Seed dispersal by ants
and its consequences for the phenology of plants
-
A study system for mutualistic animal-plant-interactions

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Wenn weder Zahlen noch Figuren
Sind Schlüssel aller Kreaturen,
Wenn die, so singen oder küssen,
Mehr als die Tiefgelehrten wissen,
Wenn sich die Welt ins freie Leben,
Und in die Welt wird zurück begeben,
Wenn dann sich wieder Licht und Schatten
Zu echter Klarheit werden gatten,
Und man in Märchen und Gedichten
Erkennt die wahren Weltgeschichten,
Dann fliegt vor einem geheimen Wort
Das ganze verkehrte Wesen fort.

NOVALIS
(FRIEDRICH VON HARDENBERG,
1772-1801)

Nach Jahren quantitativer Forschung und zahllosen statistischen Berechnungen eine Erinnerung, daß Empirie nicht alles ist, das zählt.

Contents

1. General Introduction

1.1 Community ecology and the meaning of phenology

Ecology is the study of interactions between organisms and their biotic as well as abiotic environment. Ecological studies can be performed on the individual, population and community level. In the last case, which is my area of research, the aim is to find and understand ecological patterns and mechanisms that concern interactions between organisms of different species (RICKLEFS 1990, BEGON et al. 1996). Basically, the interactions can be positive, neutral or negative for any of the interaction partners. Interactions that are positive for both partners are called mutualistic. Mutualistic interactions play an important role in nature mainly for two reasons. First, they are very common in natural communities, second, the reproduction and hence the survival of species depend - sometimes critically - on the benefit of mutualists (FEINSINGER 1983, HOWE & WESTLEY 1988, PIANKA 1994, BEGON et al. 1996). In my opinion, the study of mutualistic animal-plant-interactions is one of the most fascinating fields in community ecology.

In temperate regions two types of mutualistic animal-plant-interactions are both widespread and eye-catching: pollination and seed dispersal by animals. While animals gain food from these interactions, plants benefit from gene flow (BEATTIE 1978). With pollination, animals transfer male gametes to other plant individuals of the same species, therefore being necessary for the sexual reproduction of many plant species. Cross fertilization between plants of the same and, more rarely, of different subpopulations is assumed to be of major importance for the reproduction and fitness of plants (FAEGRI & VAN DER PIJL 1976, HOWE & WESTLEY 1988, BOND 1995). With seed dispersal, animals disperse plant embryos. Successful seed dispersal can contribute to the avoidance of predators, pathogens and competition, to increased genetic heterogeneity and successful cross-fertilization among plants of the following generations, to the spatial expansion of the plant population, and to the establishment of new plant populations (HOWE & SMALLWOOD 1982, HOWE 1986, HOWE & WESTLEY 1988, BOND 1995, BONN & POSCHLOD 1998). Besides seedling establishment, pollination and seed dispersal appear to be the dominant factors influencing reproduction in plants (FENNER 1985).

Given the importance of pollination and seed dispersal for plant reproduction, one can assume that plants have evolved special attributes and adaptations that increase the probability

for successful interactions (FEINSINGER 1983, FENNER 1985, HOWE & WESTLEY 1988). In fact, many plant attributes are discussed as adaptations to animal pollinators and dispersers. With respect to pollination, adaptive traits often appear to be tuned to particular animal species and correspondingly reflect the pollinator spectrum of a plant species. A group of different plant attributes, e.g. form, color and odor of flowers, represents the so-called pollination syndrome (HOWE & WESTLEY 1988). An example for the pollination syndrome of plants pollinated by nocturnal moths is the concurrence of nectar producing, deep flowers with bright colors and intensive odors. Corresponding syndromes are known for the seed dispersal interactions. For example, fleshy intensely colored fruits in exposed positions are typical for bird dispersed plant species (HOWE & WESTLEY 1988). In comparison to pollination, seed dispersal by animals appears to be more diffuse (JANZEN 1983, HERRERA 1985, HOWE & WESTLEY 1988, HANDEL & BEATTIE 1990), indicated by a lack of highly specialized, tight animal-plant-interactions and by species attributes which can be less obviously explained by adaptations.

Morphological adaptations, e.g. the form of flowers and fruits, as well as physiological attributes, e.g. the production of nutrients as reward for pollinators and dispersers, have been intensely studied for many decades. For seed dispersal by ants, for example, it has been known since 1873 by observations of J. F. Moggridge (see SERNANDER 1906) that some plant species have seeds with food bodies attached which represent nutrients for ants. Phenological attributes of plants, however, have been considered only for the last few decades. The phenology, the development of organisms during one season, appears to be important because selection is assumed to favor species that avoid unfavorable times (FENNER 1998). Plant phenology includes vegetative growth, flowering, fruit ripening and fruiting. As reproductively essential periods, flowering and fruiting phenology appear to be most important. Low availability of pollinators and dispersers, high competition for resources between plant species, and intensive predation on flowers, seeds or fruits are assumed as selection forces which influence and shape flowering and fruiting times (RATHCKE & LACEY 1985, RATHCKE 1988, FENNER 1998). Thus, some plant species may flower or fruit earlier or later in the season in order to reduce interspecific competition and predation or to take advantage of higher pollinator or disperses activities. In pollination ecology some evidence exists for the adaptive nature of plant phenology, in seed dispersal ecology evidence for the adaptive significance is rare and mostly appears to be speculative (see FENNER 1998).

1.2 The comparative approach in community ecology

Most of the earlier ecological studies investigated animal-plant-interactions between a single plant species and their pollinators or dispersers. These studies revealed detailed aspects for the plant species studied, but their results could at best be partly generalized. Recently, more studies investigate a group of plant species defined by their phylogenetic relationship (SMITH-RAMIREZ et al. 1998), their habitat (MURALI & SUKUMAR 1994), or their life history strategy (RATHCKE 1988). Such comparative studies which consider many different species are able to identify more universal patterns. In general, the more species are comparatively investigated in the same study the broader the view one gets from the results on possible overall patterns and relationships (BROWN 1995).

A fundamental problem arises with comparative data, e.g. the fruiting times of a list of plant species, and statistical standard procedures to test possible relationships on significance. All organisms are evolutionary related and share a common phylogenetic origin. For data on species attributes that are phylogenetically conservative, standard statistical procedures are inappropriate because the sampling units, e.g. the species, do not represent independent samples (FELSENSTEIN 1985, HARVEY & PAGEL 1991, GITTLEMAN & LUH 1992). In other words, traits of species which change very slowly in evolutionary times represent an inheritance from ancestral species. As a result, a group of species may show similar attributes only because they have the same common ancestor. In this case, the presence of similar attributes in these species does not represent an ecological but a phylogenetic pattern. For example, if a group of species features similar fruiting peaks and similar dispersal modes, this could be explained phylogenetically or ecologically. In the former case, a significant correlation between fruiting peak and dispersal mode allows no ecological implication because the sampling units do not represent independent samples and the similarities found are due to phylogenetic autocorrelation. Only in case of species attributes that are phylogenetically unrelated significant results of statistical tests can be assumed to reflect a real ecological relationship. Thus, before testing ecological hypotheses and evaluating the results obtained one has to check possible phylogenetic effects of the species attributes studied (BÖHNING-GAESE & OBERRATH 1999, BÖHNING-GAESE et al. in press).

1.3 Study system and aim of thesis

Seed dispersal by ants (myrmecochory) is an inconspicuous animal-plant-interaction. Myrmecochorous plants are usually small herbs that feature small seeds to which little food bodies are attached. These food bodies, called elaiosomes because of their fatty nutrients (SERNANDER 1906), contain special ant attractants which induce ants to collect the whole diaspore (seed plus elaiosome). Thus, the elaiosome stimulates the ants to carry the seed away which results in seed dispersal. While the benefit for ants appears obvious (i.e. food), the benefit for the plants is less clear. Five hypotheses exist to explain the benefit for plants (BEATTIE 1985). First, the dispersal of seeds from the parent plant may help to avoid seedling competition and competition between seedlings and parent plant. Second, ants may transport seeds to safe sites where the seeds suffer less predation. Third, seeds may reach nutrient rich sites by ant dispersal resulting in a higher likelihood of successful seedling establishment. Fourth, the transport below the ground may protect seeds from fire and destruction. Fifth, ant dispersal may reduce interspecific competition between plant species. Since myrmecochory is widespread both taxonomically in the plants' kingdom and geographically over the world (BUCKLEY 1982, BEATTIE 1983, HANDEL & BEATTIE 1990, PEMBERTON & IRVING 1990), different hypotheses may be true for different species in different regions. For example, fire avoidance appears not to be a selection force on myrmecochory in temperate forested habitats. However, myrmecochorous plants are abundant in North American and European forests (SERNANDER 1906, SCHEMSKE et al. 1978, PRIMACK 1985, HANDEL & BEATTIE 1990).

The dispersal syndrome of myrmecochorous plants in northern temperate regions appears to be characterized by herbaceous growth forms, small elaiosome-bearing seeds, forested habitats, and early flowering and fruiting phenologies. Assuming high ant activity when ant dispersed plants fruit and low ant activity later in the year, the early phenology of myrmecochorous plants is discussed in terms of phenological adaptations of plants to their seed dispersers (THOMPSON 1981, HANDEL & BEATTIE 1990). Following this hypothesis, the prediction exists that seed dispersal by ants works less efficient later in the season because ant activity decreases. This hypotheses, however, has not been tested rigorously and quantitative evidence does not exist.

The aim of this study was to understand a) the ecological significance of the plant phenology and b) the benefit of animal seed dispersers for plants. I determined flowering and fruiting times of ant and non-ant dispersed plants in order to quantify the period of time ant

dispersed plants flower and fruit earlier than non-ant dispersed ones. In addition, I tried to find evidence for the postulated phenological adaptation of myrmecochorous plants to their ant disperses. To reveal general patterns, I used the comparative approach to study the relationship between myrmecochory and phenology on the community level. Assuming similar niches from their dispersal syndrome, I combined all myrmecochorous plants of my study area and addressed them as the guild of ant dispersed plants. This system of ant dispersed plants and seed dispersing ants appears uniquely suited to study phenological adaptations in mutualistic animal-plant-interactions because ants - in contrast to flying animals such as birds and most insects - are active only on the spatial scale of square meters and, therefore, assumed to be easily studied. I used this advantage of the ant dispersal system to test experimentally the postulated adaptation of ant dispersed plants to flower and fruit early due to the availability of ant dispersers.

In the following chapters I discuss three major points each represented by a chapter. In chapter two, a statistical procedure is presented to test phylogenetic autocorrelation in comparative analyses. To apply this procedure I developed a computer program which performs a new kind of Mantel test. The following two chapters describe my field work. I studied the flowering and fruiting phenology of a temperate seed plant community. In 1997 I determined the flowering and fruiting phenologies for 173, in 1998 for 275 plant species. Since data for both years show similar results but with better data quality for the second year, I will present data only for 1998. Chapter three investigates whether and how the phenology of plants is correlated with other plant attributes. Traits concerning animal-plant interactions, i.e. pollination and seed dispersal mode, were explicitly tested for their significance for the plant phenology. In parallel to the botanical field work in 1998, I quantified the seasonal variation in ant activity by investigating ant seed removal during a whole vegetation period. In addition, I performed additional seed removal experiments in 1999 checking up unclear aspects such as direct observations of seed removal by ants and comparative seed removal rates of seeds from different ant dispersed plants. In chapter four, I used these zoological data together with the botanical ones of chapter three to test rigorously whether ant dispersed plants are phenologically adapted to the seasonal variation in ant activity.

2. The Signed Mantel test to cope with autocorrelation in comparative analyses

2.1 Introduction

A fundamental assumption for all analyses of variance is random sampling and, consequently, an independence of the samples within a data set (SOKAL & ROHLF 1995). This prerequisite, however, is missing when comparing evolving sampling units such as different biological taxa (e.g. populations, species, genera), medical diseases, or cultures (e.g. societies, traditions, languages). This is caused by the fact that all living things are evolutionary related and, therefore, phylogenetically not independent of each other (FELSENSTEIN 1985, HARVEY & PAGEL 1991, GITTLEMAN & LUH 1992). In addition, organisms that live in close vicinity are subject to similar environmental factors and, therefore, spatially autocorrelated (SOKAL 1979, TAYLOR & GOTELLI 1994, ROSSI 1996). As a result, we have to consider that the observed variance within comparative data may partly be explained by phylogenetic or spatial autocorrelation. Only if we can demonstrate that autocorrelation does not contribute significantly to the explanation of the observed data, we can ignore this problem and perform standard statistical analyses like regression, correlation and contingency table methods. In comparative analyses, these standard statistical procedures are called TIP analyses because they only consider the recent situation, the tip of the phylogenetic tree (RICKLEFS & STARCK 1996), and not the evolutionary history.

I suggest the Signed Mantel test to cope with possible autocorrelation in comparative analyses. The Mantel test quantifies the statistical relationships among distance measures such as phylogenetic distances or distances in traits (e.g. body size). The test's statistical principle, based on Monte Carlo randomization (for details see chapter 2.2), is recommended by DIACONIS & EFRON (1983), SMOUSE et al. (1986), SIMON & BRUCE (1991), DINIZ-FILHO & BINI (1996), LUO & FOX (1996), and ROSSI (1996). The Mantel test, originally developed by MANTEL (1967), is widely applied by scientist of various fields, for example, community ecology (DINIZ-FILHO & BINI 1996, DINIZ-FILHO et al. 1998), population ecology (DOUGLAS 1982, MANLY 1986, SMOUSE et al. 1986, LEGENDRE & FORTIN 1989), ethology (KAPSALIS & BERGMAN 1996, PEAKE & MCGREGOR 1999), evolutionary biology (LEGENDRE et al. 1994, TAYLOR & GOTELLI 1994, SOKAL et al.

1997, THORPE et al. 1996, BÖHNING-GAESE & OBERRATH 1999), soil biology (ROSSI 1996), molecular biology and genetics (HAIG et al. 1994, LU et al. 1996), systematic biology (CUERRIER et al. 1998), psychometrics (HUBERT 1979), geography (SOKAL 1979, CESARONI et al. 1997, KENT et al. 1997), medicine (MANTEL 1967, JACQUEZ 1996), and anthropology (CRAWFORD et al. 1995, WENG & SOKAL 1995, ELLER 1999).

MANTEL (1967) presented his test as an univariate analysis based on a Z-value estimating the relationship between two distance matrices but he already suggested a multivariate use. As an improvement, DIETZ (1983) calculated the PEARSON correlation coefficient to evaluate the relationship between the two matrices. SMOUSE et al. (1986) used the t-value of the linear regression analysis as test statistic and extended the test to multivariate cases. Finally, it was shown that the test can handle quantitative, semi-quantitative, qualitative data and a combination of these in multivariate analyses (LEGENDRE & FORTIN 1989).

Here, the Mantel test is extended to new metrics when calculating paired distances from trait values (raw data) of single sampling units. I do not only use the absolute value of the distances, but also their signs. Considering the sign of paired distances (i.e. which sampling unit has the larger value), one can consider the direction of possible effects found in the data. To illustrate this meaning of signed distances I will use an ecological example (see 2.3.1 to 2.3.3). Besides, I emphasize the use of categorical variables (2.4.1) and quotients to calculate the distance data for exponential or logarithmic relationships (2.4.3). Because different metrics exist to calculate the distance data and because the test results can depend on the kind of metric used I recommend to validate the transformation of the raw data into paired distances (2.5.1). To demonstrate the validation procedure most clearly, I will explain the application of the Signed Mantel test to hypothetical data (2.5.2). This data will refer to the ecological example introduced in 2.3. In addition, I will apply the Signed Mantel test to ecological field data in order to demonstrate the test's advantage coping with 'real life' problems (2.5.3). To validate autocorrelation effects, I suggest to compare three kinds of analyses: Firstly, the analysis of raw data (TIP analysis), secondly, the (Signed) Mantel test **without** the distance measure, and thirdly, the (Signed) Mantel test **with** the distance measure included (2.5.4).

I present not only the Signed Mantel test and the use of signed distances but also the *Smantel* computer program that is developed to perform this test. This computer program allows to calculate the paired distances from single trait values applying different metrics.

Additionally, the program can perform the Mantel test in different variants (based on regression, correlation or residual analysis). Finally, the program enables to explore the data visually using several kinds of graphical displays and to compare the three different analyses I recommend to validate the data transformation from raw into distance data (see 2.6).

2.2 The principle of the Mantel test

The Mantel test is designed to evaluate the relationship between a distance measure, such as the geographical (spatial) or the phylogenetic distance, and traits of sampling units, e.g. body size or food type. Consequently, the test works on squared distance matrices in which each sampling unit is compared with each other (MANLY 1986). For N sampling units the symmetrical N by N matrix consists of $\frac{N \cdot (N-1)}{2}$ different distance values. To evaluate the relationship between two or more matrices a correlation analysis (DIETZ 1983) or a linear regression analysis (SMOUSE et al. 1986) is performed. In the latter case, one has to define a regression model with a dependent variable (Y-variable) and one or more independent variables (X-variables). For each X-variable the partial t-value is computed as test statistic. Due to possible autocorrelation the significance level of this test statistic, the p-value, is of no use.

A valid significance level, the Mantel significance level, can be estimated by a Monte Carlo randomization of the test statistic (MANLY 1986, LEGENDRE & FORTIN 1989). To do this, the original t-value has to be compared with a so called Null distribution of randomized t-values constructed by Monte Carlo randomizations (SMOUSE et al. 1986). These t-values are derived from regression analyses in which the values of the Y-matrix are randomly reordered. This is realized by sorting the sampling units in both rows and columns following the same random order (MANLY 1986, LEGENDRE et al. 1994). Then, this 'rotated' Y-matrix is regressed on the unchanged X-matrices. Note that the X-matrices remain unchanged in order to preserve the original relationship between the X-variables (SMOUSE et al. 1986). The t-values of the regression coefficients are saved. One such operation for which the Y-matrix is rotated and regressed on the X- matrices is called permutation. By performing many hundreds of permutations the Null distribution of randomized t-values is obtained (Fig. 2.1). The discrepancy between the original t-value and this Null distribution is quantified by the percentage of randomized t-values that are larger than the original t-value (or smaller for a low original t-values at the lower end of the Null distribution) (MANLY 1986). For a two sided test the Mantel significance level for the original t-value of variable X_i is calculated by:

$$p(X_i) = \frac{2 \cdot N}{Num}$$

N: Number of randomized t-values larger (smaller) than the original t-value

Num: Number of permutations

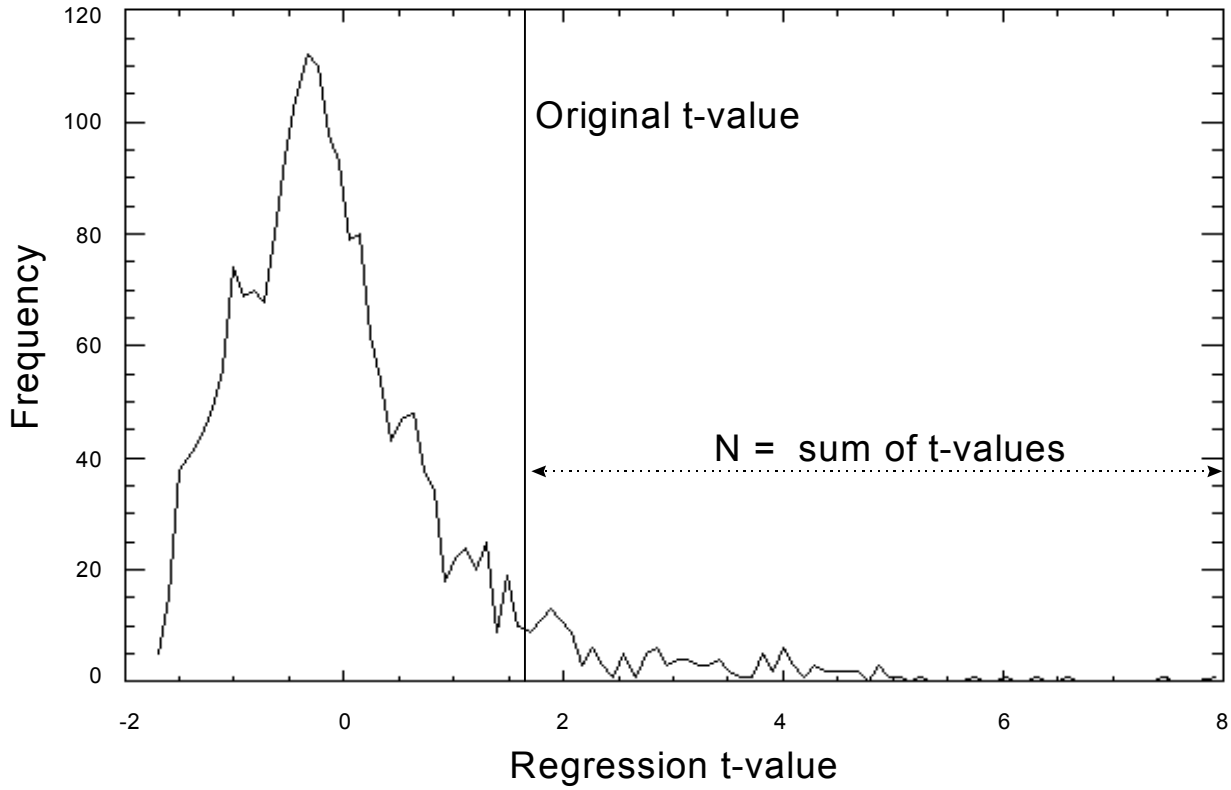

Fig. 2.1: Quantifying the discrepancy between the t-value of the original regression analysis and the Null distribution of randomized t-values. For a total number of Num = 3000 permutations and for N = 60 values of the Null distribution larger than the original t-value, the original t-value is significant with a Mantel significance level of p = 0.04.

2.3 Signed distances

The following example may illustrate the significance of signed distances and the divergence from absolute distances. Let us study a bird community and try to explain why different bird species have different bill sizes. A simple ecological explanation is that bill size

is constrained by food type. For example, fruit-eating bird species might have larger/smaller bill sizes than birds feeding on seeds or insects. When applying a t-test on the trait values of single species, we ask the question: 'Do frugivorous species have larger/smaller bill sizes than non-frugivorous ones?'. (For simplicity, we neglect the usually strong effect of body size on bill size.) If our analysis yields a significant difference in bill size between the two groups of birds, the finding of this TIP analysis, however, could rather be a phylogenetic pattern than an ecological one. In case of a strong similarity in bill size among closely related species this pattern must be explained by phylogenetic autocorrelation. Hence, we have to add the phylogeny of the bird species (i.e. the distances in their phylogenetic relatedness) to our model. Because phylogenetic data are only available as paired distances the trait values of the species (the raw data of bill size and food type) must be transformed into distance data.

2.3.1 Limitation of absolute distances

For the continuous variable bill size, the simplest way to get a distance measure is to calculate the difference (Euclidean distance) between two species. For species of the bill sizes 4, 5 and 9 cm, the paired distances are 1, 4 and 5 cm. All values of this distance measure are positive with a (theoretical) minimum of zero indicating equal bill sizes. The variable food type is nominal. Correspondingly, the distance measure consists of the two states: equal or unequal. These states can be represented by a dummy variable with zero for equal and one for unequal. Now, we have for all three variables a squared matrix in which all species are compared pairwise in regard to their distance in bill size, food type and phylogeny. The distance data of these three variables are absolute (only positive) and range from a minimum of zero (that means equal) up to a certain maximum (that means most different). Customary Mantel tests work on such absolute distance data and address the question: 'Are species that are similar in food type also similar in bill size?'. This question, however, is very different from the TIP analysis (see above), because the information about the direction of the effect is lost. In case of a significant effect, we do not know whether frugivorous or non-frugivorous bird species have larger bill sizes.

2.3.2 Advantage of signed distances

Distance matrices of Mantel tests need not necessarily be absolute (SOKAL 1979). The direction of an effect in distance data can be considered by using the additional information which species of the compared ones has the larger/smaller trait value. For that purpose, the sign information must be considered when calculating the paired distances. For

the continuous variable bill size, the primarily calculated distance data can be negative when a larger bill size is subtracted from a smaller one. The distance in bill size between species A and B can be 5 or -5 cm depending on whether A-B or B-A is calculated. The absolute difference is usually taken as the metric, because a distance measure is scaled from zero (equal) up to a certain maximum (most different). However, considering the sign of the distances we can use the information which species within a pair has the larger trait value.

The nominal variable food type has only two categories. As for the absolute distance data (see above), the two categories of the raw data, frugivorous and non-frugivorous, can be replaced by a dummy variable with 0 for non-frugivorous and 1 for frugivorous. Such a dummy variable can, for regression analyses, be handled like a continuous X-variable (see 2.4.1). Calculating the signed distance data, we obtain mathematically three distance categories which are -1 (non-frugivorous species compared with frugivorous species), 0 (both, frugivorous species compared with other frugivorous species and non-frugivorous species compared with other non-frugivorous species) and +1 (frugivorous species compared with non-frugivorous species).

For the compatibility of signs in bill size and food type, it is of prime importance that the position of the species within a matrix (i.e. the order of the sampling units in the rows and the columns of the matrix) remain equal for different matrices. Changing species positions (i.e. orders) in one of the matrices can likely result in inconsistent comparisons of sampling units. To perform the Signed Mantel test the signs of the Y- and the X-variable must be brought together (see 2.3.3). Having compatible signs in the distance matrices, we can combine the sign information of bill size (Y-variable) and food type (X-variable) by multiplying the signs. The product of these two signs results in one of the following three categories:

+1 One species within a pair has larger trait values in both Y and X.

0 Both species are equal in either Y or X.

-1 One species within a pair has a larger trait value only in Y but not in X or only in X but not in Y.

Testing the effect of the distance in bill size on the distance in food type and considering this sign information, the question our analysis addresses is now ‘Do species pairs of different food types show larger or smaller distances in bill size than species pairs of

similar food type?'. The difference between the questions asked by the different types of analyses is illustrated by the following comparison:

TIP analysis:	Are sampling units that are large/small in X large or small in Y?
Customary Mantel test:	Are pairs of sampling units that are dissimilar in X also dissimilar in Y?
Signed Mantel test	Do pairs of sampling units with large/small distances in X show large or small distances in Y?

This comparison shows that analyses of Signed Mantel tests are much more similar to TIP than customary Mantel analyses because TIP and Signed Mantel analyses reveal not only the strength of relationships but also their direction. Thus, in general, it appears useful to work on signed distance data and to use the Signed Mantel test (but see 2.5).

2.3.3 The sign transformation

How can the sign information be included in the Mantel test? In the Signed Mantel test, absolute and signed distance data must be simultaneously applied in the same analysis. This is necessary because only absolute distance data are available for variables such as phylogenetic distance, geographical distance, or niche overlaps. In our example, the signed distance in bill size is regressed on the absolute phylogenetic distance. To preserve the relationship between the signed Y-variable, distance in bill size, and the absolute X-variable, phylogenetic distance, the Y-variable must be transformed into absolute distances. This means that in this case (regressing a signed Y-variable on an absolute X-variable) the sign information of the Y-variable cannot be used. The same is true for the X-variable when regressing an absolute Y-variable on a signed X-variable.

In analyses that include both a signed Y-variable and a signed X-variable, however, the sign of the Y-variable can be transferred to the signed X-variable. This transfer is realized by multiplying the signed X-distance with the sign of the corresponding Y-distance (i.e. attributing the sign of the Y-variable to the X-variable). Then, the absolute Y-distance is taken instead of the signed one. By this sign transformation we combine and store the sign information for both the Y- and the X-variable in the X-variable as demonstrated in the previous chapter 2.3.2. Transferring the Y-sign to the X-variable results in one of the following four categories:

Positive X-distances: One species within a pair has larger trait values in both Y and X (Fig. 2.2).

Zero X-distances: Both species have the same trait value in X. Distance in X without sign, regardless of the primarily calculated sign of the distance in Y (Fig. 2.2).

Negative X-distances: One species within a pair has a larger trait value only in Y but not in X or only in X but not in Y (Fig. 2.2).

Excluded X-distances: Both species have the same trait value in Y. Distance in Y is zero and, therefore, without a sign. Hence, no sign can be transferred to the X-distance. As a special solution for this problem, the corresponding pair of sampling units will be excluded from analysis. The resulting data reduction for continuous Y-variables is usually negligible. For Y-variables with only a few different values, however, that problem can be serious, but such Y-variables should be avoided in any case (see 2.4.1).

Tab. 2.1: Illustration of the sign transformation. The table shows the trait values of single species (raw data), the distances primarily derived from the raw data, and the absolute and sign-transformed distances for six different, arbitrarily chosen pairs of species (based on 12 different species). The difference is used as the metric to calculate the distance data. For further explanations see text.

	Trait values (raw data)		Primarily derived distances		Absolute distances		Sign-transformed distances	
	X	**Y**	**ΔX**	**ΔY**	**ΔX**	**ΔY**	**ΔX**	**ΔY**
Pair	species **A** **B**	species **A** **B**	A−B	A−B	| A−B |	| A−B |	$\frac{\Delta Y}{\lvert \Delta Y \rvert} \cdot (A-B)$	| A−B |
1	1 0	10 2	+1	+8	1	8	+1	8
2	1 0	8 11	+1	-3	1	3	-1	3
3	0 1	12 3	-1	+9	1	9	-1	9
4	0 1	9 13	-1	-4	1	4	+1	4
5	1 1	4 9	0	-5	0	5	0	5
6	0 1	5 5	-1	0	1	0	-	-

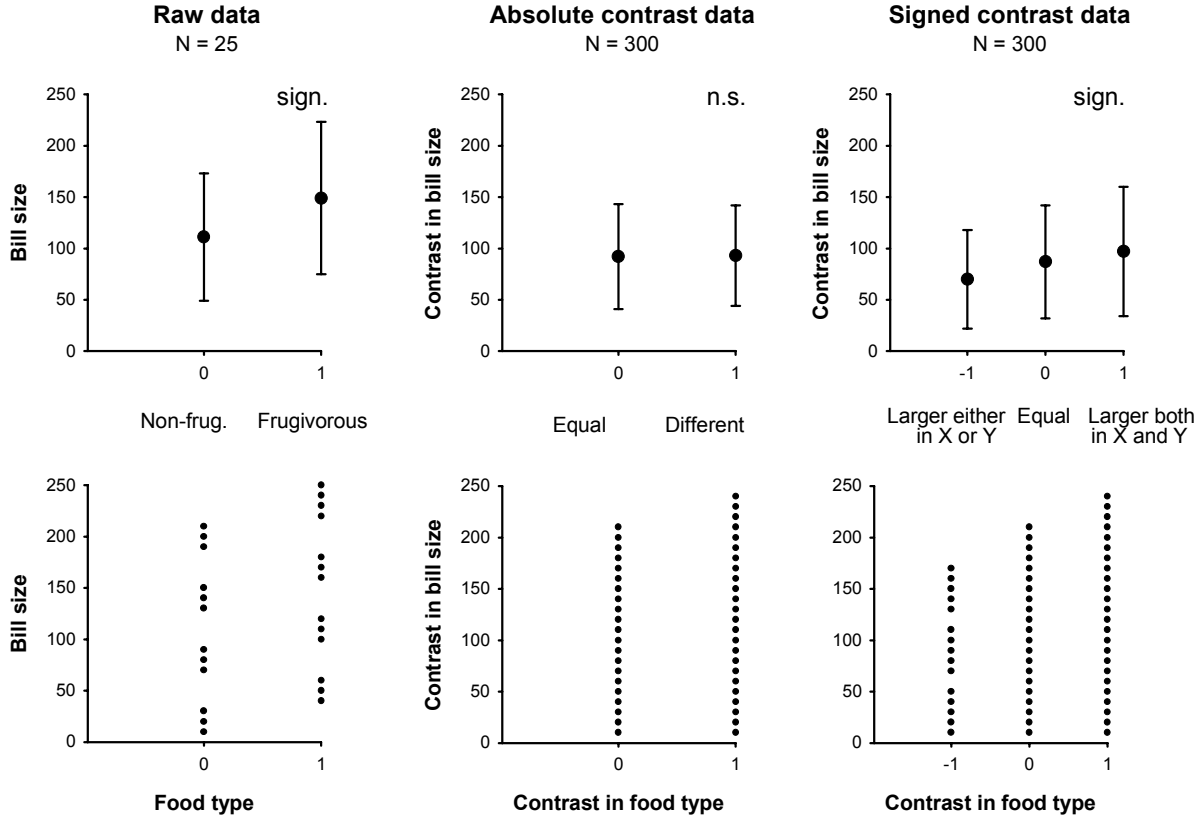

Fig. 2.2: Comparison between raw data (left column), absolute distance data (middle column) and signed distance data (right column) for the hypothetical data set explained in the text (upper diagrams: means and 95% confidence intervals; lower diagrams: data points). The absolute distance data do not preserve the relationship that is present in the raw data. In contrast, the signed distance data do show this relationship.

By this sign transformation, the information which of the sampling units compared has the larger Y(!)-value, is stored always in the X(!)-variable. In Tab. 2.1 this procedure is illustrated by six different pairs of sampling units. Calculating the absolute distances and not using the sign information, pair 1 to pair 4 would result in the same absolute value for X that is 1. Calculating the signed distances, the pairs 1, 4 and the pairs 2, 3 are separated into the two different distance categories +1 and -1. The significance of the difference between these two categories is explained in the categories described above (see positive/negative X-distances).

It is possible to combine absolute and signed distance data in the same analysis by performing the sign transformation only for signed X-variables. In our example, the distances of the Y-variable bill size become absolute because their signs are removed from Y and transferred to the signed X-variable food type. Now, the absolute Y-variable bill size is comparable with the absolute phylogenetic X-distances but the primarily calculated signs of the Y-distances are still considered in the analysis. Both the signs of the X- and the Y-variable enter the analysis with the X-variable. Analyzing our bivariate regression model based on such sign-transformed distance matrices, we ask for the X-variable phylogeny 'Are closely related species more similar in bill size than distantly related ones?'. For the X-variable food type we ask, 'Do species pairs of different food types show larger or smaller distances in bill size than species pairs of similar food type?'.

Tab. 2.2: Overview of the metric types the *Smantel* computer program offers to calculate paired distances from trait values of single sampling units (for the use of categorical variables see 2.4.1).

Type of distance	Algorithm	Range of distance values*	Application
equal/unequal (nominal)	if $x_1 = x_2$: 0 if $x_1 <> x_2$: 1	0 or 1	nominal data
absolute difference	if $x_1 > x_2$: $x_1 - x_2$ if $x_1 < x_2$: $x_2 - x_1$	• 0	linear relationships
signed difference	$x_1 - x_2$	- ∞ to + ∞	linear relationships
absolute quotient	if $x_1 > x_2$: $\frac{x_1}{x_2} - 1$ if $x_1 < x_2$: $\frac{x_2}{x_1} - 1$	• 0	exponential relationships
signed quotient	if $x_1 > x_2$: $\frac{x_1}{x_2} - 1$ if $x_1 < x_2$: $-(\frac{x_2}{x_1} - 1)$	- ∞ to + ∞	exponential relationships

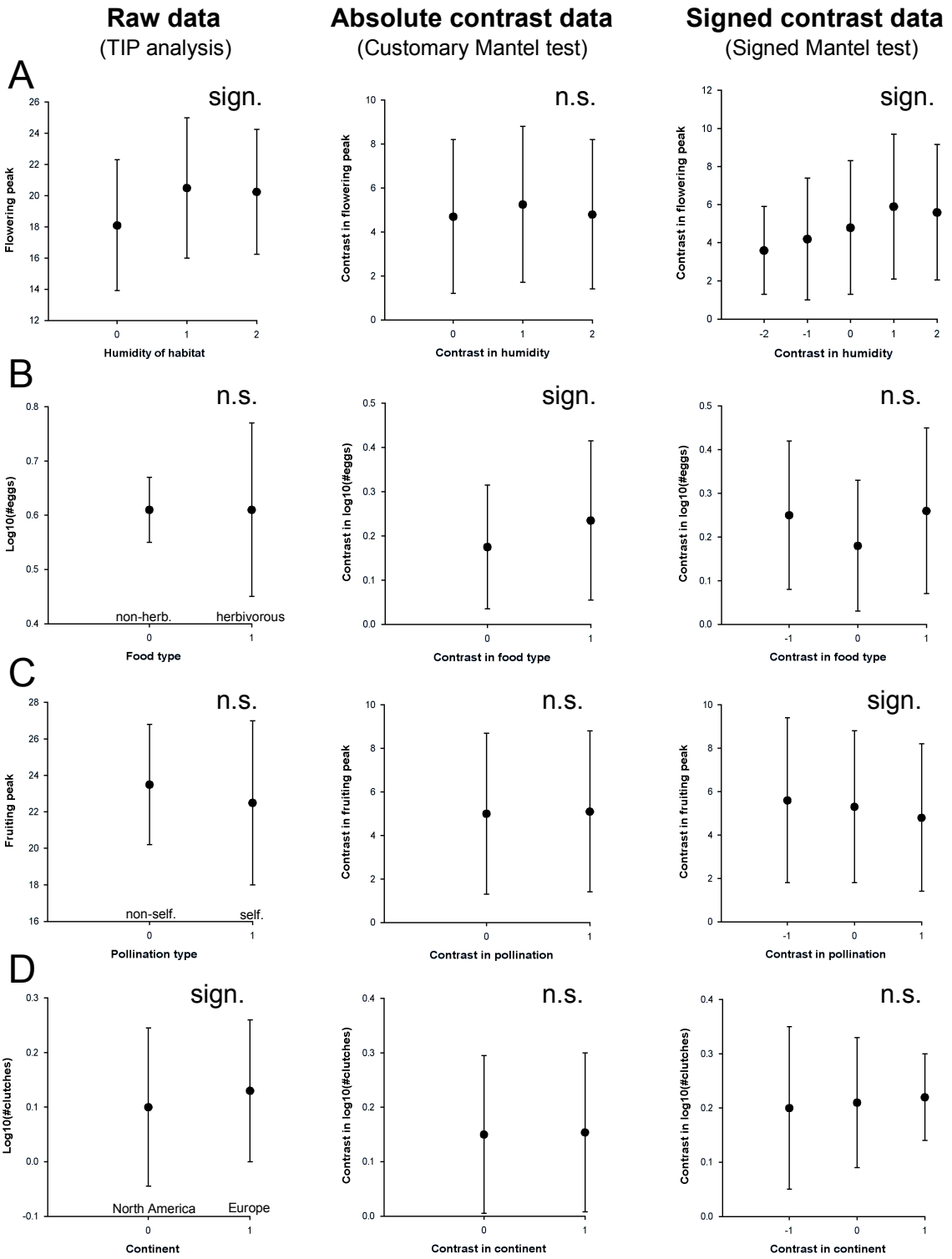

Fig. 2.3: Four examples for comparisons between raw and distance data coming from ecological data sets (see Tab. 2.3). All diagrams show mean and standard deviations. A: The signed distances did preserve the significant relationship found in the raw data (in contrast to the absolute distance data). B: The signed distance data did preserve the **non**-significant relationship found in the raw data (in contrast to the absolute distance data). C: While the absolute distance data preserve the non-significant relationship of the raw data, the signed distance data yield a significant relationship. D: Neither of the two types of distance data preserved the significant relationship found in the raw data. In contrast to C, the TIP analysis of D is significant due to a larger sample size (see Tab. 2.3).

2.4 Types of distance measures in trait variables

Different metrics exist to derive distance data from the corresponding raw data (Tab. 2.2). In this chapter I explain which metric is to be applied to calculate the paired contrasts for nominal, ordinal and continuous variables.

2.4.1 The use of categorical variables

In general, the equal/unequal measure (Tab. 2.2) can be applied to all categorical variables. This distance measure, however, does not allow to use the sign information. To consider the sign information in categorical variables alternative metrics can be applied under certain conditions. The crucial point to handle categorical variables adequately is the number of categories. I discuss this point firstly for ordinal and secondly for nominal variables.

Ordinal variables with many categories can be treated as continuous if the distances from one category to the next are roughly similar. In this case, the difference metric can be applied. This is even valid for variables with only few categories when the variable is used as X-variable (MENARD 1995). In our example on frugivorous bird species (chapter 2.3), we could define a gradient of frugivory (strictly frugivorous, mainly frugivorous, partly frugivorous, occasionally frugivorous, strictly non-frugivorous) that can be arbitrarily scaled from 5 (most frugivorous) to 1 (most non-frugivorous). However, we actually had only two categories (frugivorous, non-frugivorous) which entered the analysis as dummy variable (value 0 or 1). In this case, the X-variable food type defines two different samples and the regression analysis corresponds to the t-test (see example in chapter 2.3).

Problems exist for ordinal Y-variables with only few categories. In this case, a logistic regression analysis should be performed (MENARD 1995). However, the standard regression analysis is quite robust against the number of categories and yields often similar results. Nevertheless, Y-variables with only few categories should be generally avoided - not only to avoid statistical problem when applying standard regression analyses but also to prevent data reduction in the Signed Mantel test (see 'Excluded X-distances' in 2.3.3). Hence, most ordinal variables can be treated as continuous. As a result, the (absolute or signed) difference metric can be applied to calculate the distance data for these variables.

Nominal variables with only two categories can also be treated as dummy variables for which one category is arbitrarily addressed as 0 and the other one as 1. Thus, the same arguments apply in this case as for ordinal variables (see above). Data of such variables can be transformed using the absolute or signed difference metric (as illustrated by the example in chapter 2.3).

For nominal variables with three or more categories, two alternatives exist. Firstly, an equal/unequal measure can be applied (Tab. 2.2) to transform raw into distance data. In this case, a single analysis tests the effect of this variable but the sign information cannot be used and the direction of possible effects is not considered. Secondly, each category can be represented by an individual dummy variable. In this case, the sign information (the direction of effects) can be considered. The variable food type, for example, could have the categories 'frugivorous ', 'granivorous', and 'insectivorous'. This variable can be split up into the following three dummy variables which can be studied separately:

X-variable A:	'non- frugivorous' (value: 0)	'frugivorous' (value: 1)
X-variable B:	'non-granivorous' (value: 0)	'granivorous' (value: 1)
X-variable C:	'non-insectivorous' (value: 0)	'insectivorous' (value: 1)

2.4.2. The difference metric

The difference (Euclidean distance) is the most common metric to calculate distance data from raw data of trait variables. This metric can be applied not only to continuous variables, but also, at least in many cases, to ordinal and nominal variables (see 2.4.1). Since the difference metric is already treated intensely in the example of chapter 2.3, I give here no further information.

2.4.3 The quotient metric

The quotient is an alternative to the difference as a metric to derive paired distances from raw data of continuous trait variables (Tab. 2.2). The quotient is a valid metric for distance data of logarithmic and exponential relationships (regarding e.g. the distance between 1000 and 100 as equivalent to the distance between 100 and 10, BÖHNING-GAESE & OBERRATH 1999). Assuming positive raw data, quotients are always positive. However, the information which of the sampling units has the larger/smaller trait value is available when comparing the distance with 1. Dividing a smaller value by a larger one will yield a distance < 1. Thus, as for the difference, the quotient is available as absolute and signed metric.

Absolute quotients are obtained by always dividing the larger trait values by the smaller ones. To prevent divisions by zero, such trait values must be excluded or, alternatively, the data of the variable must be transformed (e.g. all trait values +1). Absolute

quotients range from 1 (equal) to a maximum number (most different). This range is different from the one of the difference metric (zero to maximum). To have an equivalent range using differences and quotients and to combine both metrics simultaneously in multivariate analyses, I define the absolute quotient as $\frac{larger\ value}{smaller\ value} - 1$.

Signed quotients are obtained by dividing the trait values following a fixed order. This order results from the order of the sampling units in the rows and columns of the matrix. When a smaller value has to be divided by a larger one the signed distance is computed as $-\frac{larger\ value}{smaller\ value} + 1$. Corresponding to signed differences, this metric creates signed quotients that range from a negative maximum (i.e. minimum which means that one of the sample units within a pair has a larger trait value in only X or only Y) up to positive maximum (which means that one of the sampling unit has both in X and Y a larger trait value, Tab. 2.2).

2.5 Validation Procedure for Mantel analyses

2.5.1 The validation criterion

In general, I suggest to apply the Signed Mantel test because its analysis includes, as the TIP analysis and in contrast to customary Mantel tests, the direction of possible effects (see 2.3.2). However, the Signed Mantel test is not in all cases the correct procedure (see 2.5.3). To control for incorrect analyses, I advise to validate the data transformation from raw into distance data by comparing the TIP analysis with the Mantel analyses of absolute and signed distances. For similar relationships between the raw and the distance data, the metric used to calculate the distance data is valid and the analysis correct. In other words, the congruence between the results of TIP and Mantel analysis can be used as the criterion for a valid transformation from raw into distance data.

Problems arise for divergent results between TIP and Mantel analysis. One reason for divergent results represents an invalid transformation into contrast data which do not preserve the relationship within the raw data (see 2.5.1 and 2.5.2). Such invalid data transformation can be caused by distortions of the trait values of single species by distances or distortions in the intercorrelations among the independent variables. A second reason for divergent results may be changes in the degrees of freedom and differences in the type of significance testing between TIP analysis and Mantel test which can result in a different statistical power (see also BÖHNING-GAESE et al. in press). In case of divergent results between TIP and Mantel

analysis, the (significant or non-significant) results of the Mantel analysis may not reflect the real relationship between the variables and the results have to be considered carefully (see 2.5.4). I illustrate the validation procedure by numerical examples.

2.5.2 Numerical examples: Hypothetical data

The significance of signed distances and the validation procedure are illustrated most clearly using hypothetical data. Let us pick up the example of chapter 2.3. Studying a hypothetical bird community of 25 species we want to test the ecological significance of the effect of the categorical variable food type (frugivorous versus non-frugivorous) on the continuous variable bill size. In order to control for a possible phylogenetic autocorrelation, we include the phylogenetic distance among the bird species as X-variable into the analysis. Since data on phylogenetic distances are only available as paired distances, the trait values for bill size and food type must be transformed into paired distances; absolute or signed ones.

To decide whether absolute or signed distances are valid representations of the raw data, we compare the relationship between the Y-variable, bill size, and the X-variable, food type, for the raw and distance data visually (Fig. 2.2). The raw data show a clear difference in bill size between birds of different food type (Fig. 2.2, left column). This means that frugivorous bird species have larger bills than non-frugivorous ones. The same pattern is evident in the signed distance data (Fig. 2.2, right column). Here, the mean for the X-distance 0 represents the difference between species of the same food type. The large mean for the X-distance +1 indicates larger bills of frugivorous species compared to non-frugivorous ones. The small mean for the X-distance -1 means that if non-frugivorous species do have larger bills than non-frugivorous ones, then, this difference is much smaller than in the opposite case.

The relationship found in the raw and the signed distance data is absent in the absolute distance data (Fig. 2.2, middle column). The reason for this is that the signed X-distances -1 (low mean) and +1 (large mean) are averaged in the absolute X-distance of 1. This results in an intermediate mean for the absolute X-distance 1 which is, in this example, similar to the mean of the absolute X-distance 0. Therefore, the relationship within the raw data is not preserved when transforming the raw data into absolute differences. In this example, a valid transformation into distances is obtained only when calculating signed differences.

2.5.3 Numerical examples: Ecological field data

Real ecological field data show that incorrect analyses are a frequent problem due to invalid transformations from raw into distance data. I analyzed three different data sets of different sample sizes using seven different Y-variables and 29 different X-variables. One data set is based on a macroecological study of European and North American land birds (N = 625 species, BÖHNING-GAESE & OBERRATH 1999). This data set was used to explain the migratory status, the number of clutches, the number of eggs per clutch, and the number of eggs per year by different ecological, behavioral, morphological or life history traits of the bird species. The two other data sets are based on a phenological study of a temperate community of seed plants (N = 230 and N = 45 species, see 2.4). These data sets were used to explain peak of flowering time, length of fruit production period, and peak of fruiting time by different ecological, morphological, and life history traits of the plant species. In total, I tested 98 univariate regression models (Tab. 2.3, Fig. 2.3).

Tab. 2.3: Overview of the results from 98 different univariate regression analyses of ecological data (details see text). For each analysis, a Mantel analysis of both absolute and signed distance data was compared with the corresponding TIP analysis. The results were classified as I (both distance types showed a similar relationship as the raw data), II (only signed distances showed a similar relationship), III (only absolute distances showed a similar relationship), or IV (neither of the two distance types showed a similar relationship to the raw data).

Classification		I	II	III	IV	
Absolute distance		as TIP	not as TIP	as TIP	not as TIP	
Signed distance		as TIP	as TIP	not as TIP	not as TIP	
Data set	N	Number of cases per classification				Sum
1	625	13	3	0	0	16
2	230	20	9 [A]	3 [C]	1	33
3	45	33	11 [B]	3	2 [D]	49
Sum		66	23	6	3	98
(%)		(67.3)	(23.5)	(6.1)	(3.1)	(100.0)

A, B, C, D: Categories from which the examples of Fig. 2.3 are taken.

For all models, I performed the TIP analysis (standard regression analysis or t-test applied on the raw data) and the Mantel test applied on both absolute and signed distance data. I compared these three types of analyses on congruence. From congruence between TIP analysis and Mantel test I conclude a valid data transformation from raw into distance data (see 2.5.1). The results of all 98 regression models are summarized in Tab. 2.3. In 67.3% of the analyses, absolute as well as signed distance data yielded valid results (classification I of Tab. 2.3). In 23.5% of the analyses, signed distances gave valid results while absolute distances appear to yield invalid results (classification II). Only in 6.1% of the analyses, the opposite was true (classification III). Finally, I found for 3.1% of the analyses that both types of distance data might be invalid (classification IV). Thus, signed distance data yielded valid results in 90.8% of the analyses whereas absolute distance data did so in only 73.4%. This difference illustrates the advantage of the Signed Mantel test. In a few cases, however, the signed distance data resulted in incorrect analyses. Therefore, I recommend to validate always the transformation of trait variables from raw data into distance data.

2.5.4 Validation of autocorrelation effects

In 2.5.2 and 2.5.3 I have demonstrated that for trait variables the transformation from raw data into distance data can and should be validated. However, for distance measures such as phylogenetic distances, geographic distances or niche overlaps that can cause autocorrelation (therefore, hereafter called autocorrelation variables), this does not work because for these variables only distance data are available. Nevertheless, in multivariate analyses a more indirect validation of autocorrelation effects is possible. For that purpose, the results of the following three types of analyses must be compared:

A) TIP analysis (t-test, ANOVA, standard regression analysis or ANCOVA on raw data; autocorrelation variable cannot be included)

B) Mantel test on distance data without autocorrelation variable

C) Mantel test on distance data including autocorrelation variable

If one finds an incongruity between TIP analysis (A) and the corresponding Mantel test **without** the autocorrelation variable (B), this incongruity can be caused by an invalid transformation of raw into distance data (see 2.5.1). However, if the TIP analysis (A) and the Mantel test **without** the autocorrelation variable (B) yield similar results the transformation is

valid. In this case, differences between the Mantel test with and without including the autocorrelation variable must be caused by the presence of the autocorrelation variable in the model and present a real autocorrelation effect. Therefore, I suggest the following procedure to assure correct results:

I) Test the model by a TIP analysis on the raw data (A).

II) Test the same model by the Mantel test based on paired distances (B).

III) Check the results of analysis A and B for agreement. For striking differences, the Mantel results might be invalid. The use of a different metric to calculate the distance data may solve this problem.

IV) For comparable results of A and B, include the autocorrelation variable into the model and perform the Mantel test again (C).

V) For non-significance of the autocorrelation variable in C return to the first model and take the results of A, otherwise take the results of C.

For a detailed example of this procedure see BÖHNING-GAESE et al. (in press).

2.6 The computer program

To apply the Signed Mantel test conveniently I developed the *Smantel* computer program. The program allows firstly to calculate distance matrices using one of the metrics shown in Tab. 2.2, secondly, to perform the customary Mantel test with absolute distance data, the Signed Mantel test with signed distance data, and the combination of both in multivariate analyses, and thirdly, to visualize the data and the data transformations. For TIP analyses consisting of regression analyses, the *Smantel* program is able to perform the TIP analysis as well.

2.6.1 Implementation

The *Smantel* program is written in IDL (Interactive Data Language, version 5.3, see http://www.rsinc.com). IDL is an array-oriented computer language optimized to perform matrix-operations and to visualize data (IDL 1997). Theoretically, no upper limit exists for the amount of data that can be analyzed, because IDL uses all memory resources of the system (RAM and virtual memory on hard disc). Practically, however, the computer system limits the amount of data in the analysis either due to insufficient virtual memory or due to long calculation times. Unfortunately, IDL does not allow to create executable files. Hence, the

IDL version 3.6.1 (16 bit version) or higher (32 bit versions) is a prerequisite to run the program.

The *Smantel* program reads data from one or more ASCII files. Both raw and distance data can be read. For data files containing raw data the program computes the distance data automatically. In this case, it is very easy to change the distance type of a trait variable. Tab. 2.2 lists the alternatives the program offers to construct the distance matrices for trait variables.

To run the *Smantel* program one has to specify the data file(s), the regression model, possible log-transformations of variables, the number of permutations, and the kind of output of the results (graphs, output devices). It is also possible to run the program in batch mode. For a series of tests the computer can perform these tests automatically one after the other. This is especially useful in the case of long computation time per test. In this case, I advise to run a batch file overnight.

The test results contain detailed information about the data and their transformation. For each X-variable in the model the test statistic and the Mantel significance level is presented. In addition, the r^2-values as a measure for the explained variance is available. The results can be displayed on the screen or can be written in an ASCII file. The graphs ordered when calling the Mantel command can be sent to the screen, to a file or to a printer. In addition, the program allows to save the distance data in ASCII files. So, the data can be transferred to other programs.

2.6.2 Variants of the Signed Mantel test

As an alternative to regression analyses, the program allows bivariate correlation analyses. In this case, the test statistic is the PEARSON correlation coefficient and the Null distribution consists of correlation coefficients that are derived from analyses for which one of the matrices has been randomly rotated (DIETZ 1983).

As an alternative to calculate partial regression coefficients in multivariate analyses, the program offers to perform residual regression analyses. In this case, the program first regresses the Y-variable on the X-variables for which the residuals are to be computed. The distances in the Y-matrix are then replaced by their corresponding residual values. Hereafter, the residual values are regressed on the other X-variables defined in the regression model. This variant is useful when one wishes to remove the effect of variables before performing the actual analysis instead of controlling these variables by performing partial regression analyses in which all variables are of equal weights.

2.6.3 Graphical data exploration

To explore data visually the program offers several possibilities. For a univariate regression analysis, the program shows a simple regression plot of the paired distances. For multivariate analyses, the program displays a leverage plot to show the effect of one of the X-variables on the Y-variable. The leverage plot is also used by the JMP statistical package to demonstrate the effect of one X-variable on the Y-variable when controlling for effects of the other X-variables in the model (JMP 1995). In a leverage plot, the distance of each point to a sloped regression line displays the unconstrained residuals and the distance to the X-axis displays the residual when the fit is constrained by the hypothesis (SALL 1990).

Alternatively, the program allows to create Box-and-Whisker-Plots. They are very useful for large data sets. In this plot, medians, quartiles and extreme values are presented for each of the different intervals/categories on the X-axis. As an alternative to median and quartiles, mean and standard deviation / 95% confidence interval / standard error can be displayed. Furthermore, the program can create an overview plot that visualizes the raw data, and the absolute and signed distance data of a trait variable simultaneously. Studying this plot (for example see Fig. 2.2), one can judge which kind of distance data shows similar relationships between X and Y-variable as the raw data.

2.7 Advantages and limitations

The present article stresses two new aspects in applying Mantel tests. Firstly, applying signed distance metrics and working on signed distance data, the Signed Mantel test is more similar to the TIP analysis of the raw data because the Signed Mantel test, in contrast to the customary Mantel test, considers the direction of the effect. Thus, the Signed Mantel test uses more information contained in the data and is, therefore, more meaningful than customary Mantel tests. Therefore, I suggest the Signed Mantel test as an improvement of the customary Mantel test. Secondly, it is possible to validate the analysis performed by comparing the data structures and results between the analyses of raw and distance data. For trait variables, the validation procedure is possible because raw data is available, for autocorrelation effects such as phylogenetic or geographic distance, it is possible because autocorrelation and trait variables are treated by the Mantel analyses in the same way. The validation can assure that the obtained results are no artifact caused by an invalid transformation from raw into distance data, or by the possibly different statistical power of the Mantel test.

Despite these advantages, limitations of the Mantel test exist. Two minor problems are mentioned by CHEVERUD et al. (1989). Firstly, a possible non-linearity of the relationship between matrices combined with a skewed distribution of matrix correlation values, and secondly, the possible lack of values within data matrices. The problems are solved following their instructions. To check for linearity one can examine the data visually. For a striking non-linearity the data must be transformed. The data read from file are checked for missing values which simply reduce the sample size.

A sincere problem for all analyses of comparative data can be heteroscedasticity (inequality of variances among subsamples). This is also true for all kinds of Mantel tests as well as for other methods that use distance data, e.g. the Phylogenetic Independent Constrast Procedure (PURVIS & RAMBOUT 1995). I have no procedure to control for heteroscedasticity, but one can check the data visually. For a striking heteroscedasticity the data should be transformed.

The problems mentioned above apply to all kinds of Mantel tests. Another problem exists, however, that does apply only for the Signed Mantel test. For certain data structures, the Signed Mantel test can yield significant results although no significant relationship within the raw data exists. This is due to the fact that a signed X-variable, compared to the corresponding absolute one, can be double in range. While absolute X-variables range form zero (equal) to maximum (most different), signed variables range from a negative minimum (most different with one sampling unit having either a larger values only in X or only in Y) to its positive maximum (most different with one sampling unit having larger trait values in X as well as in Y). If a slight trend in the raw data is consistent for the negative and the positive interval of the signed distance data, the Signed Mantel test can yield invalid significant results. An example for such a case is shown in Fig. 2.3C. The difference between category 0 and 1 in the raw data is also apparent in the signed distance data between category -1 and 0 and between 0 and +1. Such an circumstance caused five of the six cases of Tab. 2.3 for which the Signed Mantel test yielded wrongly significant results (classification III of the table). This circumstance, however, appears to be infrequent and can be checked.

2.8 Conclusions

With this article, I present the Signed Mantel test in combination with the *Smantel* computer program. The test is designed to evaluate the effect of distance measures for e.g. phylogenetic relatedness, spatial autocorrelation and niche overlaps on other variables and to

control for autocorrelation effects caused by these distance measures. With the *Smantel* program a highly flexible statistical tool exists for scientists of various areas to handle comparative data with the following advantages: 1. parameter free testing, 2. multivariate testing, 3. combined application of continuous and categorical variables in the same model, 4. applicable to all kinds of distance measures (e.g. distance in phylogenetic relatedness, spatial distance, or measures of niche overlap), 5. distance measures handled in the same way as other variables, which allows to validate the data transformation, the analysis, and the results, 6. applicable to tests on relatedness between organisms at all kinds of taxon levels (from population level up to kingdom level), 7. not based on any evolutionary or spatial model, 8. flexibility in applying different metrics (difference, quotient) to calculate distance data for continuous trait variables, 9. high similarity to TIP analysis by considering the direction of possible effects, and 10. amount of data to analyze actually unlimited. Thus, I suggest the Signed Mantel test and the *Smantel* computer program as helpful tools for all scientists working on comparative data.

2.9 Summary

In biology, medicine and anthropology, scientists try to reveal general patterns when comparing different sampling units such as biological taxa, diseases or cultures. A problem of such comparative data is that standard statistical procedures are often inappropriate due to possible autocorrelation within the data. Widespread causes of autocorrelation are a shared geography or phylogeny of the sampling units. To cope with possible autocorrelations within comparative data I suggest a new kind of the Mantel test. The Signed Mantel test evaluates the relationship between two or more distance matrices and allows trait variables facultatively to be represented as signed distances (calculated as signed differences or quotients). Considering the sign of distances takes into account the direction of an effect found in the data. Since different metrics exist to calculate the distance between two sampling units from the raw data and because the test results often depend on the kind of metric used, I suggest to validate the analysis by comparing the structures of the raw and the distance data. I offer a computer program that is able to construct both signed and absolute distance matrices, to perform both customary and Signed Mantel tests, and to explore raw and distance data visually.

3. Effects of pollination and seed dispersal mode on the reproductive phenology of a temperate plant community

3.1 Introduction

Reproduction in plants depends on successful pollination and seed dispersal (HOWE & WESTLEY 1988, BOND 1995). Correspondingly, pollination biology (FAEGRI & VAN DER PIJL 1976, FEINSINGER 1983, BARRETT 1992) and seed dispersal (MÜLLER-SCHNEIDER 1977, VAN DER PIJL 1982, FENNER 1985, WILLSON et al. 1990) are intensely studied. Although pollination biology represents an old discipline (SPRENGEL 1793, DARWIN 1877, KNUTH 1898, KNOLL 1926, KUGLER 1970), scientific interest in the seasonal timing of both flowering and fruiting arose only in the last few decades. The timing of flowering and fruiting is assumed to benefit the plant fitness by avoiding unfavorable times and increasing pollination and seed dispersal success (FENNER 1985, RATHCKE & LACEY 1985, LEBUHN 1997). Little is actually known about the phenology of plants (WILLSON 1992). This is especially true for the fruiting phenology which is less studied than the flowering phenology (but see FENNER 1998).

The effects of animal pollination and seed dispersal mode on the reproductive phenology of plants are of major interest because their influence implies selective forces and coadaptations between animal and plant species (FEINSINGER 1983, HERRERA 1985, HOWE & WESTLEY 1988). Flowering and fruiting patterns appear to be influenced by pollination or seed dispersal mode of the plants. For the flowering time, avoidance of competition for pollinators and of predispersal seed predation are intensely discussed (see FENNER 1998). For the fruiting time, two hypotheses exist on phenological adaptations of plants to the availability of their seed dispersers. Firstly, temperate bird dispersed plants appear to fruit late in the season when disperser activity is high due to autumnal bird migration (THOMPSON & WILLSON 1979, HERRERA 1984). Secondly, ant dispersed plants appear to fruit early in the year when ant activity is assumed to be high (THOMPSON 1981). These hypotheses imply an adaptive significance for certain plant species to flower or to fruit earlier/later in the year than others. However, both hypothesis have not been tested rigorously e.g. by controlling for other factors that might influence the phenology of plants.

Other factors that might affect plant phenology are life history traits. Both plant and seed size are found to be correlated with the flowering time in certain species (PETTERSSON

1994, OLLERTON & LACK 1998, SMITH-RAMIREZ et al. 1998, SANDVIK et al. 1999, WESSELINGH et al. 1997). Factors like growth form and length of the life cycle seem also to be important, indicated by most phenological studies working exclusively on trees, shrubs, herbs, annuals, or perennials. In addition, habitat type may be related to the phenology as many spring flowering herbs are found in forested habitats (SCHEMSKE et al. 1978, PRIMACK 1985).

Phylogenetic inertia is known to be present in plant phenology. Phylogenetic effects in the flowering time have been found (KOCHMER & HANDEL 1986, JOHNSON 1993, SMITH-RAMIREZ et al. 1998). Closely related species had more similar flowering times than distantly related ones indicating their shared origin and a possible conservatism of phenological traits.

Abiotic factors such as temperature, day length and rain fall are found to be important by restraining warmth, light and water needs of the plants (see FENNER 1998). For temperate communities with their harsh conditions in winter, photoperiodicity and temperature appear to be most important (WHITE et al. 1997, WHITE 1995, DIEKMANN 1996).

To reveal general patterns and to get a broad view of plant phenological strategies (BROWN 1995), I used the comparative approach considering many different plant species including, for example, multiple growth forms, life cycles and habitat types. Working on a temperate plant community in Central Europe, I asked whether and how the reproductive phenology of plants is related to pollination and seed dispersal mode. I studied both flowering and fruiting phenology using the same methodology because both can be constrained by each other (PRIMACK 1985, 1987, RATHCKE 1988, FENNER 1998). In addition, I investigated the period of fruit production as the time difference between flowering and fruiting peak. To study the relationship between these three phenological traits and pollination as well as seed dispersal mode I also analyzed potential influences of life history traits, phylogenetic effects and abiotic constraints.

3.2 Methods

3.2.1 Study area and plant species

The study area (in total approximately 50 ha) was located in the west of Aachen, Germany, adjoining to the border of the Netherlands and includes the Nature Reserves „Wilkensberg“ and „Schneeberg“. The area consisted of different habitat types, ruderal sites, grasslands (rich meadows and calciferous grasslands), bushes, forest edges and forests

(coniferous and deciduous). In addition, dry and wet habitats were present. For my comparative approach I tried to collect data from as many herb, shrub and tree species as possible. I investigated all seed plant species I could find on the study area, but excluded hybrids and domesticated plants. The species studied are listed in the appendix.

3.2.2 Phenological variables

Three phenological variables were studied: the flowering peak, the fruiting peak and the period of fruit production. I monitored the study area every ten days (every decade) from January to December 1998. For each census I looked for both flowering and fruiting plant species and estimated the number of open flowers and ripe fruits per plant species in four categories: absent (no flowers/fruits found), rare (< 5% of the maximum), present (5 - 95% of the maximum), maximal (>95% of the maximum). The maximum was defined as the maximum number of flowers/fruits a plant species produced in the study area in any of the decades. Criteria for fruit ripeness were color and the ability to detach fruits from the infructescence. From these data, the flowering/fruiting peak for each plant species was determined as the decade with the maximum numbers of flowers/fruits. In case the maximum number was present during more than one decade, I defined the average of these maximum decades as the peak. This was possible because species that showed two flowering and fruiting periods within the 1998 season (e.g. dead-nettle, *Lamium album*) flowered and fruited much more intensively in the first period (maximum) than in the second one. The period of fruit production was calculated as the number of decades between the flowering and fruiting peak.

3.2.3 Biotic influences

Pollination and seed dispersal mode: For pollination mode, I distinguished between wind, insect, and self-pollinated plants using data from SCHUBERT et al. (1988). For dispersal mode, I classified plants in eight different categories: dispersal by wind, water, ants, frugivorous animals (mostly birds), scatter hoarding animals (squirrels and non-frugivorous birds feeding on nutty fruits), means of barbed fruits, ballistic mechanisms, and the absence of any mechanism (data taken from SERNANDER 1906, SCHUBERT et al. 1988, and GÖTZE 1995). Species that are pollinated or dispersed in more than one way were assigned to more than one category.

Life history traits: I considered seven different life history traits: growth form, plant size, length of life cycle, seed size, fruit size, habitat openness and habitat moisture. For the growth form, I distinguished between herbaceous plants (herbs and halfshrubs), and woody species (shrubs and trees). As a measure for plant size, I used the averaged range given in SCHUBERT et al. (1988). The length of the life cycle was classified by five categories: 1-strictly annual, 2-annual or bienniel, 3-strictly bienniel, 4-perennial, or 5-persistent (SCHUBERT et al. 1988). Seed size was determined by estimating the seed volume from the length, width and height of the seeds applying the ellipsoid formula. Fruit size was estimated as the longest dimension of the fruit. To determine seed and fruit size, I collected for each plant species five fruits randomly from different individuals. For habitat openness, I assigned each plant species to one of seven categories: 1-forest, 2-forest to forest edge, 3-forest edge, 4-forest edge to grassland, 5-grassland, 6-grassland to ruderal site, or 7-ruderal site using own field data. For habitat moisture, I assigned each plant species to one of two categories: 1-dry or 2-wet using own field data.

Phylogenetic effects: Plant species may not represent independent samples due to their phylogenetic history (FELSENSTEIN 1985, HARVEY & PAGEL 1991, GITTLEMAN & LUH 1992). Closely related species may show similar characteristics as a result of their common evolutionary origin. To control for this phylogenetic autocorrelation I tested the effect of the phylogenetic distance between the species on the distance in their phenological traits using Mantel tests (see Statistics). As a measure for phylogenetic distance I used the taxonomic classification of the species. I worked on nine different taxa levels: species, genus, subfamily, family, infraorder, order, superorder, subclass and class. The data for the subfamily taxon were taken from SITTE et al. (1991), the data for the class to family level were taken from THORNE (1992). For the analysis, the phylogenetic distance between two species within the same genus was defined as one, between two species within the same subfamily as two, etc.

3.2.4 Abiotic influences

Five different abiotic factors were studied: day length, temperature, period of sunshine, wind strength, and precipitation. The data were taken as average values for each decade from the meteorological station in Aachen which is located approximately 5 km next to the study area.

3.2.5 Statistics

To analyze the effect of pollination mode, seed dispersal mode and the life history traits on the phenological variables I performed multivariate ANCOVAs. To analyze the nominal variable pollination mode, I performed two separate analyses testing self-pollinated versus non-self pollinated species and insect pollinated versus wind pollinated species. The latter analysis resulted from the fact that besides maples (genus *Acer*) all species were either insect or wind pollinated. To analyze the second nominal variable seed dispersal mode I performed for each mode a separate analysis testing wind versus non-wind, water versus non-water, etc.

To test the effect of the phylogenetic relatedness on plant phenology I performed uni- and multivariate Mantel tests which work on distance matrices (see also Phylogenetic effects). In each distance matrix, each species is compared with each other one in a certain characteristic (MANLY 1986). For seed, fruit and plant size, I calculated the distance between two species as the quotient of the two trait values (corresponding to a logarithmic relationship, see 2.4.3 and Tab. 2.2). For the other variables, I calculated the difference (corresponding to linear relationships). The phenological distances were regressed on the distances in the independent variables, pollination mode, seed dispersal mode, the life history variables and the phylogenetic distance. I computed the significance levels of the regression analyses by the permutational regression procedure of the Mantel test performing 10000 permutations (MANTEL 1967, SMOUSE et al. 1986, LEGENDRE & FORTIN 1989, BÖHNING-GAESE & OBERRATH 1999, BÖHNING-GAESE et al. in press).

To analyze the effect of abiotic factors on the plant phenology I performed partial correlation analyses between the mean values of the abiotic variables per decade and the number of species having their flowering peak, having their fruiting peak or producing fruits in the respective decade. The number of fruit producing species was calculated as the number of species per decade having past their flowering but not yet reached their fruiting peak.

I applied JMP (version 3.2.2) to perform ANCOVAs, SPSS (version 8.0) to perform partial correlation analyses and a self-developed program to perform the Mantel tests. This program is written in IDL and available from the authors (for more information see BÖHNING-GAESE & OBERRATH 1999, BÖHNING-GAESE et al. in press). I used *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, and n.s. for $p \bullet 0.05$.

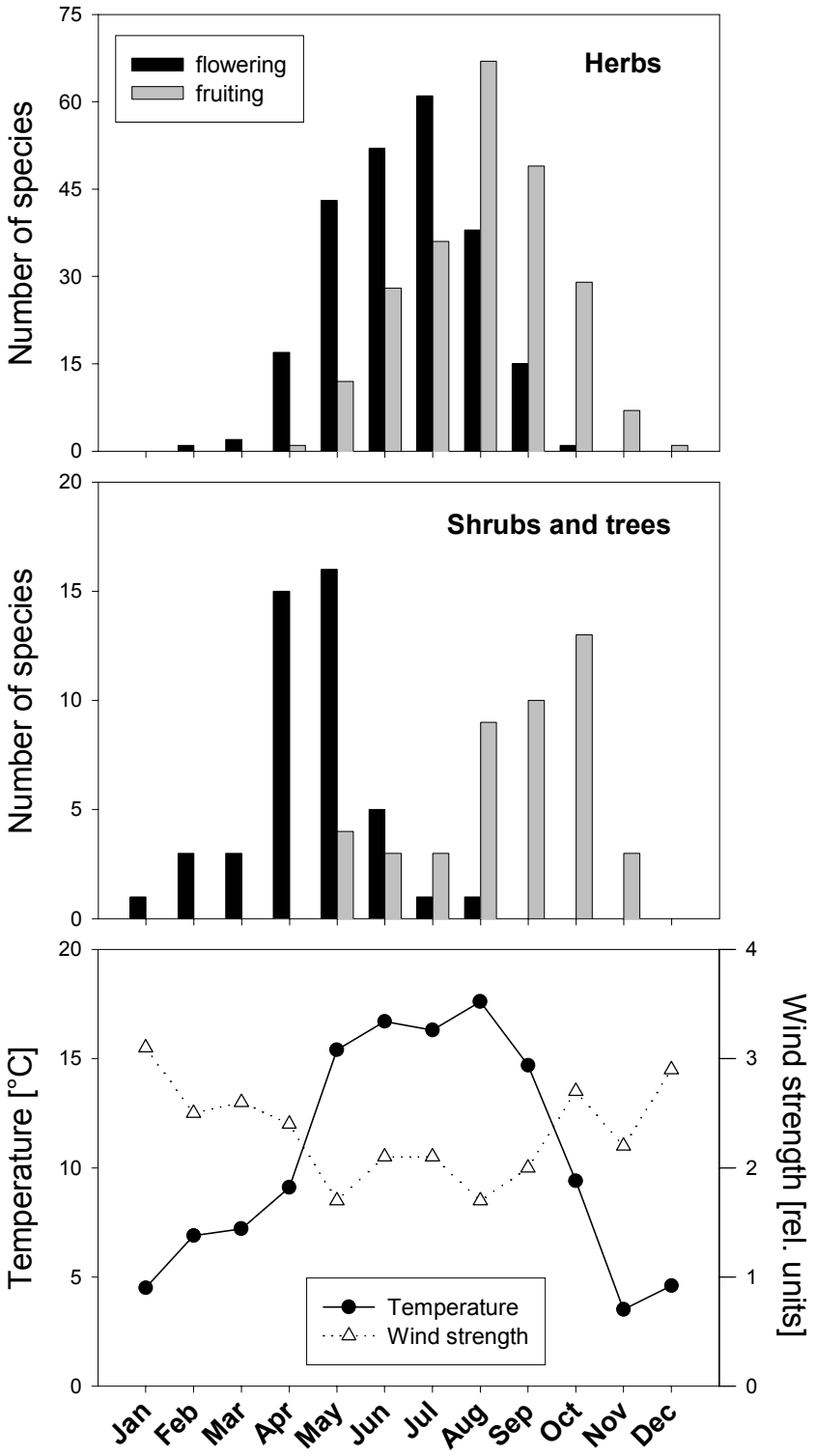

Fig. 3.1: Number of (**A**) herbaceous (n = 230) and (**B**) woody plant species (n = 45) flowering and fruiting per month. For comparison, (**C**) mean monthly temperature and wind strength are presented.

3.3 Results

3.3.1 Biotic influences

In total, I collected data for 275 species of seed plants (see appendix). The overall phenological patterns for the 230 herbaceous and 45 woody species are displayed in Fig. 3.1. Growth form was a dominant correlate of the three phenological traits (Fig. 3.2). Herbs flowered later (t-test: $t = 8.8$; $df = 273$; $p < 0.001$ explaining 22.3% of the variance within the data) but fruited earlier than shrubs and trees ($t = -2.2$; $df = 273$; $p = 0.033$ explaining only 1.7% of the variance). Correspondingly, herbaceous species needed longer to produce fruits than woody species ($t = -14.1$; $df = 273$; $p < 0.001$ explaining 42.1% of the variance). Due to these strong differences between herbaceous and woody plants I analyzed the data for these two subsamples separately.

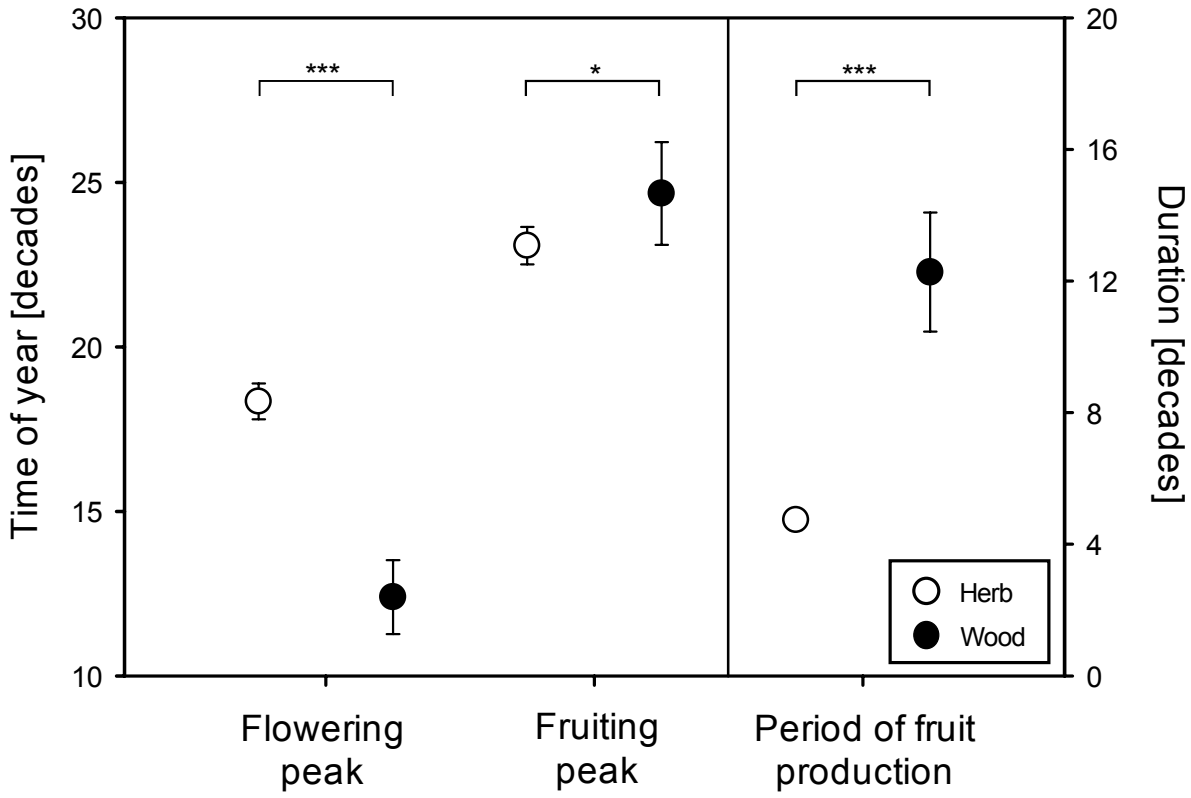

Fig. 3.2: Phenological comparisons between herbaceous (n = 230) and woody (n = 45) plant species. Mean and 95% confidence intervals are displayed.

Herbaceous species: To test effects among the phenological variables, I regressed the fruiting peak on the flowering peak (Fig. 3.3). The relationship between these variables was extremely strong ($y = 0.89x + 6.8$; $t = 23.9$; $p < 0.0001$; $r^2 = 0.72$; $n = 230$). Note, however, that this relationship is semi-dependent because flowering must occur before fruiting and all species studied flowered and fruited in the same year. As a result no data point can reside below the diagonal in Fig. 3.3. The fruit production period could not be regressed on either of the other phenological variables because flowering and fruiting peak were used to calculate the period of fruit production. Due to these reasons, I did not analyze the phenological variables as independent variables in multivariate analyses.

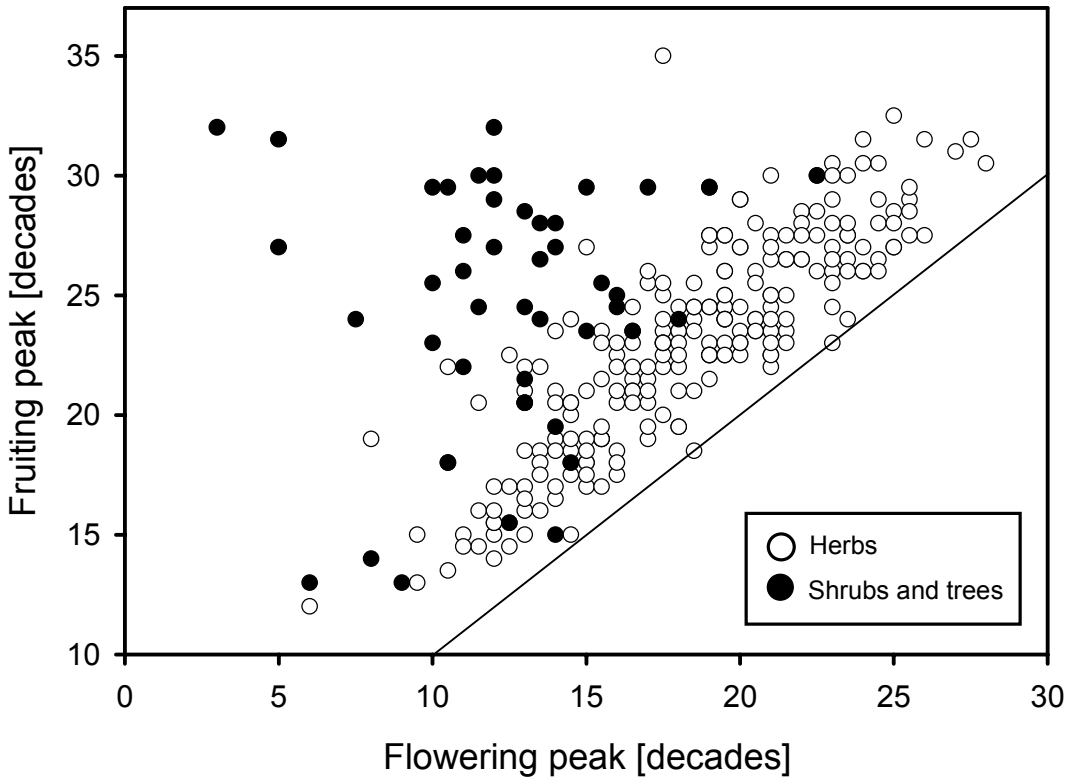

Fig. 3.3: Relationship between flowering and fruiting peak for herbaceous (n = 230) and woody plant species (n = 45).

Wind pollinated and ant dispersed herbs flowered and fruited earlier than insect pollinated and non-ant dispersed ones (Tab. 3.1). Additional weak factors of flowering and fruiting peak were self-pollination and ballistic dispersal. Of the control factors, plant size was the most import factor correlated with flowering and fruiting peak. Tall species flowered and fruited later than small-sized ones (Fig. 3.4). Other important factors were habitat openness (Fig. 3.5) and seed volume. An additional but weak control factor was the length of life cycle. Note that all factors that were correlated with both flowering **and** fruiting peak operated in the same direction. For example, plants of forested habitats flowered **and** fruited earlier than those of open habitats, large-seeded plants flowered **and** fruited earlier than small-seeded ones, and persistent herbs (species of long life cycles) flowered **and** fruited earlier than non-persistent ones (species of short life cycles). For the fruit production period I could find only three weak correlates. Self-pollinated plants fruited earlier and needed less time for fruit production than non-self-pollinated ones. Water and ballistically dispersed plants fruited later and needed longer to produce fruits than differently dispersed plants. While the models explain more than 40% of the variance observed in flowering and fruiting peak, only 9% of the variance observed can be explained in the fruit production period (Tab. 3.1).

Tab. 3.1: Overview of significant correlates of the phenology of herbaceous plants (n = 230). Displayed are the multivariate models that explained most of the variance. For significant independent variables the direction of the effect, the F-ratios of the ANCOVA, and the corresponding significance levels are presented. Control factors are marked by *.

	Dependent variables		
Independent variables (+ / – indicates)	Flowering peak (later / earlier)	Fruiting peak (later / earlier)	Fruit prod. period (longer / shorter)
Wind pollination (vs insect)	– 13.1 ***	– 19.1 ***	n.s.
Self-pollination	n.s.	– 8.1 **	– 6.4 *
Ant dispersal	– 13.3 ***	– 13.2 ***	n.s.
Water dispersal	n.s.	+ 6.8 **	+ 6.2 *
Ballistic dispersal	n.s.	+ 10.6 **	+ 7.7 **
log(plant size) *	+ 49.2 ***	+ 56.3 ***	n.s.
log(seed volume) *	– 17.8 ***	– 15.1 ***	n.s.
Length of life cycle *	– 3.9 ***	– 3.4 **	n.s.
Habitat openness *	+ 23.7 ***	+ 12.1 ***	n.s.
Explained variance (%)			
Total	42.1	42.8	9.0
Without *	14.5	18.5	9.0

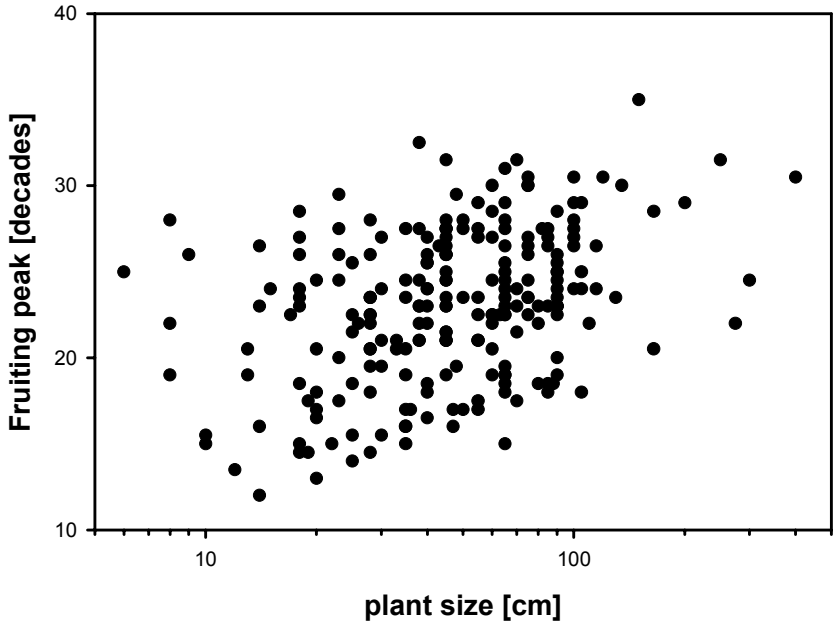

Fig. 3.4: Relationship between fruiting peak and plant size for herbaceous plant species (n = 230).

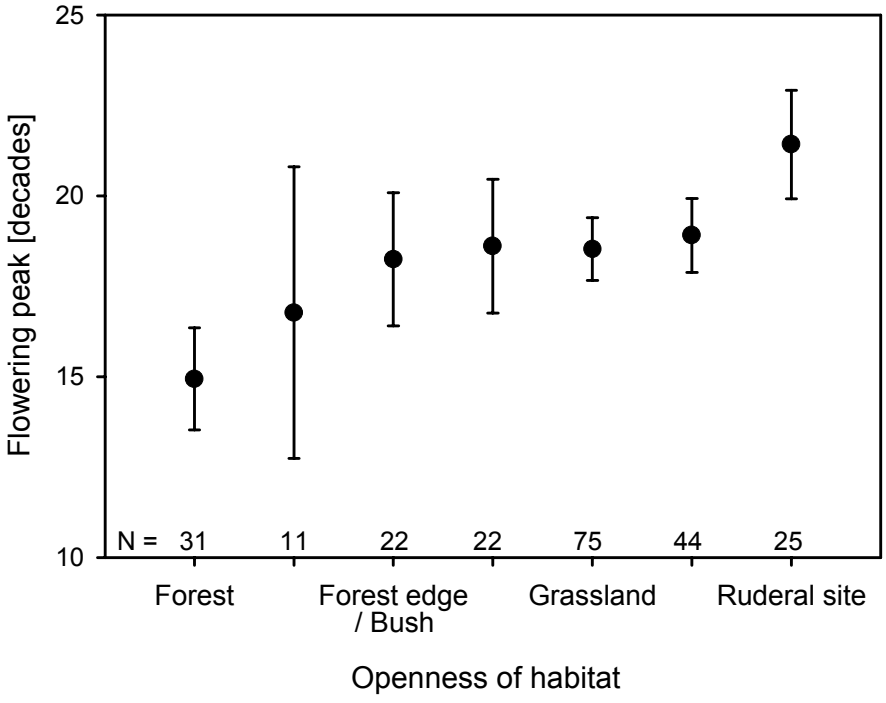

Fig. 3.5: Relationship between flowering peak and habitat openness for herbaceous plant species. Mean and 95% confidence intervals are displayed.

Woody species: I analyzed the data of woody species in the same way as for herbaceous ones. In shrubs and trees, flowering and fruiting peaks were unrelated ($y = 0.15x + 22.8$; $t = 0.7$; $p > 0.4$; $r^2 = 0.01$; $n = 45$, Fig. 3.3).

Clearly the strongest correlate of the flowering peak was pollination mode. Wind pollinated plants flowered earlier than insect pollinated ones (Fig. 3.6, Tab. 3.2). Other significant factors of the flowering peak were dispersal by scatter hoarding animals, habitat openness, moisture of habitat, wind dispersal, and seed size which were all negatively correlated with the flowering peak. For the fruiting peak, I could identify only two but strong factors, wind dispersal and seed size (Tab. 3.2). Wind dispersed and large-seeded plants fruited later than non-wind dispersed and small-seeded plants. The period of fruit production was mainly affected by the pollination mode with wind pollinated species needing longer for fruit production than insect pollinated ones. Additionally, longer periods of fruit production were related to larger seed volumes. The models of Tab. 3.2 explained for each of the three phenological variables more than 40% of variance in the data.

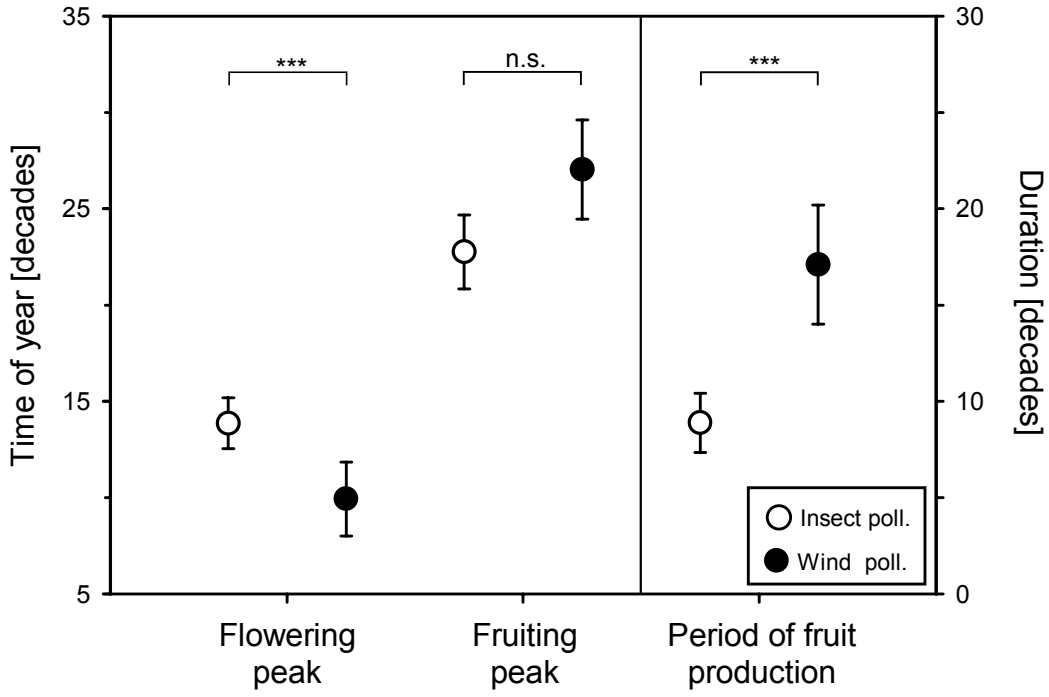

Fig. 3.6: Phenological comparisons between insect pollinated (n = 27) and wind pollinated woody plant species (n = 15, three *Acer* species were excluded from analysis because they could not be assigned clearly to either of the two categories). Mean and 95% confidence intervals are displayed. Test on differences rely on multivariate statistics of Tab. 3.2.

Tab. 3.2: Overview of significant correlates of the phenology of woody plants (n = 45). The table is designed as Tab. 3.1.

Independent variables (+ / – indicates)	Dependent variables: Flowering peak (later / earlier)	Fruiting peak (later / earlier)	Fruit prod. period (longer / shorter)
Wind. pollinat. (vs insect)	– 20.5 ***	n.s.	+ 32.3 ***
Wind dispersal	– 5.0 *	+ 21.6 ***	n.s.
Disp. by hoarding animals	– 5.7 *	n.s.	n.s.
Log(seed volume) *	n.s.	+ 39.3 ***	+ 5.3 *
Habitat openness *	– 5.6 *	n.s.	n.s.
Moisture of habitat *	– 4.7 *	n.s.	n.s.
Explained variance (%)			
Total	43.9	55.8	50.5
Without *	27.3	3.1	43.8

The phylogenetic relatedness of woody species had no effect on either of the three phenological variables both in univariate and multivariate analyses. Thus, the species were not phylogenetically autocorrelated and represented statistically independent sampling units.

3.3.2 Abiotic influences

To test the relationship between plant phenology and abiotic factors I performed partial correlation analyses between the number of flowering / fruiting / fruit producing plant species per decade and day length, temperature, period of sunshine, wind strength, and precipitation in the respective decade (Tab. 3.3). The main factor correlated with the number of flowering species was day length in both herbaceous and woody species. For the number of fruiting species, I found differences between herbaceous and woody species. Main correlates in herbs were temperature and wind strength, correlates for shrubs and trees were temperature, precipitation, wind and day length (Tab. 3.3, Fig. 3.1). For the number of fruit producing species, temperature was the main correlate in herbs, and day length the main correlate in shrubs and trees.

Temperature, period of sunshine and day length were all positively correlated with each other ($r > 0.55$, $n = 36$ decades). Wind strength was negatively correlated with these factors ($r < -0.35$; $n = 36$; see also Fig. 3.1C). Precipitation was barely correlated with the other factors ($|r| < 0.37$; $n = 36$).

Tab. 3.3: Partial correlation coefficients for the relationship between abiotic factors and the number of flowering, fruiting, and fruit producing species (n = 36 decades). For the analysis of each factor, I controlled for the effect of the four other ones.

Group of species	Factor	n flowering	n fruiting	n fruit producing
Herbs	Day length	0.697 ***	-0.443 *	n.s.
	Temperature	n.s.	0.697 ***	0.617 ***
	Period of sunshine	n.s.	0.370 *	n.s.
	Wind strength	n.s.	-0.594 ***	-0.374 *
Shrubs and trees	Day length	0.538 **	-0.462 **	0.866 ***
	Temperature	-0.422 *	0.520 **	0.364 *
	Wind strength	n.s.	-0.454 **	n.s.
	Precipitation	n.s.	0.588 ***	n.s.

3.4 Discussion

This study presents evidence for significant effects of pollination and seed dispersal mode on the reproductive phenology of plants. The flowering peak, the fruiting peak and the period of fruit production of 230 herbaceous and 45 woody species in a temperate community of seed plants were significantly correlated with pollination and seed dispersal mode. This was true even when controlling for other significant effects like growth form, plant and seed size, type of habitat and the phylogenetic relatedness of the plants.

The strongest relationship to each of the three phenological variables had growth form which indicates profound differences in the life-history strategies between herbaceous and woody plants. Woody species flowered earlier and fruited later than herbs. Correspondingly, shrubs and trees had longer fruit production periods than herbs (Fig. 3.2). Multivariate analyses explaining this difference between herbaceous and woody species in fruit production period by other life history variables (results not presented) revealed that seed size which is significantly larger in woody species contribute to the explanation of this difference. As PRIMACK (1987) I found fruit size strongly correlated with seed size (PEARSON $r = 0.40$; $p < 0.001$; $n = 275$). However, fruit size was not significant in multivariate analyses with seed size. Comparative analyses that include both herbaceous and woody species are rare, but earlier flowering times for shrubs and trees were also found by HEINRICH (1976) and by KOCHMER & HANDEL (1986). Due to the striking differences between herbaceous and woody plant species I analyzed these two subsample separately.

In herbaceous species, pollination and seed dispersal mode were significantly correlated with flowering and fruiting peak. Pollination and seed dispersal alone explained more than 14% of the observed data variance (Tab. 3.1). Wind pollinated plant species had earlier flowering and fruiting peaks than insect pollinated ones. HEINRICH (1976) also found wind pollinated species to flower earlier. Since wind is strong during winter and only weak in summer (Fig. 3.1C, which is typically in the area, at least for the last century) and since insects should be rare in early spring, the difference between wind and insect pollinated plants may indicate a phenological adaptation to the seasonal availability of the pollen vectors (SCHEMSKE et al. 1978, LACK 1982, MOTTEN 1983,MOTTEN 1986, MURALI & SUKUMAR 1994).

Ant dispersed herbs had earlier flowering and fruiting peaks than differently dispersed ones. Assuming high ant activity in early summer, the plants could use this potential of seed vectors by producing seeds early in the year. Thus, the early phenology of ant dispersed plants could be interpreted as an ecological adaptation of the plants to seasonal variation in ant activity (THOMPSON 1981, HANDEL & BEATTIE 1990, WILLSON 1992). Dispersal by water was related to late fruiting peaks and longer fruit production periods. This could be caused by the wet environment of these plants. The production and ripening of fruits depend on metabolic activity which should be lower at cooler temperatures. Wet habitats are usually cooler due to stronger evaporation. Correspondingly, I found in univariate analyses later flowering and fruiting peaks and longer fruit production periods for plants in wet habitats. Plant species dispersed by frugivorous animals, in my study area mostly birds, differed in neither of the three phenological traits from differently dispersed species. If bird dispersed plants are phenologically adapted to the high disperser activity during autumnal bird migration I would expect a significant difference in fruiting peak to differently dispersed plants. Such pattern is implied by THOMPSON & WILLSON (1979), STILES (1980) and HERRERA (1984), however, I could not find any evidence for this.

Life history traits appear to strongly influence the phenology of herbs. Plant size was most closely related to flowering and fruiting peak. Taller species flowered and fruited later than smaller ones (Fig. 3.4). This might be caused by longer periods of vegetative growth in tall plants which may delay the reproductive period. Seed size was another important morphological correlate of flowering and fruiting peak (Tab. 3.1). The larger the seeds, the earlier was both flowering and fruiting peak of the species. Correspondingly, PRIMACK (1985) and SANDVIK et al. (1999) found early flowering dates for plants with large fruits.

They explained this pattern by the need of longer seed maturation times for large seeds. However, in herbs I did not find a relationship between fruit production period and seed size. Instead, the fruiting peak was shifted in parallel with the flowering peak which resulted in earlier fruiting peaks for large seeded plants. Alternatively, early phenology of large seeded fruits could be explained by a correlation with wind dispersal. In univariate analyses, non-wind dispersed herbs flowered and fruited significantly earlier than wind dispersed ones. In addition, non-wind dispersed herbs had larger seeds than wind dispersed ones (examples for small seeded wind dispersed plants are common poppy, *Papaver rhoeas*, or mugwort, *Agrimonia vulgare*). Thus, the significant negative relationship between seed size and flowering/fruiting peak may be due to wind dispersal which is correlated with small seeds and later flowering and fruiting peaks. Later fruiting peaks for wind dispersed plants should be advantageous because wind strength increased in fall (Fig. 3.1C). However, wind dispersal was not significant in multivariate analyses that included plant size, habitat openness and seed size (Tab. 3.1).

Another important life history trait was habitat openness. Herbs of closed habitats (forests) flowered earlier (Fig. 3.4) and fruited earlier than those of open habitats (meadows, ruderal sites). This finding is consistent with HEINRICH (1976). Many spring flowering herbs are found in forested habitats (SCHEMSKE et al. 1978, PRIMACK 1985). This pattern might be caused by ant dispersed plants which are known to flower and fruit early in the year and to be common in temperate forests (ULBRICH 1919, BEATTIE et al. 1979, HANDEL & BEATTIE 1990). Another advantage of an early phenology for herbs in deciduous forests may be larger light intensity available before the foliage is fully grown (SERNANDER 1906, SCHEMSKE et al. 1978). Larger light intensity should increase photosynthetic activity which should represent an advantage for the plants (see SCHEMSKE et al. 1978).

The phylogenetic relatedness in herbaceous plant species represented to a small but significant degree phylogenetic inertia. Like KOCHMER & HANDEL (1986), JOHNSON (1993) and SMITH-RAMIREZ et al. (1998), I found a significant relationship between the phylogenetic relatedness of the plant species and their flowering times. In multivariate analyses, this effect was much weaker, presumably due to the presence of other variables in the model. In comparison to the flowering peak, the fruiting peak featured little or no phylogenetic inertia. This finding suggests that over evolutionary time scales plants can change their fruiting peaks faster than their flowering peaks. The period of fruit production showed a strong relationship to the phylogeny of the plants indicating that an evolutionary

shift of the flowering peak also causes a shift in the fruiting peak and vice versa (see PRIMACK 1985). This phylogenetic effect could contribute to the strong correlation between flowering and fruiting peaks in herbs (Fig. 3.3) and to my failure to find any strong correlate of the fruit production period in herbs (Tab. 3.1). In addition, this phylogenetic effect could explain the fact that all factors influencing both flowering and fruiting peak operated in the same direction. Flowering and fruiting peak shifted in parallel with fruit production periods remaining unchanged. This finding represent evidence that flowering and fruiting times can influence each other (PRIMACK 1985, 1987, RATHCKE 1988, FENNER 1998).

In woody species, pollination and seed dispersal mode showed very strong relations to the three phenological traits. Pollination and seed dispersal mode alone explained more than half of the variance in the flowering peak and the period of fruit production (Tab. 3.2). Wind pollinated species featured early flowering peaks which might represent a phenological adaptation. Presumably, the pollen of wind pollinated plants should be transferred before the foliage is fully developed. Otherwise the loss of pollen adhered on the surface of the leafs would be enormous and should reduce the probability of successful pollination. Additionally, early flowering wind pollinated plants should benefit from stronger winds because wind strength decreases during spring (Fig. 3.1C). In contrast to herbs, flowering and fruiting peaks of wind pollinated shrubs and trees were not shifted in parallel. Therefore, the fruiting peak, in contrast to the flowering peak, did not differ between wind and insect pollinated species. This corresponds to the longer fruit production periods in wind pollinated plants (Tab. 3.2). Wind dispersed shrubs and trees flowered early and fruited later than differently dispersed ones. However, this did not result in significantly longer fruit production periods (maybe only due to my small sample size). Late fruiting wind dispersed plants should benefit from stronger winds, because wind strength increases during fall (Fig. 3.1C).

Life history traits also affected the phenology of woody species (Tab. 3.2). Large seeded shrubs and trees fruited later than small seeded ones. Again, flowering and fruiting peak were not shifted in parallel and flowering peak remained unchanged. Correspondingly, large seeded plants needed longer times to produce their fruits than small seeded ones. This effect of seed size presumably caused the significant effect of seed volume in Tab. 3.2. In contrast to herbs, I found for shrubs and trees large variance in the fruit production period (Fig. 3.3) from which I could explain 50.5 % by only two variables (Tab. 3.2). I could not find any significance for the phylogenetic relatedness among the plants. For trees, this is consistent with KOCHMER & HANDEL (1986) but not so for shrubs. However, the difference between

my finding and those of KOCHMER & HANDEL (1986) may result from my small sample size in woody species.

Abiotic factors seem to determine start and end of a growing season. The strong correlation between the flowering peaks and day length in both herbaceous and woody species (Tab. 3.3) indicates that the reproductive period of the plant community started with an increasing amount of sunlight. For fruiting peaks and the number of fruit producing species, differences exist between herbaceous and woody species. In herbs, temperature was the dominant factor. In shrubs and trees, the relationships are less clear because four of the five abiotic factors were highly correlated with fruiting peaks. In general, the finding that the phenology of plants was strongly correlated with day length and temperature and, at best, scarcely with precipitation fit well with other studies (WHITE et al. 1997, WHITE 1995, DIEKMANN 1996). This pattern appears to be typical for temperate communities, because in temperate regions, and in contrast to the tropics, sunlight and warmth instead of water are the limiting abiotic factors.

With these study of phenological correlations within a temperate plant community, I gained new understanding about the phenology of plants. My data demonstrates the remarkable significance of pollination and seed dispersal mode for the reproductive phenology of seed plants. The simultaneous study of flowering and fruiting times represents a methodological improvement. If the same method is used to collect data of flowering and fruiting phenologies, similar data quality allows to derive a good measure for the period of fruit production. Studying this phenological link between flowering and fruiting helps to identify and to interpret phenological patterns and relationships.

3.5 Summary

I investigated the significance of pollination and seed dispersal mode for three phenological traits concerning plant reproduction, i.e. the flowering peak, the fruiting peak, and the period of fruit production as the time difference between these two peaks. Pollination and seed dispersal modes and the three phenological traits were determined for 275 different plant species within a seed plant community of Central Europe. In addition, I investigated possible effects of life history traits as well as phylogenetic and abiotic factors that may influence the plant phenology. Due to large differences between the phenology of herbaceous and woody plant species I studied the effect of pollination and seed dispersal mode on the three phenological traits for herbs and shrubs/trees separately. In both groups pollination and

seed dispersal mode showed a clear relationship to the flowering and fruiting peak also when controlling for other significant phenological influences. In herbaceous species, wind pollinated and ant dispersed species had earlier flowering and fruiting peaks than insect pollinated and non-ant dispersed ones. In woody species, wind pollinated species had earlier flowering peaks than insect pollinated ones, and wind dispersed species had later fruiting peaks than non-wind dispersed ones. The fruit production period of herbaceous species was barely correlated with any of the traits studied but showed significant phylogenetic effects. This indicates that evolutionary shifts in flowering peaks caused also shifts in fruiting peaks and vice versa. For woody species, no phylogenetic effect could be found. The results reveal that pollen and seed vectors, mainly animals and wind, play an important role for the seasonal timing of the reproductive phases within the life cycle of seed plants.

4. Phenological adaptation of ant dispersed plants to the seasonal variation in ant activity

4.1 Introduction

Reproduction in plants depends on successful pollination and seed dispersal (HOWE & WESTLEY 1988, BOND 1995). As numerous plants are pollinated and dispersed by animals, these mutualistic animal-plant-interactions are studied intensely (FEINSINGER 1983, HERRERA 1985, HOWE & WESTLEY 1988). Various adaptations of plants to animals in morphology, physiology, and behavior (*in sensu lato*) are discussed. Pollination syndromes consisting of e.g. special forms, colors, and odors of animal pollinated flowers are thought to enhance pollination success and, therefore, plant fitness (VON FRISCH 1915, ROBACHER et al. 1988, CHITTKA & MENZEL 1992, BARRETT et al. 1994). Similarly, dispersal syndromes which are beneficial for seed dispersal by animals are known in fruits (GAUTIER-HION et al. 1985, HOWE & WESTLEY 1988). However, while some highly specialized interactions between animals and plants are known in pollination ecology, seed dispersal by animals appears to be always diffuse (JANZEN 1983, HERRERA 1985, HOWE & WESTLEY 1988, HANDEL & BEATTIE 1990).

Although pollination biology represents an old area of research (SPRENGEL 1793, DARWIN 1877, KNUTH 1898), scientific interest in the seasonal timing of flowering (and fruiting) arose only in the last few decades. Recently, plant phenology has been intensely discussed because the timing of flowering and fruiting is assumed to benefit plant fitness by increasing pollination and seed dispersal success (FENNER 1985, RATHCKE & LACEY 1985, LEBUHN 1997). However, little is actually known about the phenology of plants (WILLSON 1992). This is especially true for the fruiting phenology which is less studied than the flowering phenology (but see FENNER 1998). Nonetheless, two hypotheses for phenological adaptations of plants to their animal seed dispersers exist (THOMPSON 1981). First, bird dispersed plants appear to fruit late in the season when number of dispersers increases due to autumnal bird migration. Second, ant dispersed plants appear to fruit early in the year when ant activity is assumed to be high. The second hypothesis appears to be especially suitable to test phenological adaptations because ant dispersal systems can be experimentally manipulated successfully indicated by various studies performing seed removal experiments.

The phenomenon that spring flowering plant species in temperate forests are frequently ant dispersed has been noted by many authors (e.g. SERNANDER 1906, ULBRICH 1919, MÜLLER-SCHNEIDER 1977, BEATTIE et al. 1979), but quantitative data that validate this pattern are rare. THOMPSON (1981) postulated that this pattern might represent a phenological adaptation to ant activity because ant dispersed plants do not only flower but also fruit early in the year which possibly results in higher seed removal rates concomitant with higher ant activity early in the year. In other words, ant dispersed plants 'may have altered the times at which their seeds mature' and seeds of 'plants that bear ripe elaiosomes in the spring ... may be transported more frequently than seeds presented in the summer or fall' (HANDEL & BEATTIE 1990, p. 61). However, the relationship between fruiting phenology of ant dispersed plants and the seasonal variation in ant activity has never been tested. Possibly other causes for the early phenology of ant dispersed plants exist. The phenological pattern might be explained by a habitat effect since ant dispersed plants appear to be frequent in forested habitats. Furthermore, most ant dispersed plant species appear to be small herbs which produce small seeds. Therefore, the phenological pattern might be caused by growth form, plant or seed size. Furthermore, the timing of flowering and fruiting could have influenced each other (PRIMACK 1985, 1987, RATHCKE 1988, FENNER 1998). Since ant dispersed plants are mostly insect-pollinated, the phenological pattern might be rather affected by the pollination mode of the plants. Finally, as all plant species share an evolutionary history, the phenological pattern may not represent an ecological but rather a phylogenetic pattern (FELSENSTEIN 1985, HARVEY & PAGEL 1991, GITTLEMAN & LUH 1992).

With this study I tried to find experimental evidence for the adaptation of ant dispersed plants to the seasonal activity of their seed dispersers as postulated by THOMPSON (1981) and HANDEL & BEATTIE (1990). To quantify the difference in flowering and fruiting time between the guild of ant dispersed plant species and all non-ant dispersed species of a temperate plant community, I investigated the reproductive phenology of 275 different species of seed plants. Because phenological differences between ant and non-ant dispersed plants may be affected by other plant attributes, I controlled in my analysis for these possible influences. In addition to these botanical investigations, I determined the seasonal variation of ant activity by performing repeated standardized seed removal experiments during a whole vegetation period. I used the correlation between fruiting time and seasonal ant activity as an indication for a phenological adaptation of plants to seasonal ant activity.

4.2 Methods

4.2.1 Study area and plant community

The study area (in total approximately 50 ha) was located in the west of Aachen, Germany, next to the border to the Netherlands. The area includes the Nature Reserves „Wilkensberg“ and „Schneeberg“ and consisted of different habitat types, i.e. ruderal sites, grasslands (rich meadows and calciferous grasslands), bushes, forest edges and forests (coniferous and deciduous). In addition, dry and wet habitats were present. For my community approach I collected data from as many plant species as possible. I investigated all seed plant species I could find on the study area, but excluded hybrids and domesticated plants. The species studied are listed in the appendix.

4.2.2 Plant phenology

I monitored the study area every ten days (every decade) from January to December 1998. For each census I looked for both flowering and fruiting plant species. The number of open flowers and ripe fruits per plant species were estimated in four categories: absent (no flowers/fruits found), rare (< 5 % of the maximum), present (5 - 95 % of the maximum), maximal (>95 % of the maximum). The maximum was defined as the maximum number of flowers/fruits a plant species produced in the study area in any of the decades. Criteria for fruit ripeness were color and the ability to detach fruits from the infructescence. From these data, the flowering/fruiting peak for each plant species was determined as the decade with the maximum numbers of flowers/fruits. In case the maximum number was present during more than one decade, I defined the average of these maximum decades as the peak. This was possible because species that showed two flowering and fruiting periods within the 1998 season (e.g. dead-nettle, *Lamium album*) flowered and fruited much more intensively in the first period (maximum) than in the second one.

4.2.3 Plant attributes

Plants found in the study area were classified as ant dispersed following SERNANDER (1906), SCHUBERT et al. (1988), and GÖTZE (1995). Besides dispersal mode, I investigated eight other plant attributes that might affect the timing of flowering and fruiting: pollination mode, growth form, plant size, length of life cycle, seed size, fruit size, habitat openness and habitat moisture.

For pollination mode, I distinguished between wind, insect, and self-pollinated plants using data from SCHUBERT et al. (1988). Species that are pollinated in more than one way were assigned to more than one category. For growth form, I distinguished between herbaceous plants (herbs and halfshrubs), and woody species (shrubs and trees). As a measure for plant size, I used the averaged range given in SCHUBERT et al. (1988). The length of the life cycle was classified by five categories: 1-strictly annual, 2-annual or biennial, 3-strictly biennial, 4-perennial, or 5-persistent (SCHUBERT et al. 1988). Seed size was determined by estimating the seed volume from the length, width and height of the seeds applying the ellipsoid formula. Fruit size was estimated as the longest dimension of the fruit. To determine seed and fruit size, I randomly collected five fruits from different individuals. For habitat openness, I assigned each plant species to one of seven categories: 1-forest, 2-forest to forest edge, 3-forest edge, 4-forest edge to grassland, 5-grassland, 6-grassland to ruderal site, or 7-ruderal site using own field data. For habitat moisture, I assigned each plant species to one of two categories: 1-dry or 2-wet using own field data.

4.2.4 Plant phylogeny

Plant species may not represent independent samples due to their phylogenetic history (FELSENSTEIN 1985, HARVEY & PAGEL 1991, GITTLEMAN & LUH 1992). Closely related species may show similar characteristics as a result of their common evolutionary origin. To control for this phylogenetic autocorrelation I tested the effect of the phylogenetic distance between the species on the distance in their phenological traits using Mantel tests (MANTEL 1967, SMOUSE et al. 1986, LEGENDRE & FORTIN 1989, BÖHNING-GAESE & OBERRATH 1999, BÖHNING-GAESE et al. in press). As a measure for phylogenetic distance I used the taxonomic classification of the species. I worked on nine different taxa levels: species, genus, subfamily, family, infraorder, order, superorder, subclass and class. The data for the subfamily taxon were taken from SITTE et al. (1991), the data for the class to family level were taken from THORNE (1992). For analysis, the phylogenetic distance between two species within the same genus was defined as one, between two species within the same subfamily as two, etc.

To test the effect of the phylogenetic relatedness on the plant phenologies I performed Mantel tests which work on distance matrices (BÖHNING-GAESE et al. in press, OBERRATH & BÖHNING-GAESE unpublished manuscript). In each distance matrix, each species is compared with each other one in a certain characteristic (MANLY 1986). For plant,

seed, and fruit size, I calculated the distance between two species as the quotient of their trait values (corresponding to their logarithmic relationship, see 2.4.3 and Tab. 2.2). For the other variables, I calculated the difference of the trait values as distance measure (corresponding to their linear relationships). In multivariate models, the phenological distances (flowering and fruiting peaks) were regressed on the distances of the independent variables, pollination mode, seed dispersal mode, the life history variables and the phylogenetic distance. I computed the significance levels of the regression analyses by the permutational regression procedure of the Mantel test performing 10000 permutations.

4.2.5 Seasonal variation of ant activity

I determined two different measures for seasonal ant activity, the number of seeds removed and the number of ants observed per time and area. Seed removal rates were determined 1998 using seeds of Greater Celadine (*Chelidonium majus*) which are known to be attractive to ants (ULBRICH 1919). Greater Celadine is a common herb in semi-forested habitats and produces 25-50 dark brown seeds per fruit and up to 100 fruits per individual plant. Each seed bears a white elaiosome which represents the ant attractant. To compare removal rates of ten different ant dispersed plants simultaneously Cafeteria experiments were performed in which seeds of different plant species were offered to ants (unpublished manuscript). The results demonstrated that Celadine seeds appear to be typical for ant dispersed plants due to their medium size and their representative attractiveness to ants. My results were similar to those of OOSTERMEIJER (1989) who worked only on three different plant species. To conduct seed removal rates during a whole vegetation period, fruits of Greater Celadine were collected from several different plants in 1997, shortly before releasing their seeds. After shock freezing using liquid nitrogen, I stored the fruits in a deep freezer at -75°C. Seeds of several different fruits were thawed up immediately before each seasonal experiment started. A comparative study of seed removal rates of conserved and fresh seeds revealed no significant differences in the attractiveness to ants.

To obtain seasonal seed removal rates I set five Celadine seeds on 2 x 2 cm^2 large wooden deposits. To hinder the seeds from rolling away, the deposits had a frame approximately three times higher than a seed. Cages with mesh sizes of 4.4 mm prevented access from birds and mice which are known to be possible seed predators (BEATTIE 1985). For protection of seeds against rain the cages were roofed by a transparent foil. The deposits and cages were separately fixed in the ground. In case of removed cages, I excluded the

corresponding deposit from analysis. To minimize the problem of spatial variation in ant activity on the scale of square meter (SMITH et al. 1989), I grouped three deposits to one food location. The three deposits of a food location were spaced by approximately 1 m. The minimum distance between two food locations was 10 m. In total, I worked on 18 food locations simultaneously, which were equally distributed over different habitat types (six food locations in pine and deciduous forests, six on rich meadows and calciferous grasslands, and six at the corresponding forest edges). All food locations were set up between 11 am and 2 pm at random locations which differed for each decade. I counted the seeds left after approximately six hours. From these data I calculated the percentage of seeds removed (100 % = 5 x 3 x 18 = 270 seeds) which was arcsin-transformed (SOKAL & ROHLF 1995). This procedure was repeated every decade from the second decade in March to the first decade in December in parallel to the phenological investigations of the plant species (see above).

To ascertain that no other animals than ants removed the seeds, removal of Celadine seeds were observed for 216 observation hours (unpublished data). During these observations which were performed at daytime in July 1997 and July 1998 eight different ant species were found removing the seeds (Tab. 4.1). Only in exceptional cases, harvestmen (Opiliones), ground beetles, woodlice (Isopoda), and spiders were found to be attracted by the seeds but none of these species removed or fed on the seeds repeatedly.

Tab. 4.1: Seed dispersing ant species found in the study area.

Species	Open habitats	Forested habitats
Formica cunicularia	X	
Formica rufibarbis	X	
Formica fusca	X	
Myrmica rubra	X	X
Lasius niger	X	X
Myrmica ruginodis		X
Lasius fuliginosus		X
Leptothorax nylanderi		X

The number of ant individuals present at the food locations were determined in the afternoon, between the setting up of deposits and the control for seed removal. I counted at every second food location the number of ants within a 8.24 dm^2 large circular area (diameter 32.4 cm) during a period of 2 min. For analysis, I considered only individuals of ant species which had been observed carrying Celadine seeds.

4.3 Results

4.3.1 Plant phenology

In total, I investigated 24 ant dispersed and 251 non-ant dispersed plant species (see appendix). Of the ant dispersed species, 23 were herbaceous and one woody (Tab. 4.2). Of the non-ant dispersed species, 207 were herbaceous and 44 woody. Flowering and fruiting peaks of plant species are shown in Fig. 4.1. Mean flowering peak for the guild of ant dispersed plant species was the middle of May. For non-ant dispersed plant species the mean flowering peak was the end of June. On average, ant dispersed plant species flowered 3.9 decades (5.6 weeks) earlier than the other species (t-test: $t = 4.1$; $df = 273$; $p < 0.001$). Mean fruiting peak for the guild of ant dispersed plant species was the beginning of July. For non-ant dispersed plant species the mean fruiting peak was the end of August. On average, ant dispersed plant species fruited 5.0 decades (7.1 weeks) earlier than differently dispersed ones (t-test: $t = 5.4$; $df = 273$; $p < 0.001$).

These differences in flowering and fruiting phenology may not be caused by the dispersal mode but rather by other plant attributes or by phylogenetic autocorrelations. Therefore, I performed a multivariate ANCOVA in order to control for these alternative factors. Although I could find several plant attributes influencing the phenology of the plants (Tab. 4.3), the phenological difference between ant and non-ant dispersed plants remained essentially the same (Fig. 4.2). When controlling for other influences, the difference in flowering peaks was 3.7 decades (5.3 weeks) and in fruiting peaks 3.5 decades (5.0 weeks). Due to the multivariate approach, these differences were smaller than those of the raw data (see above), but substantial and can be attributed mainly to the ant dispersal mode.

Flowering and fruiting peaks of the plant species were significantly affected by their phylogeny. When including the phylogenetic distance among the species in the multivariate models shown in Tab. 4.3, the phylogenetic effect was significant for the flowering peak (Mantel test: $t = 6.5$; $p = 0.047$; $n = 36585$ paired distances) and the fruiting peak (Mantel test: $t = 7.3$; $p = 0.026$; $n = 36585$). The presence of the phylogeny in the multivariate model,

however, yielded similar results for the effect of ant dispersal (on flowering peak: $t = 27.3$; $p < 0.001$; $n = 36585$; and on fruiting peak $t = 33.0$; $p < 0.001$; $n = 36585$). The presence of the phylogeny increased the explained variance by only 0.2 %. Thus, the phenological differences demonstrated in Tab. 4.3 and Fig. 4.2 do not represent phylogenetic effects and, therefore, cannot be attributed to phylogenetic autocorrelations.

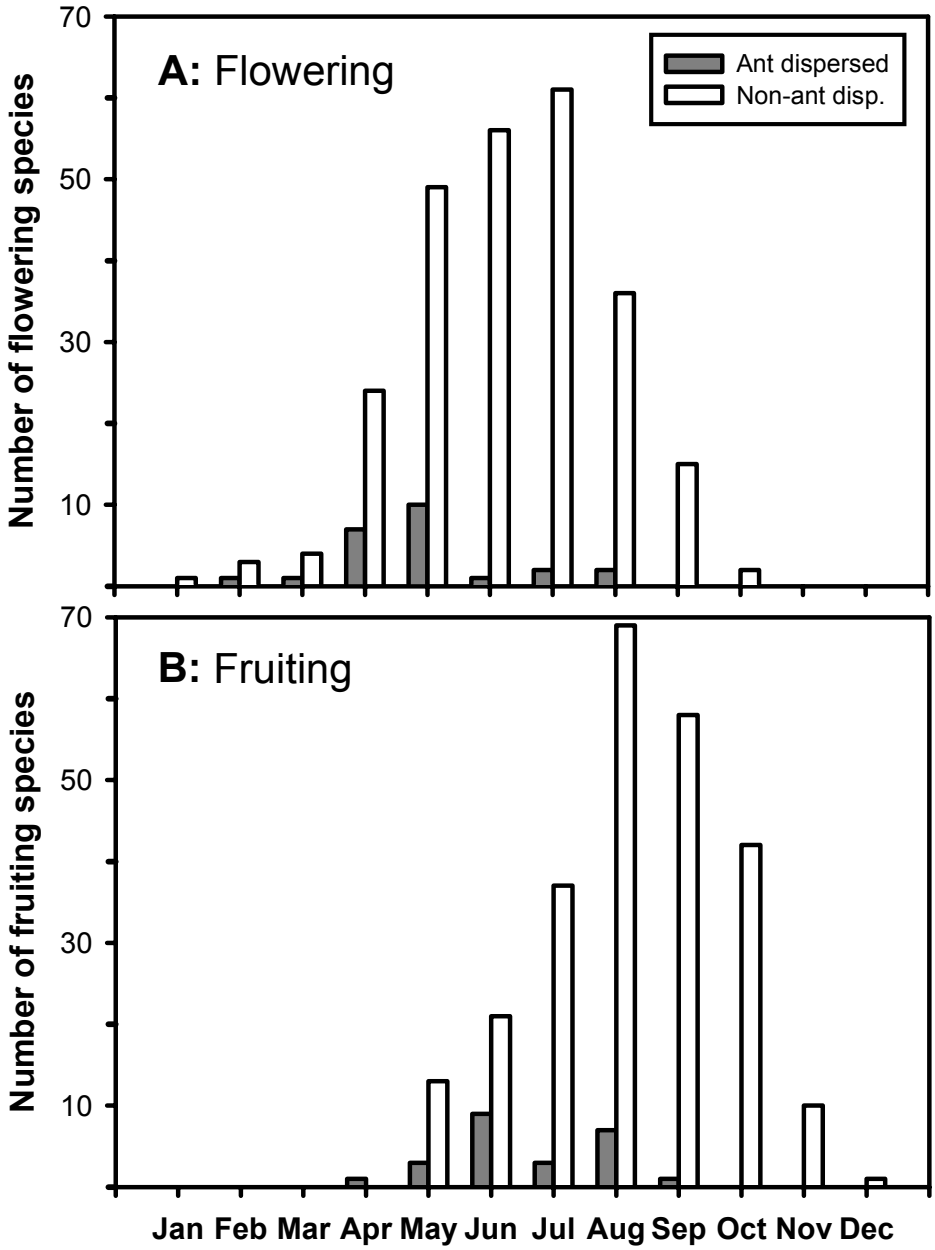

Fig. 4.1: Seasonal distribution of flowering (**A**) and fruiting peaks (**B**) of 24 ant and 251 non-ant dispersed plant species. The three decades of each month were pooled.

Tab. 4.2: List of ant dispersed plants studied. Roman numbers (I, II, III) indicate decade of month. Original categories of habitat openness which ranged from eight to one (see methods) were pooled to forest (1, 2), forest edge (3, 4) and grasslands (5, 6, 7).

Species	Growth form	Habitat	Flowering peak	Fruiting peak
Viola odorata	Herb	Forest	March I	July I
Viola hirta	"	"	April II	August I
Veronica hederifolia	"	"	April II	May III
Lamium argentatum	"	"	April III	June I
Glechoma hederaceum	"	"	April III	June I
Allium ursinum	"	"	May I	May II
Viola riviniana	"	"	May I	August II
Lamium endtmannii	"	"	May I	May II
Moehringia trinerva	"	"	May I	May III
Chelidonium majus	"	"	May II	June II
Galanthus nivalis	"	Forest edge	February III	April III
Anemone nemorosa	"	"	April III	May III
Lamium album	"	"	May I	June I
Ajuga reptans	"	"	May III	June III
Reseda lutea	"	"	June III	August III
Euphorbia helioscopia	"	Grassland	April II	June III
Lamium purpureum	"	"	May I	May III
Fumaria officinalis	"	"	May III	July II
Symphytum officinalis	"	"	May III	July I
Reseda luteola	"	"	June III	August II
Knautia arvensis	"	"	July III	August III
Centaurea scabiosa	"	"	August I	August III
Centaurea jacea	"	"	August I	September III
Sarothamnus scoparius	Shrub	Forest edge	May I	August I

Tab. 4.3: Multivariate model to determine the effect of dispersal mode on flowering and fruiting peak when controlling for the other life history traits. Displayed are the F-ratios of the ANCOVA, and the corresponding significance levels. Control factors are marked by #.

	Dependent variables	
Independent variables	Flowering peak	Fruiting peak
(Plus / Minus equals)	(later / earlier)	(later / earlier)
Dispersal mode (ant vs non-ant)	– 21.8 ***	– 14.8 ***
Pollination mode (insect vs wind) #	+ 23.3 ***	+ 4.4 *
Growth form (herbs vs shrub/trees) #	+ 69.3 ***	+ 9.3 **
Length of life cycle #	– 2.9 *	– 2.6 *
Log(plant size) #	+ 22.3 ***	+ 35.5 ***
Habitat openness #	+ 24.5 ***	+ 8.9 **
Explained variance (%)		
Total	44.8	26.1
Without #	5.7	9.5

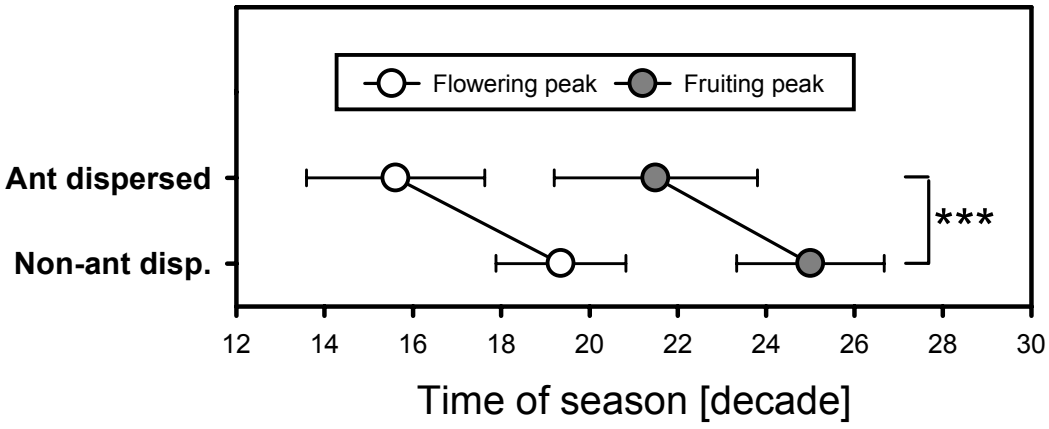

Fig. 4.2: Adjusted means and their 95% confidence interval for flowering and fruiting peaks in ant (n = 24) and non-ant dispersed plants (n = 251) when controlling for life history factors listed in Tab. 4.1.

4.3.2 Seasonal variation in ant activity

I found ten different ant species in the study area, eight of them were observed to remove seeds (Tab. 4.1). Seed removal activity of ants started in the last decade of March (Fig. 4.3). Nearly all seed removal presented in Fig. 4.3 for March occurred in the last decade. Yet, seed removal rates remained low until May (Fig. 4.3). From May to July seed removal rates were high and then decreased from August to November. The number of ants observed was already high by the end of March (data not presented) and remained high until end of July. In August the number of ants observed dropped dramatically. Latest ants were observed in October. Seed removal and number of ants observed were positively correlated (PEARSON $r = 0.461$; $p = 0.03$; $n = 22$ decades).

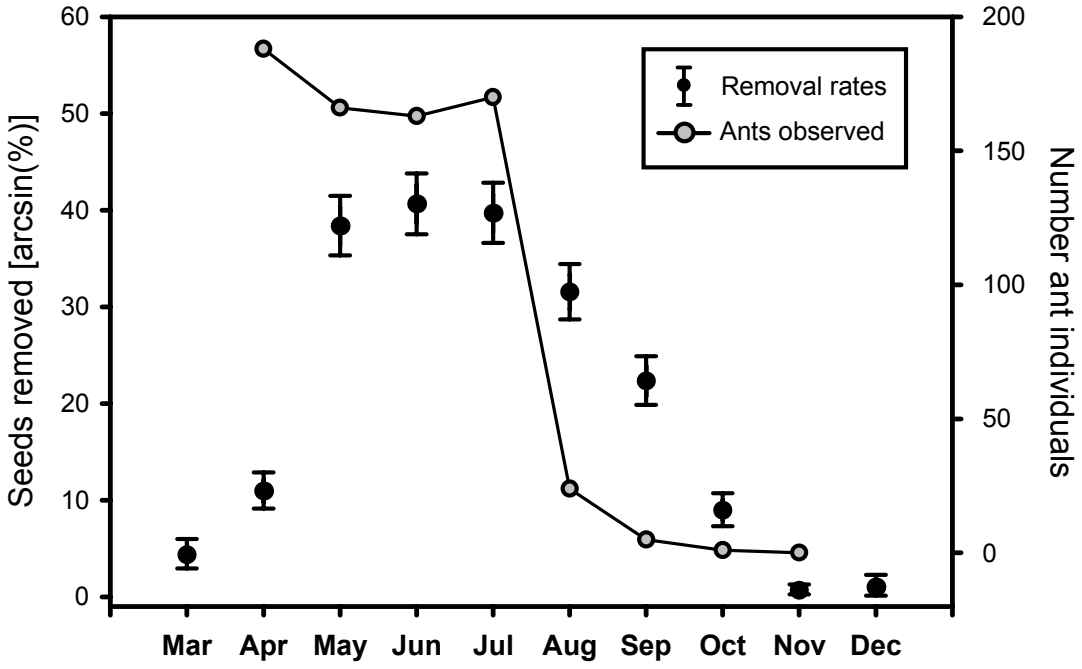

Fig. 4.3: Seasonal variation in ant activity. The three decades of each month were pooled (March two, April to November three, December one decade). For removal rates, 95% confidence intervals were calculated from a binominal distribution (SACHS 1995).

To test whether abiotic disturbances contribute to seed removal I correlated seed removal rates with mean values of wind strength and sum of rain fall per decade. Data were taken from the meteorological station Aachen which was located 5 km next to the study area. Wind was significantly anticorrelated with removal rates ($r = -0.492$; $p = 0.009$; $n = 27$) indicating that in summer, when removal rates were high, wind strength was small and that in spring and fall, when ant removal rates were low, wind strength was strong. Rain fall was non-significantly anticorrelated with seed removal rate ($r = -0.200$; $p > 0.3$; $n = 27$). Thus, the possibility of an abiotic seed removal can be ruled out. In addition, we never observed wind or rain moving the seeds.

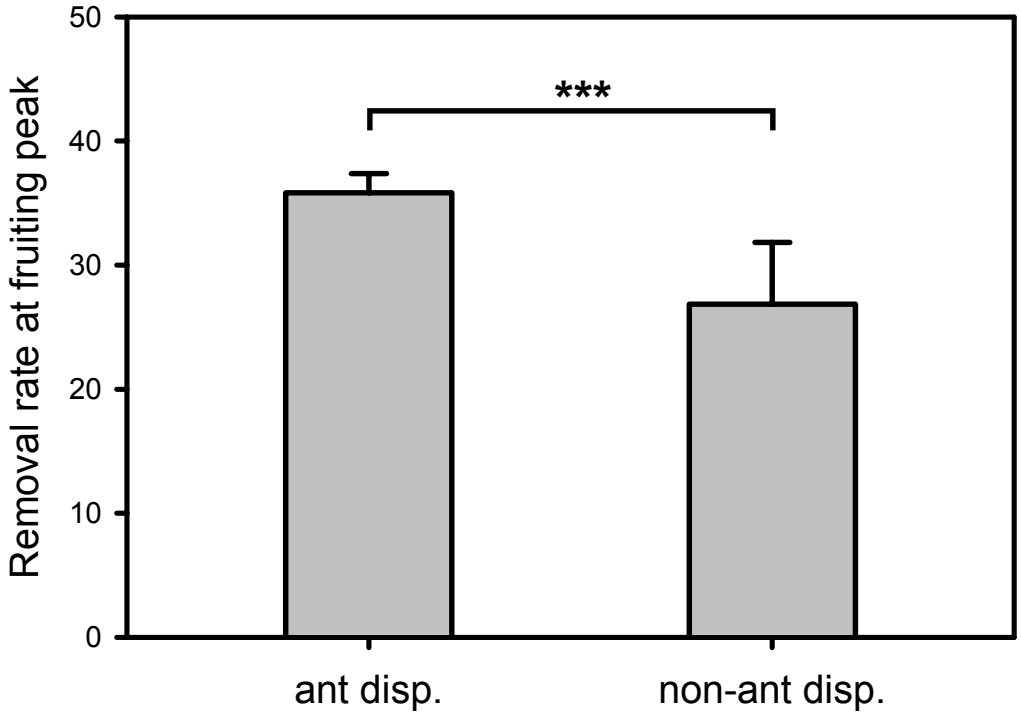

Fig. 4.4: Test on phenological adaptation. Displayed are means and 95% confidence intervals for seed removal rates at fruiting peaks. If non-ant dispersed plants were ant dispersed they would have a seed removal rate at their fruiting peak of only 74.9 % of that observed for ant dispersed plants.

4.3.3 Test on phenological adaptation

To test whether the fruiting times of ant dispersed seeds were correlated with seed removal rates, I constructed a thought concept. Given the hypothetical assumption that all 275 plant species studied were ant dispersed with similar attractiveness to ants (see discussion), I determined the seed removal rate for each plant species at its fruiting peak. Data on seed removal rates were taken from the experiments with Celadine seeds (see above). In this way I were able to test whether seed removal rates at fruiting peaks of ant dispersed plants differed from those of non-ant dispersed plants. From high seed removal rates at its fruiting peak I can conclude that a plant species got their seeds successfully dispersed. Hence, the removal rate at the fruiting peak can be interpreted as a measure for seasonal success in seed removal. The comparison of the success in seed removal between the real and the only assumed ant dispersed plants yielded a significant difference (Fig. 4.4). Real ant dispersed plants showed higher seed removal rates at their fruiting peaks than the assumed ones would have if their dispersal depended on ants (t-test: $t = 3.3$; $df = 273$; $p = 0.0008$). Since species might not represent independent sampling units I also performed a Mantel test controlling for phylogenetic effects. The results were even more significant (Mantel test: $t = 23.6$; $p < 0.0001$; $n = 35607$ paired distances).

4.4 Discussion

This study presents evidence for the early flowering and fruiting phenology of ant dispersed plants and for the phenological adaptation of these plants to their ant seed dispersers. Ant dispersed plants flowered and fruited on average more than one month earlier than plants not dispersed by ants. These differences cannot be explained by other plant attributes or by plant phylogeny, but critically depended on the ant dispersal mode of the plants. Mean fruiting peak of ant dispersed plants was in the beginning of July. In addition, I determined the seasonal variation in ant activity which was especially high from May to July. The test on phenological adaptation revealed that ant dispersed plants would suffer a decreased removal rate if they fruited as late in the season as the other plants did.

Plants adapted to ant dispersal flowered and fruited more than one month earlier than those not dispersed by ants. However, ant and non-ant dispersed plants differed in various attributes which also could have caused the phenological difference. Indeed, I were able to identify several plant attributes that correlated with plant phenology (Tab. 4.3). One of the attributes most often mentioned in the literature is habitat type (BEATTIE 1983,

PEMBERTON & IRVING 1990, HUGHES & WESTOBY 1990, BOND 1991). Ant dispersed plants of temperate regions can be found more frequently in forested habitats than in open ones (SERNANDER 1906, ULBRICH 1919, LUFTENSTEINER 1982). SERNANDER (1906) reported that ant dispersed plant species are five to ten times more abundant in forested than in open habitats. In my study, only two thirds of all ant dispersed plants came from forested habitats (Tab. 4.2), which was due to the presence of a species rich calciferous meadow within the study area. Four of the ant dispersed plants found in the grasslands were present only on this meadow. In general, many herbaceous plant species in forests of northern temperate regions are spring flowering (SCHEMSKE et al. 1978, PRIMACK 1985, HANDEL & BEATTIE 1990), and many of the these early flowering plant species are ant dispersed (ULBRICH 1919, BEATTIE et al. 1979, HANDEL & BEATTIE 1990). Interestingly, when only considering the 24 ant dispersed plants studied (Tab. 4.2), species from forested habitats flowered and fruited significantly earlier than those of closed habitats (test results not presented), which caused the bimodal distribution of fruiting peaks in ant dispersed plants (Fig. 4.1B). Possibly, the attribute of herbs to flower (and fruit) early in forested habitats could simply explain the phenological feature I found for ant dispersed plants. However, when controlling for habitat and the other significant factors correlated with plant phenology, the difference between ant and non-ant dispersed plants remained strong (Fig. 4.4). Thus, the early flowering and fruiting peak of ant dispersed plants can be explained only marginally by other factors such as habitat type, pollination mode, growth form or seed size.

Furthermore, the phenological feature of ant dispersed plants can not be addressed to phylogenetic autocorrelation. The early phenology of ant dispersed plants represented only slight phylogenetic effects. The Mantel analyses controlling for phylogenetic effects revealed a similar phenological difference between ant and non-ant dispersed plants as shown in the ANCOVA analysis of Tab. 4.3. Besides, ant dispersal is assumed to have evolved multiple times independently, as indicated by the variety of anatomical origins of the elaiosomes and by the wide taxonomic distribution of ant dispersed plants (SERNANDER 1906, BRESINSKY 1963, BUCKLEY 1982, BEATTIE 1983, HANDEL & BEATTIE 1990, HÖLLDOBLER & WILSON 1990, PEMBERTON & IRVING 1990, BOND 1991). Correspondingly, the ant dispersed plant species listed in Tab. 4.2 belong to 15 different families and 13 different orders.

All seed removing ants species found in the study area (Tab. 4.1) actually dispersed and did not prey on the seeds. Most plant species adapted to ant dispersal have evolved

elaoisomes, fatty food bodies attached to the seeds which are highly attractive to ants (BRESINSKY 1963, LISCI et al. 1996, GÓMEZ & ESPADALER 1998, MAYER & SVOMA 1998). Instead of an elaiosome, some ant dispersed plant species have fatty seed coats (e.g. wood anemone *Anemona nemorosa*). The removal of the ant attractant drastically reduces the probability that a seed will be removed (see experiments of SERNANDER 1906). Like OHKAWARA & HIGASHI (1994) and OHKAWARA et al. (1996), I found several arthropods feeding on Celadine elaiosomes: plant mites, springtails (Colembola), larvae of beetles and some (but not all) individuals of the ant species *Leptothorax nylanderi*. Most seeds whose elaiosomes were eaten remained in the caged deposits and were not removed. This finding indicates that ants of my study area were no harvester ants which do not feed on elaiosomes but eat and destroy the seeds (BEATTIE & LYONS 1975). Harvester ants were absent in the whole region (W. Kirchner, pers. comm.).

I invested much effort to assure that no other animals than ants removed the seeds. I caged every deposit to prevent large animals such as birds, mice or large ground beetles from getting access to the seeds. KJELLSON (1985), however, found ground beetles of 4 x 10 mm size that could have passed the meshes of the cages used. In addition, OHKAWARA et al. (1997) found ant dispersed seeds eaten by ground beetles of the genus *Pterostichus* which were also present in my study area although mainly active during the night (HIGASHI & ITO 1991, pers. obs.). To demonstrate that ants actually removed the seed, I correlated the number of ant individuals counted at the food locations with the corresponding seed removal rates (Fig. 4.3). Two differences were striking, first, seed removal rates were low in April although many ants were present. Presumably, in early spring ants fed on insects and shifted their preferences later to elaiosomes. Second, in August and September ant activity decreased much more than seed removal rates. This may indicate additional seed vectors or predators. However, besides ants no other animals were observed removing the seeds seed repeatedly (see methods). Since ant observations were performed in July at daytime, decreasing temperatures in fall could have caused possible nocturnal arthropods (e.g. ground beetles) to become active during daytime. Alternatively, since mice are known as seed predators (HEITHAUS 1981, HOLMES 1990), the cages may not have completely prevented mice from getting access to the seeds. In fact, seed removal from uncaged deposits increased in fall (unpublished data) most likely due to mice because in fall I often found faeces of mice on the cages. Hence, if mice were able to creep under the cages this would explain the difference between ant activity and seed removal in fall. In this case, however, the benefit in seed

removal for early fruiting ant dispersed plants would be even higher, because of escape from increasing seed predation in fall (GIBSON 1993). Finally, I can rule out the possibility of abiotic seed removal because removal rates were not positively correlated with either wind strength or rain fall. A totally different explanation for the difference between ant activity and seed removal may be that they are of different data quality. I measured ant activity only at half of the food locations. In addition, these data refer to only two minutes resulting in an total period of 9 x 2 minutes per census, whereas the seed removal rates refer to a period of six hours resulting in a total period of 18 x 6 hours! Thus, quality of seed removal data is higher than those of ant observations.

Seed removal activity of ants presumably depends on the availability of alternative food sources, honeydew of aphids and invertebrate prey. In red ants (*Formica polyctena* and allies) which were absent in my study area but which are well studied it is known that in March and April nourishment is based on fat reserves (KIRCHNER 1964). Food intake activity increases rapidly during April with its peak in May (HORSTMAN 1972) which is parallel to seed production of ant dispersed plants. Interestingly, „chemical composition and the behavioural releaser in elaiosomes have converged with the invertebrate prey of ants“ (Hughes et al. 1994, p 358). In contrast to elaiosomes, honeydew is mainly available later after the middle of May (SCHEURER 1964, 1967). Because the production of queens and drones starts in early spring (see KIRCHNER 1964) while food resources are still low, the presentation of elaiosome bearing seeds which represent attractive food for ants should result in successful seed dispersal for the plants.

As prerequisite for the phenological test, I assumed that seeds of all plant species are roughly similarly attractive to ants. Yet, ant species composition are found to influence seed removal of certain plant species (HANDEL & BEATTIE 1990, GORB & GORB 1999) indicating that certain ant species favor seeds of certain plant species (OOSTERMEIJER 1989, MIDGLEY & BOND 1995). Nonetheless, my assumption appears reasonable because all animal-plant interactions concerning seed dispersal are diffuse (BEATTIE et al. 1979, BUCKLEY 1982, JANZEN 1983). No close relationship between one ant species and one plant species are known (CULVER & BEATTIE 1978, HANDEL & BEATTIE 1990). All ant species disperse seeds of several plants that are available and seeds of a single plant species are dispersed by several ant species present (PUDLO et al. 1980, BEATTIE 1985, CUMMINGS & HEITHAUS 1992). In my approach, I pooled the activity of all eight ant species belonging to the guild of seed dispersing ants in my study area. In addition, Celadine

seeds appear to be typical for ant dispersed plants (see methods). Therefore, I assume that the seed removal rate of Celadine seeds is representative for other ant dispersed plant species.

For the test on phenological adaptation I determined the ant activity available to the plant species at their fruiting peaks. As a measure for seed removal activity of ants I used seed removal rates obtained by my experiments on Celadine seeds. When comparing the seed removal rates at the fruiting peak for the real ant dispersed plants with those of the assumed ones, a significant difference could be found (Fig. 4.4). At the fruiting peak of real ant dispersed plants 35.8 % of the seeds were removed within six hours. In contrast, for the other plant species only 26.8 % of the seeds would be removed if they were ant dispersed. On average, real ant dispersed plants fruited in the beginning of July. Seed removal activity of ants was high from May to July. Thus, if ant dispersed plants had fruited 7.1 weeks later when other plants fruited this would have been at times when ant activity had already decreased. Consequently, they would obtain only 74.9 % of their real seed removal (Fig. 4.4) which should be disadvantageous to the plants. Due to the habitat effect (see above) one could assume that the higher removal rates during early fruiting times may only exist for ant dispersed plants in forested habitats. However, when performing the test on phenological adaptation for plants of forested and closed habitats separately, I found that ant dispersed plants of both subsamples had higher seed removal rates at their fruiting peaks than non-ant dispersed plants of the same habitat. Thus, ant-dispersed plants of both open and closed habitats obtained higher removal rates by their early fruiting peak.

With this study, I present the first set of quantitative data on the early flowering and fruiting times of ant dispersed plants. In addition, I can show that due to their early fruiting peak ant dispersed plants bear ripe fruits at times when ant activity is especially high. From this correlation between fruiting times in ant dispersed plants and seasonal ant activity I derive the first experimental evidence for a phenological adaptation of plants to their animal seed dispersers.

4.5 Summary

I studied a temperate plant community in order to test whether ant dispersed plants show phenological adaptations to the seasonal variation in seed dispersal activity of ants. For plants, I investigated the reproductive phenology (flowering and fruiting peak) of 24 ant dispersed and 230 non-ant dispersed plants. For ants, I determined as a measure for their dispersal activity seasonal removal rates of Greater Celadine (*Chelidonium majus*) seeds. I

found that ant dispersed plants flowered on average 5.6 and fruited 7.1 weeks earlier than those which are non-ant dispersed. This difference did not depend on life history factors such as growth form or habitat. Mean fruiting peak of ant dispersed plants was early July. Ant activity was especially high between May and July. I were able to show that ant dispersed plants would have suffered a significant decline in seed removal if they had fruited at times when non-ant dispersed ones did. Thus, my data suggest that the early phenology of ant dispersed plants is an adaptation to their mode of seed dispersal. This is the first experimental evidence for a phenological adaptation of plants to their seed vectors.

5. General Conclusions

When mutualistic animal-plant interactions are studied, seed dispersal by ants (myrmecochory) appears to be an excellent study system to understand the beneficial effect of animals on their plant mutualists. Both, ant dispersed (myrmecochorous) plants and seed dispersing ants can be treated conveniently in observations and experiments. In contrast to vertebrate dispersed plants, myrmecochorous species are mostly herbaceous and small, making them suitable for handling. Besides, in contrast to large-sized or flying pollinators and seed dispersers, ants are active only on the scale of square meters and therefore easy to observe and to manipulate. Thus, ant dispersal systems are ideal for experimental studies of animal-plant-interactions.

I investigated the flowering peaks, the fruiting peaks and the fruit production period as difference between flowering and fruiting peak for 275 different species of a plant community in Central Europe. I asked which factors influence the reproductive plant phenology. Several life history traits as well as abiotic and phylogenetic factors appear to affect the phenological traits. In addition, animal-plant-interactions - particularly myrmecochory - seem to play an important role (see below). Unfavorable abiotic conditions in the beginning and the end and good conditions in the middle of the vegetation period strongly shape the plant phenology on the community level. In addition, several biotic factors e.g. growth form affect plant phenology. Woody and herbaceous species appeared to have totally different life history strategies which I found reflected by strong phenological differences. Other biotic effects could be identified as plant and seed size, habitat type, pollination and seed dispersal mode. The variety of factors correlated with the three phenological traits studied suggests that the plant phenology represents a plant attribute that can be influenced by several selective forces.

My results are based on comparative analyses on data of 275 different species. Because the species share an evolutionary history, my result could reflect a phylogenetic rather than an ecological pattern. In order to control for possible phylogenetic autocorrelation I applied the Signed Mantel test. Customary Mantel tests which are based on absolute distance data are not able to distinguish between positive and negative correlations. The Signed Mantel test, however, uses signed contrast data which allows to consider the direction of possible effects. The computer program I developed to perform this test and to visualize the distance data was a valuable tool to analyze my data. Applying this tool, I was able to show that the patterns I found for the flowering and fruiting times were barely due to phylogenetic effects and rather

reflect ecological relationships. In contrast, fruit production periods of herbaceous species actually appeared as strongly phylogenetically constrained.

I studied explicitly seasonal interactions between the guild of myrmecochorous plants and the guild of seed dispersing ants. Following the hypothesis that ant dispersed plants fruit early in the year when ant activity is especially high, I investigated flowering and fruiting times of plants and the seasonal pattern of ant activity. Indeed, the results obtained from my observations and experiments support this hypothesis. The data suggest that their mode of seed dispersal has selected myrmecochorous plants to flower and to fruit on average one and a half month earlier than non-myrmecochorous ones. The high ant activity in early summer appears to favor early fruiting of ant dispersed plants. This finding demonstrates that animal seed dispersers can have important effects on the plants whose seeds they disperse. However, the advantage of plants being ant dispersed is not well understood. In temperate forests where many ant dispersed herbs can be found the advantage may be the transport of seeds to nutrient-enriched as well as save sites and the lack of other suitable transport agents. Whatever the benefit may be, using ants as seed vectors in early summer myrmecochorous plants increase their seed dispersal success and, therefore, the probability of seed germination and successful seedling establishment.

Since the fruit production period (difference between flowering and fruiting) of herbaceous species seemed to be phylogenetically invariable, the shift to earlier fruiting times caused myrmecochorous plants also to flower earlier in the year. This shift appears to be constrained by limiting pollinator availability, low temperatures and little daylight in early spring. Nonetheless, many spring flowering plants are actually ant dispersed. In general, fruiting times seem to be more variable in evolutionary terms than flowering times. Therefore, when investigating possible phenological adaptations, a study of fruiting times looks more promising. Unfortunately, fruiting times have been less intensely studied than flowering times.

In general, animal seed dispersal is thought to be a more diffuse animal-plant-interaction than animal pollination. Therefore one could assume that flowering phenology and pollination is more likely to affect fruiting phenology and seed dispersal than vice versa. However, my results demonstrate that the opposite can be true. Since flowering and fruiting times seem to be evolutionary shifted in parallel, the selective force to present ripe fruits early also causes ant dispersed plants to flower early. Thus, seed dispersal by animals can have substantial and far reaching consequences for the phenology, reproduction and fitness of plants.

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7. Appendix

List of species studied following the taxonomy of THORNE (1992):

Dicotyledoneae

Asterales

Asteraceae

Achillea millefolium
Anthemis tinctoria
Arctium minus
Artemisia vulgaris
Bellis perennis
Carduus crispus
Carlina vulgaris
Centaurea jacea
Centaurea scabiosa
Chamomilla recutita
Chrysanthemum leucanthemum
Cirsium acaule
Cirsium arvense
Cirsium vulgare
Conyza canadensis
Eupatorium cannabinum
Galinsoga ciliata
Inula conyza
Matricaria maritima
Matricaria matricarioides
Senecio fuchsii
Senecio jacobea
Senecio vulgaris
Solidago canadensis
Tussilago farfara
Crepis biennis
Crepis capillaris
Crepis taraxacifolia
Hieracium murorum
Hieracium pilosella
Hieracium sabaudum
Lactuca serriola
Lapsana communis
Leontodon hispidus
Mycelis muralis
Sonchus arvense
Sonchus asper
Sonchus oleraceus
Tanacetum vulgare
Taraxacum officinale
Tragopogon pratense

Campanulales

Campanulaceae

Campanula glomerata
Campanula rapunculoides
Campanula rotundifolia

Caryophyllales

Caryophyllaceae

Arenaria leptocladus
Cerastium arvense
Cerastium fontanum
Moehringia trinerva
Stellaria holostea
Stellaria media
Silene dioica
Silene pratensis
Silene vulgaris

Chenopodiaceae

Atriplex patula
Chenopodium album

Celastrales

Celastraceae

Evonymus europaea

Araliales

Apiaceae

Aegopodium podagraria
Anthriscus sylvestris

Berula erecta
Chaerophyllum temulum
Daucus carota
Foeniculum vulgare
Heracleum mantegazzianum
Heracleum sphondylium
Pimpinella saxifraga
Torilis japonica

Cornales

Cornaceae

Cornus mas
Cornus sanguineum

Dipsacales

Adoxaceae

Adoxa moschatellina
Viburnum lantana
Viburnum opolus

Caprifoliaceae

Lonicera xylosteum
Sambucus nigra
Sambucus racemosa

Dipsacaceae

Knautia arvense
Dipsacus sylvestris

Gentianales

Gentianaceae

Centaurium pulchellum
Gentianella ciliata

Rubiaceae

Galium aparine
Galium odoratum
Gallium mollugo
Gallium verum

Scrophulariales

Lamiaceae

Ajuga reptans
Teucricum scorodonia
Clinopodium vulgare
Galeopsis tetrahit
Glechoma hederaceum
Lamium album
Lamium argentatum
Lamium endtmannii
Lamium purpureum
Lycopus europaeus
Mentha aquatica
Mentha arvense
Origanum vulgare
Prunella vulgaris
Stachys sylvaticus
Thymus serpyllum

Verbenaceae

Verbena officinalis

Oleaceae

Fraxinus excelsior
Ligustrum vulgare

Plantaginaceae

Plantago lanceolata
Plantago major
Plantago media

Scrophulariaceae

Digitalis purpurea
Euphrasia officinalis
Linaria vulgaris
Odontites ruber
Verbascum nigrum
Verbascum thapsus
Veronica arvensis
Veronica chamaedrys
Veronica hederifolia
Veronica persica
Rhinanthus minor
Scrophularia nodosa

Geraniales

Geraniaceae

Geranium dissectum
Geranium pyrenaicum
Geranium robertianum

Berberidales

Ranunculaceae

Actaea spicata
Anemone nemorosa

- *Clematis vitalba*
- *Ranunculus acris*
- *Ranunculus auricomus*
- *Ranunculus ficara*
- *Ranunculus repens*

Fumariaceae
- *Fumaria officinalis*

Papaveraceae
- *Chelidonium majus*
- *Papaver rhoeas*

Euphorbiales

Euphorbiaceae
- *Euphorbia helioscopia*

Malvales

Malvaceae
- *Malva alcea*

Tiliaceae
- *Tilia platyphyllos*

Urticales

Urticaceae
- *Urtica dioica*

Cannabaceae
- *Humulus lupulus*

Myrtales

Lythraceae
- *Lythrum salicaria*

Onagraceae
- *Circaea lutetiana*
- *Epilobium angustifolium*
- *Epilobium hirsutum*
- *Epilobium monatanum*

Betulales

Betulaceae
- *Alnus glutinosa*
- *Alnus incana*
- *Betula pendula*
- *Carpinus betulus*
- *Corylus avanella*

Fagaceae
- *Castanea sativa*
- *Fagus sylvatica*
- *Quercus petraea*
- *Quercus robur*

Rosales

Rosaceae
- *Crataegus monogyna*
- *Sorbus aucuparia*
- *Sorbus intermedia*
- *Prunus spinosa*
- *Cerasus avium*
- *Padus avium*
- *Filipendula ulmaria*
- *Fragaria vesca*
- *Geum urbanum*
- *Potentilla reptans*
- *Rosa canina*
- *Rubus idaeus*
- *Rubus spec.*
- *Sanguisorba minor*
- *Sanguisorba officinalis*
- *Agrimonia eupatoria*

Saxifragales

Crassulaceae
- *Sedum album*

Grossulariaceae
- *Ribes uva-crispa*

Rutales

Fabaceae
- *Astragalus glycophlyllos*
- *Coronilla varia*
- *Lathyrus latifolius*
- *Lathyrus nissifolia*
- *Lathyrus pratensis*
- *Lathyrus tuberosus*
- *Lotus corniculatus*
- *Medicago lupulina*
- *Medicago x_varia*
- *Melilotus alba*
- *Melilotus officinalis*
- *Onobrychis viciaefolia*
- *Ononis repens*
- *Robinia pseudacaria*

- *Sarothamnus scoparius*
- *Tetragonolobus maritimus*
- *Trifolium campestre*
- *Trifolium hybridum*
- *Trifolium pratense*
- *Trifolium repens*
- *Vicia angustifolia*
- *Vicia cracca*
- *Vicia hirsuta*
- *Vicia tetrasperma*

Sapindaceae

- *Acer campestre*
- *Acer platanoides*
- *Acer pseudoplatanus*

Solanales

Boraginaceae

- *Cynoglossum officinale*
- *Echium vulgare*
- *Myosotis arvensis*
- *Myosotis palustris*
- *Symphytum officinalis*

Convolvulaceae

- *Calystegia sepium*
- *Convolvulus arvensis*

Solanaceae

- *Solanum dulcamara*

Polygonales

Polygonaceae

- *Polygonum aviculare*
- *Polygonum hydropiper*
- *Polygonum lapathifolium*
- *Polygonum mite*
- *Polygonum persicaria*
- *Rumex acetosa*
- *Rumex obtusifolius*

Primulales

Primulaceae

- *Anagallis arvense*
- *Lysimachia nummularia*
- *Lysimachia vulgaris*
- *Primula veris*

Theales

Hypericaceae

- *Hypericum perforatum*

Brassicales

Brassicaceae

- *Alliaria officinalis*
- *Barbarea vulgaris*
- *Capsella bursa-pastoris*
- *Cardamine amara*
- *Cardamine pratensis*
- *Hesperis matrionalis*
- *Rorippa sylvestris*
- *Sinapsis arvensis*
- *Sisymbrium officinale*

Resedaceae

- *Reseda lutea*
- *Reseda luteola*

Violales

Cucurbitaceae

- *Bryonia dioica*

Salicaceae

- *Populus tremula*
- *Salix alba*
- *Salix caprea*
- *Salix viminalis*

Violaceae

- *Viola arvensis*
- *Viola hirta*
- *Viola odorata*
- *Viola riviniana*

Monocotyledoneae

Arales

Araceae

- *Arum maculatum*

Juncales

Cyperaceae

- *Carex flacca*
- *Carex muricata*

Juncaceae

- *Juncus bufonius*
- *Juncus effusus*

Poales

Poaceae

Phalaris arundinacea
Phragmites australis
Agrostis tenuis
Alopecurus myosuroides
Alopecurus pratense
Arrhenatherum eliatus
Brachypodium pinnatum
Brachypodium sylvaticum
Briza media
Bromus erectus
Bromus inermis
Bromus mollis
Bromus sterilis
Calamagrostis epigejos
Dactylis glomerata
Deschampsia cespitosa
Elymus repens
Festuca heterophylla
Holcus lanatus
Lolium perenne
Milium effusum
Phleum pratense
Poa annua
Poa nemoralis
Poa pratense
Poa trivialis

Typhales

Typhaceae

Sparganium erectum
Typha latifolia

Linales

Linaceae

Linum catharticum

Asparagales

Alliaceae

Allium ursinum

Amaryllidaceae

Galanthus nivalis

Liliales

Iridaceae

Iris pseudacorus

Orchidales

Orchidaceae

Epipactis helleborine

Pinopsida

Pinales

Pinaceae

Picea abies
Pseudotsuga menziesii
Larix decidua
Pinus sylvestris

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9. Curriculum vitae

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1995-1996	Diplomarbeit mit dem Titel „Blütenökologie beim Hügel-Lungenkraut (*Pulmonaria collina*)“ bei Prof. K. Schmidt-Koenig am Institut für Verhaltensphysiologie der Universität Tübingen
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