 2002; Sharma et al., 2003) but have become increasingly popular among plant ecologists to study phylogenetic relationships and genetic variation (Cozzolino et al., 2003), and to analyse spatial genetic structure (Brzosko et al., 2002; Chung et al., 2004). Fragmentation and isolation can cause genetic erosion and more information is needed about the long-term effects of habitat destruction on species viability. The extent of gene flow between fragmented populations, the genetic differentiation of isolated populations and the maintenance of genetic diversity within the species are important issues that need to be investigated in detail.

This dissertation is a complex approach to explain the dynamics of a plant population in the broader context of its environment. Long-term population dynamics and life history characteristics of a population of *Himantoglossum hircinum* are described and the influence

of potential controlling factors (weather, habitat structure, life cycle) on these dynamics is quantified. I used a long-term data set on the life history of more than 13000 individuals, which was recorded on a large spatial scale (1.9 ha) and over 26 years (1976 - 2001) by Dr. W. Heinrich (Heinrich, 1994; Heinrich, 2000; Heinrich, 2001; Heinrich and Voelckel, 1999), and a data set on climatic conditions provided by the weather station of Jena. I recorded further data including data on fruit set, length growth during two growing seasons (2002-2004), and spatial patterns on a local scale. I used AFLP markers to study the genetic diversity of three isolated *H. hircinum* populations growing within a maximum distance of 10 km. My dissertation will be the first to combine long-term temporal and spatial data on growth, life span, and flowering of more than 13000 plant individuals with data on climate and on genetic diversity.

This study addresses several issues thought to be important in regulating dynamics of a plant species.

A) Which factors cause the observed annual variation of characteristics of the individuals / population?

I hypothesize that individual performance and population characteristics are greatly determined by external abiotic factors, i.e. varying weather conditions over the years. There is also a lesser influence of biotic components including human activity, herbivory and other factors. In general, intrinsic constraints of population dynamics, especially life state transitions and flowering pattern, include density dependence and reproductive effort.

B) Does habitat fragmentation have an influence on genetic diversity and thus on long-term viability of *H. hircinum* populations?

Based on knowledge about orchid reproduction I hypothesize that there is high gene flow in form of long-distance seed or animal-propagated pollen dispersal between the populations resulting in low population differentiation levels on the short time-scale.

C) What factors are responsible for the current pattern of distribution?

I hypothesize that short-distance seed dispersal is responsible for the clumped pattern of distribution and the slow spatial spread of the species on a local scale. Long-distance dispersal events are important for the random distribution of populations on the regional scale.

2. Methods

2.1 Study site

H. hircinum was studied in the nature reserve “Leutratal” near Jena (Germany, Thuringia, 50°56'46” N, 11°35'35” E), which is part of a landscape characterized by calcareous grasslands along the slopes formed by the Saale river (Fig. 2.1). The landscape is of high ecological value since a variety of vegetation types occur on a rather small scale due to different geological and topographical conditions. Plant species diversity is high in the nature reserve and includes many protected species, among them about 30 orchid species.

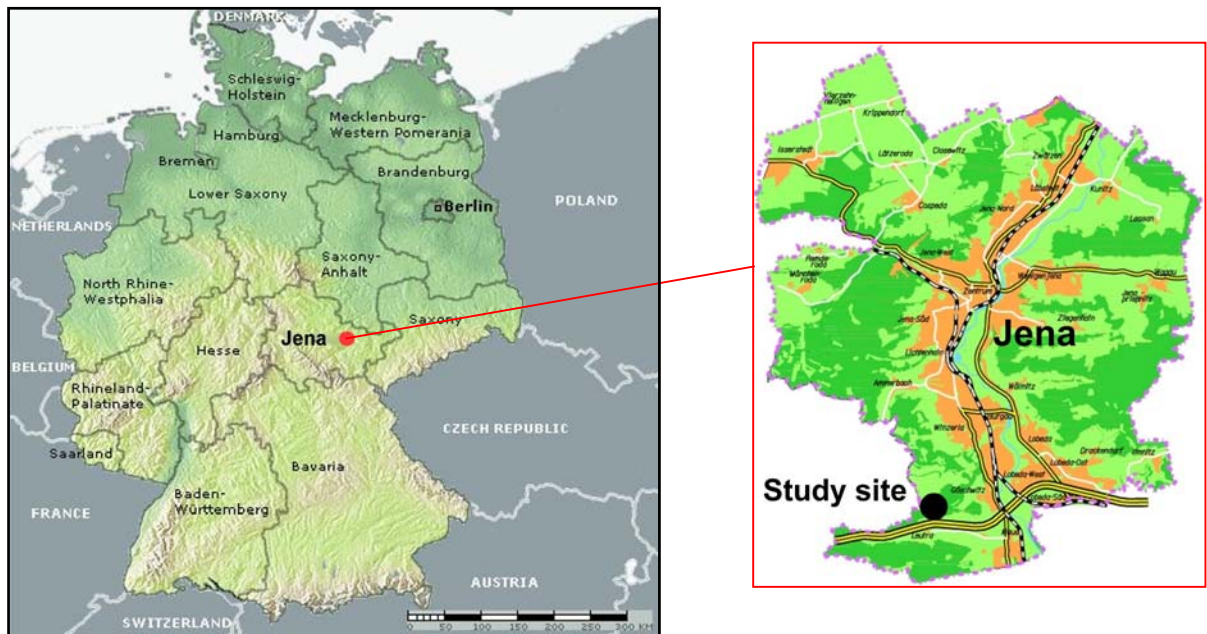


Fig. 2.1. Location of the permanent monitoring site in the nature reserve Leutratal near Jena (Germany, Thuringia, 50°56'46” N, 11°35'35” E). The study site was located in a westwards facing valley south of the town.

The vegetation in the nature reserve is characterized mainly by species-rich and diverse chalk grassland and is a result of long-time human use in the past including vine growing, agriculture, foresting and mowing. The long-term monitoring site (so-called “Katena”) was laid out by the Institute of Ecology of the Jena University in 1976. The analysis of responses of selected plant species to successional change in vegetation cover was the main target of the project (Heinrich et al., 1999). The site covers an area of 1.9ha along a south-facing slope dominated by grassland communities. The site has remained unmanaged since 1976, whereas the surrounding grassland has been mown annually (Fig. 2.2).



Fig. 2.2. Photography of the permanent monitoring site in the nature reserve Leutratal taken in January, 2003. The unmanaged monitoring site with its tall grass cover is distinctly different from the neighbouring areas that are mown annually. Scrub encroachment on the plot due to succession is obvious.

The climate is characterized by mild winters with few periods of frost and a snow cover being absent most of the time (Kluge and Müller-Westermeier, 2000). Average annual temperature between 1961 and 1990 was 9.3°C, and average yearly rainfall over these 30 years was 587mm (based on data from the university of Jena weather station). The precipitation maximum in the summer months is typical for the continental character of the regional climate (Heyer, 1993). Temperature as well as precipitation increased in the last decade, accompanied by fewer frosts and lower snow. The seasonal patterns of temperature and precipitation for the period 1961 - 1990 and 1991 - 2000 are outlined in Appendix 1, which also gives monthly values for the period of my field studies between October 2002 and July 2004.

2.2 Study species

The main area of distribution of *H. hircinum* (lizard orchid) is Southern Europe under Mediterranean conditions (Fig. 2.3). The species is likely to be limited to the North by low temperatures in both winter and summer (Carey and Farrell, 2002). In Germany, *H. hircinum* is believed to reach its northeastern limit of range. While the species is still rare, it spreads gradually in Germany (see Heinrich and Voelckel, 1999). The increase in its range to the north and to the northeast can probably be attributed to a change in climate due to global warming (Good, 1936; Carey, 1998)

The species predominantly grows on south-facing calcareous dry grasslands, or on sand and gravel, but it was also found growing on man-made habitats (Carey and Farrell, 2002). *H. hircinum* is listed in the Red Data Book (Jedicke, 1997), although new populations have been arising in Germany and UK in recent years (Carey, 1999; Heinrich and Voelckel, 1999; Carey et al., 2002).

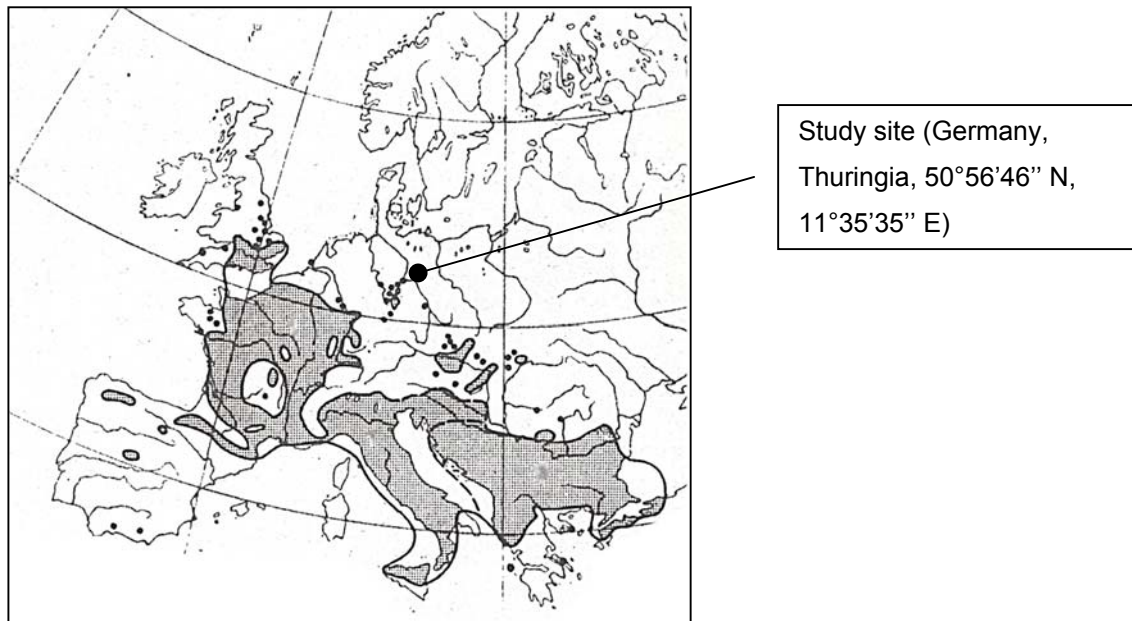


Fig. 2.3. European distribution of *H. hircinum* (L.) Sprengel (after Meusel et al., 1965). The study site lies in central Germany, where the species reaches its northeastern limit of distribution.

H. hircinum is a rare but conspicuous, long-lived terrestrial orchid species. The glabrous herb survives from one year to the next via a tuber. The growing season is from September to August and plants emerge with the autumn rains from late August onwards (Fig. 2.4). Most plants are apparent by November (Carey, 1999). Plants that appear above ground early in the season grow faster and for longer and achieve a larger size.

The leaves of vegetative plants blacken or brown and disappear from March onwards until July depending on plant size, temperature and rainfall. Mature plants flower from late May to late June / early July. Inflorescences of individuals can grow up to 90cm high (Fig. 2.5). After pollination, seeds take at least four weeks to ripen, with the first fruits forming in July. The capsule contains up to 2000 tiny seeds that are assumed to be capable of flying long distances (Carey, 1999).

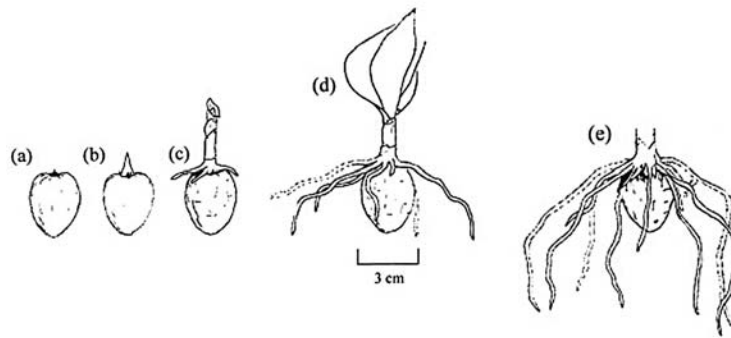


Fig. 2.4. The phases of growth of *Himantoglossum hircinum* drawn from a single plant by Richard Ward. (a) June 1983; (b) August 1983; (c) September 1983; (d) October 1983; (e) November 1983 (from Carey and Farrell, 2002).

However, Carey (1999) also reports that a large number of seeds usually remains lodged in the capsules and are dropped near the parent plant. Recruitment is mainly by seeds, and it was suggested that plants spend three to five years after germination as a protocorm stage until finally emerging above ground with green leaves.



Fig. 2.5. Pictures of the sterile and flowering state of *H. hircinum* taken in January and July 2003.

Geographical distribution, habitats, morphology and physiology of the species were described in detail by Carey and Farrell (2002).

2.3 Data set

2.3.1 Long-term data set

Between 1976 and 2001 all individuals of *H. hircinum* found at the monitoring site were labelled with wooden sticks (Heinrich, 1994; Heinrich, 2000; Heinrich et al., 1999). The 12-month period from September of the previous calendar year to August of the year of recording is referred to as a 'plant year' (autumn: September to November, summer: June to August).

In early April of each year, the site was carefully searched for *H. hircinum* individuals, even in dense grass and under shrub cover, and the number of leaves of each individual was counted. Usually all sticks of the previous year were found. The number of sticks with "missing" plants was counted. After two years absence from above ground ("dormancy"), plants were regarded as dead at the beginning of the disappearance. In May or June, depending on weather conditions and the developmental state of the plants, all flowering individuals were recorded, and their leaf number was counted again. This was done, because it was observed that flowering individuals could develop one or two additional leaves between April and June, whereas non-flowering individuals generally did not so (Heinrich, 1994, 2000).

Daily climate data were available from the nearest weather station at Jena (6 km north of the population). We used monthly, quarterly, and annual variables of mean temperature, precipitation, number of days with soil temperature below 0°C (soil frost days), and number of days with air temperature below 0°C for the plant year t , and the previous plant year $t - 1$ (see page 6).

2.3.2 Data recorded between October 2002 and August 2004

In April 2004 I randomly selected *H. hircinum* individuals in the nature reserve. I measured length and width of each leaf, and estimated the total leaf area, assuming a triangular shape ($1/2$ length *width). Presumably the number of leaves and total leaf area are positively correlated, the number of leaves can be taken as a measure of size in the regression analyses (Chapter IV).

Length growth of randomly selected individuals on the permanent monitoring site and on the managed area was measured fortnightly or monthly in the two plant years as long as climatic conditions allowed (Chapter IV). Additional plots were created to study spatial patterns on a small scale (Chapter VI).

2.4 Analysis of population dynamics

2.4.1 Multiple linear regression analysis

Multiple linear regression was applied to find a causal relationship between varying characteristics of the species and weather variables. Among the characteristics investigated were dormancy and flowering pattern of the population, annual mortality and recruitment and

transition probabilities between size based states. To find the best model, I used the Forward method in the Multiple Linear Regression modus of the statistical software SPSS 11.0. I checked the calculated models for multicollinearity and influential points.

Linear regression estimates the coefficients of the linear equation, involving one or more independent variables, that best predict the value of the dependent variable. The linear regression model assumes that i) there is a linear relationship between the dependent variable and each predictor variable, ii) the error term has a normal distribution with a mean of "0", and iii) the variance of the error term is constant across cases and independent of the variables in the model.

$$y_i = b_0 + b_1x_{i1} + \dots + b_px_{ip} + e_i \quad \text{Eq. 2.1}$$

y_i is the value of the i^{th} case of the dependent scale variable

p is the number of predictors

b_j is the value of the j^{th} coefficient, $j = 0, \dots, p$

x_{ij} is the value of the i^{th} case of the j^{th} predictor

e_i is the error in the observed value for the i^{th} case

SPSS uses an ANOVA to test the model's ability to explain any variation in the dependent variable. R^2 is the coefficient of determination, which shows how much of the variation is explained by the model.

There is often a problem with multicollinearity (statistical term for the lack of independence between variances), when too many potential predictor variables are included in the model. Collinear predictors, i.e. when linearly related, can cause problems in estimating the regression coefficients. Collinearity means, that much of the variance in the dependent variable that is explained by the independent variable is also explained by other predictor variables. Tolerances were calculated to test for collinearity among the predictor variables. When the tolerances are close to "0", there is high multicollinearity and the standard error of the regression coefficients will be inflated. A variance inflation factor greater than "2" is usually considered problematic. The collinearity diagnostics estimates the eigenvalues of the predictor variables. Eigenvalues close to "0" indicate that the predictors are highly intercorrelated and that small changes in the data values may lead to large changes in the estimates of the coefficients. SPSS computes the condition indices as the square roots of the ratios of the largest eigenvalue to each successive eigenvalue. Values greater than "15" indicate a possible problem with collinearity; values greater than "30" indicate a serious problem.

Regression diagnostics measure various ways in which a regression relation might derive largely from one or two observations. Observations whose inclusion or exclusion result in substantial changes in the fitted model (coefficients, fitted values) are said to be influential (Dallal, 2001). In linear regression, an outlier is an observation with large residual, meaning an observation whose dependent-variable value is unusual given its values on the predictor variables. An outlier may indicate a sample peculiarity or may indicate a data entry

error. While outliers in the response variable represent model failure, outliers with respect to the predictors are called leverage points (Dallal, 2001). To identify points, which have an undue influence on the outcome of the regression analysis, the leverage, values as well as Cook's distance of the cases were calculated. Cases with high leverage values and a large Cook's distance are assumed to have an undue influence on the model.

2.4.2 Transition matrix analysis

I used transition matrices to calculate several demographic characteristics of the population in the nature reserve Leutratat, including the rate of increase r , the finite rate of natural increase λ , the reproductive values of the size based states and the sensitivity-, and elasticity-, values of the matrix elements (Caswell, 1982).

The analysis of plant demographic data using matrix algebra provides a powerful tool for describing and understanding the population dynamic behaviour of organisms from differing life forms and habitats (Enright et al., 1995). Matrix models are an important tool to aid in assessing individual fitness in varying environmental conditions and to measure the most important demographic parameters of the population (Hayward and McDonald, 1997). They are often used as tools in conservation and harvesting, and to estimate the success of pest control strategies (Shea and Kelly, 1998).

In demographic models, the state vector is usually defined by the distribution of individuals among a set of categories (Caswell, 1989; Guardia et al., 2000). Individuals were often partitioned into age categories to estimate the population projection matrix (Lewis, 1942). However, this approach is difficult in plant species where some variable other than age accounts for most of the variability in survivorship and reproduction (Werner, 1975). Rather than categorizing individuals according to their age, an alternative model can be used categorizing individuals according to some arbitrary morphological or physiological feature (Lefkovitch, 1965). From a state-classified life table describing the dynamics of a population, a transition matrix can be constructed (Caswell, 1989) and various demographic parameters can be derived algebraically from the transition matrix, including the finite rate of natural increase (λ : dominant eigenvalue of the transition matrix), reproductive values (left eigenvector), the rate of increase r and the sensitivity of λ to absolute changes in elements of the projection matrix (Caswell, 1982). Elasticities can be calculated for each matrix element as a relative measure of the contribution of the elements to λ (Kroon de et al. 1986).

Short-term data are used to develop deterministic matrix models. However, long-term data allow the calculation of multiple transition matrices, which are typically analysed by stochastic matrix models under the assumption that the variation in vital rates among matrices is due to fluctuating environmental conditions (Fieberg et al., 2001). However, the general use of matrix models in conservation biology for predicting extinction (or quasi-extinction) of a population has been criticized lately.

2.5 AFLP - molecular genetic approach in plant ecology

I used AFLP markers (Amplified fragment length polymorphism) to study genetic variation of three populations of *H. hircinum* in Thuringia, to evaluate population differentiation, and genetic relationships between the isolated populations in a fragmented habitat.

2.5.1 Methods to analyse genetic diversity

The study of genetic relatedness among individuals, and the analysis of population structure, phylogenetic relationships and mapping of quantitative trait loci demand an accurate assessment of genetic diversity (Mueller and Wolfenbarger, 1999). Several techniques and genetic markers have been developed to address these questions, but each available technique exhibits both strengths and weaknesses (Mueller and Wolfenbarger, 1999). Methods used include restriction fragment length polymorphism (RFLP) techniques, random amplified polymorphic DNA (RAPD)-PCR markers, microsatellites (simple sequence repeat, SSR) and isozyme analysis.

Isozyme analysis is a traditional method and can be used to assess allelic frequencies, heterozygosity indices, the partitioning of genetic diversity using F-statistics and the degree of past inbreeding in populations (Wright, 1965; Nei, 1972). Isozymes have been used widely as a standard in studies on plant ecology and genetics (Brewbaker et al., 1968; Conkle et al., 1982; Hunziker and Schaal, 1983; Hamrick and Loveless, 1986; Wendel and Weeden, 1989; Shapcott, 1999; Stenström, A. et al., 2001). However, the quantity of information that can be obtained is low, and the resolution of genetic differences only moderate. This is due to the nature of enzymes, which are often invariant within populations (or even between species and higher taxa), or have only few variants if they are polymorphic (Mueller and Wolfenbarger, 1999).

Two molecular fingerprinting methods used frequently for investigating genetic diversity are AFLP and RAPD. RAPD analysis is fast and easy to perform, it can provide high quantity of information and yields moderate resolution of genetic differences. But its application has been questioned lately because of the low reproducibility of the results (MacPherson et al., 1993; Jones et al., 1997; Kjølnner et al., 2004). RAPD was used in studies on genetic diversity and conservation genetics of a few orchid species (Wong and Sun, 1999; Sun and Wong, 2001). Jones et al. (1997) suggest that AFLPs should be preferred over RAPDs because of their high reproducibility and the high efficiency of AFLP analysis with respect to identification of polymorphisms (Vos et al., 1995; Jones et al., 1997; Barker et al., 1999). However, Kjølnner et al. (2004) concluded from their work that both markers appear to be useful and reliable in clonal diversity studies and polyploid variable organisms, at least when a conservative scoring approach is used.

2.5.2 AFLP

AFLPs are polymerase chain reaction- based markers for the rapid screening of genetic diversity. They allow high-resolution genotyping of fingerprinting quality and because of their high replicability and ease of use, AFLP markers have emerged as a major new type of

genetic marker with broad application in systematics, pathotyping, population genetics, and DNA fingerprinting (Mueller and Wolfenbarger, 1999).

The molecular method of AFLP has been applied successfully to a variety of questions in a wide range of species (Vos et al. 1995, Paglia and Morgante 1998). AFLP can be used to investigate genetic variability in populations as well as between genera and is usually superior to similar methods (Lin et al. 1996, Powell et al. 1996, Jones et al. 1997). AFLP can be applied instantly and without previous DNA-sequence knowledge of the investigated organism. AFLP has been used repeatedly for studies on genetic analysis of populations as well as for the characterisation of genetic diversity in plant ecology (Krauss, 2000; Majer and Mithen, 1998; Alexandersson and Jon, 2000; Beismann et al., 1997; Greef et al., 1997; Paul et al., 1997; Arens et al., 1998; Qamaruz et al., 1998; Winfield et al., 1998; Barker et al., 1999; Vuylsteke et al., 1999; Kim et al., 2002).

The use of AFLP in conservation and economy

Several crops have been studied using AFLP markers to use the genetic information for breeding programs. Barker et al. (1999) investigated genetic diversity in the genus *Salix* (willow) that contains a number of species that have great potential value as biomass crops. Seefelder et al. (2000) studied genetic diversity and phylogenetic relationships among accessions of hop *Humulus lupulus*. Other important crops studied by the AFLP method include wild bean (Tohme et al., 1996), lentil (Sharma et al., 1996), soybean (Maughan et al., 1996), Indian and Kenyan tea *Camellia sinensis* (Paul et al., 1997), *Zea mays* (Vuylsteke et al., 1999), the cereal crop *Eragrostis tef* (Ayele and Nguyen, 2000), *Mangifera odorata* (Teo, 2002), *Carica papaya* (Kim et al., 2002), Italian rice varieties of *Oryza sativa* (Spada et al., 2004),

Another focus of studies with economic importance lies on genetics of principal weeds or pests causing severe damage to crop plants (Gagne et al., 2000; Danquah et al. 2002). Better knowledge of the life history and evolution of such species is essential to determine the risks of the appearance of a new race, of its spread, or of its introduction to other countries (Gagne et al., 2000).

The use of AFLP becomes increasingly popular in conservation ecology. Several studies were conducted in the last years to determine the influence of habitat loss and habitat fragmentation on genetic diversity of rare and endangered species and subspecies, including *Astragalus cremnophylax* var. *cremnophylax* in Arizona (Travis et al., 1996), *Populus nigra* subsp. *betulifolia* in UK (Winfield et al., 1998), the orchid *Orchis simia* in UK (Qamaruz-Zaman et al., 1998), and *Medicago citrina* in the western Mediterranean (Juan et al., 2004).

2.6 Spatial pattern analysis in plant ecology

I used spatial autocorrelation analysis to investigate spatial pattern and spatio-temporal spread of the study species on the long-term monitoring site. Spatial point pattern analysis was applied to analyse spatial pattern of *H. hircinum* plants on the small spatial scale

(1 - 9 m²), and to determine the level of intra-and interspecific relationships with other plant individuals.

The predictability of the physical arrangement of plants is referred to as their spatial pattern (Dale, 1999). Spatial pattern has important implications not only for the plants themselves, but also for other organisms that interact with plants, such as herbivores and pollinators (Dale, 1999). Spatial and temporal characteristics need to be described and quantified and related to underlying processes such as establishment, growth, competition, mortality, disturbance, climate, and topography.

The methods applied to study a spatial pattern depend on the time frame, the data available, the question being asked, and the scale at which we wish to answer it. Among the methods recommended by Dale (1999) to analyse the spatial pattern for one dimension (without an environmental gradient) and one species are quadrat variance methods (Kershaw, 1957; Hill, 1973; Ludwig and Goodall, 1978) and semivariograms using fractional dimension as a measure of the spatial complexity of the object (Palmer, 1988). Knowledge about the spatial relationships of pairs of species is essential for understanding plant community's structure. Within a community, natural groupings of species may arise from biological interactions or from shared and divergent responses to abiotic factors. Joint spatial pattern of pairs of species can be analysed by modified quadrat variance methods (Kershaw, 1960; Galiano, 1986), cross-covariance method and cross-spectral analysis (Jenkins and Watts, 1969).

Spatial point pattern analysis has become increasingly popular among plant ecologists. The statistical analysis of point mappings of all plant individuals within a defined area is an exact method to investigate spatial patterns in plants (Ripley, 1981; Upton and Fingleton, 1985; Stoyan and Stoyan, 1994; Dale, 1999). First-order statistics describe the intensity of a point pattern, and large-scale variation in the intensity of the points in the study area (Wiegand and Moloney, 2004). In contrast, second-order statistics are based on the distribution of distances of pairs of points (Ripley, 1981). Second-order statistics, such as Ripley's K-function or the pair-correlation function, use the information on all interpoint-distances to describe the small-scale spatial structure of the point pattern, over a range of distance scales (Wiegand and Moloney, 2004). Thus, they provide detailed information on the scale of pattern. If the pattern is not random further questions may deal with the underlying processes that give rise to these non-random patterns, e.g. regeneration behaviour and dispersal strategy of the plant species. Ripley's K function is a cumulative density function. Each larger scale investigated includes the information of the smaller scales, thereby masking positive or negative interactions that act at different scales. The pair-correlation function $\hat{g}(r)$ can be used to test for pattern at each scale.

Questions concerning the relationship between points with different characteristics (e.g. competition or facilitation between adults and seedlings, interspecific competition) can be addressed by using the bivariate alternative to the pair-correlation function (mark-correlation function).

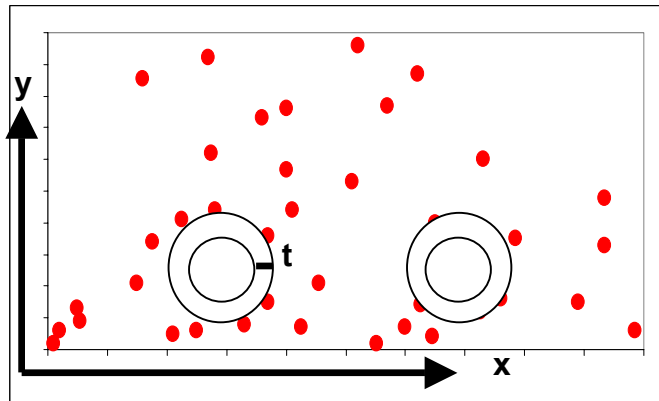


Fig. 2.6. Univariate second-order spatial statistics (pair-correlation function) of x-,y- point mappings within a rectangle. A ring of radius r is placed around each point and the number of neighbouring points within the band of the ring is counted to estimate $\hat{g}(r)$. The bandwidth of the rings t is adjusted to the mean density of the individuals (Stoyan and Stoyan, 1994). The observed pattern is often tested against a null model of complete spatial randomness (CSR), although other null models could be proposed. Under CSR conditions, it is suggested that there are no directional effects, meaning that every point on the ground within the study area is likely as every other point to be the location of an individual plant (Young and Young, 1998). Values of $\hat{g}(r) > 1$ indicate that small interpoint distances of r are more frequent, values of $\hat{g}(r) < 1$ that they are less frequent than expected under CSR.

3. High variability of life history characteristics and life state transitions of an orchid over 26 years

3.1 Abstract

Matrix models are often used to predict long-term fate of endangered and/or protected species. However, parameters of these models such as life state transitions are difficult to estimate and are often based on short-term observations only. Transition probabilities may vary depending on several external conditions further complicating model development. Based on the 26 years data set on a population of *Himantoglossum hircinum* (L) Sprengel (Orchidaceae) in a nature reserve in Germany, the variation of life-history characteristics including life span and dormancy pattern, as well as population dynamics including size structure, age structure and transition probabilities between size based life states were studied. Matrix models were used to identify the most important life state transitions for population growth. Regression analyses were used to quantify the influence of weather variables on population characteristics and transition probabilities. Population size increased exponentially and density effects could not be observed. Matrix analysis showed that flowering and large individuals had the highest and second highest reproductive value, respectively. Some transition probabilities between life states changed significantly from the first time period (1979-1989) to the second one (1990-1999). The results showed that life history traits and life state transitions were highly variable, caused in part by variability of weather conditions. Thus, matrix models used as a tool for predicting long-term behaviour of a population can give misleading results, especially when the elements of the population projection matrix are based on short-term data sets.

Additional Keywords: Matrix analysis - Protected species - Long-term predictions - Conservation

3.2 Introduction

Conservation of rare and endangered plant species demands detailed knowledge of the many facets of the life history of the species including its demography and its behaviour under different environmental conditions (habitat, microclimate, competitors). Because dynamics of plant species can be highly variable on a short time scale due to varying short-term weather conditions, long-term observations are needed to get reliable information on the life cycle as well as the population dynamics.

Plant population dynamics are constrained by the abiotic and biotic environment (Tilman, 1988). It is unclear, whether there is a dominant intrinsic mechanism causing variable patterns in the life history of a species, or whether species mainly respond to environmental factors including varying weather conditions, presence of competing species, natural disturbances and human influence. Plants differ from animals in their response since they cannot migrate in unfavourable seasons of the year and they cannot escape from places with high density of competing individuals or from places with high predation rates. They should be more vulnerable to changes in their physical or biotic environment. Furthermore, scientists start to realize that many of the same environmental constraints and tradeoffs that shape the evolution of plant morphologies and life histories also influence ecosystem functioning and the dynamics of interspecific interactions at the community level (Rees et al., 2001).

Matrix models are often used as a powerful tool for describing and understanding the population dynamics of organisms from differing life-forms and habitats (Enright et al., 1995), to aid in assessing population fitness under varying environmental conditions, to measure the most important demographic parameters of the population (Hayward and McDonald, 1997), to compare the effects of various management actions on population behaviour, and to estimate the viability of species (Fieberg and Ellner, 2001). The use of PVA by scientists and natural resource managers has increased greatly in the last decade. However, PVA is a modelling approach and its validity depends on the model structure and data quality (Reed et al., 2002). Most PVA models are based on poor data, taking into account only few years of observation, and trying to predict a population's risk of extinction (or quasi-extinction) in the long-term future.

In this study the life history traits of a plant species and their variability are investigated to quantify the importance of long-term data for the development of matrix models. The study is based on a long-term data set recorded for a population of *Himantoglossum hircinum* (Orchidaceae). The long-term monitoring covers more than 26 years and thus provides a powerful data set for studying essential questions in orchid ecology as well as regulatory mechanisms in plant population ecology. The study species is protected, but showed strong exponential increase in population size on the study site, and new populations are arising in the area, which represents the north-eastern border of the species' worldwide distribution. A single-species, state-structured projection model was used to determine the relative contribution of the defined life states to the reproduction, to indicate, which life state transitions contribute most to the growth rate of the population, and which are most sensitive

to perturbations (Shea and Kelly, 1998). Based on the assumption, that transition rates are variable, we tested, whether there is a significant, causal relationship between weather conditions in the current/previous plant years and population performance as well as life state transitions.

Two main issues were addressed in this study.

- 1) There is a high plasticity in life history characteristics of *H. hircinum* including life span and flowering pattern, as well as in population characteristics in terms of age class structure, size class structure and transition probabilities between different life states.
- 2) The high variability of life-history properties is at least in part caused by external environmental conditions (e.g. weather variability).

3.3 Methods

3.3.1 Population structure

Lifetime distribution ("half-life") was calculated for cohorts arising after 1979 because of the uncertainty of the age of individuals in the cohorts 1976 to 1978. Dormant plants may not be recorded for up to two successive years, even though they remain alive. Thus, plants first observed in 1979, could actually have been alive underground in the years before. I calculated the life span for all individuals without including plants already existing in 1976, 1977 and 1978 and without individuals still existing 1999.

3.3.2 State variables and matrix analysis

In demographic models, the state vector is usually defined by the distribution of individuals among a set of categories (Caswell, 1989; Guardia et al., 2000). Individuals are often partitioned into age categories to estimate the population projection matrix (Lewis, 1942). However, this approach is problematic in plant species where some variable other than age accounts for most of the variability in survivorship and reproduction (Werner, 1975).

Since there is a significant positive correlation between number of leaves and total leaf area in *H. hircinum*, number of leaves can be taken as a measure of size ($R^2 = 0.87$, $p < 0.001$, $N = 50$, Pfeifer et al., submitted). Based on the size and flowering behaviour of the individuals, the orchid population recorded each year was divided into five states:

1. Seeds (*S*)
2. small individuals (1 - 4 leaves) (*SI*)
3. large, sterile individuals (5 - 15 leaves) (*LI*)
4. large, flowering individuals (5 - 15 leaves) (*FI*)
5. dormant individuals (*D*)

From one year to the next, it was possible for plants to achieve any change of state (between states 2 - 5), but seeds could only be produced by flowering plants. The life cycle

of *H. hircinum* based on transitions between the states is presented in Figure 3.1. From the state-classified life table describing the dynamics of the population, a transition matrix was constructed.

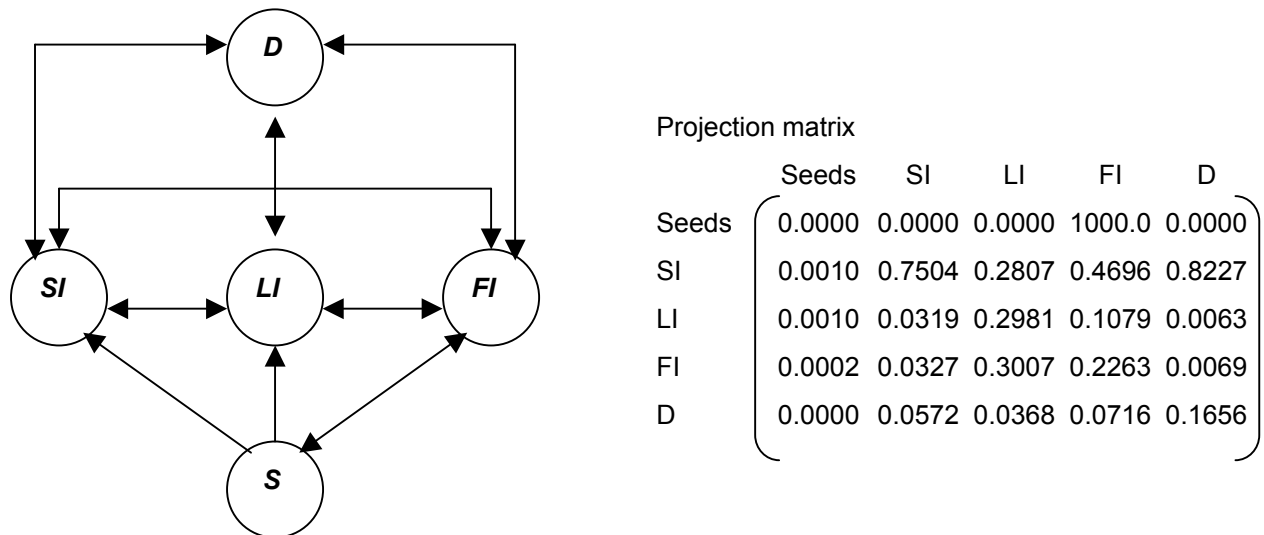


Fig. 3.1. Life-cycle graphs and projection matrix for *H. hircinum* illustrating the transitions between the size classes *S* (seeds), *SI* (small individuals), *LI* (large individuals), *FI* (flowering individuals) and *D* (dormant individuals). Transition probabilities from *S* could only be estimated, while the other transition values had been calculated and averaged. The first row in the projection matrix indicates the number of produced seeds per individual of that state. Transition to death was possible from all states but was not included.

Various demographic parameters were derived algebraically from the transition matrix including the finite rate of natural increase (λ : dominant eigenvalue of the transition matrix), reproductive values (left eigenvector), the rate of increase r and the sensitivity of λ to absolute changes in elements of the projection matrix (Caswell, 1982). I calculated the elasticities for each matrix element as a relative measure of the contribution of the elements to λ (de Kroon et al., 1986). The elasticity analysis determines the sensitivity of the dominant eigenvalue of a population projection matrix to proportional changes of each of the elements of the projection matrix.

The values in the transition matrix were the probabilities of survival and transition between states from one year to the next. We calculated mean transition probabilities for the period 1979 to 1999. Since certain average transition probabilities seemed to have changed in the last ten years, the distributions of the values of the transition probabilities in the first time period 1979 to 1990 were compared with the distributions in the second period 1990 to 1999 using Mann-Whitney U test to detect significant differences.

Two projection matrices were analysed calculating r , λ , the reproductive values of each state RV , and the elasticities of each state transition. All matrix analyses were carried out using Poptools version 2.5 (Hood, 2004). The probabilities of survival and transition from the first state to any of the other states could not be measured and were estimated. The number of produced seeds per flowering individual was estimated based on literature data and observations on the fruiting success of the individuals in 2003 and 2004.

3.3.3 Abiotic control of population characteristics

Statistical software (SPSS 11.0) was used to investigate population characteristics and to carry out regression analysis. To find out whether weather conditions have an influence on annual mortality (proportion of lost individuals in t relative to the population size in $t - 1$), recruitment (proportion of one year old individuals in t), dormancy (annual percentage of individuals that stayed belowground), and annual transition values, multiple linear regression analyses were carried out using the Forward method.

A plant year was divided into four seasons, autumn (September - November), winter (December - February), spring (March - May), and summer (June - August). Standardized weather variables of the current t and the previous plant year $t - 1$ were used. Weather variables included precipitation, temperature, number of days with air temperature below 0°C and number of days with soil temperature below 0°C. They were calculated for months, seasons and plant years. Since multiple regression is plagued by collinear relationships (Mac Nally, 2000), the resulting models were tested for collinearity among the predictor variables by calculating the variance inflation factor and the tolerance of the independent predictor variables. Only those models were considered that were not affected by multicollinearity.

3.4 Results

3.4.1 Population growth and structure

The population started with 45 individuals in 1976 and was rapidly increasing in the following years. The growth can be best described by an exponential model with an average per capita growth rate of $r = 0.19$ per year (Pfeifer et al., submitted). Population turnover was highly variable and relatively high in 1996 and 1997 (Fig. 3.2).

The population was mainly build up by one-, two- and three year old individuals (in varying proportions over the years), while the proportion of older individuals was generally low (Median 1979-2001: annual proportion of emergent individuals older than 7 years: 7.1%, 5 - 7 years: 36.8%). Especially in 1993 (proportion of one year old plants in the population: 42.5%) and 1999 (38.2%), newly recruited plant individuals highly dominated the population structure.

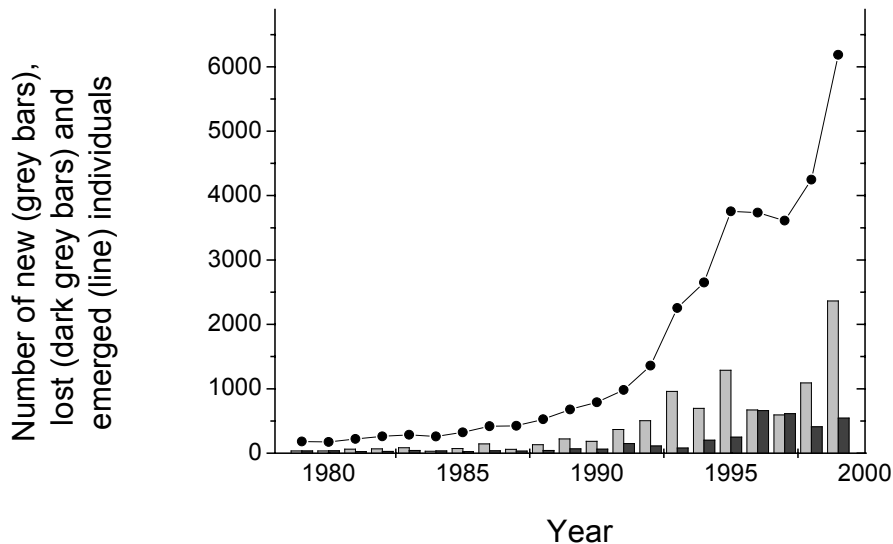


Fig. 3.2. Number of new (grey bars) and lost (dark grey bars) individuals from the population relative to the previous plant year between 1979 and 1999. The line indicates the annual number of individuals.

The state structure of the population in each year was mainly characterized by small individuals (1 - 4 leaves), which were not able to flower (Fig. 3.3). The proportion of flowering and dormant individuals showed no significant increase or decrease over the total period (Fig. 3.3; $p > 0.05$).

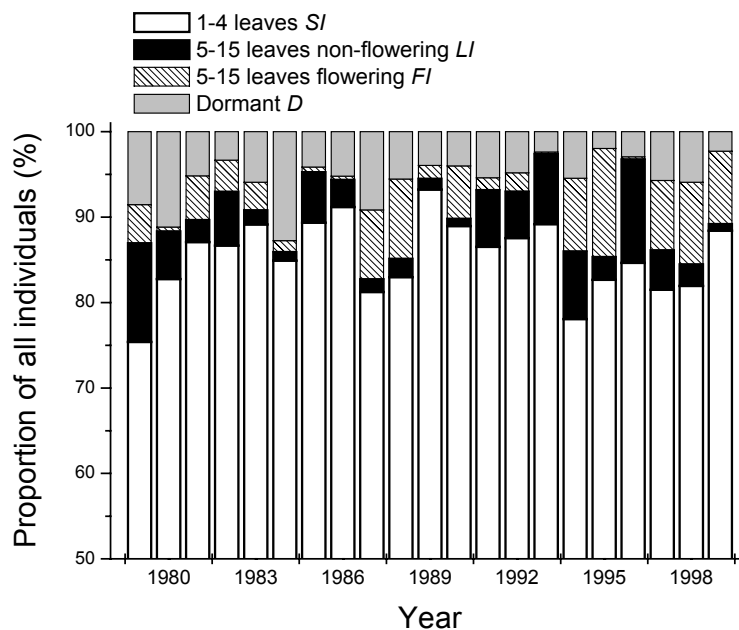


Fig. 3.3. Size class structure of the population in each year between 1979 and 1999. The scale of the y - axis starts at 50% for better visualization.

3.4.2 Survivorship curves

Logarithm of the size of the cohorts decreased linearly with age (Deevey type II survivorship curve; Deevey 1947). Half-life of the cohorts decreased with the years and ranged from 6.3 years for the 1987 cohort to 3.5 years for the 1998 cohort (Table 3.1). Mortality of the cohorts (state specific mortality rate = mean probability of an individual to die q_x , mortality rate = killing power k_x , Begon et al., 1996) varied from year to year. Mortality was high in 1996 and 1993 but low in 1992 and 1994. The risk to die was highest for one-year-old individuals and decreased for two-year-old ones, but no other consistent pattern in the cohorts could be detected.

Table 3.1. Cohort half-life values for the cohorts that established between 1980 and 1998 (log-linear regression). The first cohorts (1976 to 1979) were excluded because of the low number of individuals in this year. R^2 - strength of negative correlation between cohort size (log-scale) and cohort age.

Year of cohort establishment	Half-life	R^2	N (Initial size of cohort)
1980	5.08	0.97	35
1981	5.54	0.98	62
1982	6.08	0.98	66
1983	4.85	0.94	76
1984	4.91	0.94	30
1985	5.24	0.95	67
1986	5.76	0.98	143
1987	6.27	0.98	58
1988	5.27	0.93	131
1989	5.32	0.96	217
1990	5.32	0.95	181
1991	5.09	0.92	363
1992	4.82	0.95	503
1993	4.61	0.97	959
1994	3.96	0.98	695
1995	3.73	0.97	1287
1996	3.96	0.94	671
1997	4.45	0.95	595
1998	3.46	0.98	1093

3.4.3 Life span and dormancy/flowering pattern

There was a significant positive correlation between the average number of leaves and age of the individuals ($R^2 = 0.82$, $p < 0.001$). Most individuals never flowered at all (87%, $N = 2867$). Of these non-reproductive plants, 17.3% increased their leaf number continuously during their life (up to 9 leaves), 25.6% didn't change in size (one individual for up to 10 years) and 23.1% showed a varying leaf number (mainly between one and three leaves) until their final disappearance (Table 3.2).

Table 3.2. Growth pattern of flowering ($N = 443$) and non-flowering ($N = 2867$) individuals alive from 1979 to 1998. Individuals already existing 1976-1978 and plants still existing 1999 were not included.

Flowering individuals	Number	Proportion (%)
Increase in leaf number until first flowering event	249	56.21
Constant leaf number until first flowering event	25	5.64
Varying leaf number until first flowering event	85	19.19
One year emergent prior first flowering	56	12.64
Flowering in first year	28	6.32

Non-flowering individuals	Number	Proportion (%)
Increase in leaf number until death	497	17.34
Constant leaf number until death	734	25.60
Varying leaf number until death	662	23.09
One-year old plants	974	33.97

Many individuals died after two years of emergence. Average life span measured for all individuals observed between 1979 and 1998 was 3.4 years and ranged from 1 year ($N = 979$) to 19 years ($N = 2$).

Flowering individuals had a longer average lifetime, and the correlation between life span and number of years flowering was weakly positive ($R^2 = 0.40$, $p < 0.05$). While individuals flowering only once lived on average 5.6 years, individuals flowering more than twice lived on average 9.1 years (Table 3.3).

Table 3.3. Life span calculated for flowering and dormant *H. hircinum* individuals existing between 1979 and 1998.

	Flowering frequency			Frequency of dormancy events		
	1x	2x	>2x	1x	2x	>2x
Average Life-span (years)	5.6	7.1	9.1	5.7	7.4	10.9
Number	304	100	39	275	58	14

Flowering individuals most often started flowering in their fourth year above ground. Life span of individuals found flowering in their first year of emergence was different (Fig. 3.4). These individuals lived on average only 3.8 years, which is significantly lower than the average life span of flowering individuals, that needed one or more years above ground before flowering (6.8 years).

Average life span was also higher for individuals with at least one year of non-emergence (“dormancy”) during their life ($N = 1312$ out of 13687 (9.6%) individuals observed between 1979 and 1999). Individuals with one year of dormancy lived on average 5.7 years; individuals who did not appear above ground for more than one year throughout their life lived on average 8.1 years.

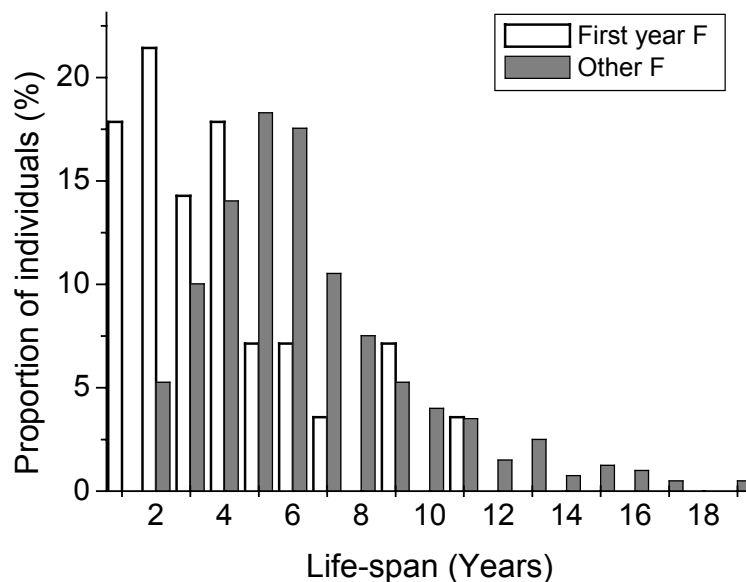


Fig. 3.4. Life span of flowering *H. hircinum* individuals that flowered in their first year of emergence ($N = 28$) and life span of individuals that started to flower later ($N = 399$). Life span was significantly lower for individuals flowering in their first year (mean 3.8 years), compared to the other flowering individuals (mean 6.0 years).

3.4.4 Transition probabilities and matrix analysis

Transitions between life states varied throughout the years. The transition probabilities for the transitions from *SI* to *LI* and from *FI* to *LI* increased significantly from the first (1979-1990) to the second (1990-1999) time period, while there was only a trend towards an increase in the transitions from *FI* to *FI*, from *LI* to *LI* and from *LI* to *FI*. The transition probability from *FI* to *SI* decreased significantly (Table 3.4).

The rate of increase r as well as the finite rate of increase λ varied in the two time periods ($r_1=0.044$, $\lambda_1=1.045$ and $r_2=0.167$, $\lambda_2=1.182$). Flowering individuals and large-sized not flowering individuals had the highest ($RV_{F1}=58.4\%$ and $RV_{F2}=254.1\%$) and second highest ($RV_{L1}=20.6\%$ and $RV_{L2}=28.9\%$) reproductive value, respectively. A sensitivity analysis of the population projection matrix (Caswell 1982) showed, that the outcome of the projection matrix strongly depended on the transitions from the seed stage to all the other stages and on the transitions from *SI* to *LI/FI*.

The elasticity analysis (Kroon de et al. 1986) revealed that the transitions *SI* to *FI* as well as the transitions from *Seeds* to *SI/LI/FI* and the number of seeds per flowering plant were most important for population growth (Table 3.5).

Table 3.4. Mean transition probabilities between the model states calculated for the two time periods 1979 to 1990 and 1990 to 1999. *SI* - Individuals with 1 - 4 leaves, *LI* - non-flowering individuals with 5 - 15 leaves, *FI* - flowering individuals with 5 - 15 leaves, *D* - dormant individuals. The distributions of the transition values between the two periods were compared using Mann-Whitney-U test. ** $p < 0.05$; * $p < 0.1$; — not significantly different.

Transition (From - To)	Mean \pm SD 79-90	Mean \pm SD 90-99	
S - SI	0.76 \pm 0.04	0.74 \pm 0.04	—
SI - LI	0.02 \pm 0.02	0.04 \pm 0.03	**
SI - FI	0.03 \pm 0.03	0.04 \pm 0.03	—
SI - D	0.06 \pm 0.03	0.05 \pm 0.02	—
LI - SI	0.36 \pm 0.19	0.19 \pm 0.09	**
LI - LI	0.26 \pm 0.19	0.35 \pm 0.24	—
LI - FI	0.24 \pm 0.28	0.37 \pm 0.27	—
LI - D	0.05 \pm 0.07	0.02 \pm 0.01	—
FI - SI	0.57 \pm 0.18	0.35 \pm 0.15	**
FI - LI	0.06 \pm 0.09	0.16 \pm 0.15	*
FI - FI	0.16 \pm 0.17	0.30 \pm 0.26	—
FI - D	0.09 \pm 0.16	0.05 \pm 0.11	—

3.4.5 Abiotic control of population trends

There was a weak but significant, positive correlation between annual precipitation in the previous plant year $t - 1$ and mortality in the current plant year t ($R^2 = 0.23$, $p < 0.05$). The years 1990 and 1979 overperformed strongly in the analysis, and if they were ignored, the correlation was much stronger ($R^2 = 0.73$, $p < 0.001$).

I found a weak but significant negative correlation between precipitation in spring $t - 1$ and recruitment in t ($R^2 = 0.21$, $p < 0.05$). The number of days with soil temperature below 0°C (Soil Frost Days) in October $t - 1$ ($R^2 = 0.26$, $p < 0.05$) and precipitation in November t ($R^2 = 0.24$, $p < 0.05$) were negatively correlated with the proportion of dormant individuals in t . A linear combination of both weather variables explained 41% of the variation in percentage of dormant plants ($p < 0.01$).

Certain transition values between the states from one plant year to the next were influenced by weather variables. A linear combination of Soil Frost Days in winter t (negative coefficient) and precipitation in autumn t (positive coefficient) explained 45% in the variation of the annual transitions from SI to FI . And there was a significant positive correlation between precipitation in December $t - 1$ and the transition from SI to FI ($R^2 = 0.38$, $p < 0.01$).

Table 3.5. Elasticity matrices based on the transition matrices of the population. The higher the elasticity value of a state transition, the higher the relative contribution of that state transition to the finite rate of increase of the population (λ). a) period 1979 to 1990; b) period 1990 to 1999.

(a)	S	SI	LI	FI	D
S	0.0000	0.0000	0.0000	0.1599	0.0000
SI	0.0393	0.3143	0.0248	0.0219	0.0296
LI	0.0770	0.0153	0.0345	0.0076	0.0000
FI	0.0436	0.0748	0.0711	0.0472	0.0016
D	0.0000	0.0256	0.0040	0.0017	0.0062
Σ	0.1599	0.4300	0.1344	0.2383	0.0374

(b)	S	SI	LI	FI	D
S	0.0000	0.0000	0.0000	0.2165	0.0000
SI	0.0395	0.1286	0.0107	0.0187	0.0082
LI	0.1288	0.0204	0.0547	0.0260	0.0002
FI	0.0482	0.0493	0.1637	0.0749	0.0009
D	0.0000	0.0074	0.0011	0.0008	0.0012
Σ	0.2165	0.2058	0.2303	0.3369	0.0105

There was a weak negative but significant correlation between precipitation in November t and the transition from SI to D ($R^2 = 0.24$, $p < 0.05$). 48% in the variation of the transition from LI to LI was explained by a linear combination of number of soil frost days in autumn t (positive coefficient) and precipitation in winter t (negative coefficient). A linear combination of number of Soil Frost Days in winter t (negative coefficient) and Soil Frost Days in autumn t (negative coefficient) explained 45% in the variation of the transition from LI to FI . A linear combination of precipitation in winter t (positive coefficient) and temperature in autumn $t - 1$ (negative coefficient) explained 56% in the variation of the transition from FI to FI . And there was a negative significant correlation between Soil Frost Days in winter t and the transition from FI to FI . A correlation between the other transition probabilities and any of the standardized monthly, quarterly and annual weather variables could not be detected.

3.5 Discussion

3.5.1 Demographic analysis

Growth and structure of the population

The monitored population of *H. hircinum* increased rapidly in size, and density effects could not be detected. This behaviour is typical for plant species that reach a new habitat or experience a change in external environmental conditions that suits their growing requirements well. Heinrich (2003) reported a similar exponential growth for a population of *H. hircinum* on a place located approximately 10 km north of our study site. The increase in the species' population size and the spatial spread of the species with new populations arising in the area can be attributed to climate change (Good, 1936; Carey and Farrell, 2002).

The population in the nature reserve Leutratal is mainly build up by newly recruited and young individuals. As in many orchids, recruitment is mainly by seeds. Hutchings (1987) estimates that less than five percent of the emergent population of *Ophrys insectifera* is recruited by vegetative propagation in each year. It has been observed at the Cambridge Botanic Gardens that *H. hircinum* can reproduce vegetatively by forming new tubers on the root system, but this could not be confirmed by field work (Carey and Farrell, 2002). Fruit-set was low in our study (2002/2003: 5.9% and 2003/2004: 7.0% of all produced flowers developed into mature fruits). However, as in many orchids, the number of seeds produced by a capsule (up to 1200, Carey and Farrell, 2002) can be very high and thus can compensate for the low fruit-set.

Most individuals were small and only few were big enough to flower. However, the chance of missing very small individuals (with one or two small leaves) can be high. Kéry and Gregg (2003) showed that even under intense survey conditions not all plants may be found, and that the detectability of vegetative plants was life-state dependent averaging only 82%. Therefore, an underestimation of annual recruitment and annual mortality is likely, as is an overestimation of the half-life of the cohorts.

Surprisingly, the annual proportion of flowering individuals, and of plants that are absent from above-ground for one or two years ("dormant"), seems to be a characteristic feature (no significant trend over time) of the population although varying strongly, probably due to variability of external factors. The proportion of dormant plants in t varied between 1.2% (1980) and 9.2% (1987), the proportion of emerged individuals flowering never exceeded 13% (Pfeifer et al., submitted).

Plasticity of half-life of the cohorts

Based on the average half-life of the cohorts (4.9 years) I suggest that *H. hircinum* is a medium to long-lived orchid species. Wells and Cox (1989) reported similar half-lives for *Acera anthropophorum* (4.0-7.8 years) and *Spiranthes spiralis* (4.6-9.2 years). The progressive decline in half-lives since 1990 might be a consequence of changing conditions in the plot to less favourable ones for survival of individuals derived from seed. The Deevey curve (Type II) implies a constant mortality risk of the individuals regardless how old they are (Deevey, 1947).

Plasticity of life span of the individuals

Individuals strongly differed in their life expectancy and this was dependent on other life history characteristics. Average life span of flowering individuals (6.3 years) was longer than that of non-flowering individuals (2.9 years), suggesting that flowering individuals appear to be physiologically/genetically fitter than non-flowering individuals despite high reproductive effort (Pfeifer et al., submitted).

Average life span was longer, when the individuals remained belowground for one or more years during their life. This suggests that during extreme weather conditions especially small individuals stay belowground thereby increasing their lifetime. Dormancy is part of the life history of several orchid species including *Spiranthes spiralis* (Wells, 1967), *Dactylorhiza incarnata* and *D. sambucina* (Tamm, 1972), *Ophrys sphegodes* (Hutchings, 1987), *Cleisthes divaricata* (Gregg, 1991), and *Cypripedium candidum* (Falb and Leopold, 1993). Some of them are absent above ground for no longer than one year (Willems and Melser, 1998), while others can stay belowground much longer (Hutchings, 1987). Carey (1999) found that it is not unusual for *H. hircinum* individuals to remain underground as tubers during any one year. Most of the as dormant recorded *H. hircinum* individuals ($N = 924$; 70.4%) in our study were marked and named and usually all sticks had been found during the census. One problem is that during extreme winter conditions leaves of especially larger plants could have died back and were consequently recorded as absent from aboveground during the census in April. This could result in an overestimation of annual percentage of dormant plants, and it could also be responsible for the observed variable dormancy pattern.

Flowering individuals that were dormant for one or more years, had a longer lifetime (9.3 years) compared to flowering individuals without a dormant phase (6.3 years). It is possible that the individuals remained belowground to store resources and avoid unfavourable environmental conditions for flowering by this increasing the chance of leaving more descendants in the future.

3.5.2 Plasticity of transition probabilities and matrix analysis

The probability of flowering in our population was greater for orchids that were large and not flowering the previous year $t - 1$ than for individuals that flowered the previous year $t - 1$. This is probably caused by high costs of reproduction (Pfeifer et al. submitted). The differences in the transition probabilities between both periods suggest that conditions for flowering improved in the second time period (1990-1999). However, the probability for flowering individuals to flower again in the following year was not significantly different. Large individuals were more likely to flower in the following year in the second time period (Table 3.4). More flowering individuals were still large in the following year, meaning that reproductive effort could be better compensated in $t + 1$.

3.5.3 Abiotic control of population trends

The annual proportion of one-year-old individuals in a year varied and seemed to be influenced by a dry spring in the previous plant year $t - 1$. However the correlation was only weak, and the time between germination of seeds and subsequent emergence of seedlings can take several years. Consequently, it is unlikely to identify a single important weather variable regulating that process. High variability of annual recruitment was also reported for *Coeloglossum viride*, a short-lived terrestrial orchid species (Willems and Melser, 1998).

A wet plant year was often followed by a year of high mortality of *H. hircinum* individuals. This could be an indirect consequence of the positive influence of high precipitation on the proportion of individuals flowering. Because of high reproduction costs causing a lower survival probability after flowering, mortality of the population is higher following a good flowering year.

Plants have been observed to emerge with the autumn rains (Carey and Farrell, 2002). Low rainfall in November was correlated with higher percentage of individuals that stayed belowground. It also appeared that a very cold October caused the plants to stay belowground the next plant year. This suggests that dormancy of *H. hircinum* is at least in part determined by extreme, external conditions (enforced *dispersal in time*; Begon et al., 1996). The ability of small individuals to grow and to flower in the following year was higher when they experienced relatively warm and wet conditions with few soil frosts in winter and high rainfall in autumn. During such weather conditions survival and especially photosynthetic activity is higher, the latter enabling the plant to store enough resources in belowground structures for reproduction. Individuals emerge with the autumn rains, and it was observed that individuals, which emerge early, grow faster, and reach a greater final size than late emerging plants. However, frosts in winter can cause the blackening and death especially of larger individuals. In winter 2002/2003 up to 50% of the individuals recorded in November in plots of one square meter, which were established to study spatial point patterns of *H. hircinum* individuals, disappeared until a second data recording in May.

A cold autumn, especially in combination with a dry or a cold winter prevents large plants from flowering. Because of high costs of reproduction in *H. hircinum* the probability to flower again immediately after flowering decreased with the number of times flowering (Pfeifer et al., submitted). However, high precipitation and warmer temperatures in winter of

the plant year after the flowering event coincides with an increased probability of the individuals to flower again without a break in the flowering sequence. Warm conditions in autumn appear to negatively affect flowering initiation.

3.6 Conclusions

Life state transitions from one year to the next proved to be highly variable and at least some of the transitions were influenced by weather variability. Especially the increase in winter temperature accompanied by fewer frost events in autumn and winter and higher precipitation in winter improved the conditions for flowering and thus for reproducing.

Already, Waite and Hutchings (1991) emphasized the importance of long-term observations to get reliable results in a matrix analysis if a projection of long-term population size is attempted. It can be concluded that the validation of the calculated dominant eigenvalue λ is difficult even when based on 26 years of observations. Any long-term projection of a matrix to predict future behaviour and fate of a plant population that is not based on long-term observations can give misleading results, be it that a population is doomed to decline or that it will increase in the future. Both conclusions would have been possible for our population of *H. hircinum* if we had used certain short time periods only (3 - 4 years).

However, the transition matrix approach remains useful for comparative studies in conservation biology to assess the effect of management activities and for the development of models of population behaviour assuming that environmental conditions would be constant on the long-term. We conclude, that because of the high variability of transition probabilities and their dependence on weather variables, matrix models and their long-term predictions regarding extinction of the populations have to be dealt with cautiously.

4. Climate, size, and flowering history determine flowering pattern of *Himantoglossum hircinum*

4.1 Abstract

Flowering behaviour of individuals of orchids as well as annual flowering frequency of an orchid population is often irregular. Various hypotheses were proposed to explain that pattern including irregularity because of costs associated with reproduction, herbivory effects, variation because of the chaotic behaviour of the system (intrinsic regulation), and external conditions (i.e. weather variability). This chapter examines the influence of age, size, reproductive effort, and climatic conditions on flowering dynamics of *Himantoglossum hircinum*. Flowering performance was highly variable and annual flowering frequency of the population never exceeded 14%. Individuals mostly flowered only once, a few flowered more than four years. Flowering probability was strongly determined by plant size in a given year, while there was no significant influence of age. Costs of reproduction were high, resulting in a decrease of leaf number in the year after flowering and a Flowering-between-Flowering value (FBF) of 24%. Weather explained about 50% of the yearly variation in proportion of plants with 8 - 15 leaves and thus had a strong, but indirect influence on flowering percentage. Drought in winter had a direct, negative influence on the flowering initial. I conclude that irregularity in flowering is caused mainly by variability of weather conditions in the previous and current plant year, and there is also an influence of costs associated with reproduction. An intrinsic regulation was not found.

Additional Keywords: Reproduction - Costs - Size class distribution - Life history - Principal component analysis

4.2 Introduction

The decline in number of populations and individuals of orchid species is of major concern in conservation biology. The exponential increase in number of individuals as well the increase in spatial distribution of an orchid is unusual. The identification of the factors that cause the strong increase in population size is important for the development of management strategies of rare plant species.

Annual flowering frequency of plant species is a main point of interest among plant ecologists and conservationists. To understand the reproductive strategy of plant species and to develop management plans for their protection, it is important to know more about determination of the flowering frequency on the population level as well as about flowering pattern and its role in the life history of individuals, especially of orchid species. Irregularity in flowering between the years appears to be widespread among terrestrial orchids. Irregular flowering may be a consequence of costs of sexual reproduction or leaf damage by herbivores influencing the flowering behaviour of the individuals (Whigham and O'Neill, 1991; Kindlmann, 1999; Kindlmann and Balounova, 1999, 2001), or of variable climatic conditions (Willems and Bik, 1991). It is even proposed that flowering frequency is unpredictable due to an intrinsically triggered irregular pattern (Inghe, 1990).

Several factors are believed to have an effect on the flowering frequency of the population and of the individuals in a given year. Age might be an important factor, but in a variety of studies plant size was described as a better predictor of flowering probability (Snow and Whigham, 1989; Wells and Cox, 1989; Zimmerman and Aide, 1989; Calvo, 1990b; Primack and Hall, 1990; Whigham and O'Neill, 1991; Carey, 1998; Willems and Melsers, 1998; but see also Hutchings, 1987a). There also is good evidence that weather affects flowering behaviour of the individuals and consequently the flowering frequency in populations of terrestrial orchids. Drought in summer had a negative effect on flowering frequency of *Herminium monorchis* (Wells, 1981) as well as on *Dactylorhiza sambucina*, and *Listera ovata* (Inghe and Tamm, 1988). After 10 years of observations, Wells and Cox (1989) suggested that flowering in *Ophrys apifera* was influenced by precipitation and availability of soil water. Almost no flowering of *Orchis simia* after severe winters was reported by Willems and Bik (1991). Carey (1999) showed that flowering probability of a *Himantoglossum hircinum* population in Britain was correlated with rainfall during the previous growing season. However, other authors found no relationship between weather conditions and flowering frequency (Wells and Cox, 1991; Whigham and O'Neill, 1991).

It has been discussed, whether costs of reproduction exist, which lead to a reduction in future growth, survival and reproduction and to what extent they may be compensated for by photosynthesis by the reproductive organs themselves, by below-ground storage of resources or increased production of carbohydrates by other organs (Primack et al., 1994). If costs arise from flowering, limiting resources used for present reproduction are not available for future use and short-term benefits may be counteracted by long-term costs including a failure to flower in subsequent years (Primack and Hall, 1990; Ackerman and Zimmerman, 1994). Negative effects of flowering (Snow and Whigham, 1989; Whigham and O'Neill, 1991;

Kindlmann 1999; Willems and Dorland, 2000) as well as of fruiting (Zimmerman and Pyke, 1988; Snow and Whigham, 1989; Zimmerman and Aide, 1989; Ackerman and Montalvo, 1990; Primack and Hall, 1990; Primack et al., 1994) on future plant growth, flowering behaviour, and survival have been found. However, several authors found no costs associated with reproduction in orchid species (Hutchings, 1987b; Horvitz and Schemske, 1988; Calvo, 1990a; Fox and Stevens, 1991; Willems and Bik, 1991; Calvo and Horvitz, 1990; Waite and Farrell, 1998).

In this study I want to find out, which factors determine the irregularity flowering in plant species, based on the long-term data set of a population of *Himantoglossum hircinum* (L.) Sprengel (Orchidaceae). The species was chosen because it is a rare and protected orchid, which can easily be identified. The species spreads in Germany and is characterized by an exponential increase in number of individuals of the population on the study site. Life history data including growth and flowering pattern from more than 13000 individuals were collected in the long-term monitoring, indicating a high variability in flowering frequency of the population between the years.

I proposed the following hypotheses using the life history data as well as climatic data of the area to test them:

- 1) Flowering pattern of the individuals is size-dependent and/or age dependent.
- 2) Weather is an important controlling factor for the size of the individuals.
- 3) Current reproduction of *H. hircinum* individuals imposes negative effects on future survival, growth and reproduction.
- 4) Flowering frequency of the population depends on the size of the individuals and weather variables.

4.3 Methods

4.3.1 Population growth and structure

To determine population growth in the 26 years and to estimate future growth I fitted the exponential growth model by using the Least Square Nonlinear Regression procedure.

$$N(t) = N_0 e^{rt} \tag{Eq. 4.1}$$

Because of the problem of possible dormancy (up to two years), the complete life cycle is strictly known only for plants first observed in 1979, and last observed in 1998. Age of the individuals already existing 1976 until 1978 could not be determined. Hence, I excluded these individuals when analysing the age structure of the population. In 1976 only the number of non-flowering and flowering individuals was recorded without counting their leaf numbers.

4.3.2 Biotic and abiotic influencing factors

To exclude that the irregularity in flowering is an intrinsically triggered phenomenon, I checked for possible autocorrelation in the time-series of proportion of individuals flowering and in the time-series of proportion of individuals with 8 - 15 leaves by calculating the autocorrelation function (ACF) and the partial autocorrelation function (PACF). Additionally, the proportion of individuals flowering in a particular year t was plotted against proportion of individuals flowering in previous years $t - x$.

Since there are many weather variables that might have an influence on flowering behaviour, and since they might be auto-correlated, a Principal Components Analysis (PCA) was carried out using PCORD 4.0. The values of selected weather variables for the years 1980 - 2001 were standardized (standardized value = $(value - mean)/standard\ deviation$) to make them comparable in their variation. The resulting new axes with their scores from the PCA were used as predictor variables in a multivariate linear regression analysis (with the Forward Method). This analysis was done separately for monthly (e.g. temperature in February), quarterly (e.g. days with soil temperature below 0°C in winter t) and yearly variables (e.g. precipitation in the previous year $t - 1$).

I assumed an influence of size on flowering probability. A first model was produced with size class as predictor variable and flowering behaviour as response variable. This model was extended by including weather variables as predictors and applying a multiple linear regression analysis. To test for a direct influence of weather variability on size, a second model was build using the annual variation in the proportion of individuals with 8 - 15 leaves (high probability to flower) as response variable and weather variables as predictor variables. To test, whether weather variables determine flowering frequency of the individuals with 8 - 15 leaves, a third model was calculated using the proportion of individuals with 8 - 15 leaves which flowered as a response variable to the weather conditions.

I used two methods to look for possible indirect costs of reproduction, which are also termed delayed or demographic costs, and which may be interpreted as the demographic consequences of resource investment in the reproductive process (Obeso 2002). In the first approach the FBF (Flowering-between-flowering) value according to Inghe and Tamm (1988) was calculated, defined as the proportion of individuals flowering in a given year t that also flowered both in the previous and subsequent years ($t - 1$ and $t + 1$). FBF values below 50% indicate high costs of reproduction. In the second approach, I compared all flowering individuals with 8 - 15 leaves with non-flowering individuals of the same size (hence potentially able to flower) with respect to their leaf number in the following year $t + 1$. Individuals were further separated according to their behaviour in the previous year $t - 1$. I used the paired samples t-test and the Wilcoxon-test to compare the size of the individuals in year t with their size in the previous year $t - 1$ and in the following year $t + 1$. A significant difference would indicate that flowering activity in any one year imposes a significant negative effect on future size of the individuals.

4.4 Results

4.4.1 Growth and flowering pattern of the population

Growth of the population followed an exponential curve with an average per capita growth rate of $r = 0.19$ per year ($R^2 = 0.97$). The slopes of the regression lines of the number of emerged individuals each year versus time and the number of flowering individuals versus time were nearly the same from 1976 to 2001 (Fig. 4.1). Although the annual growth of the population was relatively constant on the log-scale, the number of flowering individuals fluctuated greatly from year to year. Despite the fluctuations there was no significant increase or decrease of the proportion of individuals flowering with time when fitting a regression line to it ($R^2 = 0.082$, $p > 0.1$), hence the 'average' proportion of flowering individuals remained fairly constant. The proportion of emerged individuals that flowered never exceeded 14% (Fig. 4.2).

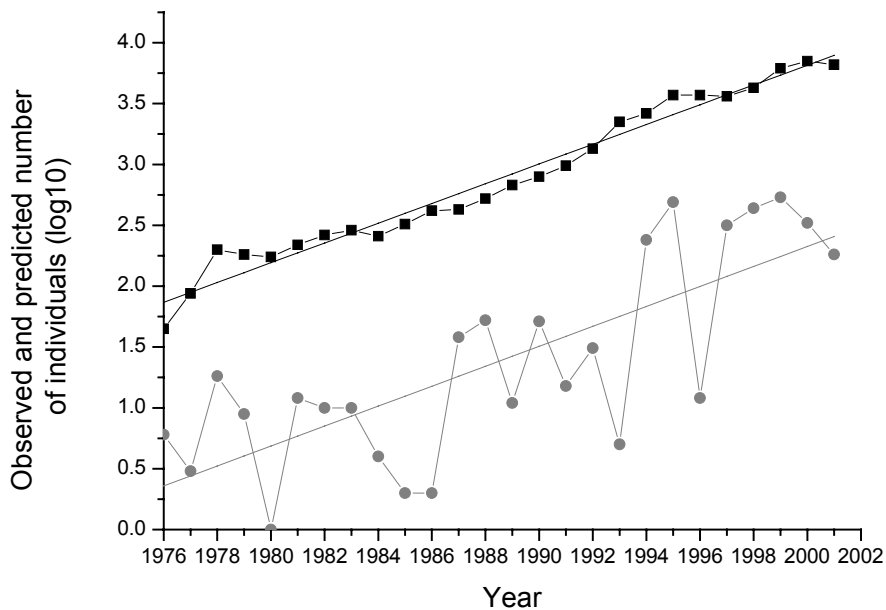


Fig. 4.1. Population growth (squares) and number of individuals flowering (dots) each year (both on a log₁₀-scale) on the long-term monitoring site between 1976 and 2001. Linear regression was used to emphasize the high growth rate of the population. The slopes of the regression lines fitted to the growth of the population and the number of individuals flowering between 1976 and 2001 were nearly the same (0.081 and 0.082 respectively), indicating a constant average proportion (approx. 5.2%) of emergent individuals flowering.

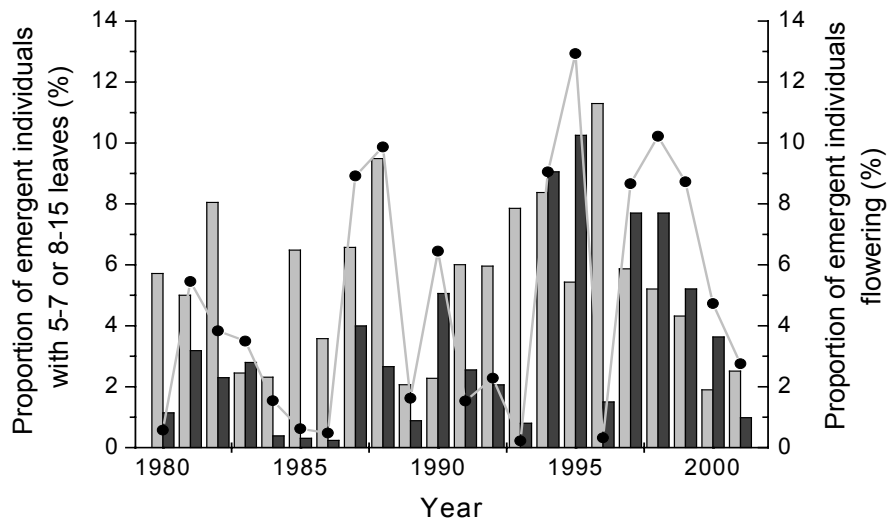


Fig. 4.2. Proportion of emerged individuals flowering (black dots) and proportion of emerged individuals with 8 - 15 leaves (black bars) as well as 5 - 7 leaves (grey bars) on the permanent site in each year between 1980 and 2001.

The number of flowering events per individual was highly variable. Only 1812 individuals out of the total of 13687 individuals observed between 1976 and 2001 ever flowered. Of the 443 flowering individuals known to have completed their life cycle, about two thirds (68.6%) flowered only once (Table 4.1).

Half of the individuals flowering twice flowered in consecutive years. However, individuals flowering more than twice often needed one or more years of regeneration between the flowering events. The higher the number of flowering events per plant the lower the probability to do so in successive years (Table 4.1). Individuals flowering three or more years in succession mostly did so in the period from 1997 to 2001.

Table 4.1. Frequency of flowering of the individuals observed between 1976 and 2001 on the permanent site. The third row represents the individuals that had their flowering in a sequence of consecutive years without any break.

No. of flowering events	1	2	3	4	5	6	7	Total
No. of individuals for which the complete life-cycle is known	304	100	29	9	1	0	0	443
No. of individuals (including individuals alive in 2001)	1150	423	149	60	15	8	1	1806
Flowering in successive years (including still existing individuals)	-	218	35	7	1	1	0	262

4.4.2 Flowering pattern and plant age

Distribution of age during first year flowering and during all flowering events followed a gamma distribution ("negative exponential) curve. Age at first flowering peaked at an age of 4 years, and the proportion of individuals flowering during all flowering events was highest for 4 and 5 year old plants. 5.1% ($N = 89$) of all flowering individuals were observed to flower in their first year of emergence. (Fig. 4.3). Flowering probability increased linearly with plant age until the individuals were seven years old, and it remained fairly constant for older individuals. Of those flowering individuals who died, 55% (of 443 flowering individuals for which the complete life cycle is known) did so after their last flowering event. This pattern was independent of plant age.

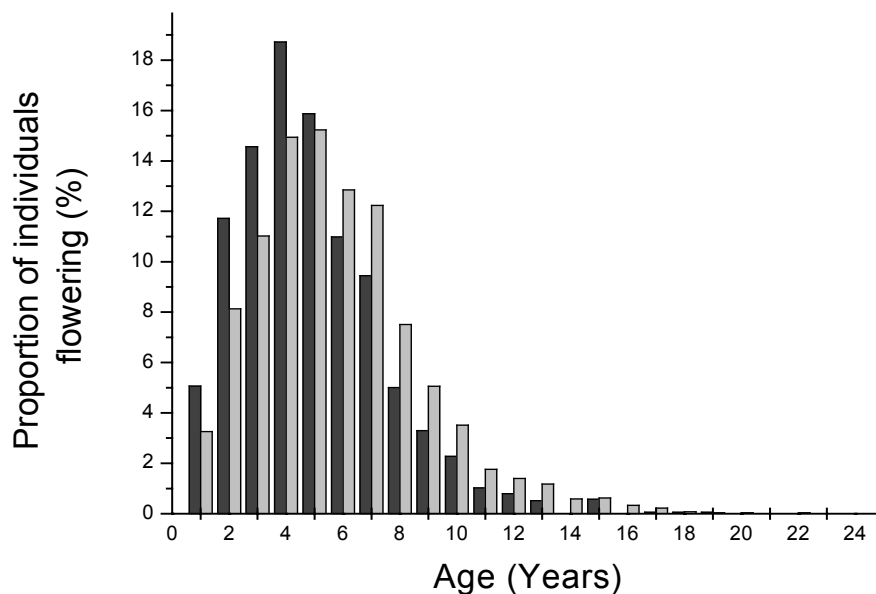


Fig. 4.3. Age of individuals at first flowering ($N = 1758$, black bars) and (because individuals might flower more than once) distribution of age of individuals during all flowering events ($N = 2731$, grey bars). Individuals already existing in 1976 - 1978 were excluded from the analysis, since their age could not be determined.

4.4.3 Flowering pattern and plant size

I found a significantly high positive correlation ($R^2 = 0.87$, $p < 0.001$) between the number of leaves and the total leaf area of the individuals. Therefore I decided to take number of leaves as a measure of plant size and photosynthetic capability. At least five leaves were necessary for the ability of a plant to flower, although one individual was found flowering with four leaves (but 1744 individuals with four leaves did not flower). Flowering individuals typically had seven, eight or nine leaves and flowering probability increased exceeding 90% for plants having more than nine leaves (Fig. 4.4). A logistic regression model, which was fitted to the data, had a strong explanatory power ($R^2 = 0.84$).

$$\text{Prob}(\text{flowering}) = 1 / (1 + e^{-z})$$

Eq. 4.2

$$z = -10.2 + 1.5 * (\text{Leaf number})$$

Flowering probability was 50% at an individual size of approx. 6.4 leaves.

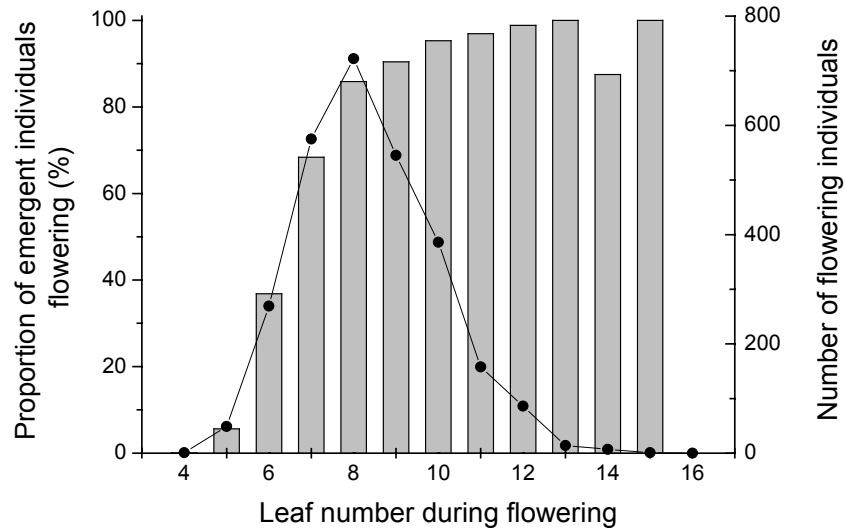


Fig. 4.4. Distribution of size of flowering individuals during all flowering events (N = 2813, black dots) and relationship between size of the individuals and flowering probability (grey bars) for all individuals observed between 1977 and 2001 on the long-term monitoring site. Flowering probability strongly increased with plant size and exceeded 80% for plants with more than seven leaves. Size of the individuals was not recorded in 1976.

4.4.4 Variability of flowering pattern between years

I tested for a relationship between annual age class distribution and flowering probability of the population. There was no significant correlation between age class distribution and proportion of individuals flowering in any year. Most individuals flowered after three or four years of emergence. Years with high proportion of flowering individuals (1994, 1995, 1997, 1998 and 1999, hereafter called 'good' years) had a low or high proportion of plants that were older than three or four years. Likewise, the proportion of plants older than three years was either small or high in years with a low proportion of flowering individuals (1986, 1993, 1996, hereafter called 'bad' years).

Since size had an influence on flowering probability of the individuals, the individuals were grouped into three size classes (1 - 4 leaves, 5 - 7 leaves, 8 - 15 leaves) to search for patterns on the population level. 'Good' and 'bad' flowering years strongly differed in their size class distribution (Fig. 4.2). There was a significant positive correlation ($R^2 = 0.75$)

between the proportion of individuals with more than seven leaves and the proportion of individuals flowering each year (Fig. 4.5).

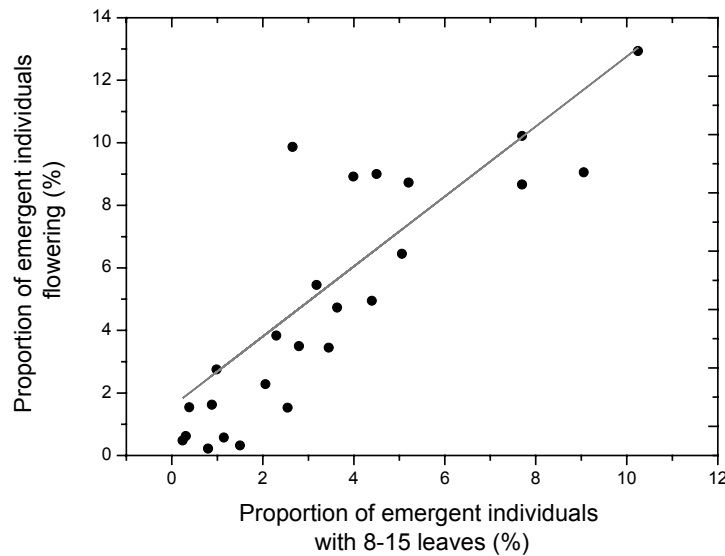


Fig. 4.5. Relationship between proportion of individuals flowering and proportion of individuals with 8 - 15 leaves on the permanent site in each year between 1977 and 2001 (black dots). There was a significant positive effect of size on flowering probability ($R^2 = 0.75$, $p < 0.001$).

I found no correlation between the proportion of flowering individuals in year t and the proportion of flowering individuals in previous years $t - x$, and no correlation between the proportion of individuals with 8 - 15 leaves in year t and in previous years $t - x$. The autocorrelation function as well as the partial autocorrelation function revealed no pattern for the time series data. Therefore, applying multiple linear regression without including autocorrelation factors was sufficient.

4.4.5 Influence of weather on flowering pattern of the population

PCA was applied for standardized weather variables (rainfall, mean temperature, number of days with air or soil temperatures below 0°C , mean minimum temperature), for annual, quarterly, and monthly values separately. The first four PCA axes explained 85.5% of the variance in the annual weather variables. The PCA axes scores represented a linear combination of correlated annual weather variables. However, the use of these PCA axes scores as predictors in the multiple linear regression resulted in only weak correlations with flowering probability and percentage of individuals with 8 - 15 leaves. The use of monthly weather variables was rejected, since at least eight PCA axes would have been necessary to explain more than 80% of the variance in the weather data. This problem was also evident when using weekly weather data.

The use of PCA axes scores calculated on the base of quarterly weather variables proved to be most successful for model development. Six axes were necessary to explain 80% in the variation of the weather variables, and their scores were used as predictor variables in the multiple linear regression. Soil temperature below 0°C and days with air temperature below 0°C in winter as well as temperature and days with soil temperature below 0°C in spring were highly correlated with axis one. Temperature and days with soil temperature below 0°C in autumn of the previous plant year $t - 1$ were most strongly correlated with axis two. Temperature in autumn, rainfall in winter of the previous plant year and days with soil temperature below 0°C in autumn were the main weather variables correlated with axis three (Table 4.2).

Table 4.2. Contribution of quarterly weather variables to the PCA axes. Values entered are from the actual plant year t and the previous plant year $t - 1$. SFD: days with soil temperature below 0°C, FD: days with air temperature below 0°C. A plant year starts, by definition, in autumn (September) and ends in August, when the leaves above ground had died.

Weather variables	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6
SFD winter t	-0.51	0.03	-0.00	0.03	0.05	-0.18
FD winter t	-0.46	-0.01	-0.03	-0.01	0.13	-0.30
SFD spring t	-0.43	-0.12	-0.09	0.19	0.05	0.25
Temperature spring t	0.41	0.09	-0.06	-0.37	0.09	-0.18
SFD autumn $t - 1$	0.02	-0.50	-0.06	0.22	0.05	-0.45
Temperature autumn $t - 1$	-0.17	0.44	-0.23	-0.02	-0.28	0.26
SFD autumn t	0.07	-0.11	-0.62	0.00	-0.12	0.04
Rain winter $t - 1$	0.01	-0.27	0.46	0.13	-0.37	0.31
Temperature autumn t	-0.04	0.36	0.49	0.17	-0.17	-0.24
Rain autumn t	-0.21	-0.27	0.17	-0.49	0.18	-0.09
Rain winter t	0.21	-0.25	-0.04	0.59	0.07	0.08
Rain in summer $t - 1$	-0.03	-0.18	0.15	-0.17	0.50	0.57
Rain autumn $t - 1$	0.20	0.24	0.17	0.16	0.45	-0.17
SFD winter $t - 1$	-0.06	0.30	-0.09	0.32	0.47	0.06

I calculated three models based on flowering percentage, proportion of individuals with 8 - 15 leaves and PCA axes scores of quarterly weather variables. Since size is known to significantly affect flowering probability, size class was included as predictor variable in the first model. Most of the variation in annual percentage flowering (Model F: dependent variable = percentage flowering) was explained by the proportion of individuals with 8 - 15 leaves of the population in each year and weather variables correlated with axis three (Table 4.3). The next step, which aimed to explain the variation in the percentage of large

individuals (8 - 15 leaves), revealed an effect of the weather variables correlated with axes two and one (Table 4.3).

The third model showed that the percentage of flowering individuals with 8 - 15 leaves was explained by quarterly weather variables correlated with axes four, six, and one.

$$\text{Model F: \% flowering} = 1.06 + (1.1 * \%8-15\text{leaves}) + (0.70 * \text{Axis 3}) \quad R^2 = 0.82$$

$$\text{Model L: \% 8-15leaves} = 3.21 + (-1.01 * \text{Axis2}) + (0.77 * \text{Axis1}) \quad R^2 = 0.47$$

$$\text{Model FL: \% flowering 8-15leaves} = 63.28 + (15.86 * \text{Axis4}) + (19.37 * \text{Axis6}) + (14.97 * \text{Axis1}) \quad R^2 = 0.75$$

All three models were significant and supported by high R^2 values (Table 4.3). The calculated values for the coefficients were significant. The tolerances for the predictor variables in each model (percentage of the variance in a given predictor that can not be explained by the other predictors) always were above 0.8. This means that more than 80% of the variance in a given predictor could not be explained by the other predictors. The collinearity diagnostics as well as the calculated variance inflation factors confirmed that there were no problems with multicollinearity in the models.

Table 4.3. Models (see Results section) explaining the variation in proportion of individuals flowering each year (F = Flowering), variation in the proportion of large individuals (with 8 - 15 leaves) each year (S = Size), and variation in the proportion of large individuals, which actually flowered. The models F, L and FL use artificial predictor variables from a PCA analysis of the weather variables. In the last model, the regression was weighted by the reciprocal of the number of large individuals, which flowered. Expl. = Explained variable, SFD = days with soil temperature below 0°C, FD = days with air temperature below 0°C, t = current plant year, $t - 1$ = previous plant year.

Model	Expl.	Important predictor variables	R^2	p	F
		Size			
F	% F	Temperature or SFD in autumn t , Rainfall in winter $t - 1$	0.82	< 0.001	41.47
L	% S	Temperature and SFD in autumn $t - 1$, SFD and FD in winter t , SFD in spring t	0.47	< 0.005	8.00
FL	% FS	Rainfall in winter t (and in autumn), Rainfall in summer $t-1$, SFD and FD in winter t , SFD in spring t	0.75	< 0.001	20.65

4.4.6 Reproductive effort

Size was the most important factor influencing flowering probability. And size was strongly weather dependent. Approximately 50% of the annual variation in the proportion of individuals with 8 - 15 leaves was explained by quarterly weather variables. To test, whether reproductive effort also had an effect on size, and thus indirectly affecting flowering probability, two approaches were used. The Flowering-between-flowerings value showed that only 24.3% of all *H. hircinum* individuals that were observed flowering in year $t - 1$ and $t + 1$ ($N = 337$) also flowered in year t ($N = 82$). However, nearly 50% of all non-flowering events in year t between two years of flowering took place in 1996. 122 individuals interrupted their flowering sequence in 1996, while only two did not ($FBF = 1.6\%$).

Flowering activity significantly affected future plant size (Wilcoxon test, $p < 0.05$). Flowering individuals experienced a significant decrease in leaf number in the year after flowering. This decrease was even stronger if the individuals also flowered in the previous year $t - 1$ (Fig. 4.6, upper row). Contrary, non-flowering individuals with 8 - 15 leaves showed on average a constant leaf number in the following year (Fig. 4.6, lower row). Figure 4.6 shows the effect of flowering of large individuals on their future size. When the previous year was included, four combinations were possible: 1) flowering in $t - 1$ and t , and leaf number in $t + 1$ (F-F-F/NF), 2) non-flowering in $t - 1$, flowering in t , and leaf number in $t + 1$ (NF-F-F/NF), 3) flowering in $t - 1$, non-flowering in t , and leaf number in $t + 1$ (F-NF-F/NF) and 4) non-flowering in $t - 1$ and t , and leaf number in $t + 1$ (NF-NF-F/NF).

4.5 Discussion

4.5.1 Flowering pattern of the individuals and the population

The population is increasing rapidly on the permanent monitoring site. The mean proportion of *H. hircinum* individuals flowering each year remained constant during the time of the study, although fluctuating largely from year to year. However, the annual percentage flowering was even at its maximum (13.0%) very low compared to other terrestrial orchids investigated (Hutchings, 1987a: 65 - 97%; Hutchings, 1987b: 70 - 100%; Inghe and Tamm, 1988: generally above 20%; Calvo, 1990b: >30%; Vanhecke, 1991: 15 - 69%; Wells and Cox, 1991: 6 - 57%; Waite and Farrell, 1998: approx. 15% - 60%).

Flowering was a rare event for *H. hircinum* plants and most individuals flowered only once during their life, if at all. Individuals which were larger at the first time of flowering were more likely to flower again, suggesting that the probability of repeated flowering depended on the size of the individuals and their ability to store resources needed for sexual reproduction.

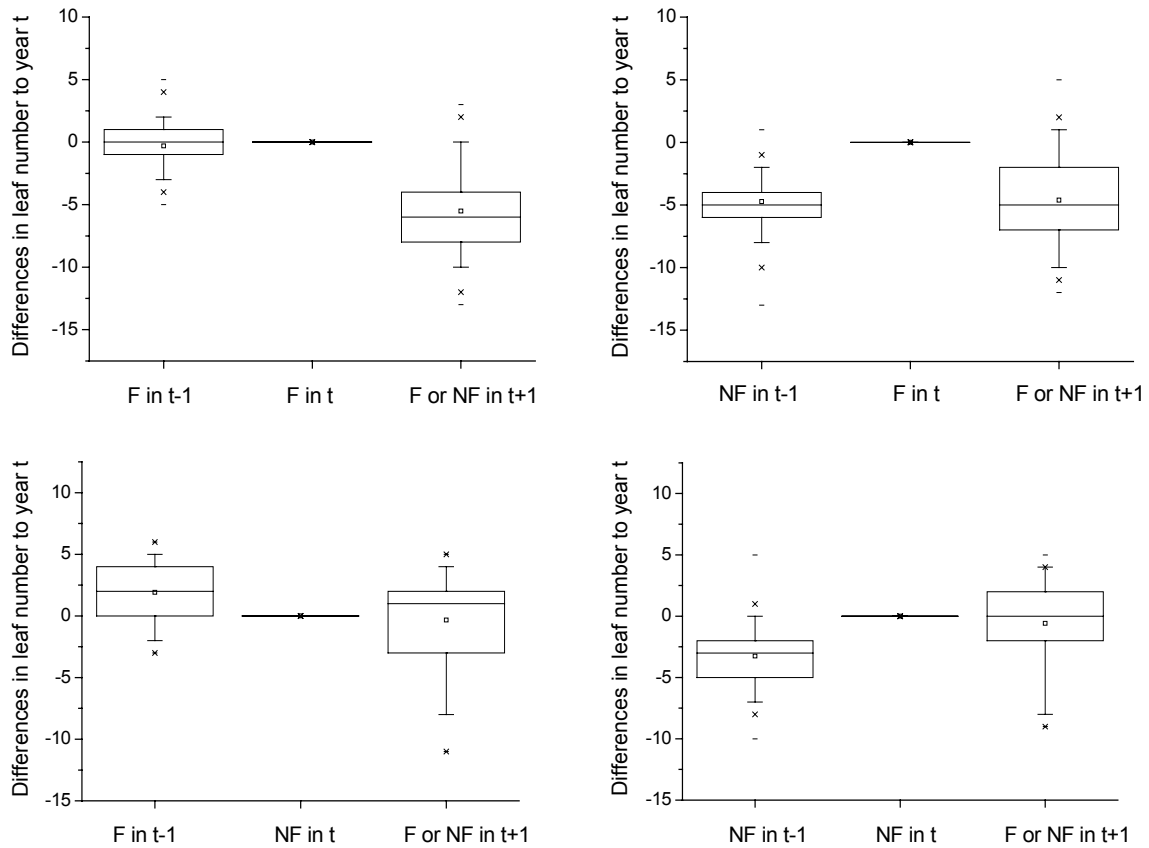


Fig. 4.6. Comparison of flowering (F) and non-flowering (NF) individuals of the same size (8 - 15 leaves) in year t regarding their future size in year $t + 1$ and dependent on their behaviour in year $t - 1$. F = Flowering, NF = Not flowering. Leaf number decreased significantly in the year after the flowering event $t + 1$ with a slightly larger decrease when the individuals also flowered in $t - 1$. Leaf number increased when the individuals with 8 - 15 leaves did not flower in t , but not significantly so.

Flowering individuals often needed one or more years before they were able to flower again, which indicates that reproductive effort was high and plants needed time to recover. However, a number of plants were able to capture enough energy to flower in consecutive years without a break (up to six times, Table 4.1).

4.5.2 Flowering pattern plant age

Flowering pattern of the individuals was irregular. Age did not have a significant effect on flowering probability of the individuals. While most individuals flowering were four years or older, a few also flowered in their first year of emergence. First year flowering was reported for several orchids, including *Ophrys sphegodes* (Hutchings, 1987a, 1989), *Orchis militaris* (Hutchings et al., 1998) and *Coeloglossum viride* (Willems and Melsner, 1998). On the other hand, first-year flowering never occurred in long-lived orchid species, including *Listera ovata*

(Willems and Melser, 1998), *Ophrys apifera* (Wells and Cox, 1991) and *Orchis simia* (Willems and Bik, 1991).

4.5.3 Flowering pattern and plant size

Plant size was an important determinant of flowering probability of *H. hircinum*. Size of the individuals might be influenced by local weather conditions, individual flowering history, herbivore damage, and further local habitat conditions. The larger a *H. hircinum* plant, measured by its number of leaves, the higher its probability to flower. Almost all plants with eight and more leaves in March were flowering in May or June. This suggests that, prior to flowering, plants need to build up sufficient energy reserves. Many individuals needed at least three years above ground to accumulate sufficient resources for flowering activity, but a few were able to store sufficient resources during the protocorm stage.

Size-dependent flowering behaviour was also reported for other orchid species (Zimmerman and Aide, 1989; Whigham and O'Neill, 1991; Carey, 1998; Willems and Melser, 1998). Calvo (1990a) not only stated a significant positive association between flowering behaviour of *Cyclopogon cranichoides* individuals and plant size, but also described plant size as the main determinant deciding whether an individual might flower or not.

It was proposed that a species-specific minimum leaf area has to be reached before flowering can take place. Above this size-threshold the intraspecific relationship between reproductive and vegetative biomass is predicted to be linear (Willems and Bik, 1991; Willems and Dorland, 2000; Obeso, 2002). *H. hircinum* individuals in our population had to reach a certain threshold in size to be able to flower, but not all plants reaching this threshold size actually started flowering. This suggests, that there were additional factors besides the storing of resources, which influenced flowering performance. Possibly, this could be a weather-dependent induction of flowering initials or growth of reproductive structures.

Flowering frequency of the population was highly variable and this variability was significantly affected by the annual variation of the population's size class structure. The proportion of large individuals that developed more than seven leaves had a major impact. Good flowering years were not only characterized by a higher number of large-sized individuals, but a higher proportion of them flowered as compared to bad flowering years.

4.5.4 Factors controlling flowering pattern

The determination of flowering probability on the individual and the population level is a complex, dynamic process. I developed a general scheme to show how the proportion of individuals flowering in any year t was influenced and controlled by external (e.g. weather) and internal (e.g. costs of reproduction) factors (Fig. 4.7).

Size of the individuals (individuals with more than seven leaves) was a crucial component in explaining flowering pattern at the population level, and size was influenced primarily by weather and flowering history. However, weather is also assumed to be an influential factor with regard to the formation of the perennating bud containing the flowering primordia (Kull and Kull, 1991).

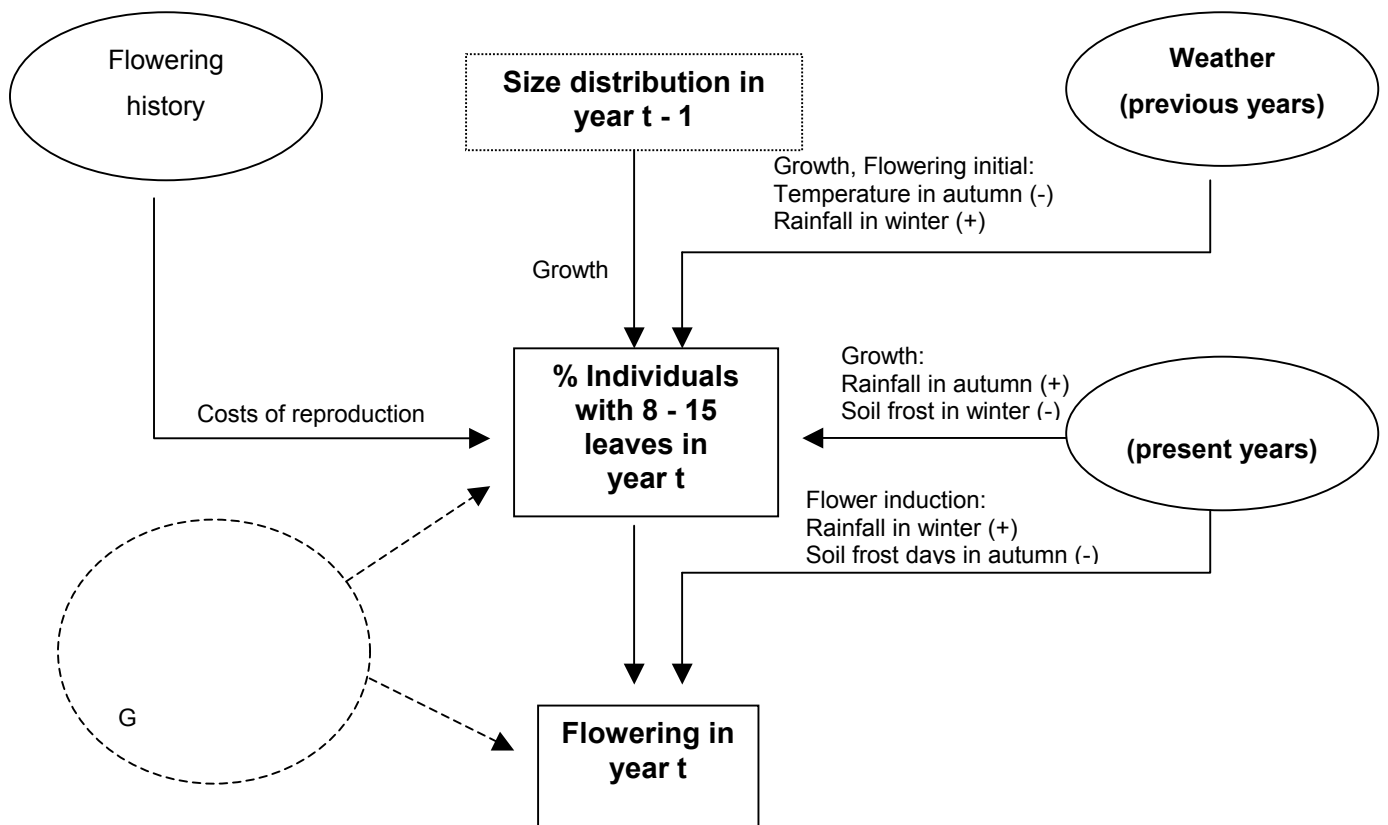


Fig. 4.7. Potential factors influencing flowering frequency of the population in each plant year. The diagram describes how flowering frequency in a year t can be controlled by a variety of external and internal factors. (+): positive effect, (-): negative effect.

I found that precipitation in winter of the current year t and in winter of the previous year $t - 1$ were important weather variables influencing flower initiation of *H. hircinum* individuals. A positive correlation between precipitation during the growing season and flowering of *H. hircinum* was also reported by Carey (1999). Winter is a critical time, since *H. hircinum* starts growing in late autumn and at least some plants have already formed larger leaves. Our results suggest that flowering initiation was negatively affected by drought in winter and also by early frosts in autumn, especially when the soil temperature was below 0°C (Table 4.3: Model F).

However, the influence of weather variables was predominantly indirect by determining plant size. The number of days with soil temperature below 0°C (SFD) in winter, especially in February, largely affected plant size (Table 4.3: Model L). *H. hircinum* plants are known to be very sensitive to temperatures below 0°C especially during winter, and leaves die back depending on the size of the plant (Carey, 1999). The frost sensitive response is further

amplified by drought stress. A longer dry period during the growing season caused leaves to turn yellow and could result in an abortion of already developed small inflorescences. I even observed that large inflorescences with fully developed flowers died from drought stress before any fruits could be produced. Plants benefited from higher rainfall and lower temperatures in autumn (especially September), i.e. the time, when leaves and roots start to grow.

The annual variation in the percentage of large individuals that actually flowered was markedly influenced by precipitation during the current year (Table 3: Model FL). Drought during winter months had a negative influence on the percentage of large individuals that flowered. The weather dependent flowering activity confirmed the suggestion of Good (1936) who already put the increasing occurrence of *H. hircinum* outside its main centre into the context of a changing climate. Warm temperature and higher rainfall in winter is typical for the Mediterranean climate, which characterizes the centre of occurrence of *H. hircinum*. Good (1936) and Carey (1996) predicted that with the change of climate (in England) towards more suitable conditions for the plant (for instance like in Western France), an northward expansion of the range of *H. hircinum* can be expected to occur. The actual increase in temperature and precipitation in the study area in the last ten years corresponds to the increase in number of populations in the area, which underlines the importance of weather for this species. Weather explained approximately 50% of the variation in the proportion of individuals with 8 - 15 leaves. However, I cannot exclude that using monthly or fortnightly weather variables or different indices of weather variability would reveal an even stronger effect.

Another important factor controlling flowering probability was the negative effect of reproductive effort on future plant size, or growth of flowering individuals (Obeso, 2002). A decrease in leaf area after flowering was also reported for *Tipularia discolor* (Whigham and O'Neill, 1991). However, our results contrast with results described by Willems and Dorland (2000), who proposed that the smaller leaf area in the year of flowering is due to the allocation of the limited underground resources to both flowering stalk and rosette at the beginning of the growing season. Because of the reproductive effort, the flowering history of the population will affect the proportion of individuals flowering in the current year t . The low FBF-value of 24.3% implied unusually high costs of reproduction for *H. hircinum*. However, as already noted by Tamm and Inghe (1988), the FBF-value is sensitive to year-to-year environmental variation, especially with respect to size or vitality distributions of mature plants, and should be used cautiously. But, flowering *H. hircinum* individuals showed a significant decrease in plant size in the subsequent year. While flowering (not only fruit production as described by Primack and Hall, 1990) had an indirect effect on the probability of flowering in the future through its direct effects on plant size, this effect was stronger for smaller plants than for larger ones. Because future growth as well as survival probability was significantly affected by the flowering process, reproduction of *H. hircinum* individuals should be resource-limited, at least on the long term (Obeso, 2002).

Flowering probability of the individuals was primarily determined by their size. While weather conditions and individual effects of reproductive effort appeared to be the main

factors responsible for the irregular flowering pattern of the *H. hircinum* population, other factors might have an influence, too. However, the potential effects of these factors, among them herbivory, intraspecific and interspecific competition, and genetic diversity, are difficult to quantify.

4.6 Conclusions

The results show that the irregular flowering pattern of *H. hircinum* was markedly dependent on size of the individuals, while age had no meaningful influence. Weather, especially extreme temperature and rainfall, is a major determinant of plant size and consequently of flowering probability of the population. However, weather also has a direct influence on the flowering initial. Reproductive effort of our *H. hircinum* population was high resulting in a decreased size after flowering. I suggest, that weather variability in combination with the individual flowering history are major factors causing the irregular flowering pattern of this orchid population.

5. Influence of geographical isolation on genetic diversity of *Himantoglossum hircinum* (Orchidaceae)

5.1 Abstract

I used AFLP markers to study variation in genetic structure within and between three populations of *Himantoglossum hircinum* (Orchidaceae) growing in central Germany. The populations were separated by a maximum distance of 10 km. The populations were separated by man-made habitat features including agricultural fields, major roads and settlements. Pairs of populations were compared to evaluate genetic variation between the populations, the genetic origin of the populations and the current level of exchange of migrants between each population. Genetic diversity was high within the populations and higher within than between the populations. Population genetic divergence was relatively high compared to other orchid species ($G_{st} = 0.20$). Gene flow between pairs of the populations differed and landscape characteristics that separate the localities appeared to be more important than geographical distance in determining gene flow. Recommendations are provided for conservation strategies for this orchid species. Management activities should concentrate on maintenance or enlargement of habitat size, and prevention of scrub encroachment.

Additional Keywords: Genetic differentiation - AMOVA - Management - Habitat fragmentation - Calcareous grassland

5.2 Introduction

When coupled with field studies, genetic studies can yield powerful insights that are relevant to conservation (Bowen, 1999). They can help in setting priorities for the protection and management of endangered species. Genetic diversity at the intra- and interpopulation levels is an important focus of interest. Factors influencing genetic differentiation between local populations and within populations include environmental-, and habitat-variability (Linhart and Grant, 1996; Mitton, 1997; Gram and Sork, 2001).

Studies of genetic diversity studies are necessary for understanding and evaluating the impact of habitat fragmentation, habitat change, and habitat management on long-term dynamics of rare species. There is special concern about conservation of rare, endangered plant species. Many endangered plants live in habitats that are becoming increasingly rare and fragmented due to human activity (Hooftman et al., 1999). Fragmentation and isolation can cause genetic erosion, and the responses of the populations regarding their genetic structure could differ as a result of differences in habitat, distance between populations, size of population, plant-pollinator interactions, and microclimatic conditions (Juan et al., 2004).

The loss of genetic variation is a major problem in conservation biology since it can prevent a species from responding to natural selection, and limit its evolutionary potential (Qamaruz-Zaman et al., 1998). In classical colonization-extinction models (MacArthur and Wilson, 1967; Levins, 1969, 1970) long-term persistence of populations in habitat fragments is maintained by regular dispersal of individuals. However, the application of the metapopulation concept is difficult for plant species. Plants not only have restricted dispersal (Ouborg, 1993), but also variability in dispersal and seed banks between remnant fragments (Freckleton and Watkinson, 2002). Several ideas have been developed from the metapopulation concept. In the source-sink model (Pulliam, 1988; Gosselin, 1996) populations that are unable to compensate for mortality by reproduction may persist in small and isolated habitat fragments if there is continued immigration from larger, so-called source populations (Pulliam, 1988). In contrast, in mainland-island models (Gotelli, 1991; Harrison and Hastings, 1996), populations on small habitat fragments maintain sufficient reproduction for survival but suffer from genetic drift and inbreeding. Dispersal of either seeds or pollen from populations in larger sites to smaller populations in marginal sites may counteract the reduction in genetic diversity, securing the long-term survival of populations in smaller fragments (Hooftman et al., 1999).

I used AFLP markers to study genetic structure of three populations of *Himantoglossum hircinum*. Amplified fragment length polymorphism (AFLP; Vos et al., 1995) is a molecular technique that is frequently used to investigate genetic diversity within and between plant species (Powell et al., 1996; Kölliker et al., 1998; Mueller and Wolfenbarger, 1999). AFLP has been used recently as an effective and efficient method of providing estimates of genetic similarity and diversity related to the current distribution among fragmented populations (Travis et al., 1996; Winfield et al., 1998; Qamaruz-Zaman et al., 1998; Richardson et al., 2003; Juan et al., 2004). The study species *H. hircinum* is a rare protected plant species that reaches the northeastern limit of its distribution in Germany

(Fig. 2.3, p. 5). It is confined to certain habitats that occur frequently in the area. A number of new populations have been arising in Thuringia, and it is of interest to know whether these populations are genetically related and whether there is still genetic exchange between them.

The following questions are addressed in this chapter. First, what level of genetic diversity is there within each of the populations of *H. hircinum*? Second, is there genetic exchange between the populations? And third, is there an influence of habitat fragmentation on population viability of the protected plant species? The answers to these questions are important for the development of management strategies for this species, since it is unclear, whether activities should concentrate on the re-establishment of suitable habitats sites as stepping stones between existing habitats, or on the maintenance of existing habitats.

5.3 Material and methods

5.3.1 Data sampling

The study sites were located within protected areas in central Germany. Three populations of *H. hircinum* were chosen, representing the main known occurrences of the species in the state of Thuringia. Geomorphological conditions at the sites were similar, consisting mainly of calcareous soils. The vegetation on the sites was calcareous grassland, growing on south-southeast facing slopes. The populations were separated by forested areas, fields, and areas settled to varying extents (Fig. 5.1). Geographical distances between the populations ranged from 3.8 km to 9.6 km.

Leaves of 18 (Site A), 19 (Site B) and 37 (Site C) *H. hircinum* individuals were randomly sampled in December 2003. Site A ("Lobdeburg") was characterized by a dense carpet of the scrub species *Prunus spinosa*, indicating an ongoing succession. The area was relatively homogenous, but I sampled individuals of *H. hircinum* from three patches within the plot (Aa, Ab, Ac). Site B ("Dohlenstein") was larger and more variable in habitat structure. Individuals were sampled from two distinct subareas (Ba and Bb), which were separated by 200 m, and one isolated plant (BIP). The largest of the three populations is found in Site C ("Leutratal"). Population C was studied extensively from 1976 to 2001 (Heinrich, 1994; Heinrich, 2000; Heinrich and Voelckel, 1999; Heinrich et al., in print) to record local spread, life history characteristics, and flowering pattern. Individuals were sampled from three distinct subareas within a permanent monitoring plot that has been unmanaged since 1976 and is characterized by an increase in shrub cover due to succession. Additionally, leaves of individuals from the neighbouring managed area were sampled.

It is recommended that DNA should be isolated from younger leaves which contain less polysaccharide-like substances. Such substances could make the DNA pellet in orchid DNA extracts more insoluble (Lim et al., 1997). Only large individuals could be sampled to minimize the impact of leaf removal on growth of the plants. Leaf samples were immediately placed in silica gel to dry prior to DNA extraction.

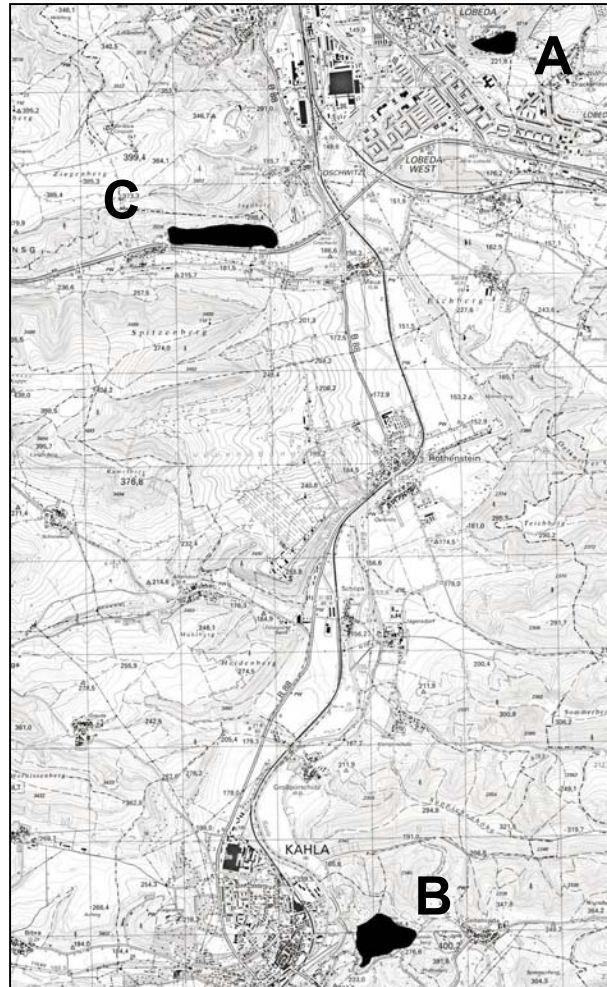


Fig. 5.1. Topographical map of the area south of Jena showing the distances between the three sampled populations as well as the River Saale (south to north), major roads and settled area (Thüringer Landesverwaltungsamt, 1993). Distances between the populations were 9.6 km (A - B), 3.8 km (A - C) and 7.9 km (B - C). The three populations are located in protected areas. Populations B and C are relatively large in size, while population A is small and covers a smaller area. One grid is equivalent to an area of one square kilometer.

5.3.2 DNA extraction and AFLP protocol

Up to 50mg of dried leaf material from each plant individual were grounded and genomic DNA was isolated using mini plasmid kits (Qiagen) according to a procedure published by Hellwig et al. (1999). DNA concentration and quality were determined by spectrophotometer and agarose gel analysis. The AFLP analysis was performed following the procedure developed by Keygene (Vos et al., 1995) with minor modifications (Krüger et al., 2002). The isolated genomic DNA 300ng / μ l was digested with a pair of restriction enzymes (EcoRI/MseI) overnight at 37°C. The genomic DNA fragments were then ligated to double-stranded EcoRI/MseI adapters for 3.5 hrs at 37°C to generate template DNA for amplification. The ligate was pre-amplified with non-selective primers (EcoRI+A, MseI+C), each having one selective nucleotide. The PCR products of the preamplification reaction

were then used as templates for selective amplification. For the final selective amplification step two AFLP primers (EcoRI and MseI) were used, which carried three selective nucleotides at the 3'-end and the EcoRI primer was fluorescein labelled (IRD 800) at the 5'-end. After randomly testing 30 different primer combinations, five primer combinations (E-ACG/M-CAC, E-ACG/M-CCA, E-ACG/M-CGA, E-ACG/M-CTA, E-ACG/M-CTT) were selected to carry out selective amplification for all individuals and for further analysis. Products from the final PCR were separated on a 6% denaturing polyacrylamide gel in 1 x TBE buffer. The amplified AFLP bands were visualized and collected with the help of SAGA^{MX}-AFLP[®] Analysis software (LI-COR Biosciences GmbH).

5.3.3 Data analysis

A data matrix was generated for each individual of the three populations in which each locus was treated as a separate character and scored as present (1) or absent (0). Only unambiguous bands were scored. The binary matrix was used to calculate genetic diversity indices of the populations and to estimate genetic distances between the populations.

Genetic structure of the populations

Gene diversity within populations and in the entire data set was calculated for each locus and averages obtained across loci using Nei's genetic diversity measure (= average heterozygosity H_e ; Nei, 1973) and Shannon's information index (Shannon and Weaver, 1949) with help of the software Popgene 1.31 (Yeh et al., 1999). Genetic diversity sensu Nei (1987), which is defined as the probability that two randomly chosen haplotypes in the same sample are different, and molecular diversity were estimated with Arlequin 2.000 (Schneider et al., 2000). TFGPA 1.3 (Miller, 1997) was used to calculate percentage of polymorphic loci both including all alleles (no criterion) and by using the 95% criterion, which treats a locus as monomorphic if the most common allele has a frequency larger than 95%.

Population differentiation

Θ ($= F_{st}$) values indicate differentiation of populations (Raymond and Rousset, 1995). This parameter ranges from "0" (absence of differentiation) to "1" (complete differentiation). To evaluate the amount of population genetic structure an AMOVA (Analysis of Molecular Variance) was carried out using Popgene 1.31 (Yeh et al., 1999). First, genetic structure indices (pairwise genetic distances; Huff et al., 1993) were estimated. The information on the difference in allelic content between haplotypes is presented in an euclidean distance metric, which was used for the subsequent AMOVA (Excoffier et al., 1992, Huff et al., 1993, Peakall et al., 1995, Michalakis and Excoffier, 1996). Nei's analysis of genetic diversity in subdivided populations (Nei, 1987) was carried out to estimate the amount of gene flow N_m between the three populations based on the G_{st} value. The amount of gene flow between pairs of populations was also estimated. The N_m value determines whether genetic drift alone can produce substantial genetic variation between locations (Slatkin and Barton, 1989).

Genetic relationships between the locations

To assess genetic relationships between the populations I calculated two genetic distances, Nei's (1978) unbiased distance and Wright's (1978) geometric average modification of Roger's distance. Distance matrices based on these metrics were produced, and they were subjected to clustering via the unweighted pair group method of arithmetic averages (UPGMA; Sneath and Sokal, 1973) using TFPGA 1.3 (Miller, 1997). The robustness of the resulting dendrograms was tested by bootstrap analysis (Felsenstein, 1985) with 1000 bootstrap re-samples to compute probabilities in terms of percentage for each node of the tree (Negi et al., 2004).

Jaccard's coefficient of similarity was calculated for all pairwise comparisons between individuals and a dendrogram was created by UPGMA cluster analysis using NTSYSpc (Rohlf, 1998). To test for the goodness of fit of the clustering of the individuals to the data set, a cophenetic value matrix was calculated and compared with the similarity matrix used for the clustering method. To measure the degree of relationship between the two matrices, the product-moment correlation, r , and the Mantel test statistic, Z , were computed.

Another method to examine separation of the individuals and populations by genetic differences is principal coordinate analysis (PCoA). This was carried out using GenAlEx (Peakall & Smouse, 2001).

5.4 Results**5.4.1 Genetic diversity within populations**

The number of fragments amplified by each AFLP primer pair varied from 37 to 65. I scored 198 bands, of which 33 were monomorphic, which means that 16.7% of all scored loci were common to all individuals analysed. Within the populations approximately 50% of the scored bands were polymorphic (Table 5.1).

Table 5.1. Genetic variation within the three populations sampled. n = number of sampled individuals; H_e = mean heterozygosity (Nei's gene diversity; Nei, 1973); S = mean Shannon's information index (Lewontin, 1972); GD = gene diversity sensu Nei (1987) = probability that two randomly chosen haplotypes from the same sample are different; MD = average gene diversity over loci = molecular diversity index; L = number of bands; PB = number of population specific "private" bands; P = percentage polymorphic loci calculated both using the 95% criterion and with all alleles included.

Population	Site	n	H_e	S	GD	MD	L	PB	$\%P$	$95\%P$
Á: Lobdeburg	A	18	0.130	0.203	1.00 ± 0.02	0.138 ± 0.07	166	4	47.5	47.5
B: Dohlenstein	B	19	0.149	0.231	1.00 ± 0.02	0.157 ± 0.08	175	13	50.0	50.0
C: Leutratal	C	37	0.140	0.221	1.00 ± 0.01	0.144 ± 0.07	170	11	56.6	43.4
Entire data set		95	0.191	0.301	1.00 ± 0.00	1.000 ± 0.00	198	-	83.3	56.1

Among the polymorphic fragments of the entire data set (165 loci, 83.3%), 28 (17.0%) were unique to one of the populations (Table 5.1).

No haplotypes were shared between all three populations. The values of average heterozygosity and Shannon's diversity index differed slightly between the populations. However, standard deviations were higher than the means, so that both values have to be used cautiously (Table 5.1).

5.4.2 Analysis of gene flow and population differentiation

AMOVA revealed that molecular variability within the populations (*d.f.* = 71; percentage of variation = 65.7) was much higher than molecular variability between the populations (*d.f.* = 2; percentage of variation = 34.3). F_{st} averaged over all loci was 0.34. A value of $F_{st} = 0.34 \pm 0.04$ ($p \leq 0.001$) was estimated by the Jackknife procedure applied across all loci (1000 replicates) to test the stability of the result.

Only a small proportion of the total variation was partitioned between populations ($G_{st} = 0.20$). Gene flow N_m within the species was 2.0. F_{st} values and gene flow between pairwise combinations of the populations varied greatly (Table 5.2). There was higher gene flow between populations A and B but low gene flow between populations A and C and populations B and C (Table 5.2).

There was a negative correlation between the genetic distance matrix based on Nei's (1978) genetic distance and the geographic distance matrix ($r = -0.82$, $p < 0.01$). However, sample number was low ($N = 3$). Gene flow between the sampled subareas within the populations was high (population A: 3.18, population B: 4.24 (the isolated plant was ignored), population C: 2.22 (between patches Ca and Cc) - 5.41 (between Ca and Cb).

Table 5.2. Pairwise genetic differences (F_{st}) between the population samples (below diagonal). Estimate of gene flow N_m from G_{st} between the populations (above diagonal). $N_m = 0.5 * (1 - G_{st}) / G_{st}$. All F_{st} values are significant (1000 permutations).

	Population A	Population B	Population C
Population A	-	3.97	1.42
Population B	0.16	-	1.55
Population C	0.39	0.37	-

5.4.3 Genetic distances between the populations

The dendrograms generated from the AFLP data using the UPGMA method based on the different genetic distance metrics varied only slightly. Clustering of populations based on Nei's (1978) distance (Table 5.3), and modified Roger's distance (Wright, 1978) revealed that population A clustered together with population B, while population C was isolated from this subcluster (Fig. 5.2).

Table 5.3. Pairwise genetic distances based on Nei's unbiased genetic distance (1978)

	Population A	Population B	Population C
Population A	-		
Population B	0.04	-	
Population C	0.11	0.11	-

The UPGMA dendrogram based on Jaccard's coefficient of similarity fitted the observed data well as indicated by the high and significant cophenetic correlation coefficient of the AFLP data ($r = 0.87$; $Z = 38.69$; $p < 0.005$). Two large clusters were revealed. Individuals of population A and B clustered together, and population C was clearly separated from them. The dendrogram furthermore showed a spatially influenced genetic separation of the individuals. Individuals which grew closer to each other were more genetically similar than individuals located further away from each other (Aa, Ab, Ac). Two subclusters were revealed within population C. Most individuals sampled from the managed area (Ca) formed a subcluster with one group of individuals sampled in the permanent plot (Cb).

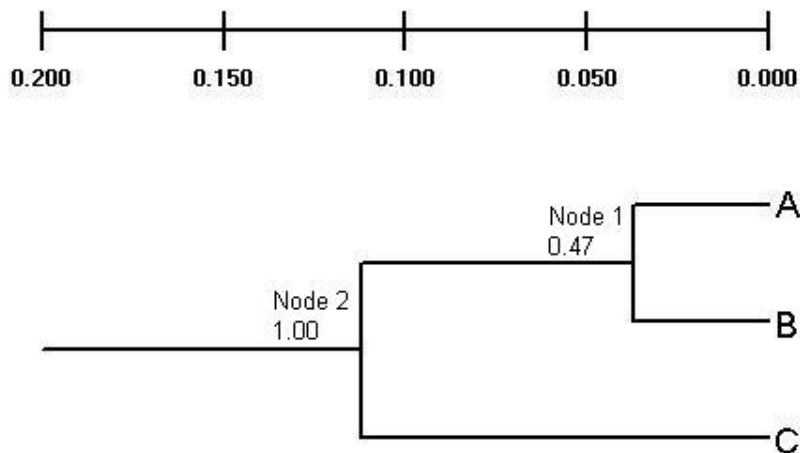


Fig. 5.2. Dendrogram of the three populations of the three sites (A-C) based on AFLP polymorphisms. Cluster analysis was performed using the UPGMA method with Nei's (1978) distance metric. No bootstrap samples (1000 permutations) resulted in trees containing ties. Number at node = Proportion of similar replicates in bootstrap procedure (1000 permutations). The scale indicates the degree of genetic distances (Nei's (1978) distance metric) between the populations.

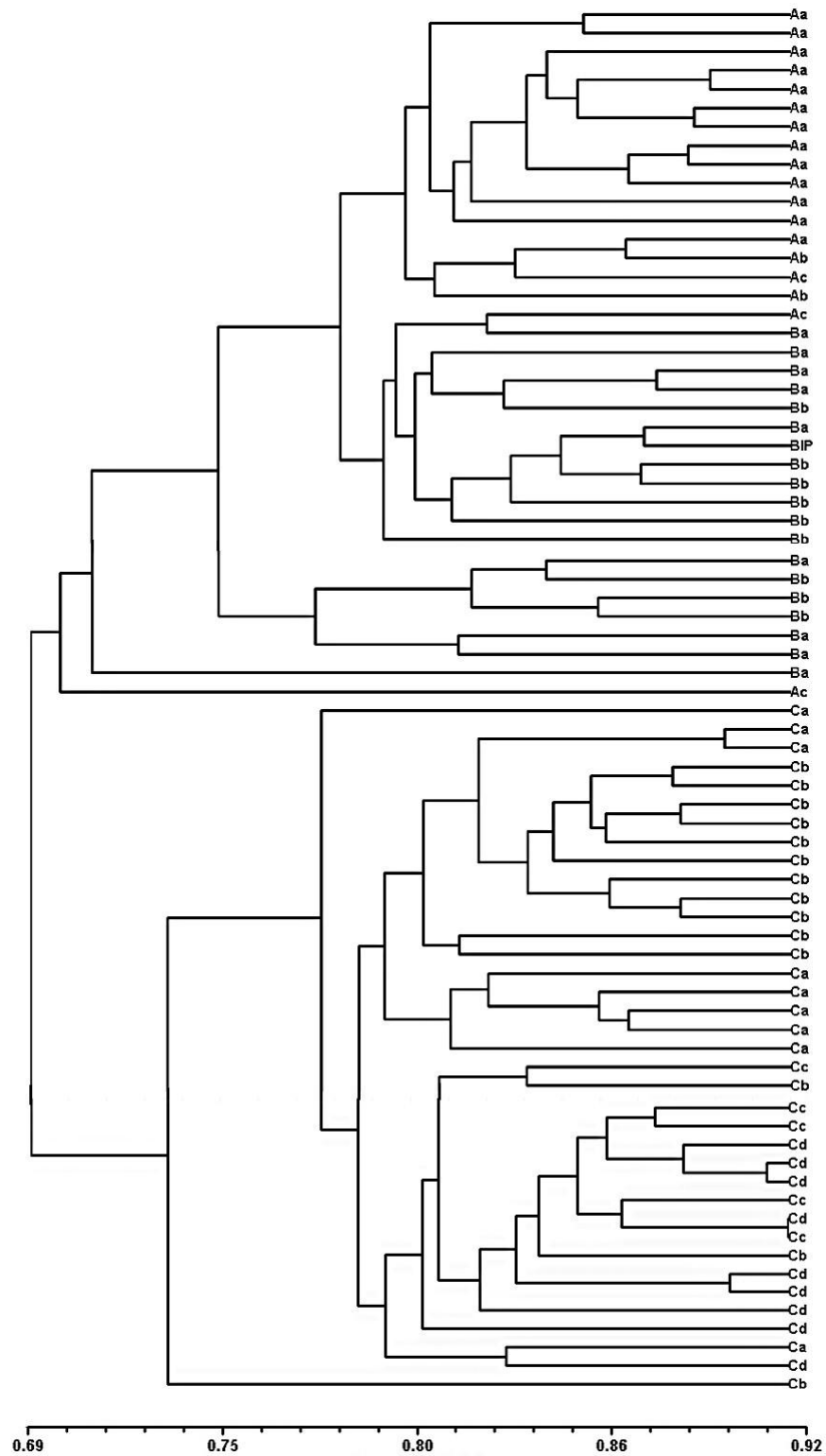


Fig. 5.3. UPGMA dendrogram based on Jaccard's coefficient of similarity for pairwise comparisons of all individuals. The individuals are named according to their localization. Three patches at site A, two patches and one isolated plant (BSP) at site B and four patches at site C are distinguished. The scale indicates the degree of genetic similarity (Jaccard's coefficient).

PCoA was applied via distance matrix with data standardization. The first three axes explained 48.6% of the variation in the distance matrix (Axis 1: 34.0%, Axis 2: 8.0%). The PCoA (Fig. 5.4) reinforces the outcome of the UPGMA analysis. Population C was clearly separated from populations B and C, which were closely related. Individuals from the other two patches sampled in the permanent plot (Cc, Cd) predominantly grouped together in a second subcluster (Fig. 5.3).

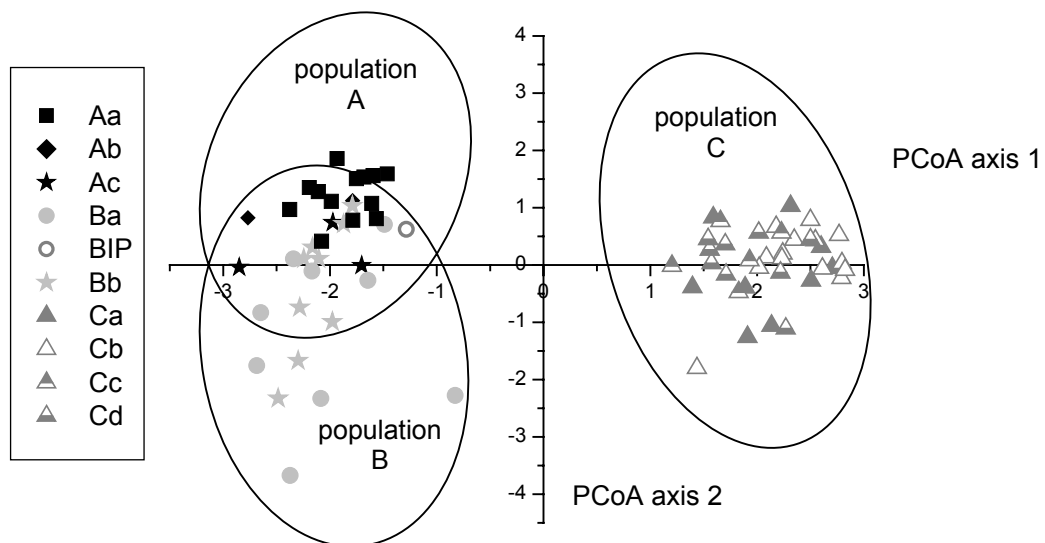


Fig. 5.4. PCoA ordination of the data set, including all individuals and 198 loci. Axis eigenvalues were calculated using GenAlEx (Peakall and Smouse; 2001).

5.5 Discussion

5.5.1 Within population genetic diversity

Moderate levels of genetic variation within populations were found. However, a selected set of primer combinations had been used, and it is possible that different primer combinations would reveal higher genetic diversity. High genetic diversity in plants is regarded as a typical characteristic of plants with wide geographical ranges, outcrossing and wind-pollination (Hamrick and Godt, 1990). Hamrick and Godt (1990) suggest that wind-pollinated species generally have higher levels of genetic variation than both animal-pollinated and selfing species. *H. hircinum* is believed to be animal-pollinated (Carey and Farrell, 2002). However, self- and wind-pollination is possible, and the occurrence of the species over a large geographical range could be a result of rare long-distance seed dispersal events by wind.

In contrast, it is assumed, that low levels of genetic variation in plant species result from high levels of asexual reproduction and self-pollination (Stenström et al., 2001). All

H. hircinum individuals were found to be different in their banding pattern, and no haplotypes were shared by the populations, suggesting that the populations are large enough and can be regarded as intact with regard to random mating and the maintenance of a genetic equilibrium (Harrison and Hastings, 1996).

5.5.2 Population differentiation and interconnectedness

Differential gene flow by seed or pollen dispersal is believed to be one of the processes, through which habitat variability can influence genetic diversity (Schaal, 1975; Waser, 1987). Therefore, by assessing the degree of genetic diversity or relatedness between the populations and subpopulations, one can estimate the extent of gene flow between them. The Analysis of Molecular Variance showed that genetic variation within a population was higher than that between the populations. Population divergence measured by F_{st} was relatively high compared to other orchid species. Published estimates of population differentiation (G_{st} and F_{st}) range between 0.015 and 0.45 with a median of 0.072 (Alexandersson and Agren, 2000). However, high levels of population differentiation were often found for populations separated by large distances. Case (1994) found a high genetic differentiation between three populations of *Cephalanthera reginae* which were separated by up to 185 km. Genetic differentiation estimated for 8 populations of *Orchis palustris* separated by up to 2150 km was 0.448 (Arduino et al., 1996). When smaller distances are involved, differentiation is often much lower, with G_{st} values ranging from 0.023 (*Orchis provincialis*; Scacchi et al., 1990) to 0.104 (*Cephalanthera longifolia*; Scacchi et al., 1991). The estimated differentiation between seven populations of the non-rewarding species *Cephalanthera rubra* (maximum distance: 17 km; $G_{st} = 0.25$; Scacchi et al., 1991) was similar to the G_{st} value of *Himantoglossum hircinum* (maximum distance 9 km; $G_{st} = 0.20$).

Greater genetic differentiation between populations is reported to be typical for rewarding animal-pollinated species. These species are often characterized by a high percentage of near-neighbor matings (Fenster, 1991), which may restrict pollen dispersal distances. In contrast, low levels of genetic differentiation ($G_{st} = 0.10$) between *Catasetum viridiflavum* populations is attributed to the joint effects of long-distance pollen/pollinator movement, and the potentially long dispersal distances of the tiny wind dispersed seeds of orchids (Murren and Ellison, 1998; Murren, 2003). It is still unclear whether *H. hircinum* flowers produce nectar, but various insects including bumble bees, hoverflies and butterflies have been seen visiting *H. hircinum* flowers, and solitary bees (*Megachile maritima*) have been observed carrying pollinia of *Himantoglossum* individuals (Willis, 1990; Carey and Farrell, 2002). Since the landscape between the orchids' habitats is highly fragmented, animal dispersal of pollen between the populations is further restricted and should decrease with distance. Additionally, mean dispersal distance of seeds is predicted to be only 1 m (Carey, 1998), although the random distribution of small populations seems to result from rare long-distance dispersal events caused by wind-updrafts or man (Carey and Farrell, 2002).

The calculated levels of gene flow between pairs of populations suggests that there is an exchange of seeds or pollen between population A and B, while population A and C and

population B and C, respectively, were more isolated from each other. Gene flow between them was restricted, probably as a consequence of the distance separating them, the structure of the habitat between them and the species' dispersal mechanism. The sampled patches within the populations were separated by 200 m within population B, and by 50 m within population A. The apparently high gene flow between the sampled patches within the populations indicates that an exchange of seeds or pollen is likely over such distances as long as there is no dispersal barrier.

Reduced pollination and seed dispersal within and between fragmented habitats are believed to cause reduced reproduction (Fischer and Matthies, 1997, 1998). Small populations are subject to increased genetic drift and reduced genetic diversity, which results in loss of fitness (Oostermeijer et al., 1994b, 1995; Koelewijn, 1998). This can lead to "senile" populations lacking juvenile plants and seedlings (Oostermeijer et al., 1994a). Sexual reproduction in all three investigated *Himantoglossum hircinum* populations appears to be high, since many seedlings have been found surrounding large individuals. All three populations contain a high proportion of juvenile plants (Heinrich et al., in print; Pfeifer et al., submitted). The extinction risk due to stochastic effects may be low, since the level of genetic diversity is moderate, permitting the possibility of evolutionary responses to changing conditions. The value of N_m was greater than "1" indicating that gene flow is sufficient to prevent substantial differentiation due to genetic drift (Slatkin and Barton, 1989).

5.5.3 Genetic relationships between the populations

The cluster analysis revealed that population A was genetically more closely related to population B than to population C, despite the larger distance between populations A and B. Both populations A and B lie on the eastern side of the Saale River, and the land between them is mainly open country. It is likely that population A was founded by seeds from population B, following the prevailing wind direction through the valley, because population B is bigger in number, covers a larger area and known in the 1930s, whereas population A has only existed since the 1960s. One would expect a higher gene flow between populations A and C than between A and B, because of the smaller distance between them. However, the land between populations A and C is mainly industrialized land including major roads. The presence of such barriers, together with including hills and a large river, presumably restricts dispersal of seeds and pollen, resulting in lower gene flow between them.

5.5.4 Implications for conservation

One goal of genetic conservation programs is to maintain natural levels of genetic diversity in order to preserve the species' evolutionary potential. The success of these programs depends on a better understanding of the level and distribution of genetic variation present in the gene pool (Qamaruz-Zaman et al., 1998). Molecular genetical methods provide us with information on the current state of populations and species. They are especially useful in combination with long-term observations regarding life-history characteristics and response

of plant individuals to a change in environmental conditions including changes in habitat characteristics, climatic conditions and presence of competing organisms.

AFLP has been used recently to study the influence of fragmentation on gene diversity of plant species. Juan et al. (2004) determined the patterns of genetic variability and gene flow among populations of the endangered shrubby species *Medicago citrina* which declines due to habitat fragmentation, and they give recommendations for management strategies to facilitate conservation effort. Qamaruz et al. (1998) studied the genetic structure of *Orchis simia*, a rare and endangered orchid species in the UK.

Our populations seem to be of sufficient size to maintain genetic variability by reproduction and by genetic exchange between patches within the populations. Rare long-distance dispersal of pollen or seeds may assist in homogenizing the gene frequencies between populations (Hooftman et al., 1999). However, since *H. hircinum* is restricted to certain habitat types, a loss of habitat can have serious effects. Therefore management activities should concentrate on the preservation or increase of the current habitat structures. The pollen exchange between populations via animals is difficult to improve, since the localities occupied by the species are separated by large distances and human-made barriers including roads, industrial area and settlements.

Another focus of interest should be an increase in flowering probability and fruiting success of the plants. It was observed that *Himantoglossum* plants show decreased flowering probability or fruit set when shrub encroachment takes place. The timing of mowing to prevent such succession should be coordinated with the flowering/fruitlet activity of the plants. Since observations in population C showed, that plants perform best in patches with high and dense grass cover, probably due to more suitable microclimatic conditions, mowing every 5 or 10 years should be sufficient.

6. Spatial pattern and dynamics of *Himantoglossum hircinum*

6.1 Abstract

In order to understand plant population dynamics, spatial pattern need to be described and quantified in addition to temporal characteristics. Because spatial pattern is the result of past processes, it can be used to test some hypotheses about how controlling or environmental processes work. In this chapter, the spatial dynamics of the *Himantoglossum hircinum* population studied between 1976 and 2001 was analysed. Spatial autocorrelation statistics was applied to investigate spatial dynamics on local scales on the permanent monitoring site over the 26 years. Spatial point pattern statistics was used to test for local distribution pattern on a small scale. And individual performance under different habitat conditions was analysed. The permanent monitoring site was divided into 10 m x 10 m "cells", when it was laid out. The positive spatial autocorrelation between the cells regarding the number of individuals in them increased during the period studied, suggesting that spread of the population is restricted by short distance seed dispersal. The results of the spatial point pattern analyses underline the result. *H. hircinum* individuals were found in small aggregates, because they emerge from seeds that often remain lodged in the capsules falling to the ground near the parent plant. Growth of the individuals did not differ between the habitats defined, suggesting that dispersal is more important. However, flowering probability and fruit set were limited in patches with high density of tall scrub plants.

Additional Keywords: Moran's I - Geary's C - Competition - Habitat heterogeneity - Long-term dynamics

6.2 Introduction

In order to understand plant population dynamics, both spatial and temporal characteristics need to be described, quantified, and related to underlying processes such as establishment, growth, competition, reproduction, and mortality (Dale, 1999). For example in nature, population dynamics underlie spatial constraints, which require further modelling approaches than describing temporal aspects only (Czárán, 1998). Such spatial constraints are often manifested in spatial patterns of plant distribution on a range of spatial scales.

Because spatial pattern is the result of past processes, it can be used to test some hypotheses about the influence of environmental processes (Ford and Renshaw, 1994; Perry et al., 2002). Spatial patterns of plants may result from different processes and forces such as seed dispersal, competition, disturbance, herbivory, or environmental heterogeneity (Sterner et al., 1986; Wiegand and Moloney, 2004). Since plants of one species can have a positive or negative effect on the occurrence and spatial arrangement of another species, one important effect of spatial pattern of one species is its effect on other plants, which can be facilitating or inhibiting (Dale, 1999). This is relevant in the analysis of the effects of vegetation succession and in management practice.

Dispersion is a concept that refers specifically to the arrangement of points in a study plot. Three patterns of dispersion can be observed. Plants can be randomly dispersed, overdispersed (regular) or underdispersed (clumped) and a range of causal mechanisms can be invoked. Clumped patterns can result from environmental heterogeneity, with organism clustering together in areas of favourable conditions, short-distance dispersal, and vegetative reproduction (Dale, 1999). Some inhibition processes such as competition for resources may lead to overdispersion of positions (Dale, 1999). The statistical analysis of point mappings of all plant individuals within a defined area is an exact method to investigate spatial patterns in plants (Ripley, 1981; Upton and Fingleton, 1985; Stoyan and Stoyan, 1994; Dale, 1999). Second-order statistics, such as Ripley's K-function or the pair-correlation function, use the information on all interpoint-distances to describe the small-scale spatial structure of the point pattern, over a range of distance scales (Wiegand and Moloney, 2004). Thus, they provide scale-dependent detailed information on pattern. If the pattern is not random at all scales, further questions may deal with the underlying processes that give rise to these non-random patterns, e.g. regeneration behaviour and dispersal strategy of the plant species.

The natural habitat of a species is often very heterogeneous. Individual performance may vary depending on habitat characteristics including soil properties, vegetation cover, slope, and inclination. To understand life history traits and their plasticity, it is necessary to compare individuals according to their localization within a network of differing habitat patches. In this work, the spatial pattern of *Himantoglossum hircinum* individuals on a range of spatial scales is described. *H. hircinum* is a rare and protected orchid species, that, contrarily to most other orchids, is increasing its range of distribution in Germany, the northeastern border of worldwide distribution. Additionally, the studied population has increased exponentially in size. Several hypotheses were proposed to explain this pattern. Individual and population performance, i.e. increase in population size and flowering probability, appeared to have

improved due to changed weather conditions (Pfeifer et al., submitted). The regional spread to the north was attributed to the change in climate conditions that allowed establishment of new populations (Good, 1937; Carey, 1999; Carey and Farrell, 2002). It has been observed that the species seemed to be clumped on the local scale (cm to ha) within a study site. Carey (1999) reported that most seeds fall to the ground beside the parent plant resulting in a clumped occurrence of seedlings. Further important determinants of seedling establishment might be the distribution of the appropriate mycorrhizal fungi, the presence of competing rosette plants, and the outshading of individuals by increasing scrub cover (Carey and Farrell, 2002).

In detail I want to test following hypotheses.

- 1) Spatial spread is slow and largely attributed to restricted seed dispersal.
- 2) Individual performance is influenced by spatial heterogeneity of the habitat.
- 3) Individuals occur in clumps on a small spatial scale and this can be explained by short distance seed dispersal.
- 4) Scrub individuals and rosette plants suppress growth of *H. hircinum* seedlings.

6.3 Methods

6.3.1 Spatial autocorrelation analysis

Spatial autocorrelation was used to find out, whether cells with relative large numbers of individuals are surrounded by similar cells, by cells with low individual number, or whether there is no pattern. Spatial autocorrelation exists whenever there is systematic spatial variation in values across a map (Upton and Fingleton, 1985). If high values at one locality are associated with high values at neighbouring localities, the spatial autocorrelation is positive, and when high values and low values alternate between adjacent localities the spatial autocorrelation is negative. In the case of a quadratic spatial grid, neighbourhood relations may be defined as Rook's case, Bishop's case or Queen's (King's) case (Fig. 6.1).

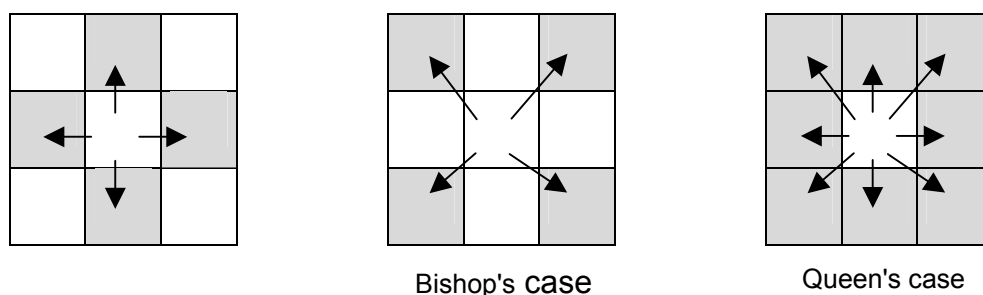


Fig. 6.1. To measure spatial autocorrelation a measure of contiguity is required: Neighbourhood relations are defined as either Rook's case (neighbourhood of four cells adjacent to each cell), Bishop's case (neighbourhood of four cells diagonally adjacent to each cell) or Queen's (King's) case (neighbourhood of all eight cells is considered).

The permanent monitoring site was divided into 10 m x 10 m squares ("cells"), when it was laid out in 1976 (Fig. 6.2; Heinrich et al., 1999). During the annual census, size and flowering of all individuals within the cells of the monitoring site was recorded. Thus, data on the annual number of emergent individuals as well as on the annual number of flowering individuals in the squares are available for the period 1977 to 2001.

Spatial autocorrelation between the cells regarding their annual number of individuals was calculated using ROOKCASE (Sawada, 1999). Two indices were measured: *Moran's I* and *Geary's C*. *Moran's I* (Moran, 1950) compares the value of the variable at any one location with the value at all other locations.

$$I = \frac{N \sum_i \sum_j W_{ij} (X_i - \bar{X})(X_j - \bar{X})}{(\sum_i \sum_j W_{ij}) \sum_i (X_i - \bar{X})^2} \quad \text{Eq. 6.1}$$

N is the number of cases,

X_i is the variable value at a particular location i ,

X_j is the variable value at another location j ,

\bar{X} is the mean of the variable, and

W_{ij} is a weight applied to the comparison between location i and location j .

(W_{ij} is a contiguity matrix and it compares the sum of the cross-products of values at different locations, two at a time weighted by the inverse of the distance between the locations.)

Moran's I values range from "-1" to "+1", with "-1" indicating a strong negative spatial autocorrelation, "0" indicating a random pattern and "+1" indicating a strong positive spatial autocorrelation. For *Geary's C* (Geary, 1954), the interaction is not the cross product of the deviations from the mean, but the deviations in intensities of each observation location with one another. *Geary's C* is inversely related to *Moran's I*. It does not provide identical inference because it emphasizes the differences in values between pairs of observations, rather than the covariation between the pairs. And while *Moran's I* gives a more global indicator, the Geary coefficient is more sensitive to differences in small neighborhoods (Lembo, 2003).

$$C = \frac{[(N-1) \sum_i \sum_j W_{ij} (X_i - X_j)^2]}{2(\sum_i \sum_j W_{ij} (X_i - \bar{X})^2)} \quad \text{Eq. 6.2}$$

Geary's C values range from "0" to "+2", with "0" indicating a strong positive spatial autocorrelation, "+1" indicating a random pattern and "+2" indicating a strong negative spatial autocorrelation. Randomization (Monte Carlo Procedure) was used to test for statistical significance of the results.

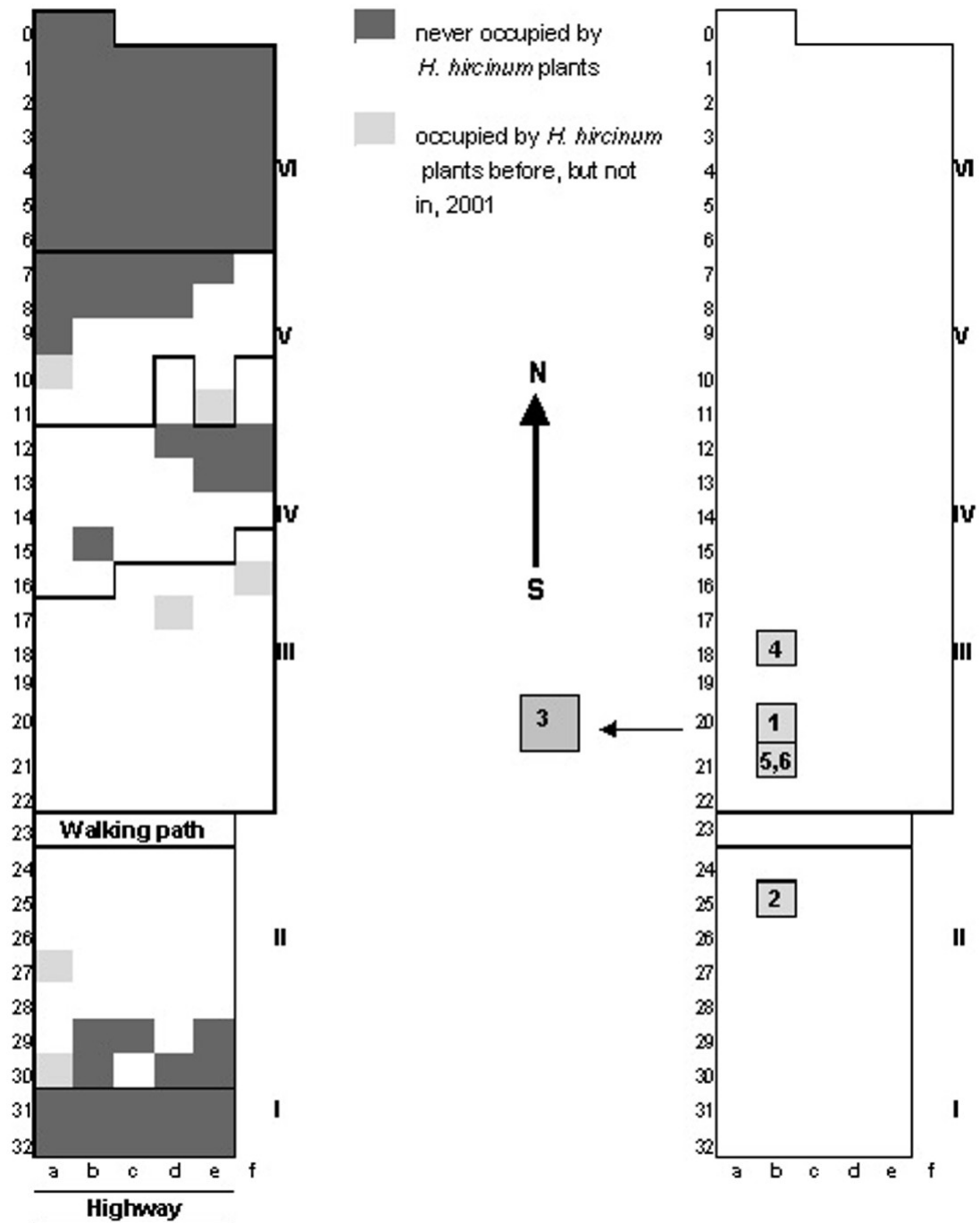


Fig. 6.2. Internal structure of the permanent monitoring site in the nature reserve Leutratal. The plot was classified into six subplots according to geomorphological differences. The subplots were further divided into 10 m x 10 m cells (0a to 32f). The localization of the six small-scale spatial plots used for the point pattern analysis (SP 1-6) is given on the right hand side (Table 6.1). SP 3 was located approx. 100 m to the west of the permanent monitoring site.

6.3.2 Spatial variability of individual and population performance

Using the long-term data set, the growth rate of the population was calculated for each cell using an exponential growth model. The flowering pattern of the individuals, e.g. flowering frequency and flowering percentage, was also determined for each cell.

Individual length growth was recorded every two weeks during two growing seasons 2002/2003 and 2003/2004. The recorded individuals were grouped according to their location in four possible habitat groups. Individuals on the annually mown area under direct sunlight (M_O) and on management patches shaded by larger shrub (M_S), individuals on the permanent plot within high grass cover (PP_O) and within dense shrub cover (PP_S). Individuals of the four habitats were tested for significant differences in length growth rate and final number of leaves using one-factorial ANOVA with habitat as grouping variable, and (whenever necessary) the non-parametric Kruskal-Wallis test and the Median-test.

In July, flowering individuals were randomly selected to record the number of flowers, the length of the inflorescence and the flowering stalk, and the number of fruits. Fruit set was calculated as the proportion of flowers that developed into mature capsules containing seeds. It was possible to investigate the fate of the flowering and fruiting individuals in the second year after the first season. One-way ANOVA was applied to test for significant differences in individual performance depending on habitat type using habitat as grouping variable.

6.3.3 Intra- and interspecific relationships: point pattern analysis

In 2003, small plots (1 m² to 9 m²) were laid out to study intra- and interspecific spatial relationships on a small spatial scale (Table 6.1). X-, Y- coordinates of all individuals of *H. hircinum* as well as of all other plant species present (except grasses and mosses) were recorded. The pair-correlation function $\hat{g}(r)$ was applied to analyse the univariate point patterns of *H. hircinum* plants to test for density effects (intraspecific competition) on different spatial scales. The bivariate mark-correlation function was used to analyse spatial point pattern relationships between *H. hircinum* plants and individuals of plant species assumed to have an influence on *H. hircinum* to test for potential effects of dispersal (spatial relationships between seedlings and large individuals), of shading by scrub plants, and of interspecific competition induced by scrub regrowth and occurrence of rosette plants (Table 6.1).

Both methods give the expected number of points at distance r from an arbitrary point, divided by the intensity of the pattern. In the univariate ($\hat{g}(r)$) and the bivariate ($\hat{g}_{12}(r)$) case distance r was increased from 0 cm to 40 cm in steps of 1 cm. Significance of the pattern in the observed data was evaluated by comparing them with a null model of complete spatial randomness (CSR; Stoyan and Stoyan, 1994). The CSR confidence envelope was calculated by Monte Carlo randomization (1000 simulations) of the location of the observed data points. Values of $\hat{g}(r) > 1$ indicate that small interpoint distances of r are more frequent, values of $\hat{g}(r) < 1$ that they are less frequent than expected under CSR. If the sample statistic was below the confidence band the pattern was assumed to be regular, if the sample statistic was above the confidence envelope, the pattern was assumed to be clumped (Schurr et al., 2002).

Table 6.1. "Spatial Plots" (SP) that were created to study intra- and interspecific competition effects using spatial point pattern analysis. Squares are labelled according to their location on the permanent monitoring site (Fig. 6.2)

SP	Size	Localisation	Vegetation	Aim
1	3 m x 3 m	Square 20b	shrub cover, high grass, shade	Influence of shade by <i>Crataegus monogyna</i> and <i>Rosa spp.</i>
2	3 m x 2 m	Square 25b	dense carpet of <i>Prunus spinosa</i> seedlings, sunny	Competition with regrowth of <i>Prunus spinosa</i>
3	2 m x 2 m	Managed area	sunny, low but dense grass, moss	density effects, interspecific competition with rosette plants
4	1 m x 1 m	Square 18b	tall shrub individuals, deep shade, leaf litter, no grass	density effects, interspecific competition with rosette plants (<i>Primula veris</i> , <i>Plantago lanceolata</i> , <i>Cirsium acaule</i>)
5	1 m x 2 m	Square 21b	high and dense grass, moss cover	spatial pattern of <i>H. hircinum</i>
6	1 m x 2 m	Square 21b	high and dense grass, moss cover	spatial pattern of <i>H. hircinum</i>

6.4 Results

6.4.1 Spatial autocorrelation analysis

Moran's I and *Geary's C* values indicated positive spatial autocorrelation when using Rook's case as contiguity measure (Fig. 6.3). The constant increase in the degree of positive autocorrelation from 1980 to 1992 has stopped in the years afterwards at an intermediate level. Values of both *Moran's I* and *Geary's C* were significant in all years ($p < 0.05$). The positive autocorrelation was higher, when Bishop's case was used as contiguity measure. However, while values of *Moran's I* were significant in most years ($p < 0.05$; not in 1977 and 1980-1983), randomization procedure of *Geary's C* values showed non-significance in all years ($p > 0.05$). A positive spatial autocorrelation pattern also was shown when using Queen's case as contiguity measure. *Moran's I* values were significant in all years, and *Geary's C* values in most years ($p < 0.05$; not in 1978 - 1986).

6.4.2 Spatial variability of population characteristics

The rate of increase in number of individuals between 1977 and 2001 differed between the squares. To find a general pattern, and to avoid errors because of too low numbers of individuals, cells were combined into larger cells and the growth rates in these new cells

were calculated (Fig. 6.4). The exponential growth curve was fitted to the data. Cells with high growth rates were separated by cells with low growth rates. There were also cells that were never occupied during the total time period (Fig. 6.2.).

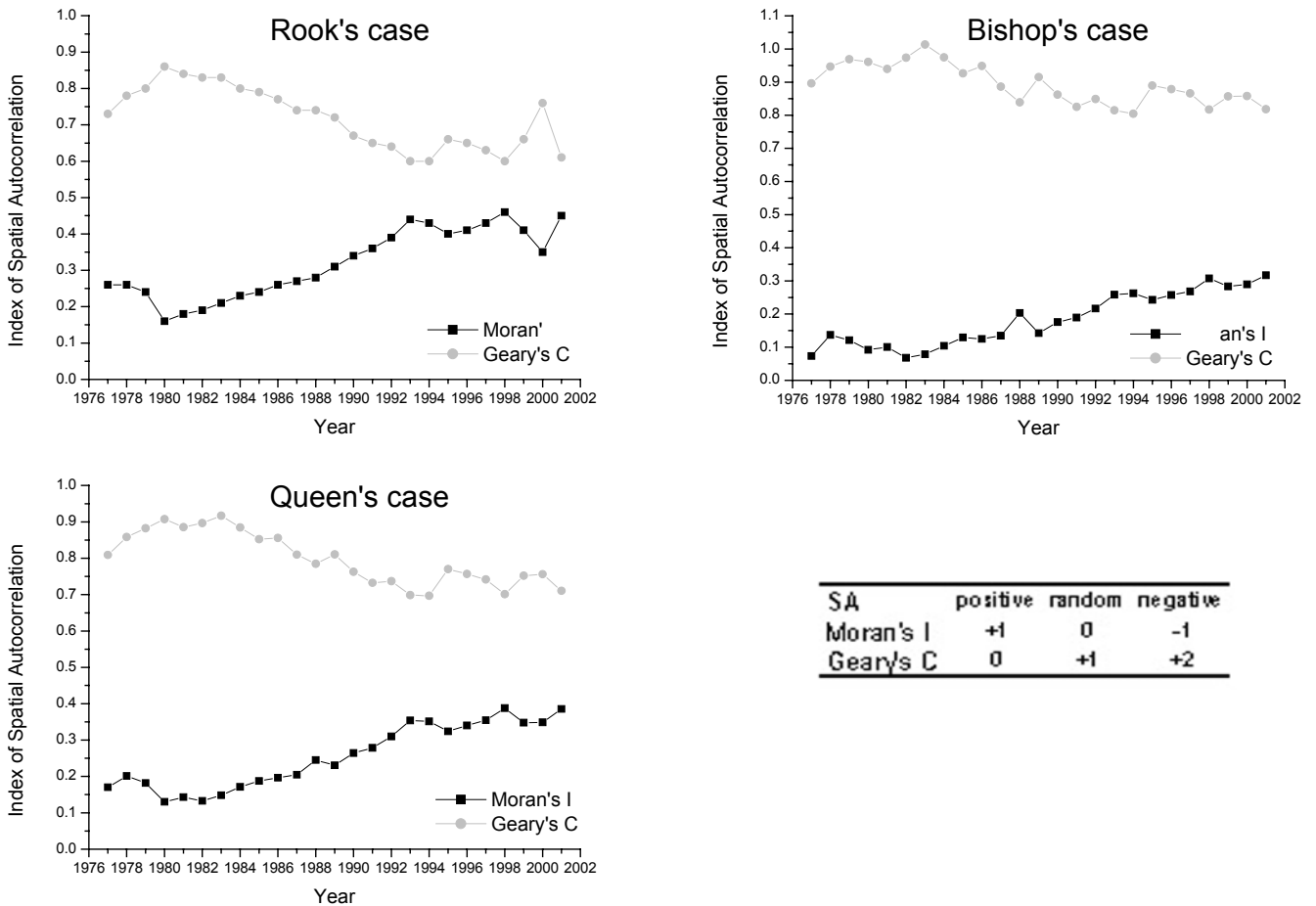


Fig. 6.3. Spatial autocorrelation (SA) between the cells (10 m x 10 m squares of the permanent monitoring site) regarding the number of individuals calculated for three different measures of neighbourhood relations (Rook's case, Bishop's case, Queen's case) between the cells.

Time series analysis revealed that the main direction of spatial spread was towards the southern-east part of the permanent plot starting at 17b.

From 1977 to 2001 flowering individuals were found mainly in subarea III (Fig. 6.2). However, in 2003 and 2004 most flowering individuals had been found in subarea II, while only few individuals were flowering in subarea III and IV. Percentage of individuals flowering varied strongly over the squares with values ranging from "0%" ($N = 1-59$) to "100%" ($N = 1 - 2$) in a year.

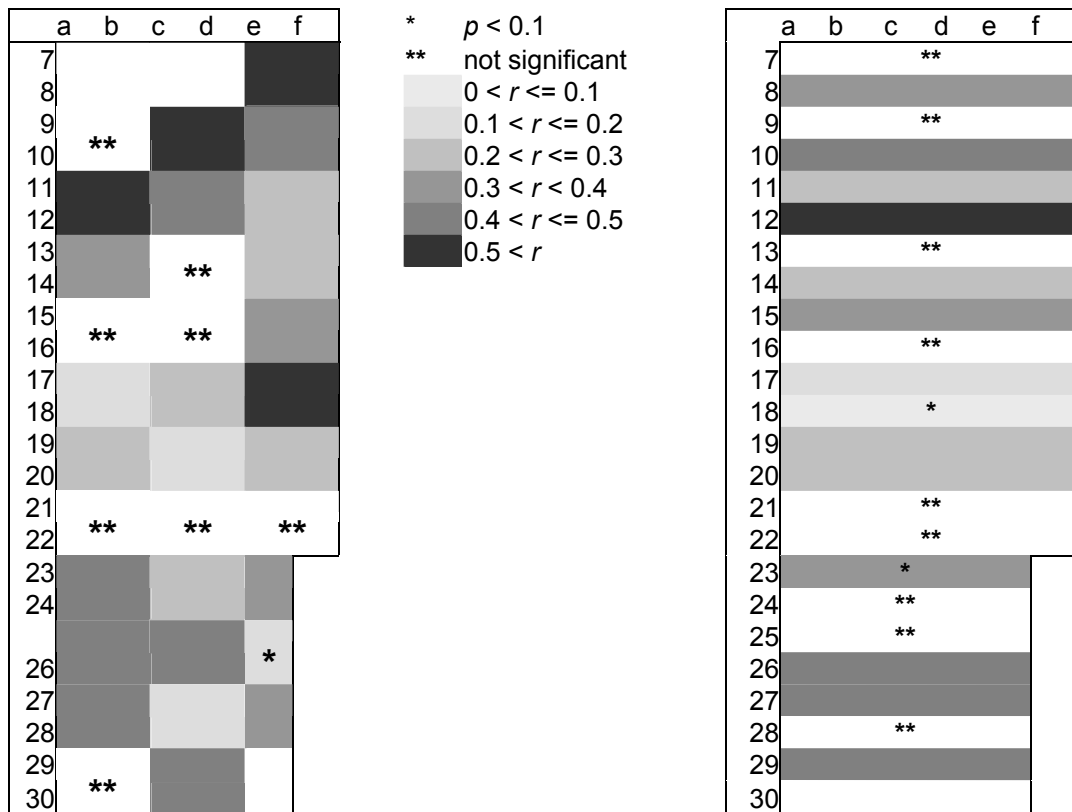


Fig. 6. 4. Spatial dependence of the increase in number of individuals with time. The exponential model fitted the data in most cases (exceptions indicated by **XX**: $p > 0.05$). In the exceptions, individual number was often low varying between one and 10 individuals. However, in some cases number of individuals was low and variable first, followed by a strong increase (right figure: row 13, 16, 21, 28), or reaching an "equilibrium" (right figure: row 22, 23) The different colours indicate the strength in growth rate with values ranging from low (0.01: light grey) to high (0.98: black). *H. hircinum* plants were not found in the squares 0a - 6f between 1976 and 2001.

6.4.3 Spatial variability of individual performance

Length growth rates varied among the individuals. Only individuals were compared, for which data were available from November 2002 onwards, for which at least three data points were available, and taking into account only data points before dieback due to drought, frost or senescence occurred. A linear regression was applied to compare the slopes of the regression lines calculated for total length of all leaves of the plant vs. time. However, the linear model did not fit in all cases, and in some cases, slow growth in winter was followed by an exponential increase in size starting in March of the next year.

Of the 60 individuals investigated in 2002/2003 (season "1") 6.7% ($N = 4$) disappeared before March, and another 21.7% ($N = 13$) disappeared from above ground before May. 14 of those were damaged by soil frost and/or drought, while the leaves of the other three ones were completely eaten by herbivores. Eight of these 17 individuals were located in the shrub regrowth patches on the permanent plot. A significant influence of habitat group on length growth rate or maximum number of leaves of the individuals could not be detected in season "1" or season "2" (2003/2004; Table 6.2).

Table 6.2. Influence of habitat characteristics on individual performance. gr_{length} = length growth rate of the leaves; MLN = maximum number of leaves; season "1" = October 2002 - August 2003; season "2" = October 2003 - August 2004; PP = permanent plot; M = Managed area; O = open, direct sunlight; S = shade by shrubs.

Habitat	$N_{recorded}$	$N_{analysis}$ season "1"	Average $gr_{length} \pm SD$ (season "1") in cm/month	Average $gr_{length} \pm SD$ (season "2") in cm/month	Average $MLN \pm SD$ (season "1")
PP_O	11	7	1.87 ± 0.13	2.63 ± 0.81	4.29 ± 1.38
PP_S	19	12	2.29 ± 1.42	1.80 ± 1.34	3.08 ± 1.98
M_O	24	12	2.11 ± 0.95	3.07 ± 1.23	3.08 ± 1.38
M_S	6	4	2.94 ± 1.11	7.75 ± 5.10	4.75 ± 1.71

The assumption of variance homogeneity was not violated when ANOVA was applied to test for significant differences in length growth rates in season "1" and to test for differences in maximum number of leaves of the individuals in season "1" (Levine's test: $p > 0.05$). However, the assumption of variance homogeneity was violated in the other two cases (Fig. 6.5), and consequently non-parametric tests (Kruskal-Wallis test and Median test) were used to test for significant differences. There was no influence of habitat on performance of the flowering individuals regarding size and number of flowers. A survey of the permanent plot and the managed area revealed that most individuals that flowered, did so outside the shrub regrowth but within the permanent monitoring site. Of the 33 individuals flowering in season "1", nine did not re-emerge in the second season, again a dependence on habitat was not found.

Of the 10 flowering individuals recorded on the managed area, only two developed any capsules, while nine individuals of the 21 individuals found flowering on the permanent plot within dense grass cover produced fruits (Table 6.3). The two individuals found flowering on patches with dense shrub cover of the permanent plot did not produce mature capsules.

Table 6.3. Performance of the flowering individuals in the different habitats. N = Number of individuals analysed; N_{leaves} = Number of leaves during flowering; $N_{flowers}$ = Number of flowers per inflorescence; N_{fruits} = total number of fruits produced; H_{i+s} = combined height of inflorescence and flowering stalk; $Fruit\ set$ = proportion of all flowers that developed into mature fruits.

Habitat	N	Average N_{leaves}	Average H_{i+s}	Average $N_{flowers}$	N_{fruits}	$Fruit\ set$
PP_O	21	8.8 ± 1.1	32.3 ± 10.2	36.2 ± 14.6	68	8.94
PP_S	2	9.5 ± 0.7	61.5 ± 12.0	53.0 ± 1.4	0	0
M_O	8	8.4 ± 0.5	31.4 ± 10.5	32.6 ± 15.0	3	1.15
M_S	2	9.0 ± 1.4	25.7 ± 0.9	37.0 ± 8.5	0	0

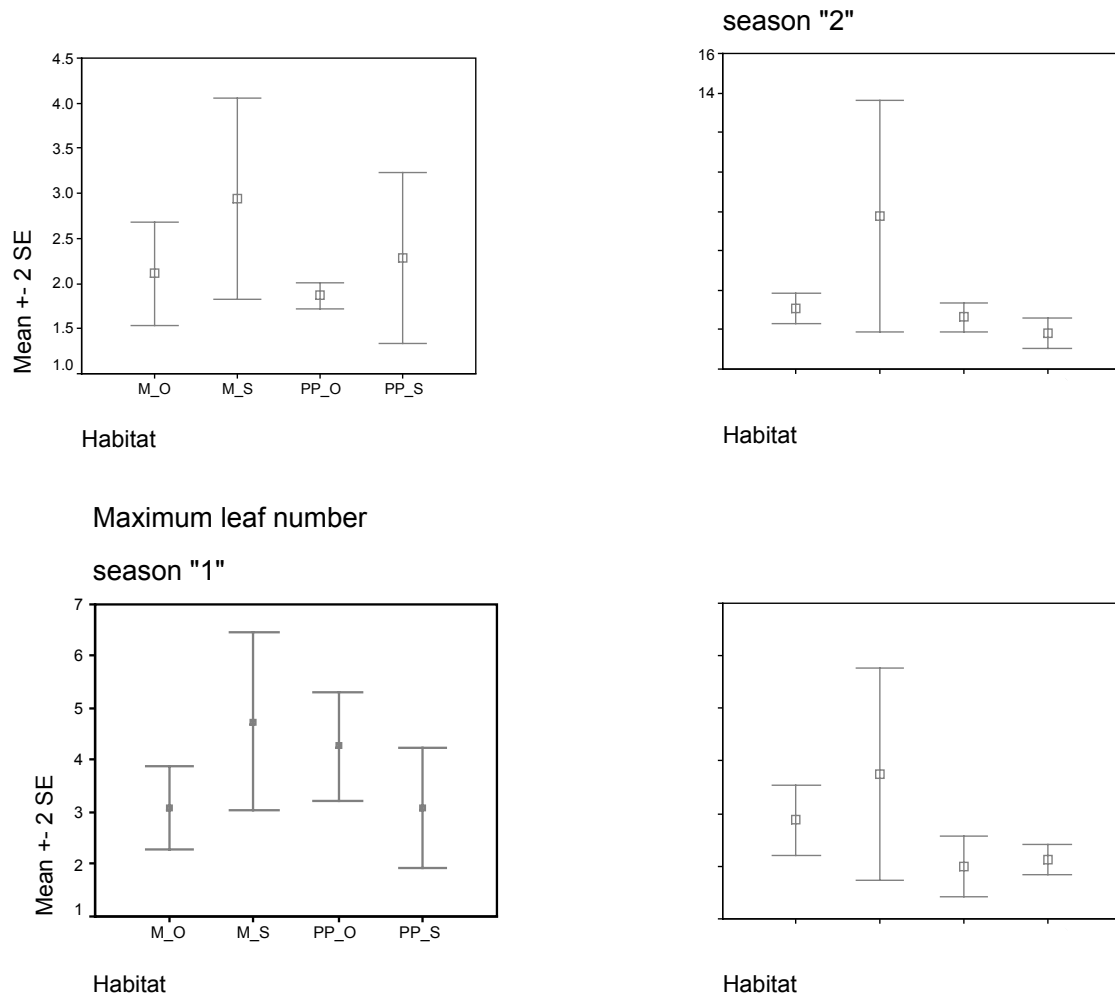


Fig. 6.5. Means and standard errors of individual performance depending on habitat structure. There is a high variation in performance suggesting that the assumption of variance homogeneity for an ANOVA analysis might be violated.

6.4.4 Spatial point pattern analysis

Intraspecific relationships

H. hircinum individuals were highly clumped on a small spatial scale (SP 1: up to a distance of $r = 23$ cm, $N = 51$; SP 2: up to a distance of 28 cm, $N = 8$; SP 3: up to 26 cm, $N = 65$; SP 4: up to a distance of 14 cm, $N = 32$). These clumps were randomly distributed in the investigated plots (Fig. 6.6). However, larger individuals were usually low in number and randomly distributed (SP 3: $N = 7$). The clumping pattern was slightly different in SP 5 ($N = 58$) with aggregation at very small distances (up to 5cm) and between 14 and 22 cm. This pattern was also found in SP 6 ($N = 49$).

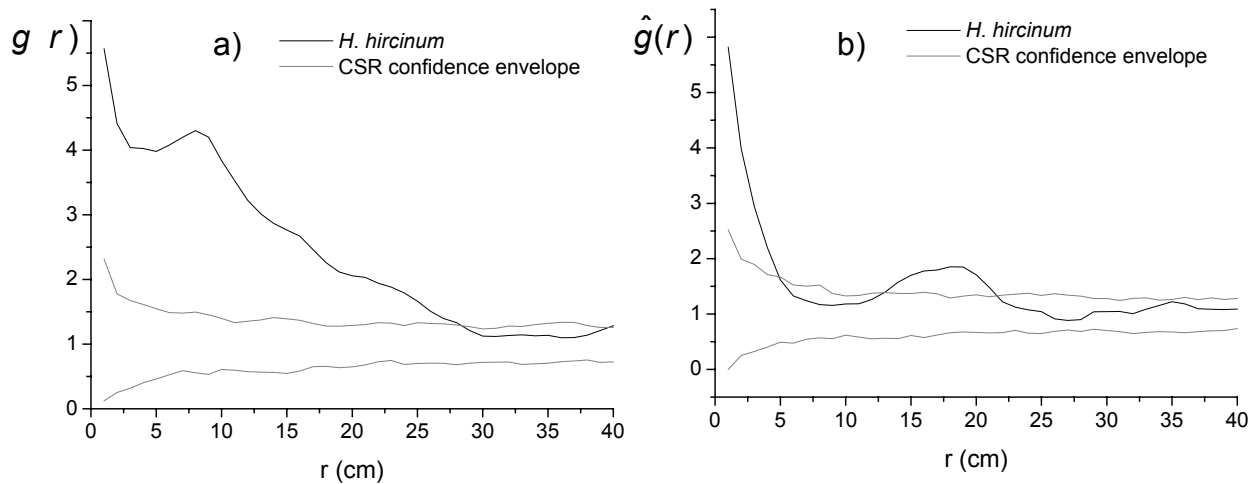


Fig. 6.6. Univariate point pattern of *H. hircinum* individuals a) on SP 3 ($N = 65$) and b) on SP 5 ($N = 58$). The CSR confidence envelope was calculated by Monte Carlo randomization of the location of the observed data points (1000 permutations).

Interspecific relationships

The bivariate point pattern analysis to test for spatial relationships between *H. hircinum* individuals and tall individuals of shrub species ($N = 46$, SP 1) showed a regular pattern at the distances 16 to 27 cm (Fig. 6.7). At these distances the positions of the individuals in the plot were more regularly distributed than expected by chance. The analysis of the point pattern to test for spatial relationships between the dense regrowth of *Prunus spinosa* and *H. hircinum* individuals revealed no significant deviation from random (SP 2), because the sample statistic was found to be within the CSR confidence envelope for each distance investigated. However, number of *H. hircinum* individuals was very low ($N = 8$), while there were many *P. spinosa* plants ($N = 111$). And the *P. spinosa* regrowth (probably clonal) was clumped on a very small scale (up to 6 cm), and at distances between 20 and 27 cm.

The bivariate point pattern analysis applied for point patterns between *H. hircinum* ($N = 65$) and *Plantago media* ($N = 52$) individuals on a 2 m x 2 m plot (SP 3) revealed a clumped co-occurrence pattern at certain scales (Fig. 6.8). Both species were relatively frequent on the spatial plot SP 3. The same pattern was shown when *Primula veris* ($N = 40$) and *H. hircinum* were compared on SP 3 (Fig. 6.8). However, a bivariate analysis to test for spatial relationships between *P. veris* and *H. hircinum* revealed no association in SP 4. The sample statistic was found to be within the CSR confidence envelope for all distances analysed.

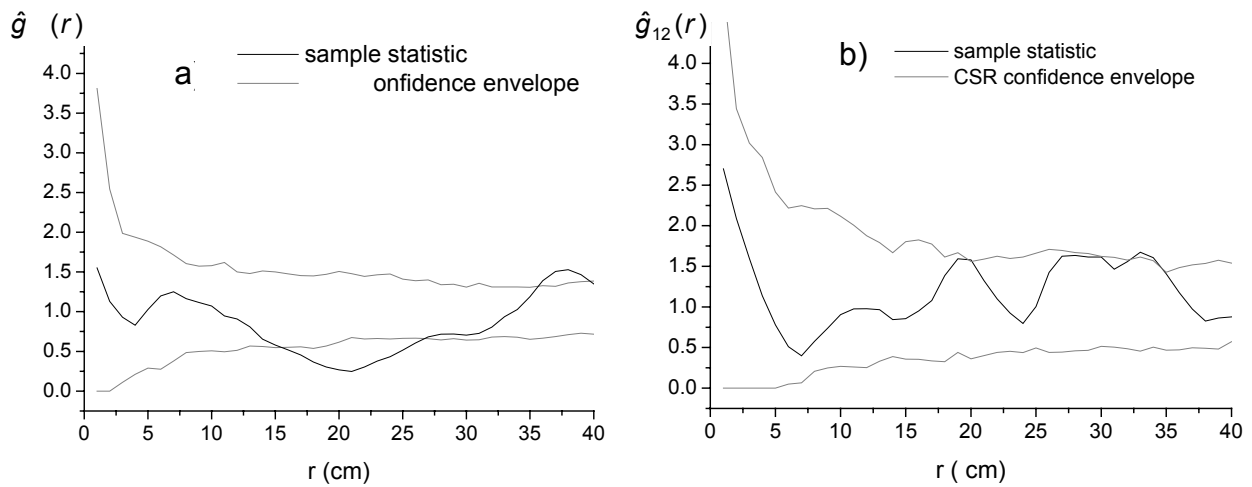


Fig. 6.7. Mark-Correlation function to test for spatial relationships a) between *H. hircinum* individuals ($N = 51$) and individuals of shrub species ($N = 46$) on SP 1, and b) between *H. hircinum* individuals ($N = 8$) and *P. spinosa* ramets ($N = 111$) on SP 2.

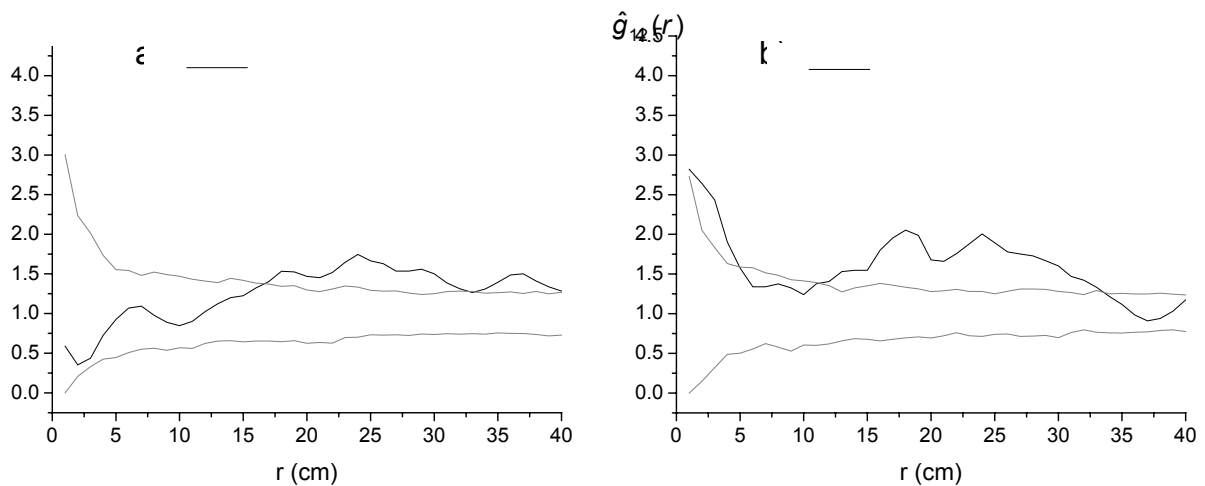


Fig. 6.8. Mark-Correlation function to test for spatial relationships a) between *H. hircinum* individuals ($N = 65$) and *P. veris* plants ($N = 40$) and b) between *H. hircinum* individuals and *P. media* ($N = 52$) plants on SP 3.

6.5 Discussion

6.5.1 Spatial spread

There was a positive spatial autocorrelation between the cells regarding the number of *H. hircinum* individuals in them, meaning that the probability to find an area with a high number of individuals was higher in the immediate neighbourhood of high-density spots than further away. The strength of autocorrelation was increasing with time, indicating that as the number of plants increased in the squares, the number of individuals in the adjacent squares increased as well. However, the strength of this trend varied depending on the contiguity measure used, suggesting that the spread of individuals into neighbouring squares followed a special pattern.

Autocorrelation results suggest that wind dispersal of the tiny seeds is not random over the permanent plot, or over larger distances, but mainly over short distances within the squares of the reproducing individuals or towards adjacent squares. There is a spread mainly southwards and downhill probably following the predominant wind direction downhill.

Observations showed that when a square was occupied by at least five individuals, it remained occupied and generally number of individuals rapidly increased in the following years. Random long-distance dispersal events allowed the establishment of new centres of high rates growth further away from cells with high number of individuals. That explains why cells with low growth rates can be found separating cells with high growth rates.

However, it was also obvious that in some squares *H. hircinum* individuals could not establish. The reason for that is unclear since the vegetation on these patches was not different to that of the patches with high number of individuals and with high growth rates. The presence of certain mycorrhiza species (*Rhizoctonia versicolor*; Carey and Farrell, 2002) might be a factor that restricts the occurrence of new plants. Germination experiments showed that most germinated seeds were infected by mycorrhizal fungi (Carey and Farrell, 2002). However, it was reported by Carey and Farrell (2002) and Heinrich (unblished) that new single individuals (probably dispersed by human activity) were found on unusual places far away from any *H. hircinum* population, on golf courses or along roads, suggesting that either a potential symbiotic partner occurs wide spread, or that a symbiotic partner is not absolutely necessary for a successful germination of the seeds and establishment of new individuals.

6.5.2 Spatial variability of performance

The proportion of individuals that flowered differed a lot among the cells and between the years. These results suggest that investigation of life history characteristics and population dynamics of plant species on small spatial scales only, might give misleading results.

The growth of *H. hircinum plants* was not influenced by habitat characteristics, at least at the scale investigated. Growth of the leaves seemed to be higher within shade, but not significantly so. Also, I could not find a significant influence of habitat differences on performance of the flowering individuals. Many individuals that flowered were located on the permanent monitoring site, on patches covered with tall grass but without shading shrub.

These results confirm the observations of Carey and Farrell (2002), who reported an exclusion of *H. hircinum* populations by scrub encroachment on the long term. Fruit set of flowering individuals on our study site was generally low and plants within dense shrub did not produce mature capsules, probably because pollinating insects had difficulties finding the flowers within the scrub patches.

However, the resolution of the habitat definitions was broad, and it is likely that studies with finer resolution would yield more information. The microenvironment of a spatial habitat is a dynamic mosaic, and identification of patch type by observation can be subjective and complex (Bazzaz, 1996).

6.5.3 Intra- and interspecific relationships

Dispersal of the tiny seeds is by wind. However, most seeds remain lodged in the capsules, which are dropped beside the adult plant, resulting in a clumped occurrence of the seedlings (Carey and Farrell, 2002). Clumping is a typical outcome of local dispersal as e.g. shown in cellular automata models. The degree of the clumping, the size of the clumps and the average distance between clumps depend on the mortality rate, the colonization rate, and the distance over which dispersal occurs (Tilman and Kareiva, 1997). Results from the point patterns for intraspecific relationships indicate that distances between clumps of *H. hircinum* plants varied and that these clumps occurred randomly in the plot, probably according to the random distribution of mature plants (Fig. 6.6). There is high mortality during the premature stage, and only a small proportion of the emergent plants ever flower (Pfeifer et al., submitted). These flowering plants occur randomly in the plot, maybe as a consequence of resource competition between seedlings before (thinning). But, an aggregation of clumps of *H. hircinum* plants at a scale of 15 - 22 cm was found on the plots SP5 and SP6, which were located close to each other on the permanent monitoring site. The reasons for that difference are unclear and might be artificial.

Individuals performed well regarding growth in patches with scrub cover on our study site, at least at this stage of shrub encroachment. However, the point pattern (Fig. 6.7) showed that there seems to be a negative effect of tall scrub individuals on the occurrence of *H. hircinum*. Both species "avoided" each other on the scale of 16 to 27 cm. This could be a consequence of the patchy distribution of openings in the shrub community providing light for establishment or growth of *H. hircinum* individuals. Summerhayes (1951) suggested that the species was associated with scrub, but Carey and Farrell (2002) assumed that individuals and populations could be wiped out by scrub encroachment.

Carey and Farrell (2002) also observed that very young plants were outcompeted by rosette plants. However, on our study site, a negative effect of the occurrence of *Plantago* spp. or *Primula veris* plants on the occurrence of *H. hircinum* individuals could not be found. The point pattern analysis revealed even a positive association between these species and *H. hircinum* on SP 3, probably because all three species require similar micro-ecological conditions for their growth.

I conclude that spread over short distances dominates the spatial dynamics. This is caused by predominantly short distance seed dispersal, which also results in a clumped

pattern of seedlings. A significant influence of habitat differences in the context of management and scrub density on growth and size of reproductive structures of *H. hircinum* could not be found on our study site at the resolution of habitat characteristics chosen. However, flowering probability appeared to be and fruiting success was higher in patches without shading scrubs. *H. hircinum* seedlings did not suffer from competition with rosette plants or seedlings from *P. spinosa*.

7. Conclusions and directions for future research

"Ecological understanding is required to develop environmental policies and to manage resources for the benefit of Humankind" (Likens, 1989)

The research presented in this dissertation exemplifies the plasticity of life history traits of individuals, and the temporal variability of population characteristics of a plant species. A diverse array of factors is believed to contribute to this variation but few studies ever tried to quantify the factors according to their influence. While some of the factors (e.g. weather variability and costs of reproduction) continuously affect plant individuals, others (e.g. management activities, herbivory, genetic variability) vary in their importance and actual presence on the study site.

Ecologists increasingly face the fact, that short-time studies will inevitably give misleading results, when they are aimed at answering questions about abiotic and biotic constraints on pattern in plant ecology and at predicting the future fate of populations. The environment of a species is variable, and conclusions about plant population dynamics depend on several factors including the habitat size and the time of study chosen. However, long-term investigations are difficult to establish for financial and logistic reasons. Therefore, the analysis of available long-term data sets has to be combined with simulation or modelling approaches.

In this final chapter, the role of factors influencing individual performance and population dynamics of *H. hircinum* is discussed and put into a broader context. An overview over long-term studies on orchid species is given. Some general remarks about the importance of long-term studies are made, and directions for future research are proposed.

7.1 Long-term studies and trends in orchid ecology

Sustained ecological research is needed to develop an ecological understanding and for predicting the direct (habitat destruction, pollution) and indirect effects (global climate change) of human activities on ecological processes. Despite the knowledge about the importance of long-term studies in that context, few changes happened in the last decades. A LTER (Long-term ecological research) program was established in 1980 by the National Science Foundation to fund research on long-term ecological phenomena in an array of ecosystems within the United States (<http://lternet.edu/>). The importance of long-term ecological studies has been recognized throughout the world. However, the coordination of long-term ecological studies on a global scale to provide greater value to individual projects has to be discussed and improved (Risser, P.G.: Scope 47 - Long-term Ecological Research, <http://data.ecology.su.se/scopesi/ScopeSI>).

Long-term studies on herbal plants are rare and were focussed on rare and conspicuous species. Plant ecologists often have been attracted to orchids, not only because they have been the flagship of nature conservation for several decades. Orchid ecology was often descriptive, analysing demographic structures based on life tables (Hutchings, 1987a; Willems and Melser, 1998). But, the importance of long-term observations to get reliable

results for predicting future fate of populations was recognized early (Wells and Cox, 1991; Carey, 1999). Due to concerns about the effects of habitat loss and habitat fragmentation on protected plant species, efforts were concentrated on finding the appropriate management schemes to preserve declining populations of orchids in their habitat refuges (Farrell, 1991; Curtis, 1946; Stuckey, 1967); Hutchings et al., 1998). In this context, the use of transition matrix models to compare population dynamics under various management schemes proved to be a successful method (Gregg, 1991; Waite und Hutchings, 1991).

Besides the efforts in searching for appropriate active management procedures, it had to be realized, that management not necessarily played an important role in the long run, and that varying climatic conditions could have a larger influence on population characteristics, including mortality and recruitment (Willems and Bik, 1991) and flowering performance (Tamm, 1991; Carey, 1999). Flowering pattern of orchids is irregular. Whether this high variability is mainly controlled by external conditions (e.g. climatic conditions, herbivore pressure, interspecific competition), life history constraints (reproductive effort) or whether it is a chaotic phenomenon (Inghe, 1991), was a major issue discussed in the last few years. Recent studies focussed on the analysis of life history traits (Willems und Melsner, 1998; Kindlmann, 1999), and their plasticity (Kéry and Gregg, 2004), and the consequences of reproductive effort (Willems und Dorland, 2000).

However, despite the arising problems of global climate change, little research was conducted in the field of spatial variability of individual performance and modelling spatial spread of orchids by including weather components (Carey, 1998; Carey, 1999).

7.2 Variability of individual characteristics and population dynamics

Long-term observational studies are not only necessary to study succession in forests, grasslands, and old fields, but also to investigate population cycles and population dynamics of long-lived higher plants (Likens, 1989). As shown by this study and other observations, life history traits (e.g. flowering) and population dynamics can be highly variable due to external and internal influences. Life span and size of the individuals, probability of life state transitions, dormancy pattern and flowering frequency of the population showed a clear relationship to weather conditions in our study. However, the study site was at the northeastern border of its distribution range. Whether characteristics of populations from the Mediterranean, which is the centre of the species' distribution, are also predominantly influenced by weather variability, has to be tested, yet.

In addition to the long-term design of observational studies, the spatial scale and spatial heterogeneity of the plot should be taken into account. On our study site, population growth rates, flowering probability of the population and individual flowering frequency strongly depended on the spatial scale studied. An "unfavourable" selection of plots "too small" could result in underestimation or overestimation of population characteristics.

7.3 Conservation issues

Population viability analysis requires information about the life history of the species. However, the prediction of the future fate of the population over the next decades without

taking into account the variability of state transitions and without including variability of external conditions can be misleading. Life state transition probabilities of the *H. hircinum* population studied in the nature reserve Leutratal differed largely between the years. However, the variation of some of the transition probabilities was not random, but influenced by the weather conditions, so that applying stochastic models might not improve the predictability of long-term persistence by matrix models.

Habitat fragmentation is another major problem that negatively affects especially rare and protected plant species, which are usually bound to certain habitat structures. The potential for evolutionary adaptation of the plants might decrease due to genetic drift and loss of genetic diversity in small fragments. While structure and dynamics might indicate a "healthy" state of the population in a habitat fragment, genetic diversity might already be low due to limited exchange of genes by dispersal of seeds or pollen. Therefore conservation ecology needs to include genetic diversity studies not only for currently endangered plant species, but also for other habitat-restricted species, which might suffer from habitat fragmentation in the future. However, their isolation did not affect genetic diversity of the populations studied. Effects of genetic erosion could not be observed with the method chosen.

7.4 Directions for future research

Several factors proved to be important in the control of individual performance, life state transitions and population dynamics. The dominant influence of weather conditions in that context is especially important when putting the knowledge of population dynamics of a plant species, which is additionally rare and protected, into the framework of global climate change. Levels of greenhouse gases in the atmosphere have been increased due to human activities, which in turn will lead to an overall global warming. Consequences of global climate change affect not only species diversity, but also humankind, because we rely on agriculture for food supply. Thus, predicting future ranges of plant species remains an urging task for conservation and economy. Migration seems to be the usual response of plants to climate change, and evolutionary adaptation seems to play a minor role (Huntley, 1991). The results of this thesis can be used to develop a more detailed model for predicting the distribution of the study species in the future under several climatic scenarios. For comparative analyses, studies on *H. hircinum* populations in the centre of its distribution, as well as further populations at the borders of its distribution range should be conducted.

The high variability of population traits complicates the use of transition matrix models for predicting (quasi) extinction of a population sensu population viability analysis. In the past, stochastic matrix models have been developed to include the stochasticity of the environment. However, they were generally used in combination with short-term data only. Depending on the species used, even 10 or 26 years are not sufficient to get reliable results on future fate of a species. Nevertheless, as suggested by others, transition models remain an important tool to compare dynamics of the species under differing management treatment plots. A stochastic transition matrix model combining weather variability and population dynamics could be developed. But effort should also be concentrated on studying individual

performance under differing environmental conditions, and under various management regimes. Again, new populations from several locations within the distribution range and at its border should be chosen.

Growth of the population followed an exponential curve. Data recording stopped in 2001. To study the theoretical assumption of logistic growth in long-lived species under natural conditions, and to search for density dependent change in behaviour of *H. hircinum* individuals, data recording on the permanent site should be continued. Additionally, fruit set and seasonal growth of a subset of individuals should be included in future measurements.

Since little is known about the speed of spread of the species studied, a spatial simulation model for the population studied could be developed. Future movement of the population could be predicted and tested by including weather parameters, vegetation change parameters, and life history characteristics (e.g. size, flowering frequency, fruit set, dispersal range) in the model. A global extension of the model to study the species' response to climate change scenarios would be a further step (see also Carey, 1999).

Conservation of the species also requires information about genetic diversity, genetic differentiation and evolutionary potential. Population genetics from several locations within the mediterranean area and at the borders of the distribution (UK, Germany) could be studied by AFLP analysis. Further, populations on small and large habitat fragments could be investigated and compared, and isozymes should be used as well, to get detailed information on the extent of genetic differentiation and possible inbreeding effects.

The main question yet to be solved is: Can we predict the future spread of *H. hircinum* under global climate change scenarios? And why should that be of any interest? An approach to the first question requires the development of a simulation model that combines population dynamics, vegetation dynamics, the influence of management, and knowledge about dispersal properties. Long-distance dispersal of seeds strongly affects spread rates of plant species. An approach that integrates dispersal with plant life history, disturbance, and habitat availability will be necessary for predicting migration rates (Higgins and Richardson, 1999). Such a model would be a step to a global 'Biodiversity Viability Analysis (BVA)' combining the information about population dynamics, species' distribution, species' migration rate and the variability of climatic conditions. The application of such simulation models to other plant (and animal) species is essential for further suggestions regarding future species loss and biodiversity under changing climatic conditions. This can only be based on a global network of long-term spatio-temporal population studies of plant (and animal) species. A database should be developed combining the efforts of plant ecologists, animal ecologists, conservation managers, and meteorologists. This data base will not only be an important reservoir for researchers searching for patterns in nature, but will also provide politicians, and managers of agriculture and forestry with essential information for their efforts to stabilize the growth of crops and the supply of food and wood resources under the unavoidable global climate change.

Summary

Plant population dynamics are often highly variable, and they are constrained by the abiotic and biotic environment and. It is unclear, whether there is a dominant intrinsic mechanism causing variable patterns in the life history of a species, or whether species mainly respond to environmental factors including varying weather conditions, presence of competing species, and human activities. This study combines descriptive and modelling approaches to gain insight into the major mechanisms causing the plasticity in patterns of life history and population dynamics of plant species. Part of the study is based on a long-term data set (1976 to 2001) on the population dynamics and life history traits of a rare and protected orchid species (recorded by Dr. W. Heinrich) and on a long-term data set on climate (measured by the weather station of Jena). Additionally, I measured spatial distribution in small plots as well as length growth, size of reproductive structures, and fruit set in two plant years between 2002-2004 to quantify spatial variability of individual performance, and to determine factors responsible for the species local distribution pattern. Genetic patterns of three isolated populations of the orchid were analysed in 2004 to determine the influence of habitat fragmentation on genetic diversity and genetic differentiation of the populations.

Variability of life history traits

- Variability of life history traits was investigated for more than 13000 plants based on the long-term data set. Size based transition probabilities were calculated and used as elements in transition matrices. Multiple Linear regression was used to test for an influence of weather variability on individual performance and life state transitions.
- Plasticity of life history traits was high with interdependence between some traits caused by intrinsic constraints (reproduction vs. mortality/ vs. size). Size-based life state transitions were highly variable, caused to a large part (up to 50% of the variation) by variability of weather conditions. Population size was increasing exponentially without density-regulation. Flowering and large individuals contributed most to population growth. Because of the high variability of transition probabilities and their dependence on weather variables, matrix models and their long-term predictions regarding extinction of the populations have to be dealt with cautiously.

Flowering pattern

- Irregular flowering pattern of *H. hircinum* was analysed. The influence of age, size, reproductive effort, and climatic conditions on flowering dynamics was investigated.
- Flowering probability of the individuals was highly variable and size-dependent. Weather had a large indirect influence on flowering via determination of plant size (about 50% of the variation). Reproductive effort of the species was high and negatively affected plant size (trade-off constraints). The combination of plant size with the direct influence of weather (on the flowering initiation) explained 82% of the annual variation in flowering percentage of the population. Weather variability in

combination with the individual flowering history are major factors causing the irregular flowering pattern of this orchid population.

Genetic pattern

- Genetic structure of three geographically separated populations of *H. hircinum* was studied by the use of AFLP markers.
- Genetic diversity was higher within than between the populations. Population differentiation was relatively high compared to other orchid species due to restricted gene flow between the populations. Populations were of sufficient size to maintain genetic variability by reproduction and by genetic exchange among patches over short distances (within a habitat). Maintenance of habitat size should be at the centre of focus of conservation programs for *H. hircinum*.

Spatial pattern analysis

- Spatial autocorrelation analysis was used to study spatio-temporal dynamics of *H. hircinum* in the permanent plot (1.9 ha). Spatial point pattern analysis was applied to investigate spatial distribution pattern of the study species on a small local scale and to test for interspecific relationships between *H. hircinum* and scrub plants as well as rosette plants. Individual performance (growth, flowering, fruit-set) under differing habitat conditions was investigated.
- Spread of the population is slow and restricted by short-distance seed dispersal. Short-distance seed dispersal dominates the local distribution pattern of the species, while density effects or interspecific competition effects could not be found. A significant influence of differences in habitat conditions on performance of the plants could not be detected at this resolution of habitat variability. Flowering probability appeared to be higher on the unmanaged permanent plot within patches with high grass cover. Plants did not develop capsules in patches with scrub cover.

Overall, weather variability was the major determinant of the variability in life history traits, life state transitions, and population characteristics. Density effects were not found with the methods used. However, reproductive effort expressed as the trade-off between future growth and current flowering was high and consequently affected other life history characteristics including life span, future growth, and future probability of flowering. Population dynamics differed among the squares, but a significant influence of shade or management on individual performance could not be found. However, fruiting success was highest for individuals located on unmanaged, open patches that were characterized by tall, dense growing grass without scrub plants. Size of the three populations investigated by AFLP markers was sufficient to maintain genetic diversity. Based on these results, conservation strategies should concentrate on preventing scrub encroachment by mowing every five years, and on maintenance or enlargement of habitat size to allow spatial spread of the population and to prevent inbreeding effects lowering genetic diversity.

Zusammenfassung

Die Populationsdynamik von Pflanzen ist zeitlich und räumlich oft variabel und limitiert durch die abiotische und biotische Umwelt der untersuchten Art. Es ist unklar, ob intrinsische Regulationsmechanismen die Muster im Lebenszyklus einer Pflanzenart dominieren, oder ob Pflanzenarten im wesentlichen auf externe Umweltfaktoren, einschließlich Wetterbedingungen und Anwesenheit konkurrierender Arten, reagieren. Diese Studie verwendet einen beschreibenden und modellierenden Ansatz, um einen Einblick zu erhalten, welche Mechanismen die Plastizität von Lebenszyklus und Populationsdynamik bestimmen. Ein Teil der Arbeit beruht auf einem Langzeitdatensatz zum Lebenszyklus und zur Populationsdynamik einer geschützten Orchideenart (aufgenommen von Dr. W. Heinrich zwischen 1976 und 2001) sowie auf einem Klimadatensatz der Wetterstation Jena. Zudem wurden von Oktober 2002 bis August 2004 weitere Daten aufgenommen bezüglich der lokalen räumlichen Verteilung sowie zum detaillierten Längenwachstum, zur Größe der reproduktiven Strukturen, und zum Fruchtansatz. Die genetische Struktur von 3 geographisch voneinander getrennten Populationen wurde analysiert, um den Einfluss von Habitatfragmentierung auf die genetische Diversität zu bestimmen.

Plastizität von Lebenszykluseigenschaften

- Basierend auf dem Langzeitdatensatz wurde die Variabilität der Komponenten des Lebenszyklus für über 13000 Individuen bestimmt. Übergangswahrscheinlichkeiten für größenabhängig definierte Lebensstadien wurden berechnet und als Elemente in Übergangsmatrizen verwendet. Multiple Lineare Regression wurde angewendet, um einen potentiellen Wettereinfluss auf die Individualperformanz und Übergangswahrscheinlichkeiten zu finden.
- Die Plastizität der individuellen Lebenszykluseigenschaften war hoch, einige Komponenten waren miteinander korreliert wahrscheinlich bedingt durch begrenzte Ressourcenverfügbarkeit (Trade-off zwischen Reproduktion und Mortalität bzw. Reproduktion und Größe). Wettervariabilität erklärte bis zu 50% der Varianz in einigen Übergangswahrscheinlichkeiten zwischen den größendefinierten Stadien. Die Population wuchs exponentiell, Dichteeffekte wurden nicht gefunden. Blühende und große Individuen leisteten den größten Beitrag zum Populationswachstum. Aufgrund der hohen Variabilität der Übergangswahrscheinlichkeiten und ihrer Abhängigkeit vom Wetter, sollten Matrix-Modelle und deren Langzeitvorhersagen zum Schicksal der Population mit Vorsicht verwendet werden.

Muster der Blühaktivitäten

- Das unregelmäßige Blühpatter von *H. hircinum* wurde analysiert. Der Einfluss von Alter, Individualgröße, Reproduktionsaufwand und Wetter auf die Blühdynamik wurde untersucht.
- Das individuelle Blühverhalten war sehr variabel und stark größenabhängig. Wetterbedingungen hatten einen großen indirekten Einfluss auf das Blühpatter

durch ihren Einfluss auf die Größe der Individuen (ca. 50% der Variation). Der hohe Reproduktionsaufwand der Art beeinflusste die Pflanzengröße negativ. Die Kombination von Pflanzengröße und direktem Wettereinfluss (auf die Blühinitiale) erklärten 82% der jährlichen Variation im Anteil blühender Pflanzen in der Population. Wettervariabilität in Kombination mit individueller Blühgeschichte sind die Hauptfaktoren, die das unregelmäßige Blühmuster der Orchideenpopulation verursachen.

Genetisches Muster

- Die genetische Struktur von drei geographisch Populationen von *H. hircinum* wurde mittels AFLP-Markern analysiert.
- Die genetische Diversität innerhalb der Populationen war größer als zwischen diesen. Die genetische Differenzierung der Populationen war relativ hoch im Vergleich zu anderen Orchideenarten, bedingt durch limitierten Genaustausch zwischen den Populationen. Die Größe der Populationen reichte aus, um die genetische Variabilität durch Reproduktion und Genaustausch zwischen Individuen innerhalb der Population aufrechtzuerhalten. Management der Art sollte sich auf die Erhaltung der Habitatgröße konzentrieren.

Räumliche Musteranalyse

- Räumliche Autokorrelationen wurden berechnet, um das Raumzeitverhalten von *H. hircinum* auf der Dauerbeobachtungsfläche (1.9 ha) zu bestimmen. Das räumliche Verbreitungsmuster der Art sowie mögliche Konkurrenzeffekte zwischen *H. hircinum* und Strauch- bzw. Rosettenpflanzen auf kleiner lokaler Skala wurden mittels räumlicher Punktmusteranalyse analysiert. Performanz der Individuen (Wachstum, Blühen, Fruchtansatz) unter verschiedenen Habitatbedingungen wurde untersucht.
- Die räumliche Ausbreitung der Population erfolgt langsam und wird gesteuert von Samendispersal über kurze Distanzen. Samendispersal über kurze Distanzen dominiert das lokale Verbreitungsmuster der Art (Klumpung), während Dichteeffekte oder Konkurrenzeffekte anderer Pflanzenarten nicht gefunden wurden. Habitatunterschiede hatten keinen signifikanten Einfluss auf die Performanz der Pflanzen, zumindest bei der gewählten Definition von Habitaten. Blühende Individuen befanden sich jedoch überwiegend auf der Dauerbeobachtungsfläche an Stellen mit hoher Grasbedeckung, aber ohne Beschattung durch Sträucher. Fruchtbildung wurde von mir nur an strauchfreien Stellen beobachtet, wahrscheinlich bedingt durch limitierte Pollinatorenaktivität innerhalb dichter Strauchbedeckung.

Wettervariabilität beeinflusste als wesentlicher Faktor die Variabilität von Lebenszyklus, Elementen der Transitionsmatrix und Eigenschaften der Population. Dichteabhängige Effekte wurden mit den verwendeten Analysemethoden nicht gefunden. Der hohe Reproduktionsaufwand führte zu einer negativen Beeinflussung von Überlebenswahrscheinlichkeit, Wachstum und Blühwahrscheinlichkeit in den dem Blühjahr

folgenden Jahren. Die Populationsdynamik war räumlich variabel. Ein signifikanter Einfluss von Beschattung oder Managementmaßnahmen auf die individuelle Performanz konnte jedoch nicht gefunden werden, obwohl blühende Individuen 2003 und 2004 meist an offenen Stellen, ohne dichten Strauchbestand, aber mit relativ hohem Grasbewuchs, gefunden worden. Die Größe der drei mittels AFLP untersuchten Populationen ist vermutlich ausreichend zur Gewährleistung genetischer Diversität. Schutzmassnahmen für diese Art sollten sich auf die Mahd zur Verhinderung der dauerhaften Strauchansiedlung konzentrieren, wobei ein Rhythmus von 5 Jahren ausreichend sein sollte. Die Aufrechterhaltung bzw. Vergrößerung der Habitatfläche wäre ein weiterer Managementansatz. Eine große Habitatfläche ermöglicht eine weitere räumliche Ausbreitung und damit ein weiteres Populationswachstum. In einer großen Population wiederum ist die Wahrscheinlichkeit von Inzuchteffekten, und damit einer Abnahme der genetischen Diversität und des evolutionären Potentials, verringert. Die Etablierung von neuen, kleinen Habitaten als "Stepping stones" in einer fragmentierten Landschaft, um den Genaustausch zwischen den Populationen aufrechtzuerhalten, ist aufgrund der geringen Dispersalreichweite von Samen und Pollen weniger geeignet.

References

- Aargaard, J.E., Harrod, R.J. and Shea, K.L. 1999.** Genetic variation among populations of the rare clustered lady-slipper orchid (*Cypripedium fasciculatum*) from Washington State, USA. *Natural Areas Journal* 19: 234-238.
- Ackerman, J.D. and Montalvo, A.M. 1990.** Short- and long-term limitations to fruit production in a tropical orchid. *Ecology* 71: 263-272.
- Alexandersson, R. and Agren, J. 2000.** Genetic structure in the nonrewarding bumblebee-pollinated orchid *Calypso bulbosa*. *Heredity* 85: 401-409.
- Arduino, P., Verra, F., Cianchi, R., Rossi, W., Corrias, B. and Bullini, L. 1996.** Genetic variation and natural hybridization between *Orchis laxiflora* and *Orchis palustris* (Orchidaceae). *Plant Systematics and Evolution* 202: 87-109.
- Ayele, M. and Nguyen, H.T. 2000.** Evaluation of amplified fragment length polymorphism markers in tef, *Eragrostis tef* (Zucc.) Trotter, and related species. *Plant Breeding* 119: 403-409.
- Barker, J.H.A., Matthes, M., Arnold, G.M., Edwards, K.J., Åhman, I., Larsson, S. and Karp, A. 1999.** Characterisation of genetic diversity in potential biomass willows (*Salix spp.*) by RAPD and AFLP analyses. *Genome* 42: 173-183.
- Bazzaz, F.A. 1996.** *Plants in Changing Environments. Linking physiological, population and community ecology.* Cambridge University Press, Cambridge, UK.
- Begon, M., Harper, J.L. and Townsend, C.R. 1996.** *Ecology.* Blackwell Science Ltd., Third edition, Oxford.
- Bowen, B.W. 1999.** Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. *Molecular Ecology* 8: 5-10.
- Brzosko, E. 2002.** Dynamics of island populations of *Cypripedium calceolus* in the Biebrza river valley (north-east Poland). *Botanical Journal of the Linnean Society* 139: 67-77.
- Brzosko, E., Wroblewska, A. and Ratkiewicz, M. 2002.** Spatial genetic structure and clonal diversity of island populations of lady's slipper (*Cypripedium calceolus*) from the Biebrza National Park (northeast Poland). *Molecular Ecology* 11: 2499-2509.
- Calvo, R.N. 1990a.** Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in south Florida. *American Journal of Botany* 77: 736-741.
- Calvo, R.N. 1990b.** Inflorescence size and fruit distribution among individuals in three orchid species. *American Journal of Botany* 77: 1378-1381.
- Calvo, R.N. and Horvitz, C.C. 1990.** Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. *American Naturalist* 136: 499-516.
- Carey, P.D. 1998.** Modelling the spread of *Himantoglossum hircinum* (L.) Spreng. at a site in the south of England. *Botanical Journal of the Linnean Society* 126: 159-172.
- Carey, P.D. 1999.** Changes in the distribution and abundance of *Himantoglossum hircinum* (L.) Sprengel (Orchidaceae) over the last 100 years. *Watsonia* 22: 353-364.
- Carey, P.D. and Farrell, L. 2002.** *Himantoglossum hircinum* (L.) Sprengel. *Journal of Ecology* 90: 206-218.

- Carey, P.D., Farrell, L. and Stewart, N.F. 2002.** The sudden increase in the abundance of *Himantoglossum hircinum* in England in the past decade and what has caused it. In: Kindlmann, P., Willems, J.H. and Whigham, D. (eds.), *Trends and fluctuations and underlying mechanisms in terrestrial orchid populations*, pp. 187-208.
- Case, M.A. 1994.** Extensive variation in the levels of genetic diversity and degree of relatedness among five species of *Cypripedium* (Orchidaceae). *American Journal of Botany* 81: 175-184.
- Caswell, H. 1982.** Stable population structure and reproductive value for populations with complex life cycles. *Ecology* 63: 1223-1231.
- Caswell, H. 1989.** *Matrix population models*. Sinauer, Sunderland, Mass.
- Chung, M.Y., Nason, J.D. and Chung, M.G. 2004.** Spatial genetic structure in populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae). *American Journal of Botany* 91: 52-57.
- Cody, M.L. and Mooney, H.A. 1978.** Convergence versus nonconvergence in Mediterranean-climate ecosystems. *Annual Review of Ecology and Systematics* 9: 265-321.
- Cozzolino, S., Noce, M.-E., Musacchio, A. and Widmer, A. 2003.** Variation at a chloroplast minisatellite locus reveals the signature of habitat fragmentation and genetic bottlenecks in the rare orchid *Anacamptis palustris* (Orchidaceae). *American Journal of Botany* 90: 1681-1687.
- Curtis, J.T. 1946.** Letter to W.S. Cooper, 12 March, *Curtis Papers*, Univ. Archives, Wisconsin, Madison.
- Czárán, T. 1998.** *SPATIOTEMPORAL MODELS OF POPULATION AND COMMUNITY DYNAMICS*. CHAPMAN & HALL, London.
- Dale, M.R.T. 1999.** *Spatial Pattern Analysis in Plant Ecology*. Cambridge University Press, Cambridge, UK.
- Danquah, E.Y., Johnson, D.E., Riches, C., Arnold, G.M. and Karp, A. 2002.** Genetic diversity in *Echinochloa* spp. collected from different geographic origins and within rice fields in Côte d'Ivoire. *Weed research* 42: 394-405.
- Deevey, E.S. 1947.** Life tables for natural populations of animals. *Quarterly Review of Biology* 22: 283-314.
- Enright, N.J., Franco, M. and Silvertown, J. 1995.** Comparing plant life histories using elasticity analysis: the importance of life span and the number of life-cycle stages. *Oecologia* 104: 79-84.
- Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* 131: 479-491.
- Falb, D.L. and Leopold, D.J. 1993.** Population dynamics of *Cypripedium candidum* Muhl. ex Willd., small white ladyslipper, in a western New York fen. *Natural Areas Journal* 13: 76-86.
- Farrell, L. 1991.** Population changes and management of *Orchis militaris* at two sites in England. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 63-68.
- Felsenstein, J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- Fenster, C.B. 1991.** Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution* 45: 398-409.

- Fieberg, J. and Ellner, S.P. 2001.** Stochastic matrix models for conservation and management: a comparative review of methods. *Ecology Letters* 4: 244-266.
- Fischer, M. and Matthies, D. 1997.** Mating structure and inbreeding and outbreeding in the rare plant *Gentianella germanica* (Gentianaceae). *American Journal of Botany* 84: 1685-1692.
- Fischer, M. and Matthies, D. 1998.** Effects of population size on performance in the rare plant *Gentianella germanica*. *Journal of Ecology* 86: 195-204.
- Ford, E.D. and Renshaw, E. 1984.** The interpretation of process from pattern using two-dimensional spectral analysis: modeling single species patterns in vegetation. *Vegetatio* 56: 113-123.
- Fox, J.F. and Stevens, G.C. 1991.** Costs of reproduction in a willow: experimental responses vs. natural variation. *Ecology* 72: 1013-1023.
- Freckleton, R.P. and Watkinson, A.R. 2002.** Large-scale spatial dynamics of plants: metapopulations, regional ensembles, and patchy populations. *Journal of Ecology* 90: 419-434.
- Gagne, G., Roeckel-Drevet, P., Grezes-Besset, B., et al., 2000.** Amplified Fragment Length Polymorphism (AFLP) as Suitable Markers to Study *Orobanche cumana* Genetic Diversity. *Journal of Phytopathology* 148: 457-459.
- Galiano, E.F. 1986.** The use of conditional probability spectra in the detection of segregation between plant species. *Oikos* 46: 132-138.
- Geary, R.C. 1954.** The contiguity ratio and statistical mapping. *Incorporated Statistician* 5: 115-145.
- Good, R. 1936.** On the distribution of lizard orchid (*Himantoglossum hircinum* Koch). *New Phytologist* 35: 142-170.
- Gosselin, F. 1996.** Extinction in a source/sink system: application of new mathematical results. *Acta Oecologica* 17: 563-584.
- Gotelli, N.J. 1991.** Metapopulation models: the rescue effect, the propagule rain and the core satellite hypothesis. *American Naturalist* 138: 768-776.
- Gram, W.K. and Sork, V.L. 2001.** Association between environmental and genetic heterogeneity in forest tree populations. *Ecology* 82: 2012-2021.
- Gregg, K.B. 1991.** Variation in behaviour of four populations of the orchid *Cleistes divaricata*, an assessment using transition matrix models. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 139-159.
- Guardia, R., Raventos, J. and Caswell, H. 2000.** Spatial growth and population dynamics of a perennial tussock grass (*Achnatherum calamagrostis*) in a badland area). *Journal of Ecology* 88: 950-963.
- Gustafsson, S. and Sjogren-Gulve, P. 2002.** Genetic diversity in the rare orchid, *Gymnadenia odoratissima* and a comparison with the more common congener, *G. conopsea*. *Conservation Genetics* 3: 225-234.
- Haase, P., Pugnaire, F.I., Clark, S.C. and Incoll, L.D. 1997.** Spatial pattern in *Anthyllis cytisoides* shrubland on abandoned land in southeastern Spain. *Journal of Vegetation Science* 8: 627-634.
- Hamrick, J.L. and Godt, M.J.W. 1990.** Allozyme diversity in the grasses. In: *Population Biology of Grasses*, ed. G.P. Cheplick, Cambridge University Press, Cambridge, pp. 11-29.
- Harrison, S. and Hastings, A. 1996.** Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution* 11: 180-183.

- Hayward, G.D. and McDonald, D.B. 1997.** Matrix population models as a tool in development of habitat models. In: USDA Forest Service (ed.), *Biology and Conservation of Owls of the Northern Hemisphere*. Second International Symposium, Winnipeg, Manitoba, Canada, pp. 205-212.
- Heinrich, W. 1994.** Betrachtungen zu einer Population der Bocks-Riemenzunge in einem thüringischen Naturschutzgebiet. *Landschaftspflege und Naturschutz Thüringen* 31: 102-104.
- Heinrich, W. 2000.** Die Bocks-Riemenzunge (*Himantoglossum hircinum*) – Langzeitbeobachtungen auf markierten Dauerflächen. In: Arbeitskreis Heimische Orchideen (ed.), *Heimische Orchideen. Artenmonitoring und Langzeitbeobachtung, Populationsdynamik und Artenschutz*, Uhlstädt, pp. 36-48.
- Heinrich, W. 2001 (unpublished).** Der Bestand der Bocks-Riemenzunge (*Himantoglossum hircinum*) in der Katena des NSG "Leutratal"-Individuenzählungen im Zeitraum 1976 - 2001.
- Heinrich, W. 2003.** Zur Ansiedlung und Wiedereinbürgerung heimischer Orchideen-die Entwicklung einer neubegründeten Population der Bocks-Riemenzunge (*Himantoglossum hircinum*). *Journal Europäischer Orchideen* 35: 455-538.
- Heinrich, W. and Voelckel, H. 1999.** Die Bocks-Riemenzunge [*Himantoglossum hircinum* (L.) Spreng.] - Orchidee des Jahres 1999. *Berichte aus den Arbeitskreisen Heimische Orchideen* 16: 83-123.
- Heinrich, W., Jetschke, G. and Pfeifer, M. 2005 (in print).** Populationsentwicklung der Bocks-Riemenzunge (*Himantoglossum hircinum*) - Untersuchungen auf Dauerflächen im NSG „Leutratal“ bei Jena (Thüringen). *Berichte aus den Arbeitskreisen Heimische Orchideen* 20.
- Heinrich, W., Marstaller, R., Bährmann, R., Perner, J. and Schäller, G. 1999.** Das Naturschutzgebiet "Leutratal" bei Jena - Struktur- und Sukzessionsforschung in Grasland-Ökosystemen. *Naturschutzreport* 14: 4-423.
- Hellwig, F.H., Nolte, M., Ochsmann, J. and Wissemann, V. 1999.** Rapid isolation of total cell DNA from milligram plant tissue. *Hausknechtia* 7: 29-34.
- Heyer, E. 1993.** *Witterung und Klima*. 9th edition. Teubner, Stuttgart.
- Higgins, S.I. and Richardson, D.M. 1999.** Predicting Plant Migration Rates in a Changing World: The Role of Long-Distance Dispersal. *The American Naturalist* 153: 464-475.
- Hill, M.O. 1973.** The intensity of spatial pattern in plant communities. *Journal of Ecology* 61: 225-235.
- Hood, G. 2003.** *PopTools version 2.5. PopTools version 2.6.2*. Available on the internet. URL <http://www.cse.csiro.au/poptools>.
- Hooftman, D.A.P., Diemer, M., Lienert, J. and Schmid, B. 1999.** Does habitat fragmentation reduce the long-term survival of isolated populations of dominant plants? A field design. *Bulletin of the Geobotanical Institute ETH* 65: 59-72.
- Horvitz, C. and Schemske, D.W. 1988.** Demographic cost of reproduction in a Neotropical herb: an experimental study. *Ecology* 69: 1741-1746.
- Huff, D.R., Peakall, R. and Smouse, P.E. 1993.** RAPD variation within and among populations of outcrossing buffalograss (*Buchlœe dactyloides* (Nutt.) Engelm.). *Theoretical and Applied Genetics* 86: 927-934.
- Huntley, B. 1991.** How Plants Respond to Climate Change: Migration Rates, Individualism and the Consequences for Plant Communities. *Annals of Botany* 67 (Suppl. 1): 15-22.

- Hutchings, M.J. 1987a.** The population biology of the early spider orchid *Ophrys sphegodes* Mill. I. A demographic study from 1975 to 1984. *Journal of Ecology* 75: 711-728.
- Hutchings, M.J. 1987b.** The population biology of the early spider orchid *Ophrys sphegodes* Mill. II. Temporal patterns in behaviour. *Journal of Ecology* 75: 729-742.
- Hutchings, M.J. 1989.** Population biology and conservation of *Ophrys sphegodes*. In: Pritchard, H. W. (ed.), *Modern Methods in Orchid Conservation*, Cambridge University Press, Cambridge, pp. 101-115.
- Hutchings, M.J., Mendoza, A. and Havers, W. 1998.** Demographic properties of an outlier population of *Orchis militaris* L. (Orchidaceae) in England. *Botanical Journal of the Linnean Society* 126: 95-107.
- Inghe, O. and Tamm, C.O. 1988.** Survival and flowering of perennial herbs V. Patterns of flowering. *Oikos* 51: 203-219.
- Inghe, O. 1990.** Computer simulations of flowering rhythms in perennials-is there a new area to explore in the quests for chaos? *Journal of Theoretical Biology* 147: 449-469.
- Jaccard, P. 1908.** Nouvelles recherches sur la distribution florale. *Bulletin de la Société Vaudoise des Sciences Naturelles* 44: 223-270.
- Jedicke, E. 1997.** *Die Roten Listen. Gefährdete Pflanzen, Tiere, Pflanzengesellschaften und Biotoptypen in Bund und Ländern.* Ulmer, Stuttgart.
- Jeltsch, F., Moloney, K. and Milton, S.J. 1999.** Detecting process from snapshot pattern: lessons from tree spacing in the southern Kalahari. *Oikos* 85: 451-466.
- Jenkins, G.M. and Watts, D.G. 1969.** *Spectral Analysis and its Applications.* San Francisco: Holden-Day.
- Juan, A., Crespo, M.B., Cowan, R.S., Lexer, C. and Fay, F. 2004.** Patterns of variability and gene flow in *Medicago citrina*, an endangered endemic of islands in the western Mediterranean, as revealed by amplified fragment length polymorphism (AFLP). *Molecular Ecology* 13: 2679-2690.
- Kershaw, K.A. 1957.** The use of cover and frequency in the detection of pattern in plant communities. *Ecology* 38: 291-299.
- Kershaw, K.A. 1960.** The detection of pattern and association. *Journal of Ecology* 48: 233-242.
- Kéry, M. and Gregg, K.B. 2003.** Effects of life-state on detectability in a demographic study of the terrestrial orchid *Cleistis bifaria*. *Journal of Ecology* 91: 265-273.
- Kim, M.S., Moore, P.H., Zee, F., Fitch, M.M.M., et al. 2002.** Genetic diversity of *Carica papaya* as revealed by AFLP markers. *Genome* 45: 503-512.
- Kindlmann, P. 1999.** Are orchid life histories really irregular? The case of *Epipactis albensis*. *Oikos* 85: 265-270.
- Kindlmann, P. and Balounova, Z. 1999.** Flowering regimes of terrestrial orchids: unpredictability or regularity? *Journal of Vegetation Science* 10: 269-273.
- Kindlmann, P. and Balounova, Z. 2001.** Irregular flowering patterns in terrestrial orchids: theories vs empirical data. *Web Ecology* 2: 75-82.
- Kluge, G. and Müller-Westermeier, G. 2000.** *Das Klima ausgewählter Orte der Bundesrepublik Deutschland: Jena.* Berichte des Deutschen Wetterdienstes 213, Deutscher Wetterdienst, Offenbach.
- Koelewijn, H.P. 1998.** Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution* 52: 692-702.

- Kölliker, R., Stadelmann, F.J., Reidy, B. and Nösberger, J. 1998.** Fertilization and defoliation frequency affect genetic diversity of *Festuca pratensis* Huds. in permanent grasslands. *Molecular Ecology* 7: 1557-1567.
- Kroon, H. de., Plaiser, A., Groenendael, J. van. and Caswell, H. 1986.** Elasticity: the relative contribution of demographic parameters to population growth rate. *Ecology* 67: 1427-1431.
- Krüger, A.M., Hellwig, F.H. and Oberprieler, C. 2002.** Genetic diversity in natural and anthropogenic inland populations of salt-tolerant plants: random amplified polymorphic DNA analyses of *Aster triploidum* L. (Compositae) and *Salicornia ramosissima* Woods (Chenopodiaceae). *Molecular Ecology* 11: 1647-1655.
- Kull, T. and Kull, K. 1991.** Preliminary results from a study of populations of *Cypripedium calceolus* in Estonia. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 69-76.
- Lefkovich, L.P. 1965.** The study of population growth in organisms grouped by stages. *Biometrics* 21: 1-18.
- Lembo, A.J. 2003.** Lecture 9. Spatial autocorrelation. Cornell University. www.css.cornell.edu/courses/620/lecture9.ppt
- Levins, R. 1969.** Some demographic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* 15: 237-240.
- Levins, R. 1970.** Extinction. In: M. Gesternhaber (ed.), *Some Mathematical Problems in Biology*, pp. 77-101. American Mathematical Society, Providence, Rhode Island.
- Lewis, E.G. 1942.** On the generation and growth of a population. *Sankhya* 6: 93-96.
- Likens, G.E. 1989.** *Long-term studies in Ecology*. Springer Verlag, Heidelberg.
- Lim, S.H., Liew, C.F., Lim, C.N., Lee, Y.H. and Goh, C.J. 1997.** A simple and efficient method of DNA isolation from orchid species and hybrids. *Biologia Plantarum* 41: 313-316.
- Linhart, Y.B. and Grant, M.C. 1996.** Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27: 237-277.
- Ludwig, J.A. and Goodall, D.W. 1978.** A comparison of paired with blocked quadrat variance methods for the analysis of spatial pattern. *Vegetatio* 38: 49-59.
- Lynch, M. and Milligan, B.G. 1994.** Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3: 91-99.
- MacArthur, R.H. and Wilson, E.O. 1967.** *The Theory of Island Biogeography*. Princeton University Press, Princeton.
- MacNally, R. 2000.** Regression and model-building in conservation biology, biogeography and ecology: The distinction between - and reconciliation of - 'predictive and 'explanatory' models. *Biodiversity and Conservation* 9: 655-671.
- Maughan, P.J., Saghai, M.A., Buss, G.R. and Huestis, G.M. 1996.** Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical Applied Genetics* 93: 393-401.
- Meusel, H., Jäger, E. and Weinert, E. 1965.** *Vergleichende Chorologie der Zentraleuropäischen Flora*. Gustav Fischer Verlag Jena, Jena.

- Michalakis, Y. and Excoffier, L. 1996.** A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142: 1061-1064
- Miller, M. 1997.** *Tools For Population Genetic Analyses (TFPGA)*. Version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff.
- Mitton, J.B. 1997.** *Selection in Natural Populations*. Oxford University Press, Oxford.
- Mooney, H.A. 1977.** *Convergent Evolution in Chile and California*. Stroudsburg, PA: Dowden, Hutchinson & Ross.
- Moran, P.A.P. 1950.** Notes on continuous stochastic phenomena. *Biometrika* 37: 17-23.
- Mueller, U.G. and Wolfenbarger, L.L. 1999.** AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution* 14: 389-394.
- Murren, C.J. 2003.** Spatial and demographic population genetic structure in *Catsetum viridiflavum* across a human-disturbed habitat. *Journal of Evolutionary Biology* 16: 333-342.
- Murren, C.J. and Ellison, A.M. 1998.** Seed dispersal characteristics of *Brassavola nodosa* (Orchidaceae). *American Journal of Botany* 85: 675-680.
- Negi, M.S., Sabharwal, V., Bhat, R. and Lakshmikumaran, M. 2004.** Utility of AFLP markers for the assessment of genetic diversity within *Brassica nigra* germplasm. *Plant Breeding* 123: 13-16.
- Nei, M. 1973.** Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* 70: 3321-3323.
- Nei, M. 1978.** Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Nei, M. 1987.** *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Obeso, J.R. 2002.** The costs of reproduction in plants. *New Phytologist* 155: 321-348.
- Oostermeijer, J.G.B., van't Veer, R. and den Nijs, J.C.M. 1994a.** Population structure of the rare, long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management in the Netherlands. *Journal of Applied Ecology* 31: 428-438.
- Oostermeijer, J.G.B., van Eijck, M.W. and den Nijs, J.C.M. 1994b.** Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia* 97: 289-296.
- Oostermeijer, J.G.B., van Eijck, M.W., van Leeuwen, N.C. and den Nijs, J.C.M. 1995.** Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe*. *Journal of Evolutionary Biology* 8: 739-759.
- Orians, G.H. and Paine, R.T. 1983.** Convergent evolution at the community level. In: Futuyma, D.J. and Slatkin, M. (eds.), *Coevolution*, pp. 431-458, Sutherland, MA: Sinauer Associates.
- Ouborg, N.J., 1993.** Isolation, population size and extinction: the classical and metapopulation approaches applied to vascular plants along the Dutch Rhine-system. *Oikos* 66: 298-308.
- Palmer, M.W. 1988.** Fractal geometry: a tool for describing spatial pattern of plant communities. *Vegetatio* 75: 91-102.
- Paul, S., Wachira, F.N., Powell, W. and Waugh, R. 1997.** Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kunze) revealed by AFLP markers. *Theoretical Applied Genetics* 94: 255-263.

- Peakall, R., Smouse, P.E. and Huff, D.R. 1995.** Evolutionary implications of allozyme and RAPD variation in diploid populations of Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). *Molecular Ecology* 4: 135-147.
- Peakall, R. and Smouse, P.E. 2001.** *GenAlEx V5: Genetic Analysis in Excel*. Population genetic software for teaching and research. Australian National University, Canberra, Australia.
- Perry, J.N., Liebhold, A.M., Rosenberg, M.S. et al. 2002.** Illustrations and guidelines for selecting statistical methods for quantifying spatial patterns in ecological data. *Ecography* 25: 578-600.
- Pfeifer, M, Heinrich, W. and Jetschke, G. (submitted).** Climate, size, and flowering history determine flowering pattern of *Himantoglossum hircinum*. Submitted to OIKOS.
- Powell, W., Morgante, M. and Andre, C., et al. 1996.** The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis. *Molecular Breeding* 2: 225-238.
- Primack, R.B. and Hall, P. 1990.** Costs of reproduction in the pink lady's slipper orchid: a four year experimental study. *American Naturalist* 136: 638-656.
- Primack, R.B., Miao, S.L. and Becker, K.R. 1994.** Costs of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*): defoliation, increased fruit production and fire. *American Journal of Botany* 81: 1083-1090.
- Pulliam, H.R. 1988.** Sources, sinks and population regulation. *American Naturalist* 132: 652-661.
- Qamaruz-Zaman, F., Fay, M.F., Parker, J.S. and Chase, M.W. 1998.** The use of AFLP fingerprinting in conservation genetics: a case study of *Orchis simia* (Orchidaceae). *Lindleyana* 13: 125-133.
- Raymond, M. and Rousset, F. 1994.** An exact test for population differentiation. *Evolution* 49: 1280-1283.
- Reed, J.M., Mills, L.S., Dunning, Jr.J.B. et al. 2002.** Emerging Issues in Population Viability Analysis. *Conservation Biology* 16: 7-19.
- Rees, M., Condit, R., Crawley, M., Pacala, S. and Tilman, D. 2001.** Long-Term Studies of Vegetation Dynamics. *Science* 293: 650-655.
- Richardson, J.E., Fay, M.F. and Cronk, Q.C.B., et al. 2003.** Species delimitation and the origin of populations in island representatives of *Phyllica* (Rhamnaceae). *Evolution* 57: 816-827.
- Ripley, B.D. 1981.** *Spatial statistics*. Wiley, New York, USA.
- Rohlf, F.J. 1998.** *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis system*, Version 2.0. Applied Biostatistics, New York.
- Sawada, M. 1999.** ROOKCASE: An Excel 97/2000 Visual Basic (VB) Add-in for Exploring Global and Local Spatial Autocorrelation. *Bulletin of the Ecological Society of America* 80: 231-234.
- Scacchi, R., De Angelis, G. and Lanzara, P. 1990.** Allozyme variation among and within eleven *Orchis* species (fam. Orchidaceae), with special reference to hybridizing aptitude. *Genetica* 81: 143-150.
- Scacchi, R., De Angelis, G. and Corbo, R.M. 1991.** Effect of the breeding system on the genetic structure in three *Cephalanthera* spp. (Orchidaceae). *Plant Systematics and Evolution* 176: 53-61.
- Schaal, B.A. 1975.** Population structure and local differentiation in *Liatris cylindracea*. *American Naturalist* 109: 511-528.

- Schneider, S., Roessli, D. and Excoffier, L. 2000.** *ARLEQUIN*, version 2.000. A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.
- Schurr, F.M., Bossdorf, O., Milton, S.J. and Schumacher, J. 2004.** Spatial pattern formation in semi-arid shrubland: a priori predicted versus observed pattern characteristics. *Plant Ecology* 173: 271-282.
- Seefelder, S., Ehrmaier, H., Schweizer, G. and Seigner, E. 2000.** Genetic diversity and phylogenetic relationships among accessions of hop, *Humulus lupulus*, as determined by amplified fragment length polymorphism fingerprinting compared with pedigree data. *Plant Breeding* 119: 257-263.
- Shannon, C.E. and Weaver, W. 1949.** *The mathematical theory of communication*. University of Illinois Press, Urbana.
- Sharma, S.K., Knox, M.R. and Ellis, T.H.N. 1996.** AFLP analysis of the diversity and phylogeny of lens and its comparison with RAPD analysis. *Theoretical Applied Genetics* 93: 751-758.
- Sharma, I.K., Jones, D.L. and French, C.J. 2003.** Unusually high genetic variability revealed through allozymic polymorphism of an endemic and endangered Australian orchid, *Pterostylis aff. picta* (Orchidaceae). *Biochemical Systematics and Ecology* 31: 513-526.
- Shea, K. and Kelly, D. 1998.** Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications* 8: 824-832.
- Silvertown, J. and Charlesworth, D. 2001.** *Introduction to Plant Population Biology*. Blackwell Science, Oxford.
- Slatkin, M. and Barton, N.H. 1989.** A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43: 1349-1368.
- Sneath, P.H. and Sokal, R.R. 1973.** *Numerical Taxonomy*, W.H. Freeman and Co. (eds.) San Francisco, CA.
- Snow, A.A. and Whigham, D.F. 1989.** Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70: 1286-1293.
- Spada, A., Mantegazza, R., Biloni, M., Caporali, E. and Sala, F. 2004.** Italian rice varieties: historical data, molecular markers and pedigrees to reveal their genetic relationships. *Plant Breeding* 123: 105-111.
- Stenström, A., Jonsson, B.O., Jonsdottir, I.S., Fagerströms, T. and Augner, M. 2001.** Genetic variation and clonal diversity in four clonal sedges (*Carex*) along the Arctic coast of Eurasia. *Molecular Ecology* 10: 497-513.
- Stoyan, D. and Stoyan, H. 1994.** *Fractals, Random Shapes and Point Field. Methods of Geometrical Statistics*. John Wiley & Sons, Chichester, UK.
- Stuckey, I.H. 1967.** Environmental factors and the growth of native orchids. *American Journal of Botany* 54: 232-241.
- Summerhayes, V.S. 1951.** *Wild Orchids of Britain*. Collins, London, UK.
- Sun, M. and Wong, K.C. 2001.** Genetic structure of three orchid species with contrasting breeding systems using RAPD and allozyme markers. *American Journal of Botany* 88: 2180-2188.
- Tamm, C.O. 1972.** Survival and flowering of some perennial herbs. II. The behaviour of some orchids on permanent plots. *Oikos* 23: 23-38.

- Tamm, C.O. 1991.** Behaviour of some orchid populations in a changing environment. Observations on permanent plots, 1943-1990. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 1-14.
- Teo, L.L., Kiew, R., Set, O., Lee, S.K. and Gan, Y.Y. 2002.** Hybrid status of kuwini, *Mangifera odorata* Griff. (Anacardiaceae) verified by amplified fragment length polymorphism. *Molecular Ecology* 11: 1465-1469.
- Tilman, D. 1988.** *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press, Princeton, New Jersey.
- Tilman, D. and Kareiva, P. 1997.** *Spatial ecology: The role of space in population dynamics and interspecific interactions*. Princeton University Press, Princeton, New Jersey.
- Tohme, J., Gonzalez, D.O., Beebe, S. and Duque, M.C. 1996.** AFLP analysis of gene pools of a wild bean core collection. *Crop Science* 36: 1375-1384.
- Travis, S.E., Machinski, J. and Keim, P. 1996.** An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Molecular Ecology* 5: 735-745.
- Upton, G.J.G. and Fingleton, B. 1985.** *Spatial Data Analysis by Example, Volume 1, Point Pattern and Quantitative Data*, Wiley.
- Vanhecke, L.E.M. 1991.** Population dynamics of *Dactylorhiza praetermissa* in relation to topography and inundation. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 15-32.
- Vos, P., Hogers, R. and Bleeker, M., et al. 1995.** AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407-4414.
- Vuylsteke, M., Mank, R., Antonise, R., et al. 1999.** Two high-density AFLP® linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. *Theoretical and Applied Genetics* 99: 921-935.
- Waite, S. and Hutchings, M.J. 1991.** The effects of different management regimes on the population dynamics of *Ophrys sphegodes*: analysis and description using matrix models. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 161-175.
- Waite, S. and Farrell, L. 1998.** Population biology of the rare military orchid (*Orchis militaris* L.) at an established site in Suffolk, England. *Botanical Journal of the Linnean Society* 126: 109-121.
- Waser, N.M. 1987.** Spatial genetic heterogeneity in a population of the montane perennial herb *Delphinium nelsonii*. *Heredity* 58: 249-256.
- Wells, T.C.E. 1967.** Changes in a population of *Spiranthes spiralis* (L.) Chevall. at Knocking Hoe National Nature Reserve, Bedfordshire, 1962-65. *Journal of Ecology* 55: 83-99.
- Wells, T.C.E. and Cox, R. 1989.** Predicting the probability of the bee orchid (*Ophrys apifera*) flowering or remaining vegetative from the size and number of leaves. In: Pritchard, H.W. (ed.), *Modern Methods in Orchid Conservation*, Cambridge University Press, Cambridge, pp. 127-139.
- Werner, P.A. 1975.** Predictions of fate from rosette size in teasel (*Dipsacus fullonum* L.). *Oecologia* 20: 197-201.

- Whigham, D.F. and O'Neill, J. 1991.** The dynamics of flowering and fruit production in two Eastern North American terrestrial orchids, *Tipularia discolor* and *Liparis lilifolia*. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 89-102.
- Wiegand, T. and Moloney, K.A. 2004.** Rings, circles, and null-models for point pattern analysis in ecology. *Oikos* 104: 209-229.
- Wiegand, T., Moloney, K.A. and Milton, S.J. 1998.** Population dynamics, disturbance, and pattern evolution: identifying the fundamental scales of organization in a model ecosystem. *American Naturalist* 152: 321-337.
- Willems, J.H. and Bik, L. 1991.** Long-term dynamics in a population of *Orchis simia* in the Netherlands. In: Wells, T.C.E and Willems, J.H. (eds.), *Population ecology of terrestrial orchids*. The Hague: SPB Publishing, pp. 33-46.
- Willems, J.H. and Melsers, C. 1998.** Population dynamics and life history of *Coeloglossum viride* (L.) Hartm.: An endangered orchid species in the Netherlands. *Botanical Journal of the Linnean Society* 126: 83-93.
- Willems, J.H. and Dorland, E. 2000.** Flowering frequency and plant performance and their relation to age in the perennial orchid *Spiranthes spiralis* (L.) Chevall. *Plant Biology* 2: 344-349.
- Willis, N. 1990.** Lizard Orchid (*Himantoglossum hircinum*) wardening at Royal St. Georges Golf Course, Sandwich Bay, Kent. *Kent Trust for Nature Conservation report*, Kent Wildlife Trust, Maidstone, Kent, UK.
- Winfield, M.O., Arnold, G.M. and Cooper, F., et al. 1998.** A study of the genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers. *Molecular Ecology* 7: 3-10.
- Wong, K.C. and Sun, M. 1999.** Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). *American Journal of Botany* 86: 1406-1413.
- Wright, S. 1978.** *Evolution and the Genetics of Populations, Vol. 4. Variability Within and Among Natural Populations*. University of Chicago Press, Chicago.
- Yeh, F.C., Yang, R., Boyle, T. 1999.** *Popgene Version 1.31*. Microsoft Window-based Freeware for Population Genetic Analysis. Department of Renewable Resources. University of Alberta, Edmonton.
- Zimmerman, J.K. and Pyke, G.H. 1988.** Experimental manipulations of *Polemonium foliosissimum*: effects on subsequent nectar production, seed production and growth. *Journal of Ecology* 76: 777-789.
- Zimmerman, J.K. and Aide, T.M. 1989.** Patterns of fruit production in a Neotropical orchid: pollinator vs. resource limitation. *American Journal of Botany* 76: 67-73.

Appendices

Appendix 1. Air temperature and precipitation data of the weather station of the University of Jena. Monthly and annual values for the period of this study (2002-2004) and for a reference period (1961-1990) are given. Monthly and annual values for a second reference period (1901-1960) are provided to indicate the overall change in weather in the 20th century.

	1901-1960	1961-1990	1991-2000	2002	2003	2004
Precipitation [mm]						
January	38.2	36.5	31.6	35.9	38.7	44.1
February	32.3	34.2	32.1	68.9	6.7	27.7
March	34.5	42.8	47.9	52.0	32.8	21.1
April	44.8	57.2	41.3	36.2	28.1	30.6
May	55.8	61.9	54.3	67.4	34.4	82.5
June	68.4	76.5	61.9	52.8	56.2	43.2
July	78.5	51.7	98.2	65.9	68.5	116.0
August	60.4	62.5	67.0	112.9	16.0	34.0
September	49.8	42.4	44.1	34.2	78.9	39.5
October	45.0	38.4	42.1	62.3	46.4	14.1
November	39.2	40.8	46.2	122.8	34.4	69.2
December	35.7	42.2	44.0	72.4	26.8	25.2
annual amount	582.5	587.0	610.7	783.7	467.9	547.2
Air temperature [°C]						
January	0.0	0.4	1.7	1.7	0.5	0.6
February	0.8	1.2	2.5	6.1	-1.6	3.7
March	4.3	4.6	5.8	6.1	6.0	5.1
April	8.4	8.6	9.6	8.5	9.1	10.3
May	13.2	13.4	14.1	15.0	15.4	12.3
June	16.3	16.7	17.0	18.3	20.3	16.4
July	18.0	18.2	19.0	19.0	20.1	18.0
August	17.0	17.4	18.6	20.1	21.7	19.8
September	13.5	14.2	14.3	14.0	14.5	14.9
October	8.9	9.9	9.5	9.1	6.6	11.0
November	4.3	5.0	4.7	6.3	6.8	5.1
December	1.3	1.7	2.1	-0.2	2.3	1.3
annual mean	8.8	9.3	9.9	10.3	10.1	9.9

Appendix 2. Mean number of days with soil temperature below 0°C (SFD) and mean minimum temperature (Tmin) of the weather station of the University of Jena. Monthly and annual values for the two periods (1961-1990 and 1991-2000/1991-1999) are given. Data for SFD values in 2000 were incomplete and excluded.

	Tmin [°C]		SFD	
	1961-1990	1991-2000	1961-1990	1991-1999
January	-12.2	-11.5	25	21
February	-10.7	-11.0	23	21
March	-7.1	-3.6	19	18
April	-2.2	-1.5	10	10
May	1.5	2.3	2	2
June	5.3	6.0	0	0
July	7.5	8.8	0	0
August	6.8	7.9	0	0
September	4.0	4.3	0	0
October	-0.9	-1.9	5	7
November	-5.9	-5.0	15	14
December	-10.9	-11.0	22	20
annual	-15.8	-14.7	121	114

Appendix 3. Number of leaves (N_{leaves}), size of reproductive structures (height of inflorescence and stalk, $\text{Height}_{\text{inf}}$, $\text{Height}_{\text{stalk}}$), and fruits set of flowering individuals in year 2002/2003 (season 1), and 2003/2004 (season 2). In season 1: * = flowered again in the successive year. Missing values are indicated by a "?".

Season 1: 2002/2003						
No.	N_{leaves}	$\text{Height}_{\text{inf}}$ [cm]	$\text{Height}_{\text{stalk}}$ [cm]	N_{flowers}	N_{fruits}	Fruit set [%]
1	7	12.8	15.9	44	0	0.0
2	7	2.5	9.5	1	0	0.0
3	7	6.7	11.5	26	0	0.0
4	8	3.6	4.0	0	0	0.0
5	8	12.0	27.0	39	0	0.0
6	8	14.6	24.7	48	0	0.0
7	8	14.7	20.9	36	0	0.0
8	8	8.3	16.5	23	0	0.0
9	8	11.9	21.3	43	0	0.0
10	8	11.9	19.2	48	1	2.1
11	8	9.2	23.1	30	1	3.3
12	8	8.6	27.6	25	2	8.0
13	8	10.3	17.0	28	2	7.1
14*	8	8.4	17.9	31	0	0.0
15	9	9.9	22.4	41	0	0.0
16*	9	14.8	25.8	36	0	0.0
17	9	11.0	19.0	29	0	0.0
18	9	13.8	20.8	46	0	0.0
19*	9	10.5	22.0	36	0	0.0
20	9	11.9	20.4	44	0	0.0
21*	9	4.5	7.7	9	0	0.0
22	9	12.8	24.7	35	1	2.9
23	9	9.0	17.3	40	1	2.5
24	9	11.7	21.1	39	5	12.8
25	9	18.3	30.0	50	16	32.0
26*	9	22.0	31.0	52	0	0.0
27	10	11.0	26.0	42	0	0.0
28	10	9.5	26.8	29	0	0.0
29*	10	10.0	24.6	36	6	16.7
30	10	16.1	28.0	68	21	30.9
31	10	8.0	17.0	43	0	0.0
32	10	27.0	43.0	54	0	0.0
33	11	19.0	34.5	51	15	29.4

Season 2: 2003/2004

No.	N _{leaves}	Height _{Inf} [cm]	Height _{stalk} [cm]	N _{flowers}	N _{fruits}	Fruit set [%]
1	7	16.0	11.0	18	0	0.0
2	7	22.0	15.0	34	0	0.0
3	7	23.0	23.0	33	0	0.0
4	7	25.0	16.0	40	0	0.0
5	7	27.0	14.0	30	2	6.7
6	7	25.0	18.0	32	4	12.5
7	7	20.0	14.0	29	4	13.8
8	7	13.0	14.0	35	8	22.9
9	8	?	?	24	0	0.0
10	8	25.5	15.5	35	0	0.0
11	8	22.0	20.0	48	1	2.1
12	8	23.0	15.0	51	4	7.8
13	8	32.0	22.0	51	6	11.8
14	8	32.0	18.0	39	8	20.5
15	9	21.0	15.0	33	0	0.0
16	9	26.0	15.0	37	0	0.0
17	9	23.2	9.8	49	0	0.0
18	9	20.0	29.0	52	0	0.0
19	9	22.5	22.5	41	1	2.4
20	9	26.0	19.0	33	2	6.1
21	9	33.0	18.0	53	5	9.4
22	10	29.0	12.0	54	2	3.7
23	10	29.0	20.0	58	16	27.6
24	12	35.0	28.0	65	3	4.6
25	12	29.0	22.0	51	12	23.5
26	?	28.0	9.0	30	0	0.0
27	?	21.0	26.0	65	0	0.0

Acknowledgements

I especially want to thank **PD Dr. Gottfried Jetschke** for providing the topic of my dissertation, helping me whenever I needed help, the freedom I had during my studies, and the fast proof-reading towards the end of this project.

I thank **Dr. Wolfgang Heinrich** for providing the long-term data set on the life the *H. hircinum* individuals recorded between 1976 and 2001, for suggestions, and for helpful comments on the project. He also provided information on the site history. I thank **Dr. Gerhard Daut** and **Prof. Gerhard Kluge** for providing the data set of the weather variables.

I thank the **federal state of Thuringia** and the **University of Jena** for their trust and financial support enabling my PhD project in time. I also want to thank the **Deutsche Forschungsgemeinschaft (DFG)** for financing my participation in the International Orchid Workshop held in Haapsalu (Estonia) in July 2004. In this context I thank **Dr. Markus Wagner**, **Prof. Dr. Wolfgang Weisser** and **Beathe Böhm** for advice on how to raise money for the participation in the workshop and the financing of the last three months of my project.

Recodings on the permanent monitoring site started under the guidance of the **Institute of Ecology**. Later in time observations were funded by the **Thüringer Landesanstalt für Umwelt und Geologie Jena** and the **Arbeitskreis Heimische Orchideen Thüringen**. I thank the **Thüringer Landesanstalt für Umwelt und Geologie** for the permits to work on the site, to use the data on *H. hircinum*, and to take leaf samples for the AFLP analysis.

I want to thank my ex-husband **Andre Pfeifer** for logistic and moralistic support, and help in the field during the first year, and for caring for our two children, by this making the project possible.

Special thanks go to a number of people who provided useful discussions and valuable proof-reading of manuscripts during the final write-up stage. **Prof. Stefan Halle**, **Dr. Andrew Davis**, and **Dr. Michael Gillman** commented on the manuscript on which Chapter four is based. **Prof. Dr. Stefan Halle**, **Prof. Dr. Pavel Kindlmann** and **Dr. Tiiu Kull** provided comments on the manuscript on which Chapter five is based. I thank **Dr. Kerstin Wiegand** for advice and co-authoring on the manuscript on which Chapter three is based and for helpful advice on Chapter six.

I thank **Dr. Jörg Perner**, **Dr. Markus Wagner** and **Dr. Jens Schumacher** for statistical help, and **Uli Möller** for solving computer problems. Parts of Chapter six would not have been possible without the software for point pattern analysis developed by **Dr. Jens Schumacher**.

I thank **Dr. Henryk Baumbach** and **Dr. Jens Pahnke** (Institute of Special Botany, Jena) for teaching me how to work with AFLP markers and I thank **Prof. Frank H. Hellwig** for permitting me to use laboratory facilities at the Institute of Special Botany. In this context I also thank **Marion Sandmann** for introducing me to good laboratory practice.

Very special thanks go to my family, and especially to my mum, **Rieta Kirchner** for encouragement and trust throughout my studies.

I also want to give my special thanks to **Dr. Michael P. Gillman** for encouragement and being there for me during the final write-up stage.

Finally I want to thank **Dr. Markus Wagner, Verena Blanke, Clemens Augspurger, Katrin Meyer, Sybille Unsicker, Dr. Kerstin Wiegand** and the other members of the Institute of Ecology for the great working atmosphere and after work enjoyment.

Selbständigkeitserklärung

Ich erkläre hiermit, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.

Jena, den 31.12.2004

Marion Pfeifer

.....

Curriculum Vitae

Dipl. Biol. Marion Pfeifer

Office: University of Jena, Institute of Ecology
Group: Plant Ecology and Modelling
Dornburger Str. 159, 07743 Jena (Germany)
Phone: (++49) 3641 949454

Home: Katharinenstr. 20, 07743 Jena
Phone: (++49) 3641 470065
pfeifermarion@web.de



Personal Details

Name: Marion Pfeifer
Date and Place of Birth: 18.12.1976 in Weimar (Thuringia, Germany)
Marital Status: Divorced

Education

1998 - 2002 Diploma degree, Biology, CSIRO Australia, University of Jena (overall mark "very good")
Concentrations: Ecology, Tropical Ecology, Botany, Zoology
Thesis: "Spatial point pattern analysis of pioneer and non-pioneer tree species in a dry tropical rainforest in northern Queensland, Australia"

1996-1998 Vordiplom (= Intermediate Degree), Biology, University of Jena, Germany (overall mark: "very good")

1991 - 1995 University entrance diploma, Grammar School, Bad Berka (overall mark "very good")

Work experience

05/2004 - 08/2002 Temporary employment as scientific assistant in the "Jena-Experiment" (Joint project between the Institute of Ecology (University of Jena) and Max Planck Institute of Biogeochemistry (Jena):
Field work and separation of the plant species (grasslands) for further biomass measurements

09/2003 - 04/2004 Temporary employment as scientific assistant in the Biotree-Project of the department "Biogeochemical processes", the Max Planck Institute of Biogeochemistry, Jena:
Treatment of soil samples, field work in planting of forest trees according to field design of the biodiversity project, translation and assistance with homepage, www.biotree.bgc-jena.mpg.de, dendro-chronological analysis

02/2000 Thuringian Institute of Agriculture and Environment, Department of Ecology and Conservation, Germany
Participation in habitat protection programs, FFH guidelines

03/1999 - 07/1999 Australia, National Parks

- 08/1999 - 10/1999 Practical course, National Park Bavarian Forest, Germany
Telemetric studies on habitat use of fox (*Vulpes vulpes*) and badger (*Meles meles*)
- 09/1998 - 10/1998 Practical course, Ecuador, Jatun Sacha Foundation
Ecological studies in a mountain rainforest in Ecuador
- 07/1997 Practical course, Institute of Biological Marine Research (IBM),
Ecology of the Baltic Sea, methods of marine research including data
sampling

Research skills

- Utilized SPSS, Canoco, PCO statistical programs extensively
- Spatial point pattern analysis using Ripley's K and O-Ring statistics
- Extensive Lab work including PCR, Primer Screening, AFLP
- Species identification and vegetational studies including conservation suggestions for larger areas
- Literature surveys
- Genetic analysis statistics

Languages

Fluent in English and German, Basic skills in Spanish

Professional experience

- 08/2004 Instructor, Institute of Ecology, University of Jena, Germany Summer
course for students: Spatial point pattern analysis of selected plant
species on a dry calcareous grassland: field work and statistics
- 08/2003 Instructor, Institute of Ecology, University of Jena, Germany Summer
course for students: Vegetation analysis - field work and statistical
analysis
- 2002 - 2004 Lectures for undergraduate students, Institute of Ecology, University of
Jena, Germany, Issues in Ecology
- 12/2003 Winter Symposium of the Institute of Ecology, University of Jena
Member of organizing committee

Research submitted and in preparation

Pfeifer M., Heinrich W. and Jetschke G.: Climate, size, and flowering history determine flowering behaviour of *Himantoglossum hircinum* (L.) (Orchidaceae). Submitted to OIKOS

Pfeifer M. and Jetschke G.: Influence of geographical isolation on genetic diversity of *Himantoglossum hircinum* (Orchidaceae). Submitted to FOLIA GEOBOTANICA

Pfeifer M., Wiegand K., Heinrich W. and Jetschke G.: Plasticity of life history characteristics and variability of life state transitions of *Himantoglossum hircinum* (Orchidaceae). In preparation.

Pfeifer M, Schumacher J, Heinrich W and Jetschke G.: Spatial pattern and spatial dynamics of an orchid. In preparation.

Conference presentations

06/2004 - 07/2004 International Orchid Workshop, Haapsalu, Estonia
Presentation of the manuscript: Climate, size, and flowering history determine flowering behaviour of *Himantoglossum hircinum* (L.). Submitted to OIKOS

Research interests

Plasticity of life history traits and causing factors in the context of evolution, statistical analysis and modelling of the relationships between plant population dynamics and weather variability, habitat fragmentation and its influence on genetic diversity of plant populations (using AFLP) - conservation genetics, spatial point patterns in plants using second-order spatial statistics (intra- and interspecific relationships, vegetation dynamics), relationship between productivity/species functional group and biodiversity

Referees

- (1) Name: Prof. Dr. Stefan Halle
Address: Institute of Ecology
University of Jena
Dornburger Str. 159
07743 Jena
Germany
e-mail: stefan.halle@uni-jena.de
Tel. ++49 3641 949400
Relation: Head of Department
- (2) Name: PD Dr. Gottfried Jetschke
Address: Institute of Ecology
University of Jena
Dornburger Str. 159
07743 Jena
Germany
e-mail: Gottfried.Jetschke@uni-jena.de
Tel. ++49 3641 949420
Relation: PhD supervisor