Wild bee communities in restored sandecosystems in north-western Germany:Community structure, population geneticsand habitat preferences

Dissertation

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Talks and poster presentations

- Exeler N. & Kratochwil A. (2005) Restitution von Wildbienen-Lebensräumen flussnaher Sandökosysteme des Emslandes (Niedersachsen). - Vortrag Symposium "Forschung und Naturschutz in Sandlebensräumen". Erlangen, Germnay
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- Exeler N. & Kratochwil A. (2007) Untersuchungen zur Besiedlung und Ressourcennutzung von Wildbienen in restituierten flussnahen Binnendünen-Komplexen im Emsland (Nordwestdeutschland). Poster Präsentation 2. Workshop Floristik und Geobotanik. Freising, München
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- Kratochwil A., Exeler N., Stroh M., Dittrich S., & Remy D. (2008) Restoration of an extensivly grazed alluvial landscape – résumé after six years. – Vortrag 6th European Conference on Ecological Restoration. Ghent, Belgium
- Exeler N., Hochkirch A. & Kratochwil A. (2008) Populationsgenetik der Heidekraut-Sandbiene Andrena fuscipes (Hymenoptera:Andrenidae). - Poster Präsentation 8. Hymenopterologen-Tagung, Stuttgart, Germany

Introduction

During the last century, many characteristic landscapes have changed rapidly in Central Europe. The intensive utilization of agricultural areas and the rapid advancement of new agricultural techniques resulted in a considerable decline of natural landscapes and traditional cultures. Numerous studies have uncovered the effects of land-use change and habitat fragmentation for plant and animal communities revealing a dramatic loss of biodiversity (Saunders *et al.* 1991; Malanson & Cramer 1999; Carvell 2002; Baguette & Schtickzelle 2003; Henle *et al.* 2004).

Efforts to conserve biodiversity comprise a variety of actions. Passive measures, for example the creation of nature reserves are important to conserve biodiversity with respect to the legal protection of areas (DeFries et al. 2005) but they often compromise on the habitat suitability if the aim is to establish certain communities. Active measures such as the restoration and management of habitats are necessary and maintain not only biodiversity but also endangered communities (Schwabe & Kratochwil 2004). While some conservation projects focus on the protection of umbrella or flagship species (White et al. 1997; Effenberger & Suchentrunk 1999; Zink et al. 2000), the restoration and conservation of complete biocenoses is much more complex and usually requires a greater effort of active conservation measures (Stroh et al. 2005). In conservation schemes of cultural landscapes, open-land management is one of the most challenging tasks. The management of abandoned areas is important as extensive land use, e.g. extensive grazing or mowing, can preserve habitats for a variety of organisms that are associated with open habitats (Bokdam & Gleichman 2000; Wallis De Vries et al. 2007). Dynamic processes such as mowing or grazing are widely used management techniques to prevent successional processes (Stroh et al. 2004; Weber et al. 2008). Other simple, but very effective measures are for instance changes of the grazing regime or the abandonment of intensive land-use practices (Kruess & Tscharntke 2002; Zehm et al. 2004).

The concept of ecological restoration is defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Society for Ecological Restoration International Science & Policy Working Group 2004). Successful restoration implies the recovery of biotic and abiotic conditions of an ecosystem to an extend that guarantees a further development independent of additional assistance. Hobbs & Norton (1996) suggest that the aim of restoration ecology should be to rebuild ecosystems to their state prior to disturbance regarding ecosystem structure, function and composition. Recent restoration projects, however, often focus mainly on structural components (e.g. species composition)

rather than include functional aspects (e.g. ecosystem function and services). A major goal of many restoration projects is the reestablishment of natural plant communities which should provide the necessary preconditions for the restoration of the rest of the community (Primack 2002). Thus, the evaluation of restoration projects often focuses on the vegetation response to such habitat modifications. However, the successful restoration of natural plant communities requires the inclusion of other structural and functional ecosystem components such as soil fauna, or the major functional components of the above-ground food-web such as herbivores, including pollinators and predators (Palmer *et al.* 1997; Lindell 2008).

Pollination is one of the key functions in ecosystems (Kevan 1999) and wild bees (Apoidea) are known to be the most important pollinators in many ecosystems (Kearns *et al.* 1998; Winfree *et al.* 2008). The maintenance of a species-rich plant community is supported by a high pollinator diversity (Fontaine *et al.* 2006), and a decline in the number of pollinators may cause increased competition among plants and reduce the reproductive success of many plant species (Vamosi *et al.* 2006). Furthermore, pollination is assumed to regulate the succession of plant communities. A deficit in pollinators during the first stages of succession could facilitate the dominance of autogamous plants and might lead to the formation of species-poor communities (Steffan-Dewenter & Tscharntke 2001). Therefore, the success of restoration projects is likely to be linked to a rapid recovery of bee communities. Moreover, native invertebrate species might react more rapid on small scale disturbances than the vegetation and thus might serve as good indicators for the successful recovery of the restored sites (Maczey *et al.* 2005).

The evaluation of wild bee responses to restoration measures thus provides the opportunity to consider structural and functional attributes of restoration at the same time. Furthermore, wild bees are a suitable indicator group for analysing the effects of restoration measures as a species-rich wild bee community is typically composed of a variety of species comprising a high morphological and behavioural diversity (Williams *et al.* 2001) and contains generalized, specialized and parasitic species. While the colonization of specialized bee species is dependent on pollen of a few plant species as larval food and/or on the availability of nesting habitats with special substrate or exposition (Kratochwil 2003), generalized bee species may readily establish as they are able to occur in a variety of habitats. On the other hand, these generalists might pollinate a high number of different plant species and thus provide important ecosystem functions in restored habitats.

The recent decline of many bee species and the potential ecological and economic consequences have become a topic of major interest (Cane & Tepedino 2001; Biesmeijer *et*

al. 2006; Butler *et al.* 2007). It is likely that the decline of bee species has been caused by a decline of suitable wild bee habitats (Steffan-Dewenter *et al.* 2002). Due to the abovementioned characteristics of specialized bee species, an appropriate habitat for species-rich wild bee communities requires two key factors: nesting sites and foraging areas (Potts *et al.* 2003b; Potts *et al.* 2005), which needs to be considered in order to achieve a successful restoration. So far, comparative studies of insect communities in restored and native ecosystems are sparse (Nemec & Bragg 2008), although they are essential for the development of restoration guidelines.

The aim of restoration projects is to establish new habitats as habitat loss is a major threat to biodiversity. However, the extinction risk is not only caused by the loss of habitats but also by their fragmentation. Many animal populations have become restricted to small and fragmented habitat patches and their viability thus depends increasingly upon their ability to move between habitat patches and interchange individuals and genetic information between populations. Hence, the dispersal ability is fundamental to the viability of populations in fragmented habitats (Haas 1995). Knowledge about plant and animal dispersal and colonization capabilities is also required for the design and evaluation of restoration projects.

The colonization of insects is assumed to take place gradually from generalists being the first colonizers followed by later stages of specialists and species with limited dispersal ability (Steffan-Dewenter & Tscharntke 1997; Tscharntke *et al.* 2002; Moir *et al.* 2005). Wild bees comprise species with a great range of different body sizes, which is assumed to be correlated with their flight radius (Gathmann & Tscharntke 2002; Araújo *et al.* 2004). The actual dispersal ability of wild bees has been discussed controversially. Some authors suggest a rather small flight radius for most species (Osborne *et al.* 1999; Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007), whereas others characterize bees as good dispersers (Cane 2001; Zayed *et al.* 2005; Beil *et al.* 2008). Furthermore, the degree of specialization of wild bees in relation to colonization and dispersal remains unclear. Some recent studies revealed a reduced gene flow for populations of specialized species and proposed a low dispersal capacity for specialists (Packer *et al.* 2005; Zayed *et al.* 2005; Zayed *et al.* 2005; Zayed *et al.* 2005; Zayed *et al.* 2007), whereas Peterson & Dennö (1998) found no difference between specialist and generalist bee species.

The objectives of this thesis are (1) to analyse the response of wild bee communities to restoration measures in order to evaluate restoration success with respect to both structural and functional aspects and (2) to evaluate the dispersal and connectivity of potential source populations. For this purpose a comparative analysis of wild bee communities in restored and

native habitats was conducted. Additionally, the population genetic structure of two model species was studied using microsatellites. These genetic aspects were analysed to obtain information on population connectivity at different spatial scales and genetic characteristics like inbreeding and genetic diversity.

Research objectives and chapter outline



Figure 1: Relationship between the objectives of this thesis. — field data approach, – – - laboratory approach, … theoretical approach.

This thesis is divided into five chapters dealing with different aspects of these objectives (Fig.1).

In chapter 1 and 2 the success of a restoration project of dry sand ecosystems in the floodplains of the river Hase was evaluated by a comparative analysis of wild bee communities at restoration and target sites. For chapter 1, two typical plant communities of dry sand ecosystems (Spergulo-Corynephoretum and Diantho-Armerietum) of this landscape were studied with a major focus on colonization patterns and succession of wild bee communities in the restored habitats in comparison with old, species-rich habitats (target sites). In general, sand-ecosystems are characterized by a high number of specialized and particularly endangered wild bee species. As potential explanatory variables accounting for differences in the community structure, environmental factors such as the vegetation composition and vegetation structure were quantified. Moreover, differences in colonization patterns in relation to life history traits such as dispersal ability (body size) and degree of specialization (generalist, specialist or parasite) have been analysed to test the hypotheses that colonization of new habitats follows a stepwise pattern dependent of body size and degree of specialization. In chapter 2 the emphasis was to analyse spatial patterns of wild bee communities in restored and target sites. Based on a grid system, two plot types (dry and moist) were analysed for differences in their wild bee community structure with respect to environmental factors such as the vegetation structure and pollen sources. A precise quantification of the entomophilous plant species throughout the vegetation period allowed the analysis of phenological differences between the plot types in restoration and target areas.

In chapter 1 and 2 special emphasis was placed on the following questions:

- How successful was the restoration of sand dune complexes for the colonization and conservation of wild bees?
- Is the colonization process of wild bees determined by characteristic traits such as the body size or the degree of specialization?
- Does the wild bee community structure of restored habitats converge to the community of target habitats and which factors determine the community structure (chapter 1)?
- Which factors determine wild bee community distribution in restored and target habitats (chapter 2)?

To gain a more precise insight into the connectivity of wild bee populations and the genetic preconditions that allow a successful colonization, the population genetic structures of two highly specialized wild bee species, *Andrena vaga* (Panzer 1799) and *Andrena fuscipes* (Kirby 1802), were analysed in **chapter 3** and **4**. *A. vaga* is a floodplain pioneer, which nests in large aggregations and is specialized on willow pollen (*Salix*) and sandy habitats with sparse vegetation. The heathland specialist *A. fuscipes* is solitary nesting and oligolectic on heather (*Calluna vulgaris*) pollen. Since the availability of suitable habitat is usually lower for specialists than for generalists, the effects of fragmentation are thought to be stronger in the former group (e. g. Kitahara & Fujii 1994; Kelley *et al.* 2000; Bonte *et al.* 2004; Polus *et al.* 2007). Hence, a reduced genetic diversity and patterns of genetic isolation by distance are assumed for both species, but these might be stronger in species associated with persistent habitats (heathland) compared to the species confined to highly dynamic habitats (floodplain). These chapters deal with the following questions:

- How is the genetic variability within and among populations of highly specialized wild bees distributed?
- Does the genetic structure of populations reflect any barriers to gene flow?

Chapter 5 aims at evaluating the general intrinsic factors that maintain the genetic diversity of wild bee populations. Although the number of studies on the genetic structure of populations is increasing (Goulson *et al.* 2008), most of these studies focus on single species, whereas comparative studies are sparse. On the basis of 23 publications on the population genetics of 52 bee species data on two major co-dominant marker systems (allozymes: 38 species; microsatellites: 18 species) were analysed.

Of particular interest were the following questions:

- Is the genetic diversity of wild bees affected by their degree of specialization, nesting strategy or family affiliation?
- Is the extent of inbreeding determined by the degree of specialization, the nesting strategy or the family affiliation?

The combination of these approaches aims to contribute to the overall understanding of colonization processes and mechanisms that determine community structure in restored ecosystems.

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RESTORATION OF RIVERINE INLAND SAND DUNE COMPLEXES: IMPLICATIONS FOR THE CONSERVATION OF WILD BEES (HYMENOPTERA, APOIDEA)



Coloured traps within a restored Spergulo-Corynephoretum site ("Wester Schleife").

Abstract

1. The evaluation of restoration measures is an important task of conservation biology. Inland sand dunes and dry, oligotraphentic grasslands have become rare habitat types in large parts of Central Europe, and their restoration and management is of major importance for the preservation of many endangered plant and insect species. As wild bees are the most important pollinators in many ecosystems, they represent a suitable key group to evaluate restoration measures. Furthermore, the recent decline of many bee species and the potential ecological and economic consequences are currently topics of strong scientific interest.

2. We studied the succession of bee communities in response to restoration measures of sand dunes and sand grasslands and compared these communities with those of old sand dune complexes as target habitats.

3. Our results show a rapid response of wild bees to restoration measures indicated by a high species richness and abundance. The community structure of bees at the restoration sites converged only slightly to those of the target sites. A higher similarity was found for the bee communities of the restoration sites (sand dunes and grasslands), indicating that their close proximity was an important determinant of species overlap. Environmental factors such as the number of entomophilous plant species and moisture had a strong influence on wild bee species composition.

4. *Synthesis and applications*. The restoration of inland sand dune complexes provides the opportunity for the colonization of a diverse wild bee community. Although it is difficult to establish a given target community, restoration measures proved to be suitable to restore community function in terms of pollinator diversity and abundance.

Introduction

Habitat destruction, alteration, degradation and fragmentation are key threats to biodiversity throughout the world (Primack 2002). Changes in land use and particularly the intensification of agricultural practices are considered to be the main reason for the decline of many plant and animal species (Malanson & Cramer 1999; Carvell 2002; Baguette & Schtickzelle 2003). In Central Europe, inland sand dunes and dry, oligotraphentic grasslands were widespread on Pleistocene and alluvial soils until the end of the 19th century (Muller *et al.* 1998; Hochkirch *et al.* 2008). These habitats have been extensively grazed by sheep and cattle for centuries and supported a highly specialized flora and invertebrate fauna, many species of which are nowadays threatened (Hochkirch *et al.* 2007). Meanwhile, oligotraphentic grasslands and inland sand dunes have become rare in large parts of Central and Northern Europe (Muller *et al.* 1998; Pywell *et al.* 2002; Riksen *et al.* 2006). Furthermore, a loss of natural dynamics due to regulation and canalization of rivers has threatened pioneer species adapted to floodplain habitats (Gröning *et al.* 2007). The restoration of such habitats and an appropriate management is, therefore, of high importance for the conservation of many endangered species.

The restoration of habitats generally follows two major axes: the recreation of ecosystem structure and the recovery of ecosystem function (Primack 2002). Pollinators are a suitable group for analysing the effects of restoration measures on both of these aspects, as they represent a species-rich and behaviourally diverse group which provides key services in ecosystems. A high pollinator diversity supports the maintenance of a species-rich plant community (Fontaine *et al.* 2006), and a decline in the number of pollinators may cause increased competition among plants and reduce the reproductive success of many plant species (Vamosi *et al.* 2006). Wild bees (Apoidea) are known to be the most important pollinators in many ecosystems (Kearns *et al.* 1998; Winfree *et al.* 2008). The recent decline of many bee species and the potential ecological and economic consequences have, therefore, become a topic of major interest (Cane & Tepedino 2001; Biesmeijer *et al.* 2006; Butler *et al.* 2007).

Habitats of wild bees have to provide two key features in order to promote a diverse wild bee community: nesting sites and foraging areas. Habitat restoration should, therefore, aim at recreating both these niche dimensions so that pollination function can be re-established. In general, a species-rich wild bee community is composed of a variety of species comprising a high morphological and behavioural diversity (Williams *et al.* 2001). A typical bee

community contains generalized, specialized and parasitic species. While generalized bee species may pollinate a high number of different plant species, specialized bee species utilize pollen of only few plant species as larval food and/or nest only in habitats with special substrate or exposition (Kratochwil 2003). The strong specialization of many wild bee species on specific pollen sources and nesting sites makes them particularly vulnerable to local extinction. Furthermore, wild bee communities are usually composed of species covering a great range of different body sizes. The body size of bees is assumed to be correlated with their flight radius (Araújo et al. 2004) and for most species a rather small foraging flight radius is considered (Gathmann & Tscharntke 2002; Greenleaf et al. 2007). Even bumblebees (Bombus) which belong to the bee species with the greatest flight capabilities prefer to forage in about 200 m distance around their nests (Osborne et al. 1999). A diverse wild bee community is a suitable indicator for a species-rich vegetation and a heterogeneous habitat structure. Although there is strong evidence for a decline of wild bees (Biesmeijer et al. 2006), only few attempts have been made to initiate restoration activities for pollinators so far (Kleijn et al. 2006; Carvell et al. 2007). Many conservation projects focus on the protection of charismatic flagship species rather than on the recreation of key ecosystem functions.

Dry, oligotrophic habitats with a high diversity of flowering plant species are among the most important habitat types for the conservation of bees. It has been shown that wild bees benefit from extensive grassland management (Carvell 2002; Kohler *et al.* 2007). Mowing and extensive grazing have proved to increase plant species richness and thus the availability of pollen and nectar (Carvell 2002). It is, therefore, likely that the restoration of extensively managed inland sand dunes and oligotraphentic grasslands is a suitable method to preserve these insects. Here, we examine the effects of a sand dune restoration project on wild bees. We compared the succession of bee communities in restored habitats with that of similar vegetation communities in an old nature reserve and a nearby sand grassland (target areas). Our aims were (1) to test whether the bee communities increase in species richness and abundance after the restoration measures and approach the level of the communities of old habitats and (2) to examine whether the bee suggested that the degree of specialization and the body size of bees might influence their dispersal ability (Tscharntke *et al.* 2002), we (3) also examined whether generalized or large-bodied species are the initial colonizers.

Methods

Study area and restoration method

The study area is located in the subatlantic zone of north-western Germany (Lower Saxony), which is characterized by temperate humid climatic conditions with mild winters and cool rainy summers (Fig. 1). The restored localities are situated at two meander cores of the river Hase close to the town of Haselünne and comprise a total area of 49 hectares. Prior to the restoration, these sites were used as maize fields and intensive grasslands. Intensive agriculture and forestry still prevail in the surroundings of the restoration sites. Restoration measures were carried out from 2001 to 2002 with the aim to convert these intensively used agricultural areas into species-rich, oligotraphentic grasslands (Diantho-Armerietum) and sand dune complexes (Corynephoretum) with an extensive land use. The measures included the removal of dikes and the restoration of a new relief composed of artificial inland sand dune complexes, fluviatile sand layers and permanent or temporarily flooded hollows (Stroh et al. 2005). The removal of dikes led to occasional flooding of the restoration sites and helped to achieve the open character of a typical alluvial landscape. In addition, the restoration sites were managed by extensive cattle grazing. In 2002, parts of the new dune complexes were treated with a hay spreading technique. Mown and raked hay from a target site was used to accelerate the establishment of a typical oligotraphentic vegetation.

A nature reserve near the river Ems (24 ha) served as target area for the Spergulo-Corynephoretum sites (C) of the restoration project. This alluvial pasture/woodland vegetation complex has been extensively grazed by cattle for centuries and is characterised by inland sand dunes, heathlands, seasonally flooded wetland and riparian willow shrubs and pasture woodlands. A second target area for the Diantho-Armerietum sites (D) was located next to the restoration sites (Fig. 1).



Figure 1: Map of the study area and the locations of restoration and target plots in north-western Germany. Target sites are indicated with black dots (CT = Spergulo-Corynephoretum target, DT = Diantho-Armerietum target), restored Spergulo-Corynephoretum sites (CR) with black triangles, restored Diantho-Armerietum sites (DR) with black squares.

Wild bee and vegetation survey

In 2002, ten permanent plots (exclosures) were established on the target and restoration sites to study the succession of the vegetation and the bee communities (Spergulo-Corynephoretum: 3 target plots (CT) and 3 restoration plots (CR); Diantho-Armerietum: 2 target plots (DT) and 2 restoration plots (DR)). The vegetation type of Spergulo-Corynephoretum is characterized by the presence of *Corynephorus canescens*, *Spergularia morisonii* and different lichen species, whereas the flower-rich vegetation type of Diantho-Armerietum is characterized by the occurrence of *Dianthus deltoides*, *Galium verum* and

Ranunculus bulbosus. From April to September 2003, 2005 and 2006, each plot was provided with two yellow and two white coloured traps (diameter: 16 cm, height: 8.5 cm) filled with ethylene glycol, and installed 0.4 meters above the ground with a distance of five meters between each. In order to assess the status quo prior to the restoration measures, four pan traps were installed at the restoration site in 2001 (two at a former dyke and two at a relict dune fragment). In the first year after the restoration (2002), the census was conducted as described above, but using a reduced set of traps (two pan traps in each plot). The traps were emptied fortnightly and the bees were identified in the laboratory. Additionally, the vegetation in each plot was quantified in a subplot of 25 m² once a year in spring (Stroh unpublished data), using the Braun-Blanquet method (Braun-Blanquet 1964). Estimates of vegetation abundance were made according to Barkman et al. (1964). Additionally, the vegetation structure (vegetation cover, bare ground cover, forb cover, grass cover, the number of plant species was used to calculate a weighted moisture indicator value (Ellenberg *et al.* 1992) for each plot.

Statistical analysis

Species richness (total number of species) and the abundance of each species were calculated for each year and plot. A repeated measures ANOVA was used to detect differences between habitat types (C versus D), treatments (target and restoration) and years in each of the following response variables: total species richness, total wild bee abundance, number and abundance of generalized, specialized and parasitic bee species, number of small (< 7 mm), medium (7-12 mm) and large species (> 12 mm) as well as abundance of single threatened bee species (according to the Red Data List of Germany). Furthermore, we tested for differences in environmental factors, such as the number of plant species and the number of entomophilous plant species, the cover of forbs, grasses and bare ground as well as moisture. These analyses were performed in the programme R 2.7.0 (R Development Core Team 2007). To determine the independent effect of each environmental variable on total species richness and abundance, the richness and abundance of specific groups of wild bees (generalists, specialists and parasites) and selected species, we performed a hierarchical partitioning analysis using the "hier.part" package for R (Walsh & Mac Nally 2003). This method is particularly suited to uncover those variables having the most independent effect on the response variable and thus avoid multicollinearity among predictor variables (Mac Nally 2002).

We calculated Renkonen's index as a measure of similarity of the wild bee communities using the programme EcoSim 7.0 (Gotelli & Entsminger 2007). We used this index for comparing pairs of restoration and target sites per year, the two types of restored sites per year and for comparing the wild bee communities of the restored sites compared to the situation prior to restoration (2001).

To identify potential indicator species, the sites were grouped according to habitat type (Spergulo-Corynephoretum and Diantho-Armerietum) and treatment (target and restoration) resulting in four groups. For these groups, an indicator species analysis was conducted as implemented in PC-ORD version 5.0 (McCune & Mefford 1999) based upon the method described by Dufrêne & Legendre (1997). This method calculates indicator values for each species in a group (IV_{ij}) as the product of the relative frequency of that species in the group (RF_{ij}) and the relative abundance in the group (RA_{ij}) (IV_{ij} = RF_{ij} * RA_{ij} *100). To test the statistical significance of the indicator species, a Monte Carlo method with 4999 randomizations was applied.

To analyse compositional differences in the wild bee community, we performed multivariate ordination analyses. Because of a long gradient length (> 2.9 SD) in the wild bee community data, estimated in a detrended correspondence analysis (DCA), we applied a canonical correspondence analysis (CCA) including the environmental variables mentioned above. CCA is a non-linear, direct ordination method that estimates the structure in the main matrix, which is usually composed of species abundance data in specific sample units, in relation to a second matrix providing environmental explanatory variables of the same sample units. The significance of the correlation with environmental variables was assessed by a Monte-Carlo permutation test (9999 permutations) as implemented in CANOCO version 4.5 (ter Braak & Smilauer 2002).

Results

Between 2001 and 2006, a total of 8735 individuals belonging to 90 wild bee species were detected at the target and restoration sites. Species of the subfamilies Andrenidae (25 species), Halictidae (25 species) and Apidae (22 species) were identified most frequently. The remaining species belong to the families Megachilidae (12 species), Colletidae (5 species) and Melittidae (1 species). A total of 17.9 % of the species were oligolectic with 9 species specialized on Asteraceae, 4 species on willow pollen (*Salix*), 1 species on *Vaccinium*, 1 on *Ranunculus* and 1 on Fabaceae.

Main effects of the vegetation type

Concerning the number and abundance of wild bee species (or subsets of this group), we found almost no significant difference between sand dunes (Spergulo-Corynephoretum) and sand grasslands (Diantho-Armerietum; Table 1, Table 2). However, the abundance of the red listed species differed between these two habitats. *L. sexnotatum* had a higher abundance at the Spergulo-Corynephoretum sites, whereas *L. quadrinotatum* was more frequent at the Diantho-Armerietum sites (Table 1, Fig. 3a & b).

Table 1: Results of the repeated- treatment (restoration or target) a specialist, generalist and parasite of the Red List species <i>Lasioglos</i> :	measures ANOVA nd year (2003, 200 species, the numbe sum sexnotatum and	s showing the effec 5 and 2006) a) on w r of large (< 13 mr 1 L. quadrinotatum.	ts of vegetation (ild bee species ri 1), medium (8-13 NS = not signific	Spergulo-Cory chness and abu mm) and smal ant; * $P \leq 0.5$; ant;	nephoretum ndance, the 1 (> 8 mm) ** $P < 0.01$;	or Diantho- A number and a species and th *** $P < 0.00$	rrmerietum), bundance of e abundance 1.
wild bee response	Error: site $(df = 1,$	(9		Error: within ((df = 2, 12)		
							vegetation x
			vegetation x		vegetation x	treatment x	treatment x
	vegetation	treatment	treatment	year	year	year	year
total sp. richness	NS	NS	NS	F = 4.18*	NS	NS	NS
total abundance	NS	F = 9.50*	F = 7.78*	F = 4.58*	NS	$F = 6.30^{*}$	NS
number of specialist sp.	NS	NS	NS	NS	NS	NS	NS
abundance of specialist sp.	NS	F = 7.5*	$F = 6.9^{*}$	NS	NS	F = 4.37*	NS
number of generalist sp.	NS	NS	F = 10.07*	NS	NS	NS	NS
abundance of generalist sp.	NS	NS	NS	$F = 7.15^{**}$	NS	NS	NS
number of parasite sp.	NS	F = 13.32*	NS	$F = 5.94^{*}$	NS	NS	$F = 5.10^{*}$
abundance of parasite sp.	NS	NS	NS	$F = 9.44^{**}$	NS	NS	NS
number of large sp.	NS	NS	NS	$F = 5.41^{*}$	NS	NS	F = 7.69**
number of medium sp.	NS	NS	F = 10.99*	NS	NS	NS	NS
number of small sp.	NS	NS	NS	F = 5.78*	NS	NS	NS
abundance of L. sexnotatum	F = 7.68*	NS	$F = 17.35^{**}$	F = 4.33*	NS	$F = 9.42^{**}$	NS
abundance of L. quadrinotatum	F = 31.69**	F = 187.03 * * *	F = 24.39 **	F = 6.33*	NS	$F = 5.96^{*}$	$F = 8.48^{**}$

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			vegetation x		vegetation x	treatment x	treatment x
	vegetation	treatment	treatment	year	year	year	year
total number of plant sp.	NS	NS	NS	NS	NS	NS	NS
number of entomophilous plant sp.	NS	NS	NS	NS	NS	NS	NS
proportion of bare ground	NS	NS	NS	F = 5.81 *	$F = 3.94^*$	NS	NS
forbs cover	F = 7.57*	NS	$F_{1,6} = 6.70^{*}$	F = 6.75*	$F = 15.01^{**}$	$F = 15.05^{**}$	F = 8.58*
grass cover	NS	NS	NS	$F = 13.22^{**}$	NS	F = 5.93*	NS
moisture	F = 18.55 **	NS	NS	F = 11.00 **	NS	NS	NS

Effects of the restoration

The differences between restoration and target sites (i.e. the effects of "treatment") were more pronounced. Treatment effects were found for the total abundance of bees and for the abundance of specialized bee species, both of which were greater at the target sites (Table 1, Fig. 2). The opposite was true for the number of parasite species and the red listed species *Lasioglossum quadrinotatum*, which occurred more frequently at restored sites (Table 1, Fig. 3a).



Figure 2 Variation of total bee abundance between the two vegetation types and restoration treatments (CT = Spergulo-Corynephoretum target, CR = Spergulo-Corynephoretum restoration, DT = Diantho-Armerietum target, DR = Diantho-Armerietum restoration) and years.

Furthermore, we found significant interactions between the vegetation type and the treatment, indicating that the different habitats responded differently to the restoration measures. At the Spergulo-Corynephoretum sites, the total bee abundance and the abundance of specialists was higher at the target sites, whereas at the Diantho-Armerietum sites the total bee abundance was similar on both restoration and target sites, while the abundance of specialists was significantly higher at the restored sites.

The number of generalist species was higher at the Spergulo-Corynephoretum target sites than at the restored sites, whereas the opposite was true for the Diantho-Armerietum sites. In addition, a significantly higher number of medium-sized bee species was found at the Spergulo-Corynephoretum target sites than at the corresponding restored sites, while the reverse was found at the Diantho-Armerietum sites. No effects were found for small-sized and large-sized species.



Figure 3a Variation in the abundance of *Lasioglossum quadrinotatum* between the two vegetation types and restoration treatments (CT = Spergulo-Corynephoretum target, CR = Spergulo-Corynephoretum restoration, DT = Diantho-Armerietum target, DR = Diantho-Armerietum restoration) and years.



Figure 3b Variation in the abundance of *Lasioglossum sexnotatum* between the two vegetation types and restoration treatments (CT = Spergulo-Corynephoretum target, CR = Spergulo-Corynephoretum restoration, DT = Diantho-Armerietum target, DR = Diantho-Armerietum restoration) and years.

Successional patterns

Significant variation in the response variables was also found among years, with a general increase in the number and abundance of wild bee species in 2005 followed by a decline in 2006. Only in few cases we found significant interactions between treatment and year, e.g. for the total and the specialists abundance, which declined gradually at the target sites. The abundance of red listed species also differed between the years. An increase was recorded for *L. quadrinotatum* at the restored sites and a decline of *L. sexnotatum* in the target areas (Table 1).

Effects on the environmental parameters

For the environmental variables, the repeated measures ANOVAs revealed a significant decline of the bare ground cover over the years, which was mainly true for Spergulo-Corynephoretum sites (Table 2). The cover of forbs and grasses changed also over time. While forb cover declined at the restoration sites, grass cover increased. Moreover, there was a significant difference in the moisture indicator values among the vegetation types, with Diantho-Armerietum sites being moister at both restored and target sites. In addition, the moisture index increased significantly over time at all sites. The hierarchical partitioning analyses revealed that moisture was the most important factor explaining bee diversity (r = -0.28, p = 0.1) and bee abundance (r = -0.35, p = 0.05) followed by the cover of bare ground (species richness r = -0.08, p = 0.7; abundance r = -0.03, p = 0.8).

response/predictor		proportion of bare	number of entomophilous		total number of	
	moisture	ground	plant species	forbs cover	plant species	grass cover
total sp. richness	26.03	13.15	7.8	44.27	3.22	5.51
total abundance	37.27	9.71	31.94	4.33	10.87	5.88
number of specialist sp.	34.55	38.12	13.7	3.67	6.62	3.33
abundance of specialist sp.	24.83	-0.27	53.23	6.22	15.94	0.05
number of generalist sp.	39.34	22.51	8.87	2.94	6.15	20.19
abundance of generalist sp.	8.73	34.61	24.75	3.63	6.08	22.2
number of parasite sp.	42.11	1.76	10.32	28.18	10.03	7.61
abundance of parasite sp.	34.43	26.29	12.16	5.56	11.14	10.42
number of large sp.	54.66	16.26	8.33	2.86	13.6	7.29
number of medium sp.	16.38	3.35	7.53	62.67	2.25	7.82
number of small sp.	14.68	34.33	6.85	17.48	2.36	14.3
abundance of L. sexnotatum	30.06	9.09	11.21	2.21	35.52	11.91
abundance of L. quadrinotatum	15.48	38.84	22.45	11.1	4.33	7.81

Table 3: Hierarchical partitioning analysis. Independent effect (%) of each predictor variable on the variance in the response variable.

The number of entomophilous plants and forb cover also had a high explanatory power for some variables (Table 3).

Similarities in wild bee assemblages

The Renkonen index of similarity in wild bee assemblage revealed a high similarity between the restoration sites as well as between the Diantho-Armerietum target sites and the respective restoration sites. In contrast, the similarities between Spergulo-Corynephoretum target and restoration and between Spergulo-Corynephoretum or Diantho-Armerietum target sites were low. The similarity index between restored sites and the situation prior to restoration increased until 2003 followed by a continuous decline (Fig. 4), suggesting an ongoing change in wild bee community composition after the restoration measures.



Figure 4 Changes in species overlap (Renkonen-index) of the wild bee communities over time. CT~CR: similarity between Spergulo-Corynephoretum target (CT) and restoration (CR); DT~DR: Diantho-Armerietum target (DT) and restoration (DR); CR~DR: comparison of the restoration sites D and C; DT~CT: comparison of target sites D and C; 2001~CR and 2001~DR: comparison of the restoration sites with the situation before the restoration measures were started (2001).

Multivariate analyses of the wild bee communities using CCA showed a clear grouping of the wild bee assemblages sampled at the Spergulo-Corynephoretum target sites. A greater variability was found for the bee communities from the restored sites and from the Diantho-Armerietum sites. However, for both habitat types a slight convergence of the restored sites with the respective target sites was found (Fig. 5). The inclusion of environmental variables in the model explained 47 % of the variance in the species data with moisture being the most important factor (Monte Carlo Permutation test, F = 3.81, p < 0.001). At the Diantho-Armerietum sites, wild bee community composition was determined by moisture, the number of entomophilous plant species and the cover of forbs , whereas at the Spergulo-Corynephoretum sites a great bare ground cover and drier conditions were more important for the wild bee species composition.



Figure 5 CCA biplot showing bee species (A. = Andrena, D. = Dasypoda, L. = Lasioglossum, P. = Panurgus) and environmental variables (first axis eigenvalue = 0.26; second axis eigenvalue = 0.07).

The indicator species analysis suggested a significant indicator value for 15 species (Table 4), most of which were indicators for the Spergulo-Corynephoretum target sites. Only one species was assigned to the Diantho-Armerietum target sites. No indicator species was found for any of the restoration sites.

Table 4: Results of an the indicator species analysis using PC-ORD. Sites are grouped according to habitat type and treatment (CT = Spergulo-Corynephoretum target, DT = Diantho-Armerietum target, Maxgrp = Group identifier for group with maximum observed iv).

		Observed indicator	iv from randomized		
species	Maxgrp	value (iv)	groups mean	sd	р
Colletes cunicularius	СТ	98.3	41.1	12.7	0.0002
Lasioglossum sexstrigatum	СТ	85.3	28.7	7.77	0.0002
Andrena praecox	CT	77.7	26	9.9	0.0002
Lasioglosum sexnotatum	CT	77	37.6	8.87	0.0006
Andrena fulva	CT	68.3	31.1	7.34	0.0002
Andrena vaga	CT	59.5	35.9	5.54	0.0004
Andrena cineraria	СТ	58.2	35.4	7.46	0.0076
Nomada flava	СТ	55.6	15.2	7.87	0.0018
Andrena nitida	СТ	48.7	32.2	7.38	0.031
Andrena helvola	СТ	47.6	20.8	7.11	0.0066
Bombus pascuorum	СТ	47	31.6	6.21	0.0208
Andrena tibialis	СТ	46.8	15.9	7.66	0.0068
Sphecodes albilabris	СТ	44.4	14.4	7.8	0.0098
Andrena nigriceps	DT	42.3	15.1	7.71	0.0086
Nomada succincta	СТ	37.4	15	7.58	0.0214

Discussion

Restoration success

Our results show that wild bee communities may change rapidly within the first years after restoration measures have been carried out. Based on hypotheses on restoration projects in general and on the succession of insect communities in particular, one would expect a gradual increase in the number of wild bee species at the restoration sites (Brown & Gange 1992), reaching the level of the target sites after some years. However, the number of wild bee species at the restored sites in any study year, and general differences between restored and target sites were mainly found in the total abundance of wild bees and in the abundance of specialist species, both of which were greater at the target sites. These results differ from those of other studies, which showed a rapid increase in flower and insect diversity during the first two years followed by a decline in species richness during

later stages (Steffan-Dewenter & Tscharntke 1997; Potts *et al.* 2003a; Tyler 2008). Studies on the succession of plant communities often show a highly dynamic vegetation composition during early successional stages (Connell & Slatyer 1977; Huston & Smith 1987) with a rapid change from annuals to perennials during the first years of succession. Similar patterns have been assumed for insect communities (Corbet 1995; Woodcock et al. 2005). Of course, a certain level of variation in bee diversity and abundance occurred at both target and restoration sites. However, this was mainly caused by a great number of species with low abundance, some of which may be not indigenous to the study sites (see also discussion in Williams *et al.* 2001; Potts *et al.* 2003b). In our study plots, 67.5 % of the species occurred with a relative abundance of less than 5 %, while the most abundant bee species were similar at restoration and target sites, a result which has also been reported by Forup et al. (2008).

The reestablishment of natural plant communities is a major goal of restoration projects as they provide the necessary preconditions for the restoration of the rest of the community (Primack 2002). On the other hand, one might argue that the restoration of vegetation is virtually impossible without the help of other community members, such as the soil fauna or pollinators. Therefore, recent studies indicate the need to include functional components in restoration efforts (Lindell 2008). Pollination is a key component of ecosystems as it regulates the succession of plant communities. A deficit in pollinators during the first stages of succession could facilitate the dominance of autogamous plants and might lead to the formation of species-poor communities (Steffan-Dewenter & Tscharntke 2001). As bees are known to be the most important pollinators in many ecosystems, a rapid recovery of bee communities is important for the success of restoration projects. Many bee species require patches of bare ground as nesting habitats, the highest diversity could thus often be found during the first successional years (Steffan-Dewenter & Tscharntke 2001; Potts et al. 2003a). In our study, we did not detect any decline in diversity, which might be explained either by the relative short time of study after the restoration or by the ongoing disturbance by cattle grazing or flooding. The co-occurrence of different successional stages and a strong heterogeneity in soil types are important components for the maintenance of a high pollinator diversity (Sjödin et al 2008).

Colonization patterns

It has been suggested that colonization patterns of bees might be influenced by their body size, which is correlated with flight radius and movement patterns (Gathmann & Tscharntke 2002). Due to their reduced dispersal abilities, a later colonization of smaller species has been

predicted (Tscharntke *et al.* 2002). Indeed, a decrease in average body size with increasing age of succession has also been reported for butterflies (Steffan-Dewenter & Tscharntke 1997). However, other authors state that bees are generally strong fliers and therefore body size would not have any influence on succession patterns (Beil *et al.* 2008). Our results support the latter findings, as we did not find any stepwise colonization of large, medium and small species. It has also been proposed that populations of strongly specialized bee species are highly fragmented (Packer *et al.* 2005; Zayed *et al.* 2005) and thus might have a reduced colonization ability. A similar pattern might also be true for parasitic bees which strongly depend on large populations of their host species. However, species richness and abundance of both groups did not differ between restored and target sites. Population genetic studies on two highly specialized *Andrena* species from the study sites revealed a high degree of genetic exchange among populations supporting the hypothesis that the degree of specialization does not influence dispersal abilities of these species.

Influence of spatial autocorrelation and environmental factors

Interestingly, our results revealed a marked difference in the response of both habitat types to the measures implemented. While the wild bee communities of the Diantho-Armerietum restoration and target sites were rather similar, the Spergulo-Corynephoretum restoration and target sites differed strongly. This might be explained by the close proximity of Diantho-Armerietum target and restoration sites leading to spatial autocorrelation patterns. The Spergulo-Corynephoretum target site was located in ca. 15 km distance to the restoration sites, whereas the Diantho-Armerietum was located in a distance of ca. 500 - 1500 m to the restoration sites. Hence, the regional species pool might influence the local species composition (Potts *et al.* 2003b). This might explain why the Spergulo-Corynephoretum restoration sites were more similar to the Diantho-Armerietum target site than to the Spergulo-Corynephoretum target site. Nevertheless, in both habitat types the wild bee communities of the restored sites converged slightly to the respective target communities.

Environmental factors had a strong influence on the structure of the wild bee communities. Wild bee species richness and abundance showed a negative correlation with increasing moisture values. Furthermore, moisture was the most important environmental factor, which explained the differences between both habitat types. Spergulo-Corynephoretum sites were characterized by drier conditions and a higher cover of bare ground, whereas Diantho-Armerietum sites were moister with a greater cover of forbs and a greater number of entomophilous plant species. It has been shown that changes in the bee community are strongly correlated to changes in the vegetation structure (Corbet 1995). In particular, the abundance of flowers and the availability of sandy soil have a strong influence on bee species richness and abundance (Sjödin *et al.* 2008).

Conclusion

In order to evaluate the success of restoration projects, it is of crucial importance to assess not only species composition but also ecosystem function. Pollinators provide key services in ecosystems (Majer *et al.* 2002). Our intention was therefore to evaluate the success of the restoration measures and to analyse which factors influence the colonisation processes of wild bees at the restoration sites. Our results show that species-rich wild bee communities establish rapidly after the restoration measures have been carried out. Although the bee communities differed structurally from the target sites, the functional aspects (number of generalists, specialists and parasites, body-size distributions) of the communities show that ecosystem function may be restored rapidly. These results should encourage conservationists to conduct similar restoration projects in order to re-establish species-rich ecosystems that contain a high number of plants and insects.

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DISTRIBUTION PATTERNS OF WILD BEE COMMUNITIES (HYMENOPTERA, APOIDEA) IN SAND-ECOSYSTEMS – A COMPARISON BETWEEN SEMI-NATURAL AND RESTORED HABITATS



Arial picture of the restoration area "Hammer Schleife" showing the situation after relief construction. (Foto: Mecklenborg/Haren)

Abstract

Habitat fragmentation enforces the vulnerability of local populations to environmental and demographic fluctuations and decreases the chance for re-colonization events. Semi-natural landscapes, such as oligotraphentic grasslands with extensive land-use, are particularly effected by habitat loss. In north-western Germany, riverine sand-dunes and natural floodplains were widespread in the past. As a result of regular flooding events, intensive agricultural land-use practices were impossible in these habitats types facilitating the formation of semi-natural landscapes with a high species richness of plants and animals. By the regulation of the natural course and building of dykes, large rivers have experienced serious anthropogenic influences resulting in a dramatic decline of adjacent inland dunes and natural floodplains. The realization of a large restoration project in north-western Germany had the aim to restore a typical floodplain composed of inland sand-dunes and seasonally flooded grasslands. Within this project we focused on the response of wild bee communities to such restoration measures. Therefore, we analysed the distribution pattern of wild bee communities in restored and target habitats and focused on inland sand-dunes and extensive grasslands. Based on a grid-system we recorded wild bee flower visitation, the quantity and phenology of pollen and nectar sources and vegetation parameters. Our results show a rapid colonization of a species-rich wild bee community reflecting a community composition which is composed of generalists, specialists and parasitic species in similar proportions expectable for this region. The studied habitat types of inland sand-dunes and moist grasslands attracted different wild bee communities but showed a similar pattern of response in restored and target sites suggesting a strong influence of habitat composition.

Introduction

The extinction of plant and animal species and the resulting decline in biodiversity is known to be mainly driven by the loss and fragmentation of natural habitats (Saunders *et al.* 1991; Primack 2002; Henle *et al.* 2004). Habitat fragmentation enforces the vulnerability of local populations to environmental and demographic fluctuations and decreases the chance for recolonization events (Lande & Orzack 1988). Especially semi-natural landscapes, such as oligotraphentic grasslands with extensive land-use practices, are particularly effected by habitat loss and experienced a dramatic decline during recent decades (van Halder *et al.* 2008).

In north-western Germany, riverine sand-dune complexes and natural floodplains were widespread in the past. As a result of regular flooding events, intensive agricultural land-use practices were impossible in these habitats types. Consequently, semi-natural landscapes with a high species richness of plants and animals were maintained by an extensive use, such as mowing or grazing. The dominance of Pleistocene sands in this region promoted the formation of large sandy grasslands and inland sand dune complexes which are species-rich and of high conservation value (Matus *et al.* 2003). Similar to other parts of Europe, large rivers have experienced a long history of anthropogenic influences in Germany, which included the regulation of the natural course and building of dykes (De Waal *et al.* 1995; Hughes *et al.* 2005). Thus, areas adjacent to rivers were converted into intensive farmland and only a few traditionally managed landscapes remained. As a consequence, inland sand-dunes and natural floodplains have become threatened habitat types. The abandonment of traditional land use and increasing atmospheric nitrogen deposition are contributing to the degradation of such semi-natural landscapes (Soderstrom *et al.* 2001).

The need to avoid a further decline of biodiversity led to the implementation of different conservation efforts comprising a variety of actions. Passive conservation measures include the creation of nature reserves that are important for the legal protection of areas (DeFries *et al.* 2005). Active measures, such as restoration and habitat management are necessary for creating new habitats and maintaining biodiversity. Some conservation projects focus on the protection of umbrella or flagship species (White *et al.* 1997; Effenberger & Suchentrunk 1999; Zink *et al.* 2000), but the restoration and conservation of complete communities is much more complex and usually requires a greater effort of active conservation measures. The establishment of extensive land use practices, e.g. extensive grazing or mowing, has proved to be an effective type of management in abandoned areas that can support a variety of

organisms associated with open habitats (Bokdam & Gleichman 2000; Wallis De Vries *et al.* 2007). Such land use techniques are necessary in order to establish dynamic processes that prevent successional processes (Weber *et al.* 2008).

Wild bees are particularly affected by the loss of open, semi-natural habitats, as they are dependent on diverse pollen and nectar sources and a variety of different nesting sites (Kratochwil 2003). In North America and parts of Central Europe, the abundance of a number of wild bee species decreased dramatically (Cane & Tepedino 2001; Biesmeijer et al. 2006), and this seems to be particularly true for species that are highly specialized on specific pollen sources as larval food or special habitat structures to build their nests. Hence, the implementation, evaluation and further development of conservation management for endangered wild bees are urgently needed. Habitat restoration is one possibility to support populations of wild bees and to improve the connectivity of existing habitat patches. However, successful restoration requires knowledge on the environmental factors that determine species occurrences. Moreover, a sound knowledge of the wild bee responses to habitat management can be obtained from comparisons of semi-natural and restored habitats. As each species responds differently to variation in environmental factors, a community approach is required in order to analyse these causal mechanisms. Based on the abovementioned habitat requirements of most wild bee species (specific pollen sources and nesting sites), it is reasonable to suggest that a direct relationship of bee communities and vegetation composition, flower phenology and management type exists.

The realization of a large restoration project in north-western Germany which had the aim to restore a typical floodplain composed of inland sand-dunes and seasonally flooded grasslands provided the opportunity to study the response of wild bee communities to such restoration measures. Our aim was to evaluate the success of habitat restoration for wild bees and to identify key factors that drive wild bee diversity and community structure in natural and restored habitats. We focus on two typical habitat types, inland sand-dunes and extensive, seasonally flooded grassland to analyze wild bee community composition and distribution. We address the following questions:

- Have the restoration measures been suitable for the establishment of diverse wild bee communities?
- Which factors determine wild bee community composition and distribution in restored and target habitats?
- Are wild bee communities in restored habitats similar to those of natural target habitats?

Methods

Study area and restoration method

The study was carried out in the Emsland (Lower Saxony, Germany), a region that is dominated by intensive land-use that also maintains some fragments of semi-natural and natural habitats. The climate in this region is characterized by temperate humid conditions with mild winters and cool rainy summers.



Figure 1: Map of the three study areas in north-western Germany. The restoration areas "Hammer Schleife" (HS) and "Wester Schleife" (WS) (red circles) are situated in two meander cores of the river "Hase", the target area "Biener Busch" (BB) (blue circle) is located next to the river "Ems".

The nature reserve "Sandtrockenrasen am Biener Busch" (from now on referred to as "target BB", Figure 1 and 2a) is situated near the river "Ems" (24 ha) and served as target (reference) area for the restoration project. This alluvial pasture-woodland vegetation complex (24 hectare) has been grazed extensively by cattle for centuries and is characterised by typical inland sand-dune vegetation (plot type "dry") complexes, seasonally flooded grasslands (plot

type "moist") and riparian willow shrubs. The restoration project was conducted at two meander cores of the river "Hase" ("Hammer Schleife - restoration HS" and "Wester Schleife - restoration WS"), which comprise an area of 49 hectare in total (Figure 1 and 2b,c). Prior to the restoration, these sites were used as intensive grasslands and maize fields. In late summer 2001, 67,000 m³ of soil and sand material were moved in order to re-establish an oligotraphentic wetland sand-dune complex low in nutrients comparable to the original alluvial situation (for further technical information see Stroh et al. 2005). A relief composed of artificial dunes and temporarily flooded hollows was modelled based on historical maps from the years 1900 and 1990 and aerial pictures taken in 1956. Dykes were removed in order to allow for seasonal flooding events that increase natural dynamics. After the restoration measures had been completed, the open character of the restoration area has been maintained by extensive cattle grazing. The establishment of the typical vegetation was promoted by hay spreading on parts of the sand-dune complexes (plot type "dry"). These plots were inoculated with mown and raked material from the target site. Moist sites were treated with a characteristic seed mixture (Seed mix N1 "Landesanstalt für Ökologie, Bodenordnung und Forsten Nordrhein-Westfalen, LÖBF") of nutrient poor dry grasslands (plot type "moist") (Remy & Zimmermann 2004).

Wild bee, pollen source and vegetation survey

A grid-based system of permanent plots was established on all study sites for vegetation and wild bee analyses (grid plot distance 50 m). In 2005, this grid system was used to study the distribution of wild bees on the sites, the quantity of pollen and nectar sources and the structure and composition of the vegetation. From late May until September, a total of 49 grid plots (Figure 2a-c) were examined weekly for flower-visiting wild bees using a net. Collection took place in a radius of eight metres around each grid plot. In the target area (BB), 16 grid plots were selected, either classified as "dry" (n = 11) or "moist" (n = 5) sites (see plot description above and figure 2a). In the restoration areas, a total of 33 grid plots was analysed, including also "dry" plots (n = 12) as well as "moist" plots (n = 21) (see plot description above and figure 2b and c). The collected wild bees were prepared and identified in the laboratory.

The quantity of entomophilous plant species (number of open flowers) was surveyed weekly during the study period within the same radius. Pollen collecting individuals of wild bees were observed at 18 flowering plant species. For the analyses, we included only those plant species that had been visited by wild bees frequently (*Hieracium pilosella*, *Leontodon*)

saxatilis, Hypochaeris radicata, Crepis capillaris, Tanacetum vulgare, Lotus corniculatus, and *Trifolium repens*). Vegetation structure and composition was determined in early June using the Braun-Blanquet method (Barkman *et al.* 1964) in a radius of 5 m around each grid plot. The cover of each plant species was used to calculate a weighted moisture indicator value (Ellenberg *et al.* 1992).



Figure 2a: Aerial picture of the grid (coloured points) at the target site "Biener Busch" (BB).



Figure 2b: Aerial picture of the grid (coloured points) at the restoration site "Hammer Schleife" (HS).



Figure 2c: Aerial picture of the grid (coloured points) at the restoration site "Wester Schleife" (WS).

Statistical analysis

Species were assigned to groups according to their life history traits, we differentiated between specialized (oligolectic species and those with a main distribution in sandy habitats), generalized and parasitic species and included body size as a measure of dispersal ability (small bees < 7 mm, medium bees 7-12 mm and large bees > 12 mm). These groups were used as wild bee response variables and were tested for differences between the treatments (target and restoration), the plot types ("dry" and "moist" plots) and the interaction of treatment and plot type with an analysis of variance (ANOVA). The wild bee records were summarized for the complete study period.

We used multiple regression analyses to identify factors that determine differences in wild bee occurrence. The environmental variables that were included as potential explanatory variables comprised the maximum flower number of the major entomophilous plant species in each plot (Hieracium pilosella, Leontodon saxatilis, Hypochaeris radicata, Crepis capillaris, Tanacetum vulgare, Lotus corniculatus, and Trifolium repens) and parameters of the vegetation structure and composition (vegetation cover, bare ground, cover of forbs and grasses, height of forbs and grasses and moisture). All variables were first checked for autocorrelation. Afterwards, the relative explanatory power of each environmental variable was determined in a hierarchical partitioning analysis using the "hier.part" package for R (Walsh & Mac Nally 2003). This method is particularly suited to uncover the average independent contribution of each explanatory variable and to decide which variables aid in explaining patterns of variation (Mac Nally 2002). All parameters with an explanatory power > 5% were afterwards included in the multiple regression analysis in the order of decreasing explanatory power. We performed model simplification using the step function based on Akaike's information criterion as implemented in R 2.7.0. Additionally, we determined the effect (positive + or negative -, table 2) of each explanatory variable on the response variables using Pearson's product moment correlation coefficient.

We tested for differences in the quantity and phenology of the most frequently visited entomophilous plant species (Hypochaeris radicata, Leontodon saxatilis, Lotus corniculatus, and Trifolium repens) between treatments (target and restoration) and between plot types ("dry" and "moist") using a repeated measures analysis of variance (rmANOVA). First the main effects of treatment and plot type on the maximum number of flowers were checked. By the inclusion of the factor time (weekly flower counts at each plot), we tested for phenological differences. These analyses were carried out in R 2.7.0 (R Development Core Team 2007).

Records of wild bee occurrences at each plot, the quantity of pollen sources and characteristics of the vegetation structure were included in a multivariate model. The correlation between wild bee community composition, flower resources and vegetation structure was studied by means of a canonical correspondence analysis (CCA). The significance of the correlation between wild bees and environmental variables (flowering plants and vegetation structure) was analysed with a Monte-Carlo-Permutation test (999 permutations) in Canoco 4.5 (ter Braak & Smilauer 2002).

Results

A total of 1334 individuals belonging to 61 species was detected in the complete study area. At the restored sites, a mean number of 9.15 (\pm 3.45 SD) species were observed per plot, whereas a mean of 11.68 (\pm 4.66 SD) species was recorded at the target plots (F_{1,45} = 6.4*). In both areas, Andrenidae (11 species in the target area, 8 species in restored sites) and the Halictidae (13 species in the target area, 9 species in restored sites) were caught most frequently.

Wild bee response to restoration measures

The number and abundance of wild bee species (or subsets of this group) differed significantly between restoration and target sites (treatment) and among "dry" and "moist" plots (Figure 3 a-f, Table 1). In both restoration and target plots, we found a similar response of wild bee diversity to dry and moist conditions with a higher diversity of wild bees in dry plots (Figure 3 a-f, Table 1). Comparing restoration and target plots, we found a significant higher number of wild bee species, total abundance, abundance of specialists, number of generalists and small species at the target plots. The number of specialist and medium species was highest (but not significant) at the dry plots within the restored areas.



Figure 3 a-f: Variation of a) wild bee species richness b) total bee abundance c) number of specialized species d) abundance of specialized species e) number of medium species and f) number of small species between dry and moist plots in target and restoration sites. Significant differences are denoted by letters.

Table 1: Results of the ANOVAs showing the effects of treatment (restoration and target), type (dry and moist)
and the interaction of these factors on wild bee species richness and abundance, the number and abundance of
specialist, generalist and parasite species and the number of large (< 13 mm), medium (8-13 mm) and small (> 8
mm) species (ns = not significant; * $P \le 0.5$; ** $P < 0.01$; *** $P < 0.001$).

wild bee response	treatment	type	type*treatment
	F _{1,45}	F _{1,45}	F _{1,45}
total sp. richness	6.4*	19.7***	ns
total abundance	8.28**	27.72***	ns
number of specialist sp.	ns	24.14***	ns
abundance of specialist sp.	6.61*	29.49***	ns
number of generalist sp.	12.42***	4.06*	ns
abundance of generalist sp.	ns	ns	ns
number of parasite sp.	ns	ns	ns
abundance of parasite sp.	ns	ns	ns
number of large sp.	ns	ns	ns
number of medium sp.	ns	22.56***	ns
number of small sp.	12.41***	27.24***	ns

Effects of environmental variables

After hierachical partitioning analyses for each wild bee response variable and the complete set of explanatory variables, we performed multiple regression analyses including variables with an independent effect of more than 5%. The resulting models comprised five to eleven explanatory variables. These analyses revealed a significant positive relationship between the number of flowers of Hypochaeris radicata and almost all wild bee response variables (Table 2). The number of flowers of Leontodon saxatilis and Hieracium pilosella was positive correlated with the total abundance of bees and the abundance of specialists. Moreover, the cover of bare ground was positively correlated with the total number of bee species and the number of small and medium species. Moisture was negatively correlated with the total number of bee species, the number of specialized and medium species.

wild bee response / environmental factor	species richness F _{1,41}	total abundance $F_{1,43}$	specialized species F _{1,44}	abundance of specialists F _{1,44}	generalist species F _{1,42}	t medium species F _{1,42}	small species F _{1,42}
bare ground	8.61**/+	ns	ni	Ni	ns	9.14**/+	6.31*/+
moisture	ns	ni	ns	Ni	ni	ns	ni
cover of forbs	ns	ni	ni	Ns	ns	ni	ns
cover of grasses	ns	ni	ns	ni	ni	ns	ns
Hypochaeris radicata	12.56***/+	-31.45***/+	18.71***/+	50.58***/+	ns	13.23***/+	6.05*/+
Leontodon saxatilis	ni	19.65***/+	7.49**/+	20.93***/+	n i	4.87*/+	ni
Hieracium pilosella	ns	9.03**/+	ni	8.57**/+	ns	ni	9.25**/+
Lotus corniculatus	ns	ns	ni	ni	ni	ni	ni
Tanacetum vulgare	ni	ni	ni	ni	ns	4.43*/+	ns
Crepis capillaris	ni	ni	ni	ni	ns	ni	ni

Table 2: Results of the multiple regression analysis showing the effects of environmental factors on wild bee response variables. ns = not significant; * $P \le 0.5$; ** P < 0.01; *** P < 0.001.

Resource availability

The repeated measures ANOVA revealed that the quantity of flowers of *Hypochaeris radicata* was significantly higher at dry sites and target sites than at moist or restoration sites (Table 3, Figure 4). Flowers of *Trifolium repens* were found more frequently at moist plots of both restoration and target areas (Table 3, Figure 7). Moreover, we found a significant interaction of treatment and time for *Hypochoeris radicata* and *Lotus corniculatus*. Both species had a flowering peak at the target plots in the middle of May, at the restoration plots these species reached a maximum flower number in August. For *H. radicata* there were also significant interactions between the type of plot and time and for treatment, plot type and time. The phenology of *Trifolium repens* was significantly different between dry and moist plots, with a longer flowering period in the latter plot type.

Table 3:	Results	of the	repeated	measures	ANOV	/As	showing	the	main	effects	s of t	the ex	planatory	varia	bles
treatment	and plo	t type c	on the qua	antity of f	lowers	and	the effec	ts of	f time	and th	ne var	iables	s treatment	and	plot
type as w	ell as the	corresp	ponding i	nteraction	s. $ns = 1$	not s	ignifican	t; * <i>1</i>	$P \le 0.5$	5; ** P	< 0.0)1; **	* $P < 0.00$	1.	

response/ effect	Hypochaeris radicata	Leontodon saxatilis	Lotus corniculatus	Trifolium repens
main effects		Sentennis		repens
treatment	$F_{1,45} = 47.35^{***}$	ns	ns	ns
type	$F_{1,45} = 30.03 * * *$	ns	ns	$F_{1,45} = 12.77 * * *$
treatment:type	ns	ns	ns	ns
time effects				
treatment:time	$F_{14,630} = 2.99 * * *$	ns	$F_{15,675} = 2.53 * * *$	ns
type:time	$F_{14,630} = 2.47 * * *$	ns	ns	$F_{14,630} = 2.88 * * *$
treatment:type:time	$F_{14,630} = 1.84*$	ns	ns	ns



Figure 4: Flower phenology of Hypochaeris radicata at moist and dry plots of target and restoration areas.



Figure 5: Flower phenology of Leontodon saxatilis at moist and dry plots of target and restoration areas.



Figure 6: Flower phenology of Lotus corniculatus at moist and dry plots of target and restoration areas.



Figure 7: Flower phenology of *Trifolium repens* at moist and dry plots of target and restoration areas.

Community structure

Despite a high variation in the wild bee communities, the canonical correspondence analysis showed a clear separation of the wild bee assemblages of dry and moist plots of the complete data set. Environmental variables explained 61.4 % of the variance in the species data with Hypochaeris radicata being the most important factor (Monte Carlo Permutation test, F = 2.96, p = 0.001). Additionally, significant effects were found for Tanacetum vulgare (F = 3.01, p = 0.001), the cover of bare ground (F = 2.27, p = 0.003) and the total vegetation cover (F = 1.87, p = 0.025). As detected by the univariate analysis, a higher wild bee diversity was found at dry plots. The most important environmental variables characterizing dry plots were a greater cover of bare ground and a high availability of the Asteraceae species Hypochaeris radicata and Leontodon saxatilis. Moreover, dry plots were characterized by the presence of some specialized species. At dry restoration plots, Panurgus banksianus and Andrena denticulata occurred in a high abundance, both of which are specialized on Asteraceae as pollen source. In contrast, moist plots had a higher vegetation cover and were dominated by generalist wild bee species (Figure 8).



Figure 8: CCA biplot showing community composition of wild bees at grid plots in target and restoration sites in relation to environmental variables (first axis eigenvalue = 0.211; second axis eigenvalue = 0.159). Species richness is reported by different symbol size.

Discussion

Our study demonstrates the importance of habitat restoration for wild bees in agricultural landscapes. In both, moist and dry plots of the restoration sites all groups of wild bee species, comprising generalists, specialists and parasitic species, were present. Dry sites had a higher wild bee diversity than moist sites in both restoration and target areas. This difference was most striking for the number and abundance of specialized and small bee species which occurred in a much higher number and abundance at dry plots. The majority of wild bee species are heliophilous and prefer warm and dry conditions predominant in open habitats with high insulation (Kratochwil 2003). The highest diversity of specialized wild bees occurs in semi-arid and mediterranean-like regions, in Germany habitat specialists prefer plant communities that are found at sites with dry and warm micro-climatic conditions (Kratochwil 2003). Ideal conditions due to a high proportion of bare ground combined with a low vegetation height at dry sites in restoration and target areas facilitate the presence of a diverse wild bee community dominated by specialized species. Moreover, a closer connection of potential nesting sites (cover of bare ground) and pollen sources might be particularly important for small wild bee species (Gathmann & Tscharntke 2002).

In contrast, moist grasslands (moist plots in target and restoration area) were dominated by a wild bee community comprising mainly generalist bee species such as different species of the genus *Bombus*. Due to the specialization on specific host plants and nesting sites, 70% of the wild bee species present in Germany can be classified as specialists, 126 of which are associated to sandy habitats (Beil & Kratochwil 2004).

Our results show a stronger effect of habitat type (dry and moist) compared to the effect of treatment (target vs. restoration) on the response of all groups of bees suggesting a rapid colonization of a typical wild bee community at the restored sites. From this we infer a high colonization ability of wild bees and a potential underestimation of the actual dispersal ability of this insect group in previous studies (Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007). It is assumed that the foraging range of most wild bee species is rather small (Greenleaf *et al.* 2007) and determined by their body size (Araújo *et al.* 2004). The estimation of foraging ranges is often used as a measure of dispersal ability (Walther-Hellwig & Frankl 2000; Gathmann & Tscharntke 2002). This comparison might be problematic as the foraging range of wild bees should be small, because of a high investment of energy and time for long distance flights (Heinrich 1976). The local availability of pollen and nectar sources is also a main determinant of foraging ranges (Walther-Hellwig & Frankl 2000; Osborne *et al.* 2008).

By the analysis of pollen loads from different wild bee species, (Beil *et al.* 2008) found comparable large foraging (up to 1250 m) distances even for small species (> 7mm body size). In order to find new suitable habitats, the dispersal ability of bees should exceed their actual foraging radius. An example for the high mobility of wild bee species is the oligolectic digger bee *Andrena vaga* (Panzer), a pioneer species of floodplains, which shows a high degree of gene flow among nest aggregations even at large spatial scales and a high interaggregation movement and emigration rate (Bischoff 2003).

Based on the assumption of reduced dispersal abilities, many authors conclude a negative influence of habitat fragmentation on many wild bee species (Gathmann & Tscharntke 2002; Kremen *et al.* 2002; Winfree *et al.* 2007a) and argue that the most important factors determining the structure of wild bee communities are the size and spatial arrangement of the habitats. The effects of habitat fragmentation and loss of natural habitats are assumed to differ between species groups. Species with low dispersal ability, food specialists or species that occur in low population densities are particularly affected by the degradation of natural habitats (reviewed in Tscharntke *et al.* 2002). Furthermore, recent studies revealed a higher extinction risk for habitat specialists when compared with generalist species (Packer *et al.* 2005; Zayed & Packer 2007). Consequently, the colonization of new habitats is also assumed to differ for these groups of species, large bee species are supposed to be the first colonizers followed by medium and smaller species (Gathmann *et al.* 1994).

Our study indicates that this is not always the case. In comparison with target areas, the restoration of different habitat types benefits a rapid colonization of a typical wild bee community. A close linkage of extensively used grasslands and dry, open habitats proved to guarantee the establishment of specialists and generalists with different habitat requirements. We found no evidence for a step-wise colonization process of large, medium and small species nor an influence of the degree of specialization.

The canonical correspondence analysis showed that the wild bee community composition differs between dry and moist habitat types but is similar for restoration and target areas. This differentiation is mainly attributable to the prevalence of different environmental conditions. While the wild bee communities of moist sites within restoration and target areas were rather attracted by a high vegetation cover and entomophilous plant species like *Lotus corniculatus*, *Senecio jacobaea* and *Tanacetum vulgare*, the dry sites of restoration and target area were dominated by specialized wild bee species using plants of the family Asteraceae as pollen and nectar source. Moreover, these sites were characterized by a high proportion of bare ground which is a key factor for the structure and composition of entire wild bee communities (Potts

et al. 2005). In our study the availability of bare ground was positive correlated with wild bee species richness and the number of medium and small species.

Another driving force for the composition of wild bee communities is the vegetation structure (Corbet 1995) and in particular the abundance of flowers as pollen and nectar source (Sjödin *et al.* 2008). The availability of flowers differed significantly between dry and moist sites in our study areas. While the Fabaceae species *Lotus corniculatus* and *Trifolium repens* had a higher density at moist plots, the Asteraceae *Hypochaeris radicata* and *Leontodon saxatilis* were dominant at dry sites, explaining the high diversity of wild bee species specialized on Asteraceae as pollen source.

Conclusions

Our aim was to analyse distribution patterns of wild bee communities in different habitat types of restoration and target areas in order to evaluate the success of the restoration measures and to identify the influence of environmental factors. Species-rich wild bee communities establish rapidly and reflect a community composition which is composed of generalists, specialists and parasitic species in similar proportions expectable for this region. Due to these results we infer a high dispersal ability for most wild bee species and a high rate of "inter-habitat-movement". The studied habitat types of inland sand-dunes and moist grasslands attracted different wild bee communities but showed a similar pattern of response in restored and target sites suggesting a strong influence of habitat composition. The most important environmental factors explaining differences in species richness and community composition were the proportion of bare ground and the phenology and quantity of specific pollen and nectar sources.

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GENETIC EXCHANGE AMONG POPULATIONS OF A SPECIALIST BEE, *ANDRENA VAGA* (HYMENOPTERA: ANDRENIDAE)



Female of Andrena vaga

Abstract

Habitat fragmentation is believed to be a key threat to biodiversity, with habitat specialists being stronger affected than generalists. However, pioneer species might be less affected by fragmentation, as their high colonization potential should increase gene flow. Here, we present an analysis of the genetic structure of populations of the solitary bee Andrena vaga, which naturally occurs in sandy habitats and is specialized on willow (Salix) pollen as larval food and sandy soils as nesting sites. While the species is widespread in the young sandy landscapes of our main study area (Emsland, northwestern Germany), it occurs less frequently in the Lower Rhine valley. Our analyses of six polymorphic microsatellite markers show that the populations are only slightly differentiated, suggesting a relatively strong gene flow. No genetic structure corresponding to the geographic origin was found as the variability within populations accounted for the major proportion of variation. F_{ST} values were higher and allelic richness was lower in the Lower Rhine valley, supporting the hypothesis that habitat availability affects the degree of genetic exchange between populations. Inbreeding coefficients were generally high and nearly all populations had a heterozygote deficiency, which could be explained by the breeding strategy of A. vaga, which nests in large aggregations.

Introduction

Habitat loss and fragmentation are major problems for the maintenance of biodiversity in modern cultural landscapes (Fahrig 2003; Henle et al. 2004; Hanski 2005). The persistence of plant and animal species within habitat fragments depends on several parameters, such as size, age, spatial isolation and the structure of the surrounding area (Tscharntke et al. 2002). Due to the disruption of the remaining suitable habitats, populations become increasingly isolated and small. Hence, they often have a reduced genetic diversity due to the reduced gene flow (Ellis et al. 2006). This reduction in genetic diversity is considered to enhance inbreeding depression and decrease the adaptability to environmental changes (Darvill et al. 2006), which often results in negative effects on the survival of populations. While the influence of inbreeding on population persistence is discussed controversially in recent literature (reviewed in Hedrick and Kalinowski 2000), it is generally acknowledged that the survival of small populations is negatively affected by the loss of genetic diversity.

Habitat specialization is generally believed to be an important trait affecting the vulnerability of species (Primack 2002) and is thus a fundamental concept explaining their extinction risk (McKinney 1997). Since the availability of suitable habitat is usually lower for specialists than for generalists, the effects of fragmentation are thought to be stronger in the former group (e.g. Kitahara and Fujii 1994; Kelley et al. 2000; Bonte et al. 2004; Polus et al. 2006). Many species of wild bees (Hymenoptera, Apoidea) are specialized on pollen resources and nesting habitats (Westrich 1989, Kratochwil 2003). Hence, the distribution of such oligolectic bees is limited by the availability of their specific floral hosts (Packer et al. 2005). The recent decline of many species of wild bees and particularly of specialised species has been attributed to habitat fragmentation (Steffan-Dewenter 2003) and the resulting ecological and economic consequences ("pollination crisis") are currently strongly debated (Cane and Tepedino 2001, Goulson 2003, Biesmeijer et al. 2006, Butler et al. 2007). While the degree of specialization is probably an important factor influencing the genetic population structure of a species, the life history strategy might substantially influence the consequences of fragmentation. Pioneer species with a high dispersal capability and comparatively large population sizes might be less prone to fragmentation effects as they should be adapted to dynamic processes in their natural habitats. Hence, it could be proposed that populations of pioneer species might be strongly connected despite a strong degree of specialization. Moreover, the availability of habitat is probably more important than the degree of specialization. Thus, it is reasonable to suggest that highly specialized species are able to persist as long as the habitat availability remains high.

Here we test the hypothesis that populations of pioneer species are strongly connected. We analyzed the genetic differentiation among populations of a widespread specialist bee species (*Andrena vaga* Panzer, 1799) using six polymorphic microsatellite loci. *A. vaga* is a floodplain pioneer with a high dispersal capability, which is specialized on willow pollen (*Salix*) and sandy habitats with sparse vegetation. It is widespread in northern Germany due to the predominance of sandy soils and a high availability of suitable nesting habitats but more restricted in the Lower Rhine valley, where the landscape structure is strongly shaped by intensive agriculture and urbanisation.

Methods

Study area and sampling

The main study region is located in the Emsland area in northwestern Germany (Lower Saxony, Fig. 1), which is mainly characterized by alluvial soils and utilized as arable farmland and pine plantations. Due to the dominance of Pleistocene sands in northern Germany, the availability of nesting habitats for A. vaga in the Emsland is relatively high. A total of 32 nest aggregations of Andrena vaga were located in the floodplains of the rivers Ems and Hase, eleven of which were large enough to be selected for further analyses. While the watercourse of the Ems is characterized by a high amount of adjacent forests and arable fields, the floodplain of the Hase is dominated by open habitats due to restoration measures, which were carried out between 1998 and 2001. These measures included the removal of dikes, the restoration of inland dunes and the reconnection of oxbows.

Samples of Andrena vaga were collected from 2002 to 2006. We analyzed a total of 201 females from eleven nest aggregations (12-25 individuals per aggregation). The distance of nests within aggregations (ca. 10 cm, max. 1 m) was considerably smaller than the distance between aggregations (> 750 m). The individuals were sampled as they were haphazardly encountered. To study the influence of geographical distance on the genetic differentiation, we included eight nest aggregations (156 individuals) with increasing distance to the study area. Three of them were situated in the surroundings of Osnabrück (Lower Saxony), further four from the Lower Rhine valley (North Rhine-Westphalia) and another one near Darmstadt (Hesse; see Table 1 and Fig. 1). The Lower Rhine valley is characterized by intensive agriculture and urbanisation (van Dijk et al. 2006). While in the Emsland many sandy paths

exist which are frequently colonized by A. vaga, paved tracks predominate in the Lower Rhine valley. Hence, the availability of nesting habitats for A. vaga is rather low compared to the Emsland. The minimum distance between nest aggregations was 750 m, the maximum distance 330 km. The number of nests per aggregation varied from 100 to more than 5000 nests.



Figure 1 Map of the locations of the 19 *Andrena vaga* populations studied within Germany. A) Map of the complete study area in Germany, B) main study site in the Emsland region of Lower Saxony (note change of scale between A and B).

DNA extraction and amplification

Genomic DNA was extracted from abdominal or thoracic tissue using the DNeasy Tissue Kit[™] (Qiagen), following the manufacturer's protocol. Each sample was typed at six microsatellite loci (vaga01, vaga02, vaga05, vaga08, vaga09 and vaga13) developed by Mohra et al. (2000). The loci were amplified separately using the HotMasterMix[™] (Eppendorf). The 5'-end of each forward primer set was labelled with a fluorescent marker, either 5-FAM, JOE or TAMRA. The products were genotyped on an ABI PRISM 377

automated DNA sequencer (Applied Biosystems Inc.). Fragment lengths were determined using GENESCAN and GENOTYPER 2.5 (Applied Biosystems Inc.).

Statistical analysis

GENEPOP 3.4 (Raymond and Rousset 1995) was used to calculate genotypic linkage disequilibrium, using Fisher's exact test and the Markov-chain method. With the same program a global test for departure from Hardy-Weinberg equilibrium was performed with the null hypothesis of random mating and an alternative hypothesis of heterozygote deficiency. The significance of departure from HWE was estimated using the Markov-chain method (1000 iterations). Since the data departed significantly from HWE, they were inspected for the presence of null alleles using MicroChecker 2.2.3 (Van Oosterhout et al. 2004).

FSTAT 2.9.3 (Goudet 1995) was used to estimate the mean number of alleles, the allelic richness R and the inbreeding coefficients F_{IS} for each nest aggregation. The measure of allelic richness R (El Mousadik and Petit 1996) was used as it is independent of sample size. The expected (H_E) and observed (H_O) heterozygosity for each locus and each nest aggregation was determined using GenAlEx 6.0 (Peakall and Smouse 2006), which performs a Chi-square test to asses the significance of a departure from HWE. The same program was also used to inspect the occurrence of private alleles (alleles which can only be found in one population) for each nest aggregation.

To examine the genetic structure within and between nest aggregations an analysis of molecular variance (AMOVA) was performed in GenAlEx 6.0 (Peakall and Smouse 2006) based upon Wright's F-statistics (Wright 1951). F_{ST} values are based on the variance in allele frequencies among the nest aggregations. For this purpose, aggregations belonging to the main study region (Emsland) were assigned to two groups corresponding to their origin from the floodplains of the rivers Hase and Ems. The genetic structure was tested at three different levels: floodplains (groups of nest aggregations), nest aggregations within floodplains and individuals within nest aggregations. The same analysis was performed for the complete data set, in which nest aggregations were grouped according to their geographic origin (Emsland, Osnabrück and Rhineland together with Darmstadt).

To examine the pairwise population differentiation within each floodplain (Ems, Hase) we performed a log-likelihood based exact test (G-test), which tests the distribution of genotypes between each pair of aggregations (Goudet et al. 1996) as implemented in FSTAT (Goudet 1995). The significance of these tests was adjusted using standard Bonferroni corrections. This method seems to be particularly efficient for non-random mating populations (Goudet et al. 1996) and a low overall populations structure (Petit et al. 2001).

To test for isolation-by-distance, pairwise genetic distances (F_{ST} calculated in GenAlEx 6.0 as described above) and geographical distance matrices were checked for a correlation assuming that F_{ST} is linearly related to the geographical distance between populations. We employed a Mantel-test of matrix correlations and used a reduced major axis (RMA) regression to estimate the intercept and slope of the isolation-by-distance relationship within the program IBD 1.52 (Bohonak 2002). This procedure was first applied for the eleven Emsland populations. Afterwards, the populations from further south were included to assess the influence of increasing distance on the genetic differentiation using the complete data set.

Moreover, the relationship between genetic and geographical distance was checked for spatial autocorrelation of the genetic distance estimates as implemented within GenAlEx 6.0 (Peakall and Smouse 1999). This method is based on a multivariate technique combining alleles and loci to reduce stochastic noise. The theory of spatial autocorrelation is based on the assumption that samples collected at any locality will have a greater similarity to those from locations in their vicinity. Thus, positive correlation coefficients should occur between populations from neighbouring areas whereas a negative correlation coefficient is expected for populations separated over a greater spatial scale. No spatial autocorrelation, indicated by values close to zero, gives evidence for a random pattern of genetic distance over the studied spatial scale.

Since the availability of habitat differs among the studied areas (more potential habitats in the Emsland than in the Rhineland), we tested our data for effects of the sampled regions. We performed ANOVAs to test for differences in allelic richness, expected (H_E) and observed heterozygosity (H_O) and inbreeding coefficients (F_{IS}) using "region" as the explanatory variable and the average values across all loci as response variables. In case of significance, we conducted pairwise t-tests with Bonferroni correction. These analyses were carried out with the program "R 2.5.1" (R Development Core Team 2007).

Region	Population	N	А	R	$\mathbf{F}_{\mathbf{IS}}$
Emsland - Hase	1	21	7.17	6.11	0.26
	2	23	8.67	7.30	0.26
	3	11	7.50	7.50	0.42
	4	19	7.67	6.82	0.33
	5	22	8.33	6.85	0.29
Emsland - Ems	6	12	7.17	6.95	0.30
	7	17	6.17	5.68	0.36
	8	19	6.67	5.94	0.26
	9	18	8.33	7.53	0.36
	10	20	8.17	7.21	0.28
	11	19	7.17	6.39	0.34
Osnabrück	12	18	6.83	6.06	0.40
	13	18	7.50	6.50	0.48
	14	17	6.67	5.94	0.35
Rhineland	15	25	7.00	6.10	0.51
	16	22	5.83	5.41	0.57
	17	21	7.33	6.18	0.43
	18	15	5.00	4.90	0.52
Darmstadt	19	20	8.17	7.03	0.45

Table 1 Characteristics of the analysed *Andrena vaga* populations (N = sample size; A = mean number of alleles per locus; R = mean allelic richness; F_{IS} = inbreeding coefficient)

Results

The complete data set contained 19 populations with a total of 357 females of *Andrena vaga* (Table 1). A global test of genotypic linkage disequilibrium across all populations revealed no significant departure for any combination of microsatellite loci. A global test for departure from Hardy-Weinberg equilibrium revealed a significant deviation from random mating (P < 0.001) with an excess of homozygotes. Null alleles were detected in nearly all populations in at least one locus, but all loci did amplify in all individuals (and also in 12 males, which were not included in the statistical analyses).

Region		Emsl	and-F	Hase			Emslé	and-E	ms				Osnab	rück		Rhine	eland			Da
Locus/Pop.		1	2	З	4	5	9	Г	8	6	10	11	12	13	14	15	16	17	18	19
Ν		21	23	11	19	22	12	17	19	18	20	19	18	18	17	25	22	21	15	20
vaga01	H_{O}	0.52	0.9	0.6	0.5	0.6	0.25	0.4	0.8	0.5	0.7	0.3	0.22	0.2	0.4	0.25	0.2	0.3	0.3	0.5
	H_E	0.77	0.8	0.8	0.8	0.8	0.63	0.8	0.8	0.8	0.8	0.8	0.68	0.7	0.7	0.54	0.6	0.6	0.6	0.8
	Р	0.16	0	0.6	0	0	0.08	0	0.1	0	0.8	0	0	0	0	0	0	0	0	0.1
	þĄ																			
vaga02	H_{0}	0.43	0.5	0.6	0.5	0.5	0.5	0.5	0.4	0.6	0.7	0.6	0.61	0.3	0.4	0.6	0.4	0.4	0.4	0.6
	H_E	0.83	0.8	0.9	0.8	0.8	0.88	0.7	0.8	0.8	0.9	0.8	0.7	0.7	0.8	0.85	0.8	0.8	0.8	0.8
	Р	0	0	0.1	0	0	0.02	0	0	0	0.1	0.3	0.45	0	0	0.01	0	0	0	0.1
	þĄ			Π										0				1		
vaga05	H_{0}	0.75	0.7	0.5	0.6	0.6	0.42	0.6	0.7	0.4	0.6	0.5	0.39	0.5	0.7	0.32	0.4	0.4	0.7	0.6
	H_E	0.78	0.8	0.8	0.8	0.7	0.77	0.8	0.8	0.8	0.8	0.8	0.81	0.7	0.8	0.77	0.8	0.8	0.8	0.9
	Ρ	0.38	0.2	0.3	0	0	0.12	0	0.2	0	0	0	0	0.4	0.3	0	0	0.3	0.2	0
	рA										1									-
vaga08	H_{O}	0.62	0.6	0.5	0.5	0.5	0.75	0.5	0.5	0.4	0.5	0.4	0.56	0.6	0.6	0.36	0.4	0.5	0.5	0.5
	H_{E}	0.77	0.9	0.8	0.8	0.7	0.69	0.7	0.6	0.9	0.8	0.8	0.77	0.9	0.8	0.79	0.8	0.8	0.7	0.7
	Ρ	0	0	0.1	0	0	0.98	0.2	0.2	0	0	0.1	0.11	0.1	0.1	0	0	0.1	0.1	0
	рA								-			1			1		1			-

Table 2 Hardy-Weinberg equilibrium and private alleles (Pop. = population; H_0 = observed heterozygosity; H_E = expected heterozygosity. P = sionificance of a chi- square test of Hardy-Weinberg equilibrium nA = number of mivate alleles).

Table 2 (continued H_E = expected hete private alleles).	 Hardy-Weinberg equilibr. erozygosity; P = significan 	ium and private alleles (Pop. = pc ce of a chi- square test of Hardy	pulation; H ₀ = - -Weinberg equil	observed heterozygos ibrium, pA = number	ity; r of
Region	Emsland-Hase	Emsland-Ems	Osnabrück	Rhineland	Da

-	`																			
Region		Emsl	and-F	Hase			Emslé	and-E	sm				Osnat	rück		Rhine	land			Ja
Locus/Pop.		1	2	3	4	. 5	9	7	8	6	10	11	12	13	14	15	16	17	18	19
Z		21	23	11	19	22	12	17	19	18	20	19	18	18	17	25	22	21	15	20
vaga09	H_{O}	0.62	0.6	0.5	0.7	0.7	0.67	0.4	0.8	0.6	0.5	0.7	0.44	0.6	0.4	0.36	0.2	0.6	0.1	0.1
	H_E	0.76	0.9	0.9	0.9	0.8	0.82	0.7	0.9	0.9	0.7	0.8	0.69	0.8	0.8	0.72	0.8	0.8	9.0	0.8
	P	0.75	0	0	0.5	0.1	0.21	0	0.4	0	0	0.6	0.05	0	0	0	0	0.1	0	0
	рA																			7
vaga13	H_{O}	0.62	0.6	0.5	0.6	0.5	0.75	0.6	0.1	0.8	0.7	0.6	0.56	0.3	0.7	0.36	0.4	0.4	0.2	0.4
	H_E	0.75	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.8	0.8	0.7	0.78	0.7	0.7	0.77	0.5	0.7	0.7	0.8
	P	0.48	0.8	0.3	0.3	0.4	0.62	0.1	0	0.9	0.8	0.2	0	0	0.4	0	0.2	0	0	0
	рA			-						0			2							
Genetic diversity

In all populations the observed heterozygosity was lower than the expected heterozygosity with 66 of 114 tests for each locus showing a significant departure from HWE. This means that each of the 19 populations had a heterozygote deficiency for at least one locus (Table 2). Inbreeding coefficients (F_{IS}) within populations were high, ranging from 0.26 to 0.57 (mean 0.38 ± 0.095 SE; Table 1). Nevertheless, we detected a high genetic variability: The average number of alleles per population (7.23 ± 0.93 SE) and the resulting allelic richness (6.44 ± 0.73 SE) was high for all populations, with the lowest values observed in a population within the Rhineland (population 18) and the highest number of alleles detected within the Emsland area. The average gene diversity was also high ranging from 0.74 to 0.86 (0.79 ± 0.04 SE). Private alleles occurred in a low number and frequency in eleven populations.

Genetic differentiation

Assigning nest aggregations to the river catchments revealed no genetic structure corresponding to their origin from the floodplains of the rivers Hase and Ems. The highest genetic variance detected by the AMOVA was measured among individuals within aggregations (95%, variance component 2.4, P = 0.01). Pairwise estimates of population differentiation revealed a stronger differentiation among aggregations located at the Ems than at the Hase (Table 3).

		Hase					Ems					
		1	2	3	4	5	6	7	8	9	10	11
Hase	1	/	NS	**	*	***						
	2	0.026	/	NS	NS	NS						
	3	0.052	0.012	/	NS	NS						
	4	0.038	0.016	0.020	/	NS						
	5	0.052	0.027	0.026	0.035	/						
Ems	6						/	**	***	*	**	**
	7						0.092	/	**	**	**	**
	8						0.083	0.052	/	**	**	**
	9						0.059	0.050	0.069	/	NS	**
	10						0.059	0.087	0.097	0.033	/	**
	11						0.066	0.104	0.109	0.065	0.050	/

Table 3 Pairwise F_{ST} for populations located at the river Hase (1-5) and the river Ems (6-11) below diagonal, significance of pair-wise population differentiation above diagonal.

Although the average F_{ST} values within these regional groups were generally low (Hase: $F_{ST} = 0.03 \pm 0.01$ SE; Ems: $F_{ST} = 0.07 \pm 0.02$ SE), the values were significantly higher for nest aggregations belonging to the Ems group (ANOVA, $F_{1,23} = 26.71$; P < 0.001). The highest F_{ST} values were detected in the Rhineland (0.09 ± 0.02).

Analysing the combined dataset of all nest aggregations revealed also no genetic structuring corresponding to the spatial arrangement of populations. The results of the AMOVA showed that 92% of the molecular variance was explained by variation within nest aggregations (variance component 2.4; P = 0.01). The remaining variance is partitioned among aggregations within regions (6%, variance component 0.17, P = 0.01) and variation among regions (2%, variance component 0.05, P = 0.01). Pairwise population differentiation was significant for many populations and global F_{ST} was 0.07 (± 0.03) across all loci.



Figure 2 Isolation-by-distance-plot for the entire study area. Note that the variability of F_{ST} values is high across the complete range of distances ($R^2 = 0.12$; P = 0.02).

Geographical effects

Within the main study region (Emsland) there was no significant correlation between the genetic distance (pairwise F_{ST}), and the pairwise geographical distance (Mantel-test, r = 0.17, P = 0.11; intercept = 0.01 ± 0.007 , slope = 0.004 ± 0.001). The Mantel-test for the entire study area revealed an r-value of 0.34 (P = 0.02; intercept = 0.05 ± 0.003 , slope = 0.0003 ± 0.00002 ; Fig. 2), indicating a weak but significant isolation-by-distance. Further analysis of spatial autocorrelation revealed no linear relation to geographic distance (Fig. 3).



Figure 3 Genetic autocorrelation (r) as a function of distance, with a null hypothesis of a random pattern of genotypes (error bars are +/- SE for r determined by bootstrapping). The 95% confidence interval (CI) is depicted by a dotted line (note the increase in CI with larger distances due to fewer replicates).

Significant positive correlation coefficients were only detected within a range of 20 kilometres (10 km r = 0.15, P = 0.002; 20 km r = 0.11, P = 0.001). With increasing distance, correlation coefficients differed not significantly from zero.

Analysing the genetic diversity and inbreeding coefficients of aggregations according to their geographic origin revealed significant differences in allelic richness ($F_{2,15} = 5.3$; P = 0.018), H_E ($F_{2,15} = 5.54$; P = 0.016; Fig. 4), H_O ($F_{2,15} = 27.45$; P < 0.001; Fig. 4) and F_{IS} ($F_{2,15} = 20.86$; P < 0.001). The aggregations from the Rhineland had the lowest allelic richness (pairwise t-test with Bonferroni correction, P = 0.02), H_E (P = 0.02) and H_O (P < 0.001), as well as the highest F_{IS} (P < 0.001).



Figure 4 Mean expected heterozygosity (H_E , black bars) and observed heterozygosity (H_O , white bars) in the three sampled regions (Emsland: n = 11, Osnabrück: n = 3, Rhineland: n = 4). Note that both, H_E and H_O are significantly higher in the Emsland than in the Rhineland (error bars are +/- SE).

Discussion

Our data show that the nest aggregations of *A. vaga* are only slightly differentiated, although the species is strongly specialized on willow (*Salix*) pollen as larval food and sandy soils as nesting habitats. These results support the hypothesis that pioneer species are able to maintain a high level of gene flow despite a strong degree of specialization. Due to its high dispersal capability and large population sizes, *A. vaga* is well adapted to the natural floodplain dynamics. Similar low levels of population differentiation were found in highly specialized, but dispersive fig wasps (Zavodna et al. 2005) and bark beetles (Sallé et al. 2007). Nevertheless, we found a stronger degree of genetic differentiation in regions with stronger anthropogenic impacts, indicating that a reduced habitat availability increases the fragmentation of populations even in this highly dispersive species.

The AMOVA indicated that the highest variance occurred among individuals within aggregations and was only slightly influenced by the affiliation to or spatial arrangement of nest aggregations. Even if the Rhine populations were included, only a weak influence of the geographical distance on the genetic differentiation was found. Within a small spatial scale (< 20 km), there was a significant positive spatial autocorrelation, indicating that nest

aggregations within this distance are of greater genetic similarity (Harper et al. 2003). However, our results show no linear decline in the correlation coefficient suggesting a random pattern of differentiation.

The low F_{ST} values at the river Hase (average F_{ST} : 0.03) compared to the Ems (average F_{ST} : 0.07) and Lower Rhine valley (average F_{ST}: 0.09) might be caused by the differing landscape structure of these areas. The restoration measures at the river Hase created new habitats (Stroh et al. 2005), which might serve as stepping stones between populations resulting in genetic homogenisation. The Ems area is stronger fragmented by pine forests (Pinus sylvestris), which might increase the separation of populations of A. vaga. These populations might also be of higher age than at the Hase, leading to a stronger degree of differentiation (Le Corre and Kremer 1998). Compared to the Emsland, the Lower Rhine valley is characterized by intensive agriculture and urbanisation (van Dijk et al. 2006). Hence, the availability of habitats for A. vaga is rather low and the existing populations are stronger fragmented than in the Emsland, which has a much (four times) lower human population density. Moreover, the availability of habitats for A. vaga is positively influenced by the Pleistocene sands which dominate northern Germany. The degree of differentiation in the Rhine valley is comparable to the pattern found in a rare Bumblebee (Bombus muscorum) which seems to be particularly affected by habitat loss and isolation (Darvill et al. 2006). This is also reflected by the fact that A. vaga is red listed in Westphalia (Kuhlmann 1999), while it is widespread in Lower Saxony (Theunert 2002). These conclusions are supported by the reduced genetic diversity (allelic richness and H_E) and higher levels of inbreeding in the Rhineland populations compared to the Emsland (Fig. 4).

Habitat fragmentation is known to have a substantial influence on pollinator communities and floral reproductive success, but detailed studies on specific responses to fragmented nesting sites and floral resources are sparse (Cane 2001). The effects of habitat fragmentation are believed to be strongly related to the degree of specialization (Packer et al. 2005; Zayed et al. 2005). In fact, *A. vaga* is strongly specialized and not polylectic as proposed by Packer et al. (2005). Some authors (e.g. Sallé et al. 2007) even suggest that strong food specialization acts as a selective force favouring individuals with high dispersal capabilities. Moreover, specialization does not represent a threat *per se* as long as the availability of nesting habitats and pollen resources is high (Peterson and Denno 1998). This conclusion is rather logical if one considers the high number of specialized but common butterfly species feeding on widespread plants (e.g. *Inachis io* feeding on *Urtica dioica*). Furthermore, highly specialized pioneer species should be able to react rapidly on changes in their dynamic habitat and,

therefore, should maintain high levels of genetic variability. Flooding events are known to have dramatic consequences for populations of *A. vaga* as the brood cells are not water resistant (Fellendorf et al. 2004). Hence, a high rate of dispersal might compensate for local extinctions. This hypothesis is supported by the fact that about 50% of the emerging females of *Andrena vaga* are known to emigrate (Bischoff 2003).

Despite the comparatively weak genetic differentiation of A. vaga populations compared to other insects (Darvill et al. 2006; Repaci et al. 2006), most populations had rather high inbreeding coefficients and a deficiency of heterozygotes for most microsatellite loci. In the Emsland populations heterozygote deficiency occurred only in single loci, but nevertheless inbreeding coefficients were positive, ranging from 0.26 to 0.42 (Rhineland: 0.43 to 0.57). Deviations from HWE can generally be caused by a variety of factors including non-random mating, population subdivision and the presence of null alleles (Callen et al. 1993). Although Microchecker detected null alleles, all loci were successfully amplified in all individuals, suggesting little importance of null alleles in our analysis (see also Stahlhut and Cowan 2004 for discussion). Homozygote excess has also been reported from other Hymenoptera (Paxton et al. 1996; Danforth et al. 2003; Zayed et al. 2005, Stow et al. 2007). It is usually ascribed to inbreeding events within populations and seems to be strongly related to the life history strategies of the species involved (Paxton et al. 1996; Zavodna et al. 2005; Stahlhut and Cowan 2007). One explanation for this general excess of homozygotes in Hymenoptera might be found in the lower effective population sizes of haplo-diploid organisms (Packer and Owen 2001). However, it is likely that the deviation from HWE in A. vaga is also influenced by its breeding system and nesting behaviour. The high nest densities of A. vaga increase the possibility of inbreeding as males wait at nest aggregations for females, whereas in solitary nesting species males patrol flowers or landmarks seeking for mates (Paxton 2005). The latter is suggested to support random mating (Stahlhut and Cowan 2004). In fact, we found lower degrees of inbreeding in a solitary nesting species, Andrena fuscipes (unpublished data), while Paxton et al. (1996) documented even higher degrees of inbreeding in the communal nesting species Andrena carantonica Pérez, 1902. In this species, 70% of the females mate with nestmates before emerging from their natal nest (Paxton and Tengö 1996).

Although the populations of *A. vaga* seem to be highly inbred, they are characterized by a high allelic richness (4.9-7.5) compared to other studies on bees (Darvill et al. 2006; Francisco et al. 2006). This pattern might be influenced by the wide distribution and the high mobility of this species. A high gene diversity and low degree of differentiation has also been recorded for other widespread insects (Vandewoestijne et al. 1999; Schmitt and Hewitt 2004).

Solitary bees have a rather small foraging range, therefore local habitat structure is believed to be of particular importance for the maintenance of viable populations (Williams and Kremen 2007). Detailed studies on the dispersal ability of wild bees are still needed, as the existing studies mainly concern bumblebees (Osborne et al. 1999, Chapman et al. 2003, Knight et al. 2005), while our knowledge of the dispersal ability of solitary wild bees is sparse. The foraging range of pollinators is considered to be positively correlated with body size (Gathmann and Tscharntke 2002). Hence, a limited foraging range makes small species stay in one habitat (Tscharntke and Brandl 2004). However, Zayed et al. (2005) point out that the distance wild bees cover during their foraging trips are not necessarily related to the dispersal ability and can not be used to predict gene flow. Hence, the dispersal capability of wild bees might often be underestimated substantially.

Conclusions

Our results show that populations of the specialist bee *A. vaga* exhibit a high genetic diversity associated with a low overall population differentiation. Thus, we can not support current theories describing a generally higher threat of specialized wild bee species (Packer et al. 2005; Zayed et al. 2005). The population genetic structure rather seems to be dependent on the availability of the floral host and nesting habitats. As floodplain pioneer species can be severely affected by unpredictable events, such as flooding, a lower genetic variability might decrease the adaptability of the populations substantially. Although the populations of *A. vaga* seem to be still connected in large parts of the Emsland, our results show that highly specialized species might become threatened when habitat availability is decreasing. During the last century the natural habitat of *A. vaga* has been degraded by canalisation of rivers, drainage of wetlands, and cultivation of dry grasslands resulting in decreasing availability of the floral host *Salix sp.* and nesting sites (Winfree et al. 2007). This process has probably affected the populations in the Lower Rhine valley, but not the populations in the Emsland, as the availability of suitable habitats is still high.

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POPULATION GENETIC STRUCTURE OF A HEATHLAND SPECIALIST, ANDRENA FUSCIPES (HYMENOPTERA: ANDRENIDAE)



Flowering stands of Calluna vulgaris in the nature reserve "Borkener Paradies"

Abstract

Habitat fragmentation is believed to be a key threat to biodiversity as it decreases the probability of survival of populations, reduces gene flow among populations and increases the possibility of inbreeding and loss of genetic diversity within populations. Heathlands represent excellent systems to study fragmentation effects as the spatial and temporal course of fragmentation is well documented for these habitats. At the beginning of the 19th century, heathlands were widespread in northern Germany, but they became increasingly fragmented at the end of the 19th century until only few fragments had been left. As many insect species are strongly specialised on heathland habitats, they represent ideal study systems to test the genetic effects of such recent fragmentation processes. The solitary bee Andrena fuscipes is strongly specialised on heather (Calluna vulgaris) and, therefore, occurs exclusively in heathland habitats. The species is red-listed in Germany and other parts of Europe. Here, we present an analysis of the genetic structure of twelve populations of Andrena fuscipes using eight microsatellite loci. The populations showed little geographical structure and the degree of genetic differentiation was low. Compared to related bee species, inbreeding coefficients were relatively low and seem to be mainly affected by the bees' solitary nesting behaviour.

Introduction

Habitat loss, degradation and fragmentation of habitats are major factors influencing the maintenance of biodiversity (Fahrig 2003; Tscharntke & Brandl 2004). Habitat fragmentation is believed to reduce gene flow among populations, leading to a loss of genetic diversity within populations and increased population differentiation (Fischer & Lindenmayer 2007). During the last century, the characteristic cultural landscape of Central Europe experienced a rapid transformation caused by socio-economic changes. Many semi-natural and traditional cultural habitats have been destroyed due to the intensification of agricultural land-use practices, including the use of fertilisers and new cultivation techniques (Bakker & Berendse 1999). Some traditional agricultural habitats, which had been shaped by centuries of human influence (e.g. extensive hay meadows, dry oligotrophic grasslands or heathlands), have meanwhile become rare and fragmented. Species confined to such habitat types are, therefore, among those listed under the highest red list categories.

Heathlands have been created by human land use on oligotrophic sandy soils, including sheep grazing, burning and sod cutting. These open habitats reached their maximum distribution during the 18th and early 19th century and became particularly widespread in the Netherlands, parts of Great Britain and northern Germany (Fig. 1a). They rapidly declined at the end of the 19th century and meanwhile have become restricted to small, isolated patches (Webb 1989; Fig. 1b, 1c). At present, heathland habitats are listed as critically endangered in Germany and other parts of Europe (Riecken et al. 2006) and are protected by the EU Habitats Directive (EU directive 92/43/EEC). However, heathlands are still threatened by changes in land-use, but also by the increasing nutrient deposition and rapid succession by shrubs and trees (Sedláková & Chytrý 1999). The remaining heathland fragments often represent local hot spots of biodiversity, as many endangered plant and animal species are adapted to the ecological conditions of these open, xerothermic habitats (Usher 1992; Dupont & Nielsen 2006). The prevailing warm and dry conditions particularly support a species rich insect fauna.

Given that heathland specialists are often unable to survive in other habitat types and the rapid fragmentation of their habitat is very well documented, populations of heathland species represent excellent model systems to study the genetic effects of habitat fragmentation. We studied the genetic effects of heathland fragmentation on *Andrena fuscipes*, a solitary bee which is specialized on heather pollen (*Calluna vulgaris*). Our main objective was to examine whether the relatively recent fragmentation influenced the genetic variability, the population

genetic structure or the magnitude of gene flow among populations of this highly specialized species.

Methods

Study object

Andrena fuscipes is a solitary nesting bee species, which is univoltine with a flight period from August to September. In Central Europe, the species is specialized on heather pollen (Calluna vulgaris) as larval food. Females build single nests in the ground and prefer dry, sandy soils (Westrich 1989). Although A. fuscipes is widespread in Central Europe, it has become rare and is listed as vulnerable in countries where red lists for Hymenoptera exist, such as Germany (Westrich et al. 1998), the Netherlands (Peeters & Reemer 2003) and Ireland (Fitzpatrick et al. 2006).



Fig. 1 Decline of heathland habitats (grey) in north-western Germany and location of the study sites (black triangles) (a) Distribution of heathland at the beginning of the 19th century (b) during the 1930s and (c) in 1980 (modified from Heckenroth 1985).

Study area and sampling

Females of A. fuscipes were sampled on C. vulgaris during peak flight activity (between 11.00 and 5.00 pm) in August and September 2005 and 2006. Twelve locations in north-western Germany were sampled (Fig. 1), comprising a total of 195 individuals and spanning a geographical distance from less than 4 kilometres to 150 kilometres (Tab. 1). We tried to collect at least 20 specimens in each population. However, A. fuscipes does not nest in high densities and some populations were too small to achieve this number. The study area is located in Lower Saxony and North Rhine-Westphalia. The sampling sites have been assigned to four regions. Five sites were located in the "Emsland", four sites in the "Nordhorn" area

and two in "Steinfurt". In addition, we sampled one population at a greater distance ("Senne"). The whole study region is strongly characterized by intensive agricultural land use.

DNA isolation and amplification

Genomic DNA was extracted from thoracic muscle tissue using the DNeasy Tissue Kit (Qiagen, Hilden, Germany), following the manufacturers' protocol. Each sample was genotyped at eight microsatellite loci (vaga01, vaga02, vaga08, vaga12, vaga13, vaga18, vaga20 and AJ01) developed by Paxton et al. (1996) and Mohra et al. (2000). The loci were amplified separately using the HotMasterMix (Eppendorf, Hamburg, Germany). The 5'-end of each forward primer set was labelled with a fluorescent marker, either 5-FAM, JOE or TAMRA. The products were genotyped on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Fragment lengths were determined using GENESCAN 3.1 and GENOTYPER 2.5 (Applied Biosystems, Inc., Foster City, CA, USA).

Statistical analysis

Tests for genotypic linkage disequilibrium were calculated with Fisher's exact test and a Markov-chain method using GENEPOP 3.4 (Raymond & Rousset 1995). The presence of null alleles was inspected using MicroChecker 2.2.3 (Van Oosterhout et al. 2004). Allelic richness (R) and observed and expected heterozygosity for each population were calculated in GenAlEx 6.0 (Peakall & Smouse 2006), which performs a Chi-square test to asses the significance of a departure from Hardy-Weinberg-Equilibrium (HWE). The measure of allelic richness R was used as it is independent of sample size (El Mousadik & Petit 1996). To evaluate inbreeding effects, the inbreeding coefficient (F_{1S}) was calculated for each population within FSTAT 2.9.3 (Goudet 1995). To test whether the populations passed a recent genetic bottleneck we used the program Bottleneck 1.2.02 (Cornuet & Luikart 1996). A two-phased model of mutation (TPM) was applied in this analysis and the significance was determined by a Wilcoxon signed-rank test (1000 replications). Since the size and the type of management (grazing, military, no management) varied between the sampled locations, we tested the effects of these traits on the allelic richness (R) and the inbreeding coefficient (F_{IS}) with an analysis of covariance (ANCOVA) in the program "R 2.7.1" (R Development Core Team 2008).

The population genetic structure among the study region was quantified with different methods. We performed an initial comparison of the genetic structure within and among the sampled regions (Emsland, Nordhorn and Steinfurt together with Senne) using analysis of

molecular variance (AMOVA) in GenAlEx 6.0 (Peakall & Smouse 2006). Furthermore, we analysed the genetic structure among the sampled regions with a Bayesian model of population structure as implemented in BAPS 3.2 (Corander & Marttinen 2006). For this purpose we used an admixture based clustering method regarding the sampled regions (admixture based on pre-defined populations/regions). BAPS uses a stochastic optimization algorithm which runs considerably faster than MCMC-based algorithms and has increased power to detect differentiation at small geographical distances (Corander & Marttinen 2006; Latch et al. 2006).

Chord distances (D_C) between the sites were calculated in Microsat 1.5b (Minch 1997) and the program Phylip 3.57c (Felsenstein 1993) was used to visualize these as an unrooted UPGMA tree. Differentiation between pairs of populations was examined with a loglikelihood based exact test (G-test), which tests the distribution of genotypes between each pair of population as implemented in FSTAT (Goudet 1995). The significance of these tests was adjusted using standard Bonferroni corrections. This method seems to be particularly efficient for non-random mating populations and a low overall populations structure (Petit et al. 2001).

To test for isolation-by-distance, we calculated pairwise F_{ST} distances based upon Wright's Fstatistics (Wright 1951) with the program GenAlEx 6.0 and tested these for a correlation with geographical distances. A Mantel-test of matrix correlations and a reduced major axis (RMA) regression to estimate the intercept and slope of the isolation-by-distance relationship was performed within the program IBD 1.52 (Bohonak 2002).

Results

Genetic diversity

A departure from Hardy-Weinberg expectations (excess of homozygotes) was found in 37.5% of the 96 locus-population combinations. A potential presence of null alleles was determined in 23 of the 96 cases, but all loci did amplify in all individuals. Tests for linkage disequilibrium were not significant after Bonferroni correction. Overall microsatellite variability was high (2-17 alleles per locus and population). Allelic richness ranged from 4.6 to 6.7 (averaged over all loci) and mean expected heterozygosity (H_E) ranged from 0.38 to 0.87 (Tab. 1).

sample site	region	size (ha)	Ν	management	R	F _{IS}	$H_{\rm E}$	Ho
A1	Emsland	1.51	20	grazing	5.65	0.196	0.68	0.56
A2	Emsland	0.03	8	none	4	0.04	0.59	0.61
A3	Emsland	1.74	23	grazing	4.94	0.133	0.60	0.53
A4	Emsland	2.88	15	grazing	5.03	0.268	0.58	0.44
A5	Emsland	1.31	8	grazing	5.38	0.285	0.62	0.48
B1	Nordhorn	25.84	20	grazing	4.76	0.35	0.56	0.38
B2	Nordhorn	2.96	10	military	4.57	0.259	0.63	0.50
B3	Nordhorn	3.90	20	none	5.66	0.191	0.66	0.55
B4	Nordhorn	24.48	16	military	5.81	0.167	0.69	0.59
C1	Steinfurt	1.39	26	grazing	5.56	0.153	0.67	0.58
C2	Steinfurt	1.20	21	none	5.27	0.123	0.63	0.57
D1	Senne	9.12	8	grazing	4.63	0.176	0.58	0.52

Table 1 Characteristics of the analyzed *Andrena fuscipes* populations (size (ha) = size of the sampled heathland fragment; N = sample size; R = mean allelic richness; F_{IS} = inbreeding coefficient; H_E = expected heterozygosity; H_O = observed heterozygosity)

Positive inbreeding coefficients (F_{IS}) were detected in all populations, ranging from 0.04 to 0.35. We detected no effects of fragment size or the management type (grazing, military, no management) on allelic richness or inbreeding coefficient (Tab. 2). The bottleneck analysis revealed a significant excess of heterozygosity for one population (A2; p = 0.039), indicating a recent bottleneck event.

Table 2 Results of the ANCOVA for effects of management type, heathland size or study region on the inbreeding coefficient (F_{IS}) and allelic richness (R).

	management	size	region
F _{IS}	$F_{1,2} = 2.01; p = 0.19$	$F_{1,10} = 2.05; p = 0.18$	$F_{3,8} = 0.7; p = 0.58$
R	$F_{2,9} = 0.1; p = 0.91$	$F_{1,10} = 0.2; p = 0.67$	$F_{3.8} = 0.49; p = 0.7$

Differentiation among populations

The AMOVA revealed that 94 % of the variance was explained by variation within populations, 6 % among populations within regions. No variance was explained by the regional arrangement of populations. Genetic differentiation was generally low (Tab. 3) with the highest pairwise population differentiation in the Emsland area (average $F_{ST} = 0.054$).

	A1	A2	A3	A4	A5	B1	B2	B3	B4	C1	C2	D1
A1	Х	*	***	NS	*							
A2	0.07	Х	**	***	NS	**	NS	NS	*	*	***	**
A3	0.07	0.06	Х	**	***	***	**	NS	***	***	***	NS
A4	0.03	0.07	0.05	Х	***	NS	NS	NS	NS	NS	NS	*
A5	0.01	0.06	0.07	0.04	Х	NS	NS	*	NS	NS	NS	*
B1	0.03	0.10	0.04	0.02	0.05	Х	NS	NS	NS	NS	NS	*
B2	0.01	0.03	0.04	0.05	0.03	0.04	Х	NS	NS	NS	NS	NS
В3	0.04	0.04	0.01	0.04	0.06	0.03	0.01	Х	*	NS	**	NS
B4	0.02	0.08	0.06	0.01	0.02	0.04	0.03	0.04	Х	NS	NS	NS
C1	0.01	0.05	0.03	0.03	0.02	0.02	0.00	0.01	0.02	Х	NS	NS
C2	0.02	0.06	0.04	0.04	0.02	0.03	0.01	0.03	0.03	0.01	Х	***
D1	0.06	0.10	0.05	0.06	0.09	0.06	0.06	0.03	0.03	0.05	0.08	Х

Table 3 Pairwise population F_{ST} below diagonal, significance of pairwise population differentiation after Bonferroni correction above diagonal.

Genetic admixture analysis using BAPS revealed different degrees of admixture for the presumed clusters (Fig. 2) with cluster 2 (Nordhorn) showing the highest rate of admixture supporting a high genetic exchange. In cluster 1 and 3, 42% of the individuals were assigned to the corresponding region with a probability of more than 0.75.



Fig. 2 Assignment probabilities of individual genotypes from 12 populations to predefined clusters using BAPS.

These results were also supported by an UPGMA tree (Fig. 3), in which the arrangement of populations reflects their geographic origin to some extent. While populations located in the Emsland area were differentiated (Fig. 3), the Steinfurt populations C1 and C2 clustered together with populations from the "Nordhorn" region. Population D1 (Senne) branched of basally with the greatest genetic distance to all other populations. We found a weak but non-significant isolation-by-distance effect among the populations (Mantel test, r = 0.103, P = 0.134, Fig. 4).



Figure 3 Unrooted UPGMA tree based on Cavalli-Sforza & Edwards' (1967) chord distance (D_c) derived from allele frequencies at eight microsatellite loci. Branch length reflects the genetic distance between populations.



Fig 4 Relation between gene flow and geographic distance among sites (F_{ST} vs. geographic distance; $r^2 = 0.103$ p = 0.13)

Discussion

Habitat fragmentation and genetic differentiation

Our analyses revealed a weak but significant genetic differentiation among the populations of A. fuscipes, indicating that the relatively recent fragmentation of heathland habitats has reduced gene flow only marginally. The Emsland samples were stronger differentiated than populations in the Nordhorn or Steinfurt area despite the greater geographical distance between some of the latter populations. These results indicate that geographical distance per se could not explain the degree of genetic differentiation. In fact, we only found a weak (and non-significant) isolation-per-distance. Similar results have been found in other bee species, such as the bumblebees Bombus pascuorum and B. sylvarum and the sand bee Andrena vaga, where differences in the landscape structure accounted for a restricted gene flow rather than geographical distance (Widmer & Schmid-Hempel 1999; Ellis et al. 2006). It is likely that the mobility of a species strongly influences the genetic structure among populations. Strong gene flow and genetic admixture across populations is often found among strong flyers, such as bees (Chapman et al. 2003), while populations of flightless insects often exhibit a clear

geographic structure (Witzenberger & Hochkirch unpublished data). A strong degree of admixture might be particularly true for solitary nesting bee species, which need to be more mobile in order to find mates. In addition, small populations of A. fuscipes might persist at forest edges or other places with local occurrence of C. vulgaris. Such sites might serve as stepping stones increasing the dispersal among populations.

A high number of studies on the effects of landscape structure and fragmentation on population differentiation have been carried out during the last decades, many of which revealed conflicting results (Keyghobadi et al. 2005). The degree of specialization is often assumed to have negative effects on dispersal ability and, consequently, the effects of fragmentation are believed to be stronger for habitat specialists than for generalists (e.g. Kitahara & Fujii 1994; Kelley et al. 2000). This has been particularly proposed for specialized wild bee species which are often specialized on single plant species or genera as larval food (Packer et al. 2005; Zayed et al. 2005). On the other hand, one might argue that specialized species may be adapted to the sparse occurrence of their resources and may avoid negative effects of a patchy habitat structure by a higher dispersal ability or a higher effective populations size (Peterson & Denno 1998; Sallé et al. 2007). It is likely that heathland habitats were strongly fragmented before they were spread by human activities. Species adapted to such naturally fragmented habitats might have stronger dispersal abilities than those occurring naturally in strongly interconnected habitat types (e.g. forests). Interestingly, the genetic differentiation of A. fuscipes populations was even lower than in a related bee species, Andrena vaga, which is adapted to highly dynamic floodplain habitats. Both species are specialized on a single pollen resource (Calluna and Salix, respectively), showing that a high degree of specialization is a poor predictor of genetic divergence between populations.

Due to the loss of heathland habitats, A. fuscipes has become threatened in parts of Europe. However, the extinction of populations of this bee is not only caused by the decrease of its floral host. A recent study of the bee communities in the Emsland has shown that A. fuscipes is often absent even if its host, C. vulgaris, is present (von der Heide & Metscher 2003). This illustrates that other factors than host plant occurrence are important predictors of the occurrence of this species, such as the availability of nesting sites or the size of the habitat, which must sustain a minimum viable population (Tscharntke 1991). Indeed, many heathland patches in northern Germany are small and dominated by old, degenerated and even-aged stands of heather. The dense vegetation reduces the availability of suitable nesting sites. It has also been shown in other insect species that vegetation structure is often a more important component of the habitat than vegetation composition (e.g. Gröning et al. 2007). Thus, the suitability of heathlands for sustaining a diverse insect fauna is particularly influenced by habitat management (Usher, 1992; Assmann & Jansen 1999). Sheep grazing, burning and sod cutting are important management types in heathland habitats as they create a high structural heterogeneity. Non-managed heathlands may lose many insect species as the heather stands become dense and high, shading open sand patches, which are particularly important for insects adapted to xerothermic conditions (Wuellner 1999). Although we did not find any effects of management types on the genetic diversity of A. fuscipes populations, local extinction processes of this species might be affected by the reduction of potential nesting sites or decreasing nectar and pollen availability due to the aging of heather plants (von der Heide & Metscher 2003).

Genetic variability and inbreeding

Compared to microsatellite analyses in other bee species, A. fuscipes populations had a similar or even higher genetic variability (Paxton et al. 1996; Beveridge & Simmons 2006). These results indicate that the populations of A. fuscipes are either still large enough to maintain a relatively high genetic diversity or that the high variability is a consequence of the former widespread distribution of Calluna heathlands in northern Germany (Fig. 1), which might have supported large populations of A. fuscipes for a long time. The first hypothesis is supported by Peeters (pers. comm.), who found A. fuscipes still rather common in the Netherlands, which is adjacent to our study sits. We also found only little evidence for genetic bottlenecks. This possibility was suggested only for one population in the Emsland (population A2). This population occurred in a small heathland fragment with old stands of heather plants and was one of the smallest populations studied. It is possible that the relatively recent habitat fragmentation might reduce the chance to detect bottlenecks (Ellis et al. 2006).

In all populations, we detected significant reduced heterozygosities compared to HWE and positive inbreeding coefficients (ranging from 0.04 to 0.27). Nevertheless, deviations from HWE were rather sparse compared to other Hymenoptera (Paxton et al. 1996; Danforth et al. 2003; Zayed et al. 2005; Stow et al. 2007). A reduced heterozygosity might be caused by several factors, including non-random mating, population subdivision and the presence of null alleles (Callen et al. 1993). Although the program MicroChecker suggested the occurrence of some null alleles in our data, all loci were successfully amplified in all individuals. Hence, they might be of little importance for our analyses (see also Stahlhut & Cowan 2004 for discussion). It seems to be more likely, that the excess of homozygotes is a result of high levels of inbreeding which has been reported for several bee species as a result of their nesting

strategies (Paxton et al. 1996; Danforth et al. 2003). As Hymenoptera are haplodiploid organisms, inbreeding might also be a result of reduced effective population sizes (Packer & Owen 2001). Nevertheless, the inbreeding coefficients of A. fuscipes were relatively low compared to other bee species. This might be the consequence of the solitary nesting behaviour of A. fuscipes. Males of solitary nesting bee species patrol at flowers to search for females, whereas males of species nesting in aggregations often stay at the nesting site or even in the natal nest to mate (Paxton 2005).

Conclusions

Studies on fragmentation effects on the genetic structure and diversity of populations are common, but heathlands have rarely been studied in this context, although they are highly suitable for such analyses. Due to the strong losses of heathland habitats in northern Germany, we expected a high degree of genetic differentiation and a low degree of gene flow among the remaining heathland fragments. However, our results show that strong flyers, such as bees, might be only little affected by recent fragmentation events of former continuous habitats. The variable genetic differentiation observed among A. fuscipes populations might reflect different stages of an ongoing process of genetic isolation. The relatively high levels of genetic variability and relatively low levels of inbreeding might be affected by its solitary nesting behaviour.

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GENETIC DIVERSITY AND INBREEDING IN BEES (HYMENOPTERA, APOIDEA) - A COMPARISON OF ALLOZYME AND MICROSATELLITE DATA



Nomada lathburiana (left side) cuckoo bee of Andrena vaga (right side)

Abstract

Understanding the factors that maintain the genetic diversity of populations is an important task in evolutionary and conservation biology. An increasing number of studies on the genetic structure of populations has been published. Most of these studies focus on single species, whereas comparative studies are sparse. We present an analysis of existing allozyme and microsatellite studies on 52 bee species (Apoidea) to identify factors influencing inbreeding coefficients (F_{IS}) and expected heterozygosities (H_E). Bees are particularly suited for such analysis, as they exhibit a high variability of life history traits. In allozyme studies, nesting behavior, food specialization, family affiliation and the number of polymorphic loci affected H_E . These effects disappeared, if only polymorphic loci were included. Microsatellite data revealed no significant effects for any explanatory variable. Our results suggest that polymorphism and not H_E is affected by life history traits of bees. The differing results between the two marker systems seem to be mainly caused by the fact that allozyme studies usually include mono- and polymorphic loci, whereas microsatellite studies generally use only polymorphic loci. Future studies should focus on the effects of extrinsic factors such as habitat fragmentation or population size.

Introduction

The maintenance of genetic variability within and among populations is of major interest for understanding evolutionary processes as well as for the conservation of species (Lande 1995; Hedrick & Kalinowski 2000). Loss of genetic diversity is believed to be a key threat to biodiversity as it decreases the adaptability of populations and increases the rate of inbreeding, which might subsequently lead to inbreeding depression (Frankham et al. 2002). Hence, there is a high interest in identifying factors that determine genetic variability and rates of inbreeding in certain organisms. During recent decades, an increasing number of studies on the genetic structure of populations have been published (Hedrick 2001). This process has been accelerated by the development of new molecular techniques and new statistical methods, which offer a variety of sophisticated analyses (Excoffier & Heckel 2006). Codominant marker systems, such as allozymes or microsatellites, represent ideal tools for estimating rates of inbreeding (Hedrick & Kalinowski 2000). The application of such methods across a wide range of species might enable us to uncover principal determinants of genetic variability and rates of inbreeding. However, most population genetic studies focus on a single species or compare a few related species, whereas comparative studies across a wide range of species are sparse (Pamilo et al. 1978; Owen et al. 1992).

The recent decline and increasing extinction risk of many wild bee species (Hymenoptera, Apoidea) and the resulting ecological and economic consequences ("pollination crisis") are currently strongly debated (Biesmeijer et al. 2006; Butler et al. 2007) and gave rise for conservation genetic studies of bees (Goulson et al. 2008). Many species of wild bees are specialized on certain pollen resources and nesting habitats (Kratochwil 2003). The degree of pollen specialization has, therefore, been assumed to affect the genetic diversity and levels of inbreeding in bee populations (Packer et al. 2005; Zayed et al. 2005). Oligolectic bees which are restricted to specific host plants, have been proposed to be particularly prone to inbreeding and loss of genetic variability (Zayed & Packer 2007). However, recent studies suggest that food specialization alone is a bad predictor of genetic variability (Peterson & Denno 1998). Habitat availability and persistence, dispersal capacity, mating behavior and nesting strategies might also influence the genetic structure of wild bee populations (Carvell et al. 2006; Herrmann et al. 2007). Moreover, bees have become classic study systems in sociobiology as they exhibit different levels of social behaviour within and between species (Packer 1991; Chenoweth et al. 2007; Stow et al. 2007; Wilson & Wilson 2007). It is reasonable to suggest that the evolution of social behavior has been strongly influenced by genetic constrains

(Linksvayer & Wade 2005) and, therefore, one might also expect an effect of the social status of a species on its rate of inbreeding (Michod 1993; Soucy & Danforth 2002).

Nesting strategies are highly variable among bees. Some species build single nests in strong distance to conspecifics, whereas other species nest in aggregations or even in the same nest (communal nesting). Communal nesting behavior occurs among various bee families. In andrenid bees it represents the most complex form of social behavior (Michener 1974), whereas other bee families (such as halictine bees) consist of solitary, communal and eusocial species (Kukuk & Sage 1994). Nest members of communal nesting species often cooperate during nest building (Evans & Shimizu 1996). Hence, these species may benefit from rapid nest foundation during short vegetation periods and a better defense against parasitoids (Danforth et al. 1996). On the other hand, group nesting might increase the risk of inbreeding (Paxton & Tengö 1996), a problem which might be solved in eusocial species by excluding a high number of individuals (the workers) from reproduction. Bee species strongly differ in their mating behavior and particularly in their strategy to search for females (Thornhill & Alcock 1983). Encounter sites may be the sites of female emergence, sites where females forage or special landmarks. As females are not randomly distributed (Parker 1978), males often concentrate their efforts to find a mate at locations where females occur in higher frequency. Hence, male strategies of mate acquisition seem to correlate with female nesting behavior (Paxton 2005). Males of species which nest in aggregations often use the nesting sites for mating, whereas males of solitary nesting species search at foraging sites for females. It has been suggested that inbreeding is strongly linked with such different mating systems (Paxton 2005) as the communal bees Andrena carantonica and Macrotera portalis exhibit high levels of inbreeding possibly as a consequence of intra-nest mating (Paxton et al. 1996; Danforth et al. 2003).

Here, we review existing studies on the population genetics of several bee species. We were particularly interested in the question which factors determine inbreeding and expected heterozygosity in these species. For this purpose, we created a database containing 23 publications on the population genetics of 52 bee species. We analyzed the two major co-dominant marker systems (allozymes: 38 species; microsatellites: 18 species) separately, as variability is known to be substantially higher in microsatellites than in allozymes. Our analysis included the degree of specialization, nesting strategy and family affiliation as potential explanatory variables, as well as the phylogenetic history of the species involved.

Methods

We analyzed publications (Supplementary table S1) dealing with the population genetics of different bee species using either microsatellite or allozyme markers. We particularly searched for studies presenting estimates of expected heterozygosity (H_E) averaged over multiple loci and/or inbreeding coefficients (F_{IS}). If data on inbreeding coefficients were not provided but measures of the observed and expected heterozygosity (Ho and HE) were given, we calculated values for F_{IS} according to Wright (1931): $F_{IS} = H_E - H_O/H_E$. Since microsatellites usually reveal much higher levels of genetic diversity than allozymes (Frankham et al. 2002), we analyzed each marker system separately. Moreover, we performed two analyses for the allozyme data set, as authors may either include all loci in their analyses (e. g. Pamilo et al. 1978; Packer et al. 2005) or only polymorphic loci (Zayed et al. 2005), while microsatellite studies usually only consider polymorphic loci. A study was only included, if sample size (number of individuals and populations) and the population genetic parameters mentioned above were presented. In order to avoid pseudoreplication, we used only one study per species (the one with the greatest sample size). We included family affiliation, nesting behavior (composed of single, aggregated, communal and social nesting strategies) and food specialization as explanatory variables for the variation in inbreeding coefficients and expected heterozygosities. We used the number of polymorphic markers, the number of sampled localities and the number of haploid genomes (i.e. the total number of males and 2 times the total number of females) as covariates. If information on any explanatory variable was not provided in the original publications, we gathered information from the literature (e. g. Michener 1974; Westrich 1989; Michener 2000).

All statistical analyses were carried out in R 2.5.0 (R Development Core Team 2007). We first performed hierarchical partitioning to uncover the relative explanatory power of each variable using the "hier.part" package for R (Walsh & Mac Nally 2003). This method is particularly suited to uncover the average independent contribution of each explanatory variable and to decide which variables aid in explaining patterns of variation (Mac Nally 2002). All parameters with an explanatory power > 5% were afterwards included in an ANCOVA in the order of decreasing explanatory power. If necessary, data were Box-Cox-transformed using Venables and Ripley's MASS library for R (Venables & Ripley 2002) to comply with the model assumptions. To test for phylogenetic contrasts, we constructed matrices of genetic distances between bee families estimated from Danforth et al. (2006) and differences in heterozygosity and F_{IS} between each family pair. We applied Mantel tests for

different matrix pairs to test for a significant relationship between the phylogenetic relations and the population genetic parameters using the package "ade4" for R with 999 randomizations (Chessel *et al.* 2004).

Results

Allozymes

Hierarchical partitioning revealed that four factors (nesting strategy, food specialization, family affiliation and the number of polymorphic loci) were the most important parameters affecting H_E . These factors were included in an ANCOVA and most of them were significant (Tab. 1; Figs 1-4). The highest heterozygosity was found for single nesting species followed by those nesting in aggregations and social species. Furthermore, H_E values were significantly lower for oligolectic than for polylectic species.

If only polymorphic loci were included in the analyses, the same parameters were chosen after hierarchical partitioning, but none of them was significant (Tab. 1; Figs 1-4). For both data sets (all loci and polymorphic loci), the Mantel test for a relationship between phylogenetic distances and differences in mean values for H_E among bee families showed no significant correlations (all loci: r = -0.081; p > 0.05; polymorphic loci: r = -0.269; p > 0.05).

Microsatellites

No phylogenetic trend was found for inbreeding coefficients F_{IS} and genetic variability H_E estimated from microsatellite data (F_{IS} : r = -0.48 p > 0.05; H_E : r = -0.51 p > 0.05). For inbreeding coefficients, hierarchical partitioning identified "family affiliation", "food specialization" and "nesting strategy" as potential predictors with the highest explanatory power. However, the ANCOVA revealed no significant effect for any of the variables (Tab. 1). Four factors (family affiliation, nesting strategy, number of localities, number of individuals) were proposed as potential explanatory variables for the variation H_E using hierarchical partitioning. Again, none of these predictors had a significant effect on the variation in H_E (Table 1).



Figure 1 Average expected heterozygosities of different bee families and marker systems.





Figure 2 Average expected heterozygosities of oligolectic and polylectic bee species revealed from different marker systems.



Figure 3 Average expected heterozygosities of bee species with different nesting strategies revealed from different marker systems.



Figure 4 Correlation between average expected heterozygosity (across all loci) and the number of polymorphic markers used in allozyme studies.
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Table 1 Results c polymorphic loci, inbreeding coeffici	f the ANCOV ₁ number of popu ents.	As on the ef alations studi	fects of nesting ed and number	g strategy, f of haploid ε	ood specializa genomes includ	tion, family led on expe	/ affiliation, r cted heterozy	number of gosity and
	Allozyme	s (H _E) ci	Allozymes polymorphi	(H _E) c loci	Microsate (H _E)	llites	Microsat	ellites
nesting strategy	$F_{2,29} = 15.14$	P < 0.001	$F_{2,28} = 0.29$	P > 0.05	$F_{3,9} = 0.48$	P > 0.05	$F_{3,8} = 0.99$	P > 0.05
food specialization	$F_{1,29} = 32.54$	P < 0.001	$F_{1,28} = 0.74$	P > 0.05	ı	I	$F_{1,8} = 0.35$	P > 0.05
family affiliation	$F_{4,29} = 2.50$	P = 0.064	$F_{4,28} = 0.50$	P > 0.05	$F_{3,9} = 0.30$	P > 0.05	${\rm F}_{3,8}=2.21$	P > 0.05
polymorphic loci	$F_{1,29} = 4.44$	P = 0.044	$F_{1,28} = 0.73$	P > 0.05	ı	ı		
populations	ı	·			$F_{1,9} = 0.02$	P > 0.05		
haploid genomes	I	ı	·	·	$F_{1,9} = 0.32$	P > 0.05		
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Discussion

The main objective of our study was to identify intrinsic factors that influence the genetic variability H_E and the inbreeding coefficient F_{IS} in wild bee species. Previous analyses suggested that food specialization might affect both the genetic diversity and levels of inbreeding in bees (Packer *et al.* 2005; Zayed *et al.* 2005). Other authors argued that nesting strategies and mating behavior might influence the genetic structure of wild bee populations (Paxton & Tengö 1996; Herrmann *et al.* 2007). Our analyses show that the results are strongly determined by the data included. If all allozyme loci were used, we found significant effects of both nesting strategy and food specialization on expected heterozygosities. However, if only polymorphic markers were analyzed, no significant effects of any potential explanatory variable were found for both allozyme and microsatellite studies. The reason behind this discrepancy might be that polymorphism and not H_E directly is influenced by food specialization and nesting strategy. This conclusion is supported by a significant effect of the number of polymorphic loci on H_E (Fig. 4).

Our results also show that a major problem in comparing microsatellite and allozyme studies is the different way in which both marker systems are often analyzed. While microsatellite studies usually apply only polymorphic loci, many allozyme studies use a high number of markers and also analyze monomorphic loci. These differences seem to have stronger consequences for our results than the marker system itself. After we removed the monomorphic loci from the allozyme data set, both marker systems revealed no significant results. Since the use of allozymes requires at least 10-20 polymorphic loci to assure a minimal statistical confidence (Parker *et al.* 1998) the results of studies including monomorphic markers should be considered with caution. On the other hand, some authors propose that allozyme loci should not be preselected for polymorphism (Lester & Selander 1979; Packer & Owen 2001).

Comparisons of microsatellites and allozymes within species usually report a lower variability for the latter marker system (Estoup *et al.* 1998; Gao *et al.* 2002). Indeed, our study shows that the same is true for interspecific comparisons (Figs 1-3). On average, H_E was 0.615 for the microsatellite loci, 0.025 for all allozyme loci and 0.173 for polymorphic allozyme loci. As many species are monomorphic for most allozymes and the number of loci that can be screened per species is limited (Parker *et al.* 1998), this marker system is not always useful for population genetic studies. In contrast, microsatellites often have a stronger discriminative power as they detect more genetic variation (Estoup *et al.* 1998). Microsatellites are more suitable for the assignment of individuals to populations, but scoring errors and homoplasy more often affect the results of this method (DeWoody *et al.* 2006).

In bees, the low heterozygosity revealed by allozyme studies has often been ascribed to the lower effective population size of Hymenoptera, which is caused by haplodiploidy (reviewed in Packer & Owen 2001). However, the relatively high values uncovered in microsatellite studies suggest that haplodiploidy is not a good predictor of H_E . Although the phylogenetic relationships and family affiliation had no significant effect in our analyses of polymorphic loci, hierarchical partitioning revealed that family affiliation explained a high percentage of the variance in all data sets. Thus, the phylogenetic classification should not be excluded as a potential explanatory variable. The analysis of phylogenetic correlations requires an adequate sample size to guarantee statistical validation (Freckleton *et al.* 2002) which was not the case for our data sets. Some families have rarely been studied (e.g. Anthophoridae have only been studied once, Beveridge & Simmons 2006), while others (particularly Apidae) are well studied (Pamilo *et al.* 1978; Owen *et al.* 1992; Repaci *et al.* 2006; Stow *et al.* 2007). Although the number of microsatellite studies increased rapidly during the last decade, only 18 studies were available for our analysis. It is likely that more microsatellite studies on population genetics of bees are needed to draw confident conclusions.

A topic which is closely related to the phylogenetic relationships is the evolution of social behavior. The influence of sociality on population genetics is discussed controversially. Some authors report similar levels of H_E in social and solitary bee species (Kukuk & Sage 1994), whereas others expect a decreasing H_E with increasing complexity of sociality (Packer & Owen 2001). In our study, nesting strategy explained a significant part of the variance in H_E only in the analysis of all allozyme loci, but not in the analyses of polymorphic loci. In the first case, species with a more complex social behavior had on average lower expected heterozygosities than single or aggregated nesting species. It has been suggested that relatedness among nestmates in communal nests provides the condition for the development of social behavior. Hence, communal nesting behavior of closely related individuals might be a preliminary stage of eusociality (Abrams & Eickwort 1981). In allodapine bees, Langer et al. (2004) revealed a positive effect of relatedness on reproduction and concluded that this represents the most important factor for the shift from solitary to social organization. In contrast, Kukuk et al. (2005) found in the halictine bee Lasioglossum hemichalceum that female biased dispersal may function as a mechanism to decrease the degree of inbreeding. High intranest relatedness and significant inbreeding coefficients have been reported for many communal species (Paxton et al. 1996; Danforth et al. 2003) leading to the assumption that inbreeding within the natal nest might be underestimated (Paxton 2005). In our analyses of polymorphic loci the effect of nesting strategy was not significant, but it should be noted that only three studies of communal nesting species were available (Paxton *et al.* 1996; Kukuk *et al.* 2002; Danforth *et al.* 2003). It is assumed that the complementary sex determination system (CSD) that occurs in four hymenopteran superfamilies (Apoidea, Vespoidea, Ichneumonoidea and Tenthredinoidea) has evolved as a mechanism to reduce inbreeding (Cook & Crozier 1995). In CSD species, individuals that are hemizygous (haploid) or homozygous (diploid) at the sex determining locus develop as males (Stahlhut & Cowan 2004). As a consequence of inbreeding the number of diploid males increases due to matched mating between females that are homozygous at the sex locus and males that carry an identical sex allele (Cook & Crozier 1995; van Wilgenburg *et al.* 2006). These highly inbred diploid males are excluded from further reproduction as they are sterile and have a reduced viability (Heimpel & de Boer 2008).

Altogether, the evidence for intrinsic effects on levels of heterozygosity and inbreeding is still sparse. Two major drawbacks of our analysis should be considered. First, the number of available studies is still sparse, and the sample size for some factor levels was probably to low to reveal a significant effect. Second, it is possible that extrinsic effects may blanket the effects of intrinsic factors on the rates of inbreeding and expected heterozygosities. It is well known that genetic bottlenecks and founder events may have strong effects on the genetic variability within populations (Nei *et al.* 1975). Hence, age, size and fragmentation of populations should be considered as potential explanatory variables. However, theses variables are often difficult to quantify reliably and such values are rarely presented in population genetic studies.

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Supplementary material

Table S1 (supplementary table): Publications included in the database

Reference	Species	Family	Marker system
Packer et al. 2005	Acamptopoeum submetallicum	Andrenidae	allozyme
Pamilo et al. 1978	Andrena clarkella	Andrenidae	allozyme
Pamilo et al. 1978	Andrena lapponica	Andrenidae	allozyme
Owen et al. 1992	Bombus bifarius	Apidae	allozyme
Owen et al. 1992	Bombus centralis	Apidae	allozyme
Owen et al. 1992	Bombus flavifrons	Apidae	allozyme
Owen et al. 1992	Bombus frigidus	Apidae	allozyme
Owen et al. 1992	Bombus huntii	Apidae	allozyme
Pamilo et al. 1978	Bombus hypnorum	Apidae	allozyme
Pamilo et al. 1978	Bombus lapidarius	Apidae	allozyme
Pamilo et al. 1978	Bombus lucorum	Apidae	allozyme
Owen et al. 1992	Bombus melanopygus	Apidae	allozyme
Owen et al. 1992	Bombus moderatus	Apidae	allozyme
Pamilo et al. 1978	Bombus pascuorum	Apidae	allozyme
Owen et al. 1992	Bombus sylvicola	Apidae	allozyme
Owen et al. 1992	Bombus ternarius	Apidae	allozyme
Pamilo et al. 1978	Bombus terrestris	Apidae	allozyme
Owen et al. 1992	Bombus terricola	Apidae	allozyme
Packer et al. 2005	Cadeguala occidentalis	Colletidae	allozyme
Packer et al. 2005	Caupolicana quadrifasciata	Colletidae	allozyme
Packer et al. 2005	Centris chilensis	Apidae	allozyme
Packer et al. 2005	Centris mixta	Apidae	allozyme
Packer et al. 1995	Coelioxys funeria	Megachilidae	allozyme
Packer et al. 1995	Coelioxys moesta	Megachilidae	allozyme
Packer et al. 2005	Colletes seminitidius	Colletidae	allozyme
Pamilo et al. 1978	Colletes succinctus	Colletidae	allozyme
Kukuk & Sage 1994	Lasioglossum hemichalceum	Halictidae	allozyme
Blanchetot & Packer 1992	Lasioglossum marginatum	Halictidae	allozyme
Packer et al. 2005	Leioproctus rufiventris	Colletidae	allozyme
Packer et al. 1995	Megachile inermis	Megachilidae	allozyme
Packer et al. 1995	Megachile relativa	Megachilidae	allozyme
McCorquodale & Owen 1997	Megachile rotundata	Megachilidae	allozyme
Packer et al. 2005	Neofidelia longirostris	Megachilidae	allozyme
Packer et al. 2005	Nolanomelissa toroi	Andrenidae	allozyme
Lester & Selander 1979	Nomia melanderi	Halictidae	allozyme
Owen et al. 1992	Psithyrus insularis	Apidae	allozyme
Owen et al. 1992	Psithyrus suckleyi	Apidae	allozyme
Packer et al. 2005	Trichothurgus aterrimus	Megachilidae	allozyme
Beveridge & Simmons 2006	Amegilla dawsoni	Anthophorini	microsatellite
Paxton et al. 1996	Andrena carantonica	Andrenidae	microsatellite
unpublished data	Andrena fuscipes	Andrenidae	microsatellite
unpublished data	Andrena vaga	Andrenidae	microsatellite
Darvill et al. 2006	Bombus muscorum	Apidae	microsatellite
Herrmann et al. 2007	Bombus pascuorum	Apidae	microsatellite
Ellis et al. 2006	Bombus sylvarum	Apidae	microsatellite
Estoup et al. 1996	Bombus terrestris	Apidae	microsatellite
Stow et al. 2007	Exoneura nigrescens	Apidae	microsatellite

Repaci et al. 2006	Exoneura robusta	Apidae	microsatellite
Kukuk et al. 2002	Lasioglossum hemichalceum	Halictidae	microsatellite
Zayed 2006	Lasioglossum leucozonium	Halictidae	microsatellite
Paxton et al. 2002	Lasioglossum malachurum	Halictidae	microsatellite
Zayed & Packer 2007	Lasioglossum oenotherae	Halictidae	microsatellite
Danforth et al. 2003	Macrotera portalis	Andrenidae	microsatellite
Green et al. 2001	Trigona carbonaria	Apidae	microsatellite

Conclusions

The colonization success of wild bees in restored ecosystems is affected by a number of factors. On the one hand, the dispersal ability of bee species and the connectivity of wild bee populations are key factors for colonization processes; on the other hand the quality of the colonized site (e.g. availability of pollen, nectar and nesting sites) is of high importance for the establishment of a species-rich community. The aim of this thesis was to analyze both, the dispersal ability of wild bees as well as the environmental factors that determine colonization patterns in restored ecosystems. These analyses might enable us to evaluate the success of the restoration measures with respect to the conservation of wild bees.

Monitoring of restoration projects is essential to evaluate the success of such measures and improve the quality of future restoration projects. During recent decades, the number of restoration projects has been rising, but our knowledge of successful habitat restoration is still incomplete (Forup *et al.* 2008). In general, the definition of clear restoration goals is crucial for evaluating the success of a project. Ehrenfeld (2000) discusses three different objectives of restoration projects: restoration of species, of whole ecosystems and of ecosystem functions. He points out a strong need of flexibility during restoration planning. Using reference (target) sites as restoration goals is often proposed, but this method has also been discussed controversially. The main obstacle to this approach is the amount of spatial variation in communities of different habitats *per se* which might be more important as a determinant of differences in the community structure than the restoration process (Potts *et al.* 2003). However, the same problem might occur with the use of historical data as restoration target, as range shifts or population fluctuations caused by the variability of environmental factors such as the climatic conditions may account for community differences (Ehrenfeld 2000).

The realization of a large restoration project in north-western Germany had the aim to restore a typical floodplain composed of inland sand-dunes and seasonally flooded grasslands (Stroh *et al.* 2005). In the context of this project, both approaches were taken into account. On the basis of historical maps a typical landscape relief was re-modelled, a target area served as a model to define objectives for the development of a typical vegetation (Remy & Zimmermann 2004). The results of this projects provide evidence for the high efficiency of both approaches (see Schwabe & Kratochwil 2004). This project provides the opportunity to study the response of wild bee communities to such restoration measures from the beginning.

Through the analysis of wild bee colonization patterns for monitoring of restoration success it is possible to evaluate both, ecosystem function and structure. So far, analyses regarding wild bee community response to restoration measure are sparse. Many studies focus either on groups of species or refer to simple management tools such as sowing of entomophilous plant species or mowing (e.g. Carvell *et al.* 2004; Russell *et al.* 2005; Carvell *et al.* 2007).

In chapter 1 and 2 of this thesis, the succession and distribution of bee communities in response to restoration measures of sand dunes and sand grasslands was studied and compared to the communities of old sand dune complexes as target habitats. Previous studies on the effects of habitat management on pollinators report a fast response of this highly diverse group (Carvell et al. 2004; Forup & Memmott 2005; Carvell et al. 2007; Forup et al. 2008). The data presented in the first two chapters support these findings and illustrate the high potential of wild bees to colonize new habitats. Immediately after restoration measures have been carried out, a highly diverse wild bee community established at the restored sites. This community was characterized by a high total species richness as well as a high number of specialists, generalists and parasites. Hence, the restoration measures proved to be successful for the conservation and establishment of wild bees. Contrary to general hypotheses on the correlation of flight radius and colonization ability of bees with their body size (Gathmann & Tscharntke 2002; Greenleaf et al. 2007), the results do not indicate a stepwise colonization process of large, medium and small species. This result suggests that the actual colonization capability of wild bees might be underestimated. Based on the analysis of pollen loads, Beil et al. (2008) observed large flight distances even for small bee species. Similar conclusions can be drawn from the results of the population genetic analyses of this thesis, performed for two oligolectic bee species of the genus Andrena. The large (11-15 mm body length) specialist A. vaga (Panzer), is oligolectic on willow (Salix) pollen and lives as a pioneer species in floodplain habitats in large nest aggregations. Populations of the heathland specialist A. fuscipes (Kirby) (8-11 mm body length) are rather small. The species is specialized on heather pollen (Calluna vulgaris) and is solitary nesting. Despite their different life history strategies, both species showed a low genetic differentiation among populations even at great geographical distances and a high genetic diversity within populations (chapter **3 and 4**).

Despite a high variability of community composition throughout the years, the community structure of bees at the restoration sites converged to those of the target sites. Differences in the community structure of wild bees were mainly caused by a great number of species with low abundance. A typical wild bee community is assumed to be composed of a few species occurring in a high abundance, whereas the majority of species is represented by a few

individuals with highly fluctuating populations (Williams et al. 2001; Potts et al. 2003; Cane et al. 2005; Larson et al. 2006; Forup et al. 2008). In this study, the most abundant species occurred at almost all sites throughout the years indicating the presence of stable populations. In contrast, greater fluctuations were observed for rare species adding to the evidence supporting this pattern as indeed a typical structure of wild bee communities. In comparison with the target sites, a higher variation in the community structure was found at the restored sites. This might be an effect of the ongoing succession of the restored sites, which is characterized by a high rate of colonization events resulting in a temporary presence of species that later become extinct. Environmental factors had also a strong influence on wild bee species composition, particularly the number of entomophilous plant species and moisture conditions of the soil. While wild bee communities of Spergulo-Corynephoretum sites were related to a greater proportion of bare ground and dryer conditions, Diantho-Armerietum sites offered a higher diversity of entomophilous plant species. The question whether the structure and composition of communities is driven by "niche-assembly" or "dispersal-assembly" is discussed controversially (Gilbert & Lechowicz 2004; Ostling 2005). The niche-assembly theory predicts that the coexistence of species is determined by the availability of specific resources and environmental conditions to which they are best adapted (Brandle et al. 2002). In contrast, the theory of dispersal-assembly emphasises the influence of chance events, history and dispersal ability for the explanation of community composition (Chave & Leigh 2002). However, both theories are not mutually exclusive and the results of chapter 1 indicate that indeed both these theories play a role for explaining the oberserved succession patterns. While the differentiation of wild bee communities of both habitat types (Spergulo-Corynephoretum and Diantho-Armerietum) provides evidence for the theory of niche assembly, the high rate of fluctuation suggests a strong impact of chance events and dispersal processes. The analysis of distribution patterns of wild bee communities within restoration and target areas (chapter 2) also suggests an interaction of environmental conditions and dispersal processes. The habitat types of inland dunes (dry sites) and seasonally flooded grasslands (moist sites) are occupied by different wild bee communities. While dry sites attract a high diversity of specialists and small species, moist sites are characterized by a wild bee community composed of a large number of generalist species (mainly species of the genus Bombus) in both, target and restoration area. This pattern is consistent with the structure of a typical wild bee community. Thus, the highest diversity of specialized wild bees occurs in semi-arid and mediterranean-like regions with prevailing dry and warm microclimatic conditions (Kratochwil 2003). Generalist species and in particular social species (e.g.

bumble bee species), rather rely on an extensive flower availability. The consistence of wild bee communities between target and restoration areas indicates the high ability of the regional wild bee species pool to respond to such restoration measures. The data presented in the first two chapters support these findings and illustrate the **high potential of wild bees to colonize new habitats**. Immediately after restoration measures have been carried out, a highly diverse wild bee community established at the restored sites. This community was characterized by a high total species richness as well as a high number of specialists, generalists and parasites. Hence, the restoration measures proved to be successful for the conservation and establishment of wild bees.

The questions of what determines wild bee diversity at different spatial scales and the effects of the changing landscape are of major importance as a decrease of wild bee diversity has been reported from different regions throughout the world (Cane & Tepedino 2001; Biesmeijer et al. 2006; Butler et al. 2007). Several studies propose that landscape heterogeneity, or more specifically a combination of agricultural areas and other habitat types, is the most important factor promoting a high diversity of wild bee communities in agricultural landscapes (Tscharntke et al. 2005; Winfree et al. 2007; Winfree et al. 2008). In this context, the concept of metacommunities was introduced to explain linkages between local and regional community structures (Wilson 1992; Mouquet & Loreau 2003; Leibold et al. 2004). Wilson (1992) defined a metacommunity as an assemblage of communities of local habitats that are connected by the dispersal of different species. In their discussion of four paradigms to model metacommunity structure, Leibold et al. (2004) demonstrated that it is problematic to transfer these models to natural ecosystems as the models either assume identical habitat conditions among patches or different degrees of adaptations among species (no variations in ecological traits vs. various traits). A combined approach that considers habitat variability as well as a wide range of species responses seems to provide the most realistic explanation for metacommunity dynamics.

Closely related to the theory of metacommunities is the metapopulation model (Levins 1969), which implies that a metapopulation is composed of several interacting local subpopulations. Hanski & Gilpin (1991) stress the need to combine both theories since the existence of a metacommunity would hardly be possible without the presence of interacting subpopulations. By the combination of population genetic studies and community surveys of wild bees, the results of this thesis provide evidence for an actual relation between these models. The rapid colonization of all groups of wild bees (generalists, specialists and parasitic species) suggest

the existence of many local communities with a high connectivity. Additionally, the population genetic analyses based on two specialized wild bee species confirm the existence of a metapopulation structure.

However, the maintenance of stable metacommunities and metapopulations presumes on the one hand the existence of diverse habitats for all groups of wild bees and on the other a high inter-habitat movement and dispersal ability of wild bees.

In **chapter 3 and 4**, the genetic structure of wild bee populations was studied at different spatial scales. The consideration of population genetic analyses in the context of restoration evaluation provides the opportunity to draw conclusions about the stability of immigrating populations. Due to the existence of genetically diverse populations in the surroundings of the restoration area it seems likely that immigrating individuals have a high potential to establish stable populations.

Controversially to existing hypotheses on a reduced genetic variability of specialized wild bees (Packer et al. 2005; Zayed et al. 2005; Zayed & Packer 2007), the results indicate a high genetic variability within populations of two specialized wild bee species (Andrena vaga, Andrena fuscipes) in the study region. These findings suggest either an underestimation of the actual dispersal ability and population connectivity of specialized wild bee species or the presence of suitable preconditions such as large or well connected habitats. In combination with the results obtained in chapter 1 and 2, both explanations seem to be likely. The rapid colonization of the restored sites indicates a high migration rate of wild bees, while the genetic patterns of both species studied, indicate that the landscape characteristics influence gene flow at a regional scale. Genetic diversity and population connectivity of Andrena vaga were reduced in regions with strong human impact caused by a higher degree of habitat fragmentation. Moreover, differences in the rate of inbreeding as a consequence of different degrees of sociality in A. vaga and A. fuscipes were observed. This relation was also found in many other wild bee species (chapter 5) and might indicate an underlying mechanism for the evolution of social interactions (Langer et al. 2004). Other factors that are assumed to explain variation in the genetic diversity and different rates of inbreeding in wild bees such as the degree of specialization (Packer et al. 2005; Zayed et al. 2005; Zayed & Packer 2007), the mating behaviour (Paxton 2005) and nesting strategies (Paxton et al. 1996) were taken under consideration in chapter 5. Actually, none of these factors itself revealed significance for the explanation of genetic variation. It seems rather likely that population genetic parameters are highly dependent on extrinsic factors such as the connectivity of the habitat or the size and age of populations. To draw general conclusions the actual availability of population genetic data for wild bees is insufficient.

In conclusion, the analysis of restoration success at different scales revealed the significance of both, habitat conditions and dispersal ability as determinants of community structure. At a local scale a strong influence of environmental factors was detected. Considering temporal aspects of community structure a high dynamic for wild bee communities was found, indicating a strong influence of fluctuation due to migration processes. This combination of mechanisms is consistent with both dispersal- and niche-assembly processes. Dispersal is assumed to be one of the main drivers of biodiversity at different spatial scales. The fragmentation of natural habitats by changing land-use practices is significantly affecting different levels of biodiversity such as the genetic diversity of populations and the diversity of populations is guaranteed. The diversity of communities is supported by heterogeneity at different spatial scales providing a variety of potential niches.

Wild bees appear to be useful indicators for monitoring the effects of restoration projects. The combination of population genetic analyses and community monitoring proved to be particularly suitable for the documentation of restoration success.

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Summary

Habitat fragmentation enforces the vulnerability of local populations to environmental and demographic fluctuations and decreases the chance for re-colonization events. Semi-natural landscapes, such as oligotraphentic grasslands with extensive land-use, are particularly effected by habitat loss. In north-western Germany, riverine sand dunes and natural floodplains were widespread in the past. As a result of regular flooding events, intensive agricultural land-use practices were impossible in these habitat types facilitating the formation of semi-natural landscapes with a high species richness of plants and animals. By the regulation of the natural course and building of dykes, large rivers have experienced serious anthropogenic influences resulting in a dramatic decline of adjacent inland sand dunes and natural floodplains.

The realization of a large restoration project in north-western Germany had the aim to restore a typical floodplain composed of inland sand-dunes and seasonally flooded grasslands. Within this project, the response of wild bee communities to such restoration measures was evaluated and is subject of this thesis.

Therefore, an analysis of the succession and distribution patterns of wild bee communities in restored and target habitats was conducted including population genetic studies which had the aim to estimate wild bee dispersal and movement patterns. In chapter 1 and 2 the success of the restoration measures was evaluated by a comparative analysis of wild bee communities at restoration and target sites. For chapter 1, two typical plant communities of dry sand ecosystems (Spergulo-Corynephoretum and Diantho-Armerietum) of this landscape were studied with a major focus on colonization patterns and succession of wild bee communities in the restored habitats in comparison with old, species-rich habitats (target sites). Wild bee surveys were conducted by the use of coulered traps in the years 2001-2003, 2005 and 2006. In chapter 2 the emphasis was to analyse spatial patterns of wild bee communities in restored and target sites. Based on a grid system, two plot types (dry and moist) were analysed for differences in their wild bee community structure with respect to environmental factors such as the vegetation structure and pollen sources. A precise quantification of the entomophilous plant species throughout the vegetation period allowed the analysis of phenological differences between the plot types in restoration and target areas. The results of these chapters show a rapid colonization of a species-rich wild bee community reflecting a community composition which is composed of generalists, specialists and parasitic species in similar proportions expectable for this region. The studied habitat types of inland sand dunes and

moist grasslands attracted different wild bee communities but showed a similar pattern of response in restored and target sites suggesting a strong influence of habitat composition. In particular, the quantity and phenology of entomophilous plant species, the moisture conditions of the soil and the proportion of bare ground had a strong influence on wild bee species composition. The structure of bee communities over time was highly variable in both restoration and target sites, indicating a high influence of migration processes. Moreover, the results indicate neither a stepwise colonization process of large, medium and small species, nor a correlation of colonization ability with the degree of specialization suggesting that the actual colonization capability of wild bees might be underestimated.

To gain a more precise insight into the connectivity of wild bee populations and the genetic preconditions that allow a successful colonization, the population genetic structures of two highly specialized wild bee species, *Andrena vaga* (Panzer) and *Andrena fuscipes* (Kirby), were analysed in **chapter 3** and **4**. Additionally, general intrinsic factors that maintain the genetic diversity and influence the degree of inbreeding were evaluated in **chapter 5** on the basis of an extensive literature survey.

These results also reflect a high dispersal ability and inter-population movement of wild bees. For both species a high genetic diversity within populations and a low genetic differentiation among populations was found even at great geographical distances. The consideration of population genetic analyses in the context of restoration evaluation provides the opportunity to draw conclusions about the stability of immigrating populations. Due to the existence of genetically diverse populations in the surroundings of the restoration area, it seems likely that immigrating individuals have a high potential to establish stable populations. Other factors that are assumed to explain variation in the genetic diversity and different rates of inbreeding in wild bees such as the degree of specialization, the mating behaviour and nesting strategies were taken under consideration of genetic variation. It seems rather likely that population genetic parameters are highly dependent on extrinsic factors such as the connectivity of the habitat or the size and age of populations. To draw general conclusions the actual availability of population genetic data for wild bees is insufficient.

Since a decrease of wild bee diversity has been reported from different regions throughout the world, the question of what determines wild bee diversity at different spatial scales and the effects of the changing landscape are of major importance. So far, landscape heterogeneity or a combination of agricultural areas and natural habitat types, are assumed to be the most important factors promoting a high diversity of wild bee communities in agricultural

landscapes. In this context, the concept of metacommunities was introduced to explain linkages between local and regional communities. Closely related to the theory of metacommunities is the metapopulation model, which implies that a metapopulation is composed of several interacting local subpopulations. Both theories are necessarily linked to each other, since the existence of a metacommunity would hardly be possible without the presence of interacting subpopulations. By the combination of population genetic studies and community surveys of wild bees, the results of this thesis provide evidence for an actual relation between these models. The rapid colonization of all groups of wild bees (generalists, specialists and parasitic species) suggest the existence of many local communities with a high connectivity. Additionally, the population genetic analyses based on two specialized wild bee species confirm the existence of a metapopulation structure.

In conclusion, wild bees proved to be useful indicators for monitoring the effects of restoration projects. The combination of population genetic analyses and community monitoring provides the opportunity to evaluate different aspects of restoration success.

Zusammenfassung

Die Zerstörung und Fragmentierung natürlicher Lebensräume gefährdet den Erhalt lokaler Populationen und macht diese anfälliger für negative Umwelteinflüsse. Halb-natürliche Lebensräume, wie zum Beispiel extensiv genutzte Magerrasen sind besonders von Flächenverlusten betroffen. Im Nordwestdeutschen Tiefland waren flussnahe Binnendünen-Flutmulden Komplexe ehemals weit verbreitet. Als Folge regelmäßiger Überflutungen war eine intensive landwirtschaftliche Nutzung dieser Bereiche unmöglich wodurch die Entstehung artenreicher halb-natürlicher Lebensräume begünstigt wurde. Durch umfangreiche Flussbegradigungen und Eindeichungen sind viele große Flüsse starken anthropogenen Einflüssen unterworfen, wodurch eine Vielzahl angrenzender Flussauen zerstört wurde. Die Umsetzung eines großflächigen Restitutionsprojektes im Nordwestdeutschen Tiefland hatte das Ziel einen typischen Binnendünen-Flutmulden Komplex wiederherzustellen. Im Rahmen dieses Projektes ist die Frage nach der Besiedlungs- und Etablierungsfähigkeit von Wildbienen von besonderem Interesse und bildet das zentrale Thema dieser Arbeit.

Ziel der Arbeit ist die Untersuchung der Kolonisations- und Etablierungsfähigkeit von Wildbienen in neu geschaffenen Sandökosystemen mit Hilfe verschiedener methodischer Ansätze.

Die Kapitel 1 und 2 befassen sich mit der Analyse von Wildbienen-Gemeinschaften in Restitutions- und Leitbildgebieten im Hinblick auf zeitliche und räumliche Muster. In Kapitel 1 wurden zwei typische Pflanzengesellschaften trockener Sand-Ökosysteme (Spergulo-Corynephoretum and Diantho-Armerietum) in Leitbild- und Restitutionsgebieten auf ihre Wildbienen-Gemeinschaften hin analysiert. Der Schwerpunkt lag hier zum einen auf der Erfassung räumlicher Verteilungsmuster von Wildbienen-Gemeinschaften und zum anderen auf der Analyse von Sukkzessionsmustern über mehrere Untersuchungsjahre. Besonders auffällig ist die schnelle Besiedlung der neu entstandenen Lebensräume. Entgegen der Erwartungen sind bereits nach kurzer Zeit viele hochgradig spezialisierte Arten in den Restitutionsflächen zu finden. Die Gemeinschaftsstruktur schwankt in ihrer Artenzusammensetzung zwischen den Jahren, was einerseits auf eine hohe Kolonisationsrate schließen lässt, andererseits aber auch ein Hinweis darauf ist, dass sich nicht alle einwandernden Arten etablieren können. Die Verteilung von Wildbienen über die Untersuchungsgebiete wird von bestimmten Umweltfaktoren, wie z.B. das lokale Blütenangebot, die Verfügbarkeit offener Bodenstellen und die Feuchteverhältnisse des Bodens bestimmt.

Am Beispiel der spezialisierten Sandbienen Arten *Andrena vaga* und *Andrena fuscipes* sind in **Kapitel 3 und 4** die populationsgenetischen Voraussetzungen für das hohe Kolonisationspotential von Wildbienen und insbesondere spezialisierter Arten untersucht worden. Bei der Sandbiene *A. vaga* handelt es sich um eine auf Weiden (Salix) als Pollenquelle spezialisierte Art die als Pionier in den Auenlandschaften Norddeutschlands häufig ist. Die auf Heide als Pollenquelle spezialisierte Sandbiene *A. fuscipes* gilt dagegen als gefährdete Art deren Lebensraum, die trockene Sandheide, stark von Fragmentierung betroffen ist. Für beide Arten konnte eine hohe genetische Variabilität innerhalb der Populationen festgestellt warden und die Struktur der Populationen lässt auf hohen Genfluss schließen.

Ein negativer Einfluss zunehmender Habitatfragmentierungen kann jedoch bei beiden Arten nicht ausgeschlossen werden. In der Populationsstruktur der Heidekraut-Sandbiene äußert sich dieses zum einen durch eine Populationsstruktur, die sehr deutlich die geographische Anordnung der Populationen widerspiegelt und zum anderen durch Hinweise auf genetische Flaschenhals-Ereignisse. Für die Weiden-Sandbiene ist vor allem eine Abnahme der genetischen Variabilität in stark fragmentierten Lebensräumen zu finden.

Unterschiede zwischen den beiden Arten konnte in der Inzuchtrate festgestellt werden. Während *A. vaga* in Aggregationen nistet und eine höhere Inzuchtrate aufweist, ist diese bei der solitär nistenden *A. fuscipes* deutlich geringer. In **Kapitel 5** steht die Frage nach den generellen Faktoren für die Steuerung von genetischer Diversität und Inzucht in Bienen im Vordergrund. Als Grundlage neben den eigenen Daten sind hier populationsgenetische Studien weiterer Arten eingeflossen. Die Analyse gibt Hinweise auf einen Zusammenhang zwischen dem Pollensammelverhalten und der Nistweise und der genetischen Diversität der Nistweise (solitär - aggregiert – communal) zu. Die bisherige Datengrundlage ist jedoch nicht ausreichend um gesicherte Aussagen treffen zu können. Insbesondere Untersuchungen an solitären Wildbienenarten sind im Gegensatz zu Studien an sozialen Arten deutlich unterrepräsentiert.

Die Ergebnisse dieser Arbeit zeigen, dass die Kombination verschiedener Methoden zur Evaluation von Restitutionsmaßnahmen die Möglichkeit bietet, ein vollständiges Bild über den Erfolg der Restitution zu gewinnen. Darüber hinaus haben sich die Wildbienen als Indikatorarten zur Bewertung des Restitutionserfolgs als besonders geeignet herausgestellt. Sie sind in der Lage besonders schnell auf Veränderungen des Lebensraumes zu reagieren. Durch die Verknüpfung der einzelnen Kapitel zeigt sich, dass die tatsächliche Ausbreitungsund Etablierungsfähigkeit von Wildbienen bisher unterschätzt wurde. Der weltweite Rückgang zahlreicher Wildbienenarten führt zu der Frage nach den Faktoren die eine hohe Wildbienendiversität auf verschiedenen landschaftlichen Skalen begünstigen. Bisher gilt die Heterogenität der Landschaft, bzw. die Verknüpfung von landwirtschaftlichen Nutzflächen und natürlichen Lebensräumen als besonders wichtig für die Förderung von Wildbienen-Gemeinschaften. In diesem Zusammenhang wurde der Begriff der Metacommunity eingeführt, um die Vernetzung lokaler Wildbienen-Gemeinschaften zu erklären. Demnach setzt sich eine Metacommunity aus verschiedenen lokalen Communities zusammen die durch Ab- und Zuwanderung miteinander verknüpft sind. In engem Zusammenhang mit diesem Konzept steht das Modell der Metapopulation, nachdem die Populationen einer Art durch den Austausch einzelner Individuen miteinander vernetzt sind. Die Gesamtheit dieser Subpopulationen wird als Metapopulation bezeichnet. Die Daten dieser Arbeit belegen, dass beide Modelle tatsächlich nicht voneinander trennbar sind. Die schnelle Besiedlung der neu entstandenen Lebensräume wäre nicht möglich gewesen, ohne die Präsenz lokaler Gemeinschaften als Besielungsquellen. Die starke Vernetzung einzelner Populationen in der Region, belegt durch die Analyse populationsgenetischer Daten, deutet auf die Existenz einer Metapopulationsstruktur hin.

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Erklärung über die Eigenständigkeit der erbrachten wissenschaftlichen Leistung

Gem. § 8 Abs. 2 Buchstabe b der Promotionsordnung der Fachbereiche Physik, Biologie/Chemie und Mathematik/Informatik der Universität Osnabrück

Ich erkläre hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet.

Bei der Auswahl und Auswertung folgenden Materials haben mir die nachstehend aufgeführten Personen in der jeweils beschrieben Weise unentgeltlich geholfen.

- Prof. Dr. Anselm Kratochwil stand mir als Betreuer in allen Phasen der Arbeit beratend zur Seite und war behilflich bei der Vorbereitung der Studien, der Datenaufnahme, der statistischen Auswertung und dem Verfassen der Manuskripte.
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- Jun. Prof. Dr. Till Eggers gab Ratschläge zur statistischen Auswertung der Daten und zur Verfassung der Manuskripte.

Weitere Personen waren an der inhaltlichen, materiellen Erstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe von Vermittlungsbzw. Beratungsdiensten (Promotionsberater oder andere Personen) in Anspruch genommen. Niemand hat von mir unmittelbar oder mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

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