The relationship between genetic diversity and species diversity – impact of parallel processes in isolated plant populations

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Dissertation zur Erlangung
des Doktorgrades
der Naturwissenschaften (Dr. rer. nat.)

dem Fachbereich Biologie
der Philipps-Universität Marburg
vorgelegt von

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Halle/Saale, im August 2012

Vom Fachbereich Biologie
der Philipps-Universität Marburg als Dissertation am 25.10.2012 angenommen.

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Tag der mündlichen Prüfung am 30.10.2012

List of Articles

This thesis is based on the following publications and manuscripts. They will be referred to in the text by the term "chapter" (1-3). The author's and co-authors' contributions to the manuscripts are listed below:

Paper I: Connection between species diversity and genetic diversity: an empirical assessment in eight dry grassland species

together with Walter Durka and Stefan G. Michalski

Manuscript

The study was designed by Walter Durka and me. I did the field work, the genetic analyses in the laboratory, analyzed the data and wrote the manuscript. Walter Durka and Stefan Michalski helped analyzing and discussing the data and commented on earlier versions of the manuscript.

Paper II: Short-term fitness and long-term population trends in the orchid *Anacamptis morio*

together with Walter Durka and Stefan G. Michalski

Plant Ecology (2012), DOI: 10.1007/s11258-012-0113-6 (in press)

The study was designed by Walter Durka and me. I conducted the data sampling in the field, performed the AFLP analyses, analyzed the data and wrote the manuscript. Walter Durka and Stefan Michalski helped analyzing and discussing the data and commented on earlier versions of the manuscript.

Paper III: Year to year variation overrides relationships between reproductive fitness, population size and genetic variation in the rare *Muscari tenuiflorum* (Hyacinthaceae)

together with Walter Durka and Gabriele Weiss

Flora (2012), vol. 207 (in press)

The study was designed by Walter Durka and me. I performed the AFLP analyses and censused actual population sizes in the field. I analyzed this data in combination with data previously collected by Gabriele Weiss and student helpers. Walter Durka helped analyzing and discussing the data and commented on earlier versions on the manuscript.

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Zusammenfassung

Die Porphyrkuppenlandschaft bei Halle (Saale) bietet ideale Bedingungen um natürliche und langfristige Habitatisolation und deren Bedeutung für Pflanzenpopulationen zu untersuchen. In diesem System hatten wir die Möglichkeit die grundsätzlichen Prozesse zu untersuchen, die Beziehungen zwischen den Biodiversitätslevels schaffen können.

Auswirkungen von Habitatisolation auf die genetische Diversität in- und zwischen Populationen

Die langfristige Habitatisolation der Porphyrkuppen sollte erwartungsgemäß die genetische Diversität innerhalb und zwischen Populationen von Trockenrasenarten beeinflussen. Bisherige Studien aus dieser Region haben gezeigt, dass einerseits die Habitatisolation die genetische Diversität einiger Pflanzenarten beeinflusst (Dannemann et al., 1999; Peterson et al., 2008; Hensen et al., 2010), andererseits wurden auch lediglich geringe Effekte berichtet (Bachmann und Hensen, 2007).

Wir konnten nun in unseren Analysen bestätigen, dass die Habitatisolation den Genfluss zwischen den Populationen beeinflusst. Die Effekte wurden hauptsächlich zwischen den Populationen sichtbar, d.h. sie betreffen die genetische Differenzierung. Diese war in den Versuchsarten generell hoch (globaler F_{ST}: 0,081 – 0,403) und ein positiver Zusammenhang zur geographischen Distanz wurde in den meisten Arten deutlich. Demnach erfolgte der Genfluss hauptsächlich zwischen benachbarten Standorten, was starke Drift-Effekte auf größeren räumlichen Skalen bestätigt. Außerdem, zeigten einige Arten extrem geringen Genfluss schon auf kurzen Distanzen (mittlerer Abstand zwischen den Flächen: 3 km). In diesen Fällen waren die grundsätzlich hohen F_{ST}-Werte unabhängig von der räumlichen Distanz.

Generell kann geringer Genfluss zwischen Populationen die genetische Diversität innerhalb von Populationen reduzieren (Young et al., 1996). Eine Verminderung der genetischen Diversität aufgrund von genetischer Drift würde sich in kleinen Populationen zuerst zeigen (Leimu et al., 2006). Demzufolge würde man unter Bedingungen, die Genfluss limitieren eine positive Beziehung zwischen Populationsgröße und genetischer Diversität erwarten. Dieser Zusammenhang zeigte sich nur in einer Versuchsart (*Muscari*) und nur geringe Effekte traten in der Vielartenstudie auf. Darüber hinaus zeigten alle Versuchsarten eine hohe mittlere genetische Diversität (H_E: 0,160 – 0,245). Sowohl die genetische Diversität innerhalb der Arten als auch die verschiedenen Differenzierungsmuster könnten, wie im Folgenden beschrieben, prinzipiell auf deren biologische Artmerkmale („life history traits“) zurückzuführen sein (Loveless und Hamrick, 1984):

Erstens kann die hohe genetische Diversität, die in fast allen Arten vorherrschte (außer bei *Spergula*) dem vorwiegend xenogamen Befruchtungssystem zugeschrieben werden (Nybom, 2004). Jedoch ist in auskreuzenden Arten auch die Verringerung der genetischen Diversität ausgeprägter, die als Folge von Isolation, Fragmentierung oder Abnahme der Populationsgröße auftreten kann (Aguilar et al., 2008). Solche Effekte konnten wir in unseren Versuchsarten und für unser System nicht bestätigen. Im Gegensatz zu auskreuzenden Arten tendieren selbstende Arten zu geringerer genetischer Diversität (Hamrick und Godt, 1996). In unseren Analysen war nur eine selbstende Art vertreten (*Spergula*). Erwartungsgemäß, fanden wir hier – im Vergleich zu den anderen eher fremdbestäubten Arten – die geringste genetische Diversität.

Zweitens begünstigt die lange Lebensdauer der Versuchsarten (außer bei der annuellen *Spergula*) eine hohe genetische Diversität, auch unter Bedingungen von limitiertem Genfluss zwischen den Populationen. Mehrjährige Arten leiden eventuell weniger unter den negativen Folgen von Habitatisolation und kleiner Populationsgröße, weil weniger Generationen bestimmte Zeiträume durchlaufen. Folglich treten negative Drift-Effekte in mehrjährigen Arten geringer und verzögert auf (Hartl und Clark, 1989). Außerdem haben mehrjährige Arten in dynamischen Systemen, in denen sich die Umweltbedingungen oft ändern, eine geringere Wahrscheinlichkeit auszusterben. Eine lange Lebensdauer kann einen geringen reproduktiven Erfolg oder begrenzte Ausbreitungsmöglichkeiten kompensieren (Bossuyt und Honnay, 2006). In annuellen Arten hingegen kann variierender reproduktiver Erfolg einerseits problematisch sein, weil die Etablierung vom Fortpflanzungserfolg des Vorjahres abhängt. Andererseits sind Samenbänke und die Samenbildung über Selbstbefruchtung in solchen Arten häufig. Beides sind Absicherungen gegen geringen Reproduktionserfolg in einzelnen Jahren (Thompson und Grime, 1979; Coffin und Lauenroth, 1989).

Drittens können effektive Ausbreitungsmechanismen eventuelle Genfluss-Hürden (z.B. große Ackerflächen oder Monokulturen zwischen Habitat-Inseln, Siedlungen, Flüsse oder Straßen) ausgleichen. Ein effektiver Mechanismus ist beispielsweise die Windausbreitung von Pollen und Samen (Salisbury, 1976; Ozinga et al., 2004; Ghazoul, 2005). Im Gegensatz dazu ist die Abhängigkeit von bestimmten Ausbreitungsvektoren nachteilig, wenn die Häufigkeit oder die Fortbewegung dieser Vektoren beeinträchtig ist. Entsprechend hatten in unseren Studien solche Arten mit geringer Ausbreitungslimitierung, wie z.B. *Anacamptis morio* (windverbreitete Samen), *Carex humilis* (windbestäubt) und *Dianthus carthusianorum* (viele und große Populationen, Bestäuber mit großen Flugdistanzen) die geringste genetische Differenzierung.

Dies weist auf einen hohen Genaustausch zwischen den Populationen hin. Im Gegensatz dazu hatten die Arten mit höherer Ausbreitungslimitierung – *Muscari tenuiflorum* (schwere Samen), *Spergula morisonnii* (kleinwüchsig, schwere Samen) und *Silene otites* (spezialisierte Bestäuber) – eine höhere genetische Differenzierung. Dennoch muss erwähnt werden, dass die räumliche Isolation auf eher kleiner Skala existiert. Ausbreitung kann auch sporadisch und zufällig erfolgen, unabhängig von der Distanz oder den jeweiligen Ausbreitungsmechanismen. Dies könnte eventuelle Isolationseffekte abschwächen.

Zusammenfassend lässt sich sagen, dass der Genfluss in unserem Untersuchungssystem limitiert ist. Dies gilt für nahezu alle untersuchten Arten. Obwohl die Reaktion auf limitierten Genfluss stark von den biologischen Artmerkmalen abhängt, konnten wir keinen starken Hinweis auf genetische Verarmung feststellen.

Korrelationen zwischen genetischer und Artendiversität – parallele Effekte

Verschiedene Prozesse wie Drift und Selektion (Habitatheterogenität) wirken theoretisch parallel auf genetische Diversität (GD) und Artendiversität (SD; species diversity) und führen so indirekt zu positiven Korrelationen zwischen beiden Ebenen (GD-SD Korrelationen) (Vellend, 2005). Unter fehlendem Genfluss (z.B. Migration) führt genetische Drift zu einer Reduzierung von GD und SD insbesondere in kleinen Populationen beziehungsweise in kleinen Habitaten (Barrett und Kohn, 1991). Auch Selektion oder heterogene Umweltbedingungen können GD und SD positiv beeinflussen. Wenn zeitlich oder räumlich variierende Selektionsdrücke verschiedene Allele bzw. Arten begünstigen, dann ist die Aussterbewahrscheinlichkeit für einzelne Allele oder Arten geringer (Vellend und Geber, 2005). Demnach sollten heterogene Habitate höhere SD und höhere GD innerhalb der Arten aufweisen.

Theoretisch bot unser Untersuchungssystem kleiner isolierter Trockenrasen die Umweltbedingungen, die den Einfluss von Drift und Umweltheterogenität auf GD und SD begünstigen (Vellend, 2005). Erstens schränkt die räumliche Isolation die Ausbreitung von Pollen und Samen ein. Zweitens begrenzen die meist kleinen Habitate die Populationsgrößen und erhöhen somit die Anfälligkeit für genetische Drift. Drittens stellen die Porphyrkuppen sehr heterogene Habitate dar. Mehr als 50 verschiedene Pflanzengesellschaften wurden hier beschrieben und charakterisieren somit die hohe Vielfalt an unterschiedlichen Umweltbedingungen (Mahn und Partzsch, 1996). Schließlich sind die Kuppen, ihre Isolation und die Gesellschaften sehr alt (Bliss et al., 1996). So sollten Drift und Selektion die Biodiversität auf den verschiedenen Biodiversitätsebenen während der letzten Jahrtausende

geformt haben. Kurzfristigere Habitatveränderungen könnten jedoch auch die Effekte der Langzeitbedingungen beeinflussen.

Wie erwartet haben die oben erwähnten Umweltbedingungen des Untersuchungsgebiets den Genfluss zwischen den Flächen beeinflusst und somit die genetische Struktur der Populationen geprägt. Obwohl die Effekte stark artspezifisch sind, ist der Genfluss begrenzt und dies bemerkenswerterweise schon auf dieser eher kleinen räumlichen Skala (innerhalb von etwa 20 km²). Der parallele Einfluss von Umweltbedingungen auf GD und SD wurde zuvor bereits auf kleineren räumlichen Skalen beschrieben (< 4 km²): Beispielsweise hat bei *Banksia attenuata* die Dünenhöhe einen positiven Effekt auf GD und SD bewirkt (He et al., 2008). Parallele Reaktionen von GD und SD auf Umweltbedingungen wurden auch bei *Daviesia triflora* beobachtet und demnach zeigten sich hier positive GD-SD Korrelationen (He und Lamont, 2009).

Wir haben wenig Hinweise darauf gefunden, dass parallele Prozesse zu positiven GD-SD Korrelationen führen. Auch hatte, entgegen früherer Studien (e.g. Solbrig und Simpson, 1974; Morishima und Oka, 1979; Bruun, 2000), Habitatheterogenität bei uns kaum Einfluss auf die Biodiversität. Wie oben erwähnt könnten die biologischen Merkmale der Versuchsarten selbst (d.h. ihre geringe Anfälligkeit für Habitatisolation und geringen Genfluss) dafür verantwortlich sein, dass keine GD-SD Korrelationen gefunden wurden. Das Fehlen solcher Korrelationen wurde auch schon in anderen Studien berichtet, die parallele Effekte erwartet hatten. Beispielsweise haben Odat et al. (2004) GD und SD in *Ranunculus acris*-Populationen auf vergleichbarer räumlicher Skala untersucht. Im Gegensatz zu unseren Flächen haben sie generell große und zusammenhängende Grasländer untersucht. Ähnlich wie wir, haben sie keine positiven GD-SD Korrelationen gefunden. Dennoch entdeckten sie einen positiven Zusammenhang zwischen genetischer Distanz und der Verschiedenheit der Lebensgemeinschaften bezüglich der Evenness. Verschiedene Selektionsdrücke und geringer Genfluss zwischen den Populationen könnten diese Muster geschaffen haben aber mündeten nicht in GD-SD Korrelationen.

Das Fehlen von GD-SD Korrelationen könnte auch andere Ursachen haben. Beispielsweise könnten sich die Arten unserer Studie nicht in Gleichgewichtsbedingungen befinden. Theoretisch könnte dies für Arten gelten, die sich erst kürzlich hier etabliert haben und bei denen demnach die genetische Diversität durch Gründereffekte bestimmt ist. Auch wäre es denkbar, dass Drift die genetische Diversität reduziert hat, sich jedoch Auswirkungen auf die

SD noch nicht zeigen weil es die Arten noch gibt. Eine Vielartenstudie von Fady und Conord (2009) über mediterrane Baumarten zeigte ebenfalls unterschiedliche Reaktionen von GD und SD in Bezug auf Umweltbedingungen. In ihrer Studie haben klimatische Einflüsse zwar die GD beeinflusst (Wirkung über die Populationsgröße) jedoch keinen Effekt auf die SD gehabt. Jedoch kann eine Verringerung der GD zu einer späteren Abnahme der SD führen, wenn z.B. über verminderte Fitness oder verringertes Anpassungspotential die Aussterbe-wahrscheinlichkeit einzelner Arten steigt („extinction dept"; Gilpin und Soulé, 1986).

Frühere Studien ließen vermuten, dass – über verschiedene räumliche Skalen – positive GD-SD Korrelationen in natürlichen und künstlichen Ökosystemen vorkommen. Oft haben parallele Effekte zu solchen Mustern geführt, obwohl die einzelnen Mechanismen zum einen sehr unterschiedlich sein können (z.B. lokale Umweltbedingungen, Landnutzung, Populationsgröße) und zum anderen auch artspezifisch unterschiedlich sind (Fady und Conord, 2009). Weil die Ansätze früherer Studien sehr divers sind können generelle Schlussfolgerungen kaum aus der Literatur gezogen werden. Deshalb leistet unsere Vielartenstudie einen wertvollen Beitrag zur anhaltenden Diskussion über den Einfluss paralleler Effekte auf GD-SD Korrelationen.

Der Einfluss abiotischer Umweltbedingungen und des Klimawandels auf die reproduktive Fitness

Klimawandel, Landwirtschaft und damit verbundene Habitatveränderungen sind die wichtigsten Risikofaktoren für die Biodiversität (Salafsky et al., 2008). Pflanzen reagieren als immobile Organismen besonders empfindlich auf Umweltveränderungen. Insbesondere haben Habitatspezialisten keine Möglichkeit alternative Flächen zu besiedeln, weil passende Habitate schlichtweg fehlen oder zu weit entfernt liegen. Trockenrasenarten sind dieser Problematik ausgesetzt. Trockenrasen gehören zu den am meisten bedrohten Lebensräumen Mitteleuropas und sowohl ihre Anzahl als auch ihre Größe nehmen kontinuierlich ab (Riecken et al., 1994; Poschlod und Schumacher, 1998). Verschiebungen der Verbreitungsgebiete von Arten und daraus resultierende Veränderungen in der Artzusammensetzung der Lebensgemeinschaften werden für die Zukunft prognostiziert (Parmesan, 2006). In Deutschland gilt dies insbesondere für Habitattypen mit hohem Naturschutzwert, wie nährstoffarme Trockenrasen, die einen hohen Anteil an gefährdeten Arten beherbergen. Dies kann sich auf Populationsgrößen der Einzelarten und auch auf interspezifische Interaktionen auswirken, da die Reaktionen meist von Art zu Art unterschiedlich sind (Pompe et al., 2011). Zusätzlich zum direkten Verlust von Habitaten und

Populationen könnten die restlichen Populationen unter negativen Konsequenzen der Habitatisolation und kleiner Populationsgröße leiden ("extinction dept"; Gilpin und Soule, (1986)).

Durch das Nutzen von Langzeit–Beobachtungsdaten konnten wir den Einfluss sich annuell verändernder Wetter– und Klimabedingungen auf Orchideenpopulationen in isolierten Trockenrasen untersuchen. Zusätzliche Analysen der genetischen Diversität und der reproduktiven Fitness erlaubten es ein umfassendes Bild der Prozesse zu erlangen, die in den Populationen des Kleinen Knabenkrauts (*A. morio*) agieren. Unsere Ergebnisse unterstreichen die Bedeutung die typischen Trockenrasen-Bedingungen zu bewahren um das kurz- und langfristige Bestehen der Populationen zu gewährleisten. Die xerotherme Habitatqualität fördert die Individuenzahlen und die Reproduktion. Beides beeinflusst die zukünftige Entwicklung der Populationen, beispielsweise durch Effekte auf genetische Diversität, Ausbreitung und Besiedlung neuer Habitate. Im Vergleich zur Pollenausbreitung hat die Windverbreitung der Orchideensamen große Bedeutung und wirkt somit dem möglichen Genverlust durch Drift entgegen. Die untersuchten Orchideenpopulationen konnten trotz der Isolation eine hohe genetische Diversität bewahren. Außerdem können sich die im letzten Jahrhundert berichteten Populationsrückgänge möglicherweise nicht fortsetzen, solange die Trockenrasen durch angemessenes Management bewahrt werden. Folglich zeigen die existierenden Populationen aktuell positive Trends oder zumindest stabile Individuenzahlen.

Zusätzlich zu den lokalen Umweltbedingungen können auch langfristige Veränderungen, wie etwa der Klimawandel die Entwicklung der Pflanzenpopulationen beeinflussen. Zum Beispiel wurde gezeigt, dass die im letzten Jahrhundert steigenden Frühjahrstemperaturen in Mitteleuropa mit einer Verschiebung der Blühphänologie in einigen Arten einher geht (Menzel und Fabian, 1999; Menzel et al., 2001). Solche Veränderungen könnten Konsequenzen für ökologische Interaktionen und demnach für die Reproduktion nach sich ziehen (Bartomeus et al., 2011). Auch in unserer Region konnten wir in den letzten Jahrzehnten einen Anstieg der mittleren Apriltemperaturen beobachten. In anderen Monaten hingegen gibt es keine solchen Trends. Gleichzeitig sind, unseren Analysen zufolge, hohe Apriltemperaturen förderlich für das Blühverhalten der Orchideen. Es scheint also als würde der aktuelle klimatische Wandel positiv auf *A. morio* wirken, indem dadurch vorteilhafte Bedingungen für die Pflanzenentwicklung und Reproduktion entstehen.

Reproduktive Fitness, Populationsgröße und der Effekt der interannuellen Variation

Die Beziehung zwischen reproduktiver Fitness und Populationsgröße ist oft positiv (Leimu et al., 2006), was eine Vielzahl sich nicht gegenseitig ausschließender Gründe haben kann. Grundsätzlich ziehen große Populationen mehr Bestäuber an: Ein größerer Teil der Blüten wird bestäubt, was dann zu höherem Samen- und Fruchtansatz führt (Knight et al., 2005). Außerdem zeigen große Populationen oft eine höhere genetische Diversität als kleine Populationen (Frankham, 1996). Die Wahrscheinlichkeit der geringeren reproduktiven Fitness durch Inzucht ist in kleinen Populationen höher, weil mehr schädliche rezessive Allele exprimiert werden (Ellstrund und Elam, 1993). Diese Beziehungen können durch Bestäuberdichte und -verhalten beeinflusst werden. Zum Beispiel lockten große Populationen von *Lychnis viscaria* mehr Hummeln an. Jedoch war die Besuchsrate und reproduktive Fitness in lichteren Populationen höher, womöglich weil längere Flugdistanzen zwischen den Individuen die Verweildauer an den Blüten erhöhen (Mustajärvi et al., 2001). Zusätzlich zur Populationsgröße kann eine Vielzahl an interagierenden Faktoren zur reproduktiven Fitness beitragen, wie z. B. Ressourcenverfügbarkeit, Wetter, Klima oder Bestäuberverhalten (Wilcock und Neilund, 2002). Solche Faktoren haben auch die Reproduktion unserer Untersuchungsarten *M. tenuiflorum* und *A. morio* beeinflusst. Letztlich war jedoch die Populationsgröße bestimmend für hohen Reproduktionserfolg, was generelle Muster in natürlichen Pflanzenpopulationen bestätigt.

Unsere beiden Fallstudien zeigten, dass sowohl Individuenzahlen als auch reproduktive Fitness stark zwischen den Jahren variieren können. Möglicherweise sind dafür variierende Umweltbedingungen verantwortlich, die z.B. die Wasser- und Nährstoffverfügbarkeit (Bengtsson, 1993), Bestäuber (Price et al., 2005), Pathogene (Scherm und Yang, 1995) oder Herbivoriedruck (English-Loeb und Karban, 1992) beeinflussen. Sicherlich beeinträchtigen diese Fluktuationen kaum die absoluten Populationsgrößen in mehrjährigen Arten. Viele Arten sind an solche Bedingungen angepasst und haben eine lange Lebensdauer, Samenbänke oder zeigen Dormanz (Dalgleish et al., 2010). Jedoch kann eine Variation der jährlichen Individuenzahlen und Fitness die Ergebnisse von Einjahresstudien (über den Zusammenhang zwischen Populationsgröße und Fitness) maßgeblich beeinflussen. Entsprechend waren bei *M. tenuiflorum* Populationsgröße und Fitness in zwei Jahren positiv verbunden, während zwei andere Jahre keinen Zusammenhang zeigten. Bei *A. morio* (Einjahresstudie) hatten, wie

erwartet, große Populationen eine höhere reproduktive Fitness. Obwohl wir hier keine wiederholten Messungen in mehreren Jahren durchführten kann davon ausgegangen werden, dass sich die Schwankungen zwischen den Jahren auch in schwankendem Reproduktionserfolg niederschlagen.

Fazit

Das Ziel der vorliegenden Arbeit war es, die Faktoren zu untersuchen, die die genetische Diversität und die Artenvielfalt in isolierten Trockenrasenarten beeinflussen. Ein besonderer Schwerpunkt lag in der Untersuchung einer möglichen Korrelation zwischen beiden Biodiversitätsebenen, die sich theoretisch unter den gegebenen Umweltbedingungen einstellen kann. Unser Ansatz viele Arten gleichzeitig zu untersuchen erlaubte es die Verallgemeinbarkeit von parallelen Effekten auf die beiden Biodiversitätsebenen zu testen. Wir fanden jedoch nur begrenzte Hinweise auf eine positive Korrelation zwischen genetischer und Artendiversität. Zusammenfassend lässt sich sagen, dass die Habitatisolation den Genfluss zwischen den Populationen schon auf sehr geringer räumlicher Skala begrenzt. Die genetische Resonanz darauf ist stark artabhängig und durch die jeweiligen biologischen Artmerkmale bestimmt. Die Mehrzahl der acht untersuchten Arten besitzt Eigenschaften, die sie weniger anfällig für genetische Verarmung durch Habitatisolation und kleine Populationsgröße machen. Dies betont die Bedeutung für Vielartenstudien, wenn Umwelteinflüsse auf Pflanzenpopulationen untersucht werden sollen. Die beiden Fallstudien in *A. morio* und *M. tenuiflorum* ergaben zusätzliche wertvolle Informationen über reproduktive Fitness und zeigten, dass Individuenzahlen und Reproduktion stark zwischen den Jahren variieren können. Darüber hinaus konnten wir einer der Versuchsarten zeigen, dass auch globale Veränderungen (Klimawandel) Pflanzenpopulationen beeinflussen können, indem sie auf das Blühverhalten wirken. Ob jedoch solche, auf den ersten Blick förderlichen Effekte, auch in einer verbesserten langfristigen Entwicklung der Pflanzenpopulationen münden, bleibt offen. Entsprechend unserer Ergebnisse hängt die Entwicklung von Populationen und Arten von einer Bandbreite vieler verschiedener Faktoren deren Interaktion ab. Diese müssen berücksichtigt werden, wenn es darum geht den momentanen Zustand und auch mögliche Zukunftsperspektiven von Pflanzenpopulationen zu beschreiben.

General introduction

Biodiversity and biodiversity research

Terminology and history of biodiversity research

Biodiversity (short for biological diversity; from the Greek word *bios* = life and Latin word *diversitas* = variety or difference) is the variability at the different levels of biological organization and describes the variation of genes, species, communities and ecosystems. The term “biodiversity” was first introduced at the “National Forum on Biodiversity" in 1986 in Washington, DC. The contributions from this conference are found in the book "Biodiversity" by E.O. Wilson (1988). Just some years later, in 1992, the Convention on Biological Diversity was opened for signature at the United Nations Conference on Environment and Development in Rio de Janeiro. Today, 168 states and the EU have signed the convention which main three goals are: (1) conservation of biological diversity, (2) sustainable use of its components, and (3) fair and equitable sharing of benefits arising from genetic resources (www.cbt.int).

Research on the topic of biodiversity has gained strong scientific interest since the mid-twentieth century but already dates back to Darwin. As quoted in Hector and Hooper (2002), in *The Origin of Species* Darwin (1859) writes: “It has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised.”. Here, Darwin describes (probably the worlds first ecological) experiments from the beginning of the 19th century in England (Hector and Hooper, 2002). Today biodiversity studies often focus on conservation issues and global change and investigate the reasons or consequences of biodiversity loss. Also, much attention has been put on the relationship between biodiversity and ecosystem functioning because of the high value for human well being (Tilman, 1997; Loreau et al., 2001; Díaz et al., 2006; Hector and Bagchi, 2007). It is now widely accepted that biodiversity positively affects ecosystem functioning (Hooper et al., 2005), but see (Schwartz et al., 2000). Important ecosystem functions are for example, pollination and seed dispersal, regulation of climatic conditions, biomass production, nutrient and water cycling and soil formation (Díaz et al., 2006). It has been shown that diverse ecosystems can have higher resistance or resilience towards temporal changes in environmental conditions, biological invasions or disturbance and thus have a higher overall stability (McCann, 2000; Hooper et al., 2005). Thus, humankind depends to a large extent on intact and biodiverse ecosystems. However, at the same time anthropogenic impacts threaten biodiversity (Chapin et al., 2000).

Biodiversity loss

Globally, declining biodiversity has been recognized as major environmental problem. Declines are reported at the level of genes, populations, species and ecosystems. According to the International Union for Conservation of Nature and Natural Resources (IUCN) "Direct Threats" to biodiversity are: residential and commercial development, agriculture and aquaculture, energy production and mining, biological resource use, natural system modifications, invasive species, pollution and climate change (Salafsky et al., 2008). A further increase of the known pressures on biodiversity (e.g. invasive species, nitrogen pollution, overexploitation, and climate change) is predicted for the future (Butchart et al., 2010) and thus, the current rate of biodiversity loss appears to continue.

Habitat loss is listed as one major reason for the loss of biodiversity. Habitats, and thus species and *genotype*s, can directly get lost due to human activities such as the conversion of natural ecosystems into arable land, mining or urbanization (McKinney, 2002). Habitat loss is closely connected with fragmentation of remaining habitats. In addition to the direct impact of habitat loss, fragmentation can have important indirect ecological consequences by affecting species migration, dispersal or abiotic environmental conditions within fragmented or isolated habitats (Lienert, 2004). Furthermore, the most basic level of biodiversity, genetic diversity within species, is often lower in fragmented and isolated habitats or small populations (Young et al., 1996). As a consequence, a lower fitness of inbred individuals and a lower adaptation potential lead to a higher extinction risk of genotypes and species in the long term (Leimu et al., 2006). Furthermore, **agricultural activities** have negative impacts on biodiversity. First, this is caused by the direct loss of habitat and second, by the intense use and release of nitrogen, phosphorous and water, the application of pesticides or the conversion of natural ecosystems into agriculture (Tilman et al., 2001). Especially the increases in N and P fertilization are projected to cause intense losses of biodiversity in both, terrestrial and marine ecosystems, for example, because of eutrophication of surface waters (Carpenter et al., 1998) or changes in species compositions (Vitousek et al., 1997). **Climate change** is another major challenge to biodiversity. For example, for a large fraction (one fifth to one third) of European species an increased extinction risk is projected if mean temperatures rise more than 2 to 3 °C (Lovejoy and Hannah, 2005). If possible, i.e. if suitable habitat is available, species have to shift their ranges (Parmesan, 2006). This may be impossible for species at their latitudinal and altitudinal range margins and for species with limited dispersal abilities. Furthermore, ecological

communities and interactions, e.g. plant-pollinator interactions, may be disrupted (Feehan et al., 2009).

Certain species are at higher risk than others to be affected by the direct and indirect consequences of habitat loss, habitat deterioration or climate change. The probable losers and winners of global change differ for example in their life history traits. Thus, species with a high extinction risk are, for example, long lived, poor dispersers and resource use specialists (Díaz et al., 2006).

The two basic levels of biodiversity - species diversity and genetic diversity

Species diversity (SD) is the traditional measure of biodiversity and describes the variety of different species with in a region. It can be defined, for example, as species richness or evenness. Species richness, simply, is the number of species whereas species evenness a measure of the homogeneity of abundances in a sample or a community (Colwell, 2009). Accordingly, two communities may have the same number of species but differ in relative abundance. SD within a given habitat depends on a variety of factors. On a global scale high SD is connected with high temperature, longer growing seasons, or higher climate predictability, conditions which are met in the tropics, where highest diversity is found on earth (Myers et al., 2000). Generally, positive species-area relationships exist. First the "habitat-diversity hypothesis" predicts that larger areas tend to contain larger numbers of species because of higher habitat diversity or environmental heterogeneity (Williams, 1964). Second, the "equilibrium theory of island biogeography" explains species number as a function of immigration and extinction rates. Hence, immigration depends on the distance between the site and a species source pool, whereas extinction is largely affected by site are per se via effects on population size (MacArthur and Wilson, 1963). Thus, local and regional environmental conditions can determine SD within a habitat by affecting species migration and selection.

Genetic diversity (GD) is the most fundamental component of biodiversity. It includes all genetically determined variation between individuals within a species. Genetic diversity is found within and among populations and is – similarly to SD – determined by (a) *mutation*, the basis of genetic diversity because new alleles are formed by changes in the genomic sequence, (b) *migration*, the movement of genes or alleles into or out of a population, (c) *selection*, the preference of particular alleles over others and (d) *drift*, the random loss of alleles because of a limited number of breeding individuals (Hartl and Clark, 1989). These forces act together and

lead to evolutionary adaptation of populations to their environments. Thus, changes in natural or anthropogenic environmental conditions can also affect genetic diversity of species or populations. Vice versa, genetic diversity can affect the species ability to respond to changing environmental conditions (Jump et al., 2009). Assessing GD within or among populations can give valuable information, for example about geographic structure, connectivity or gene flow among populations (Hughes et al., 2008) and can thus be used as independent indicator of environmental conditions.

Genetic diversity determines the extinction risk of a species or population in the short and long term. First, low genetic diversity can increase inbreeding effects and thus increase the extinction probability of a species (Booth and Grime, 2003). Second, genetic diversity is the prerequisite for a species to adapt to changing environmental conditions and thus it is a key factor long-term survival of a species (Frankham et al., 2002). In this context population size plays an important role because small populations are at higher risk to lose genetic diversity via drift and inbreeding (Leimu et al., 2006). Genetic diversity can be assessed by using morphological traits (Storfer, 1996). However, often these traits result from genes, the expression of which can be strongly affected by the environment (Falconer, 1989). Largely independent of environmental factors, neutral molecular markers can provide accurate assessments of genetic diversity within and among populations (Nybom, 2004).

However, at this point it is important to point out that there are two types of genetic diversity – neutral and adaptive genetic diversity (Holderegger et al., 2006). Neutral diversity refers to genes or alleles that have no influence on fitness and natural selection does not act upon these. However, information on neutral diversity can be used to gain information about gene flow, migration or dispersal. A wide array of molecular markers is available to assess neutral diversity. These are for example allozymes, microsatellites, RAPD, ISSR or AFLP. In contrast, adaptive genetic diversity has an effect on fitness and thus natural selection will act on genotypes (Connor and Hartl, 2004). Adaptive diversity can be investigated in quantitative genetic experiments, by, for example, growing individuals with a know genetic relationship under constant environmental conditions (Holderegger et al., 2006). Neutral and adaptive diversity are not necessarily related, although it can not be excluded that some neutral markers might be linked to functional genes. Generally, neutral and adaptive genetic diversity have been shown not to be directly correlated, although some studies report a positive relationship between neutral and adaptive genetic diversity (Reed and Frankham, 2001).

Relationship between GD and SD – theory

Several hypotheses on possible relationships between both biodiversity levels have been developed and predict positive but also negative GD-SD correlations (Vellend, 2005; Vellend and Geber, 2005). On the one hand **parallel effects** on both levels may indirectly create a positive connection between GD and SD (Figure 1). In particular, habitat isolation and environmental heterogeneity might act similarly on both levels of biodiversity. As stated above, species and genetic diversity depend on comparable processes, such as habitat size/population size, migration/gene flow or selection. For example, a habitat with many different ecological niches may contain a large number of species. Similarly, this environmental heterogeneity may allow different genotypes of a species to coexist if several genotypes differ in their use of resources. Thus, the separate positive effect of environmental heterogeneity on both diversity levels will result in an indirect positive GD-SD correlation. Among populations and among habitats migration can act on both diversity levels. Accordingly, limited gene exchange (e.g. pollination) and species migration (e.g. seed dispersal) can lead to random losses of alleles and species. Such negative drift effects decrease with habitat size, habitat connectivity and population size.

Besides parallel effects also direct **causal effects** between GD and SD have been described. A number of, however closely related, hypotheses have been proposed to account for correlations between GD and SD. First, GD and SD may most principally be positively related because GD is the prerequisite for speciation and thus for SD. Second, GD can favor SD because it enhances population fitness and thus can decrease the extinction probability (Reed and Frankham, 2003; Frankham, 2005). Higher fitness of genetically diverse populations may for example arise if genotypes differ, for example, in their resource use (Antonovics, 1978) or enemy resistance (Burdon, 1987). Third, the "coexistence hypothesis" also predicts a positive GD-SD correlation (Silvertown et al., 2009). According to that genetic diversity favors coexistence among species because every species can contain genotypes that are competitive and thus, competition occurs among individual genotypes and not only among species.

In contrast to the previous hypotheses, the "niche-variation hypothesis" predicts a negative GD-SD correlation. Accordingly, populations with few interspecific competitors and hence broader niches are expected to contain greater genetic diversity (Van Valen, 1965).

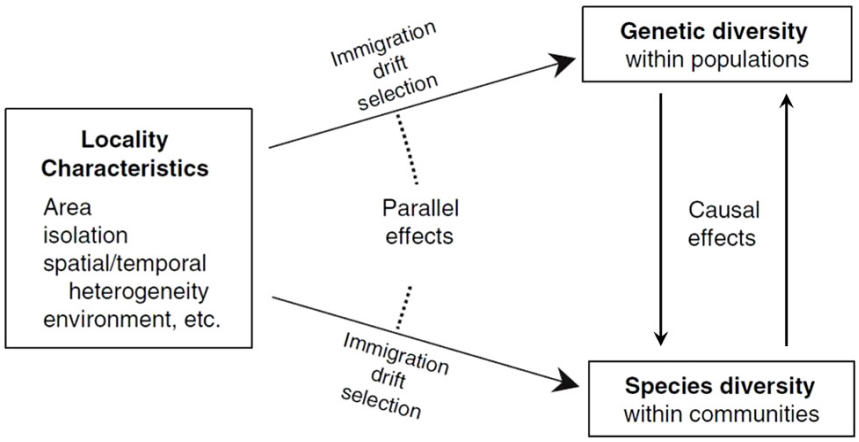

Figure 1: Potential connections between genetic diversity and species diversity. Modified from Vellend & Geber, 2005.

Relationship between GD and SD – empirical assessments

Some empirical studies have confirmed the abovementioned hypotheses on the impact of parallel processes (Table 1). From single species studies there is evidence for the parallel impact of local and regional habitat characteristics on both diversity levels. For example, altitude (He et al., 2008; Wei and Jiang, 2011), land use (Vellend, 2004), abiotic conditions (He et al., 2008; Odat et al., 2010) or drift (Nestmann et al., 2011) have been shown to create positive GD-SD correlations. However, no clear evidence exists, because also the absence of such effect is frequently reported (e.g. Odat et al., 2004; Rudmann-Maurer et al., 2007; Puşcaş et al., 2008). Often species specific traits (He et al., 2008), characteristics like population history (Puşcaş et al., 2008) or different habitat specific features (Odat et al., 2004) influence the existence or detectability of GD-SD correlations.

A few multiple species studies which focus on the relationship between GD and SD are available (Table 1). However, they have different approaches. For example, either GD is treated as dependent variable (Wehenkel et al., 2006; Fady and Conord, 2009) or – in the experimental studies – GD is manipulated and used as independent variable (Booth and Grime, 2003; Fridley and Grime, 2010). The former two studies mainly examine the impact of parallel processes on larger scales and find either no or only weak evidence for GD-SD correlations. The experiments by Booth & Grime (2003) and Fridley & Grime (2010) focus on direct effects of GD on SD and both find positive correlations between the two diversity levels. For example, the rate at which SD declined during the study period was reduced in genetically diverse plots (Booth and Grime, 2003). Fridley and Grime (2010) later showed that GD can decrease competition among species and thus, high GD promotes species coexistence.

Table 1: Summary of relevant empirical studies on the relationship between genetic diversity and species diversity.

Study type	Species	System	other variables studied	GD Marker	Spatial scale [a]	GD-SD pattern	Process	Reference
Single species – GD as dependent variable	*Euptelea pleiospermum*	riparian mountain forests	disturbance	MS	regional (< 100 km)	pos: in natural forests, no: disturbed forests	parallel effect of altitude	Wei & Jiang, 2011
	Banksia attenuata	patchy sand dunes	dune area, dune height	MS	3 x 4 km²	pos	parallel env. effect of dune height	He et al., 2008
	Plantago lanceolata	montane hay meadows	-	AFLP	regional (< 28 km)	pos	parallel effects of abiotic habitat conditions	Odat et al., 2010
	Trillium grandiflorum	forest herb community	land use history (primary vs secondary forest); environmental conditions, landscape context	allozymes, cpRFLP, MS	1300 km²	pos	parallel effects of land use history and population/community size	Vellend, 2004
	Brassica nigra	nartural and artificial communities	fitness	secondary compound (sinigrin)	n.g.	pos	GD in sinigrin promotes coexistence among species; trade-off between intra- and interspecific competitive ability	Lankau & Strauss, 2007
	Solidago altissima	experimental plots	aboveground primary productivity (APP), arthropod diversity	nr of genotypes (AFLP)	n.g.	pos, high GD in *S.altissima* caused high arthropod diversity and APP	APP effect: increased niche complementarity; SD effect: caused indirectly by APP effect and by positive effect of GD on resource availability/diversity	Crutsinger et al., 2006
	Daviesia triflora	sclerophyll shrubland, sand dunes	13 traits of 16 co-occurring species, dune area + height,	MS	3 x 3 km²	pos: within functional group (legumes)	effect of rhizobia - within functional group shared range of microsymbionts that respond to environmental properties	He & Lamont, 2009
	Oryza glaberrima and *O. sativa*	rice fields	water condition, disturbance, grazing	seed-type diversity	regional	pos	Eventually effect of habitat heterogeneity	Morishima & Oka, 1979

	Lolium perenne	experimental grasslands	-	SNP	local	weak pos	drift	Nestmann et al., 2011
	Ranunculus acris	grasslands	land use	AFLP	< 17 km	no	different habitat specific selective forces	Odat et al., 2004
	Carex cuvula	alpine grasslands	-	AFLP	large scale (> 2000 km)	no	GD shaped by location of glacial refugia; no similar response of GD and SD to post glaciation habitat dynamics	Puşcaş et al., 2008
	Poa alpina	grasslands	land use, altitude, reproduction	MS	large scale (< 180 km)	no	n.g.	Rudmann-Maurer et al., 2007
	Anthoxanthum odoratum	experimental grasslands	resource and soil pH gradients	AFLP	local	no	resource addition increased GD but decreased SD; indication of "niche variation hyp.", resource competition	Silvertown et al., 2009
Multiple species – GD as dependent variable	Mediterranean tree species	various ecosystems	elevation, longitudinal gradients	various (from literature)	large scale (Mediterranean)	no	LGM climate affected GD (via effects on population sizes) but not SD	Fady & Concord, 2009
	10 tree species, (climax, pioneer, admixed)	forest	-	Isozymes	n.g.	pos (for transpecific div), no (single species)	effect of successional stage	Wehenkel et al., 2006
Multiple species – GD as independent variable	11 herbs	artificial	-	1, 4, 16 genotypes	local	pos	GD reduces SD decline	Booth & Grime, 2003
	8 species, (grass/herb/sedge)	artificial	community composition, aboveground productivity	1, 4, 8 genotypes	local	pos	GD decreased competition among species, competition among indiv. Genotypes	Fridley & Grime, 2010

[a]Scale: (largest distance between sites)
n.g. = information not given
AFLP = amplified fragment length polymorphism
MS = microsatellites
SNP = single nucleotide polymorphism
RFLP = restriction fragment length polymorphism

Study system: The porphyry hilly landscape of Halle (Saale)

The porphyry hilly landscape of Halle (Saale) is located between 51°53' - 51°63' N and 11°82' - 11°94' E at an average altitude of ca 110 m a.s.l.. The region, is part of the "Semiarid region of central Germany" ("Mitteldeutsches Trockengebiet"), lies in the rain shadow of the Harz Mountains and arid conditions arise temporally (Bliss et al., 1996). The climate of the area is characterized by low annual rainfall of approximately 451 mm and mean annual temperature of 9.1 °C (local weather station Halle-Kröllwitz; German Weather Service).

During the last Ice Age the region remained ice free. Under dry conditions and bioturbation very fertile soils (chernozem) developed. A part of the area has never been wooded since the Ice Age. Especially the outcrops and here the south/south-west facing slopes are naturally free of woody species because of the dry and shallow soils. They are covered by open dry grasslands (e.g. Thymo-Festucetum). At other sites agricultural activities, mainly cutting and grazing, prevented succession and the growth of woody plant species and thus led to the creation of secondary semi-dry grasslands. The outcrops eroded from the landscape due to intense agricultural techniques such as ploughing (Bliss et al., 1996). Still this is an ongoing process and new outcrops emerge (Partzsch et al., 2003).

Today these isolated outcrops form a heterogeneous mosaic landscape which is of high value for nature conservation. (Mahn and Partzsch, 1996) describe 50 different plant communities here with very high species richness (> 600 vascular plant species in total, mean number of species per site = 108) and a high proportion of rare and vulnerable species (Richter et al., 2003). The vegetation of the outcrops is characterized by extra-zonal continental dry and semi-dry grasslands and atlantic/subatlantic dwarf shrub communities. The typical plant communities and the vegetation structure of the porphyry outcrops have been characterized by (Mahn and Partzsch, 1996) and are shown in Figure 2.

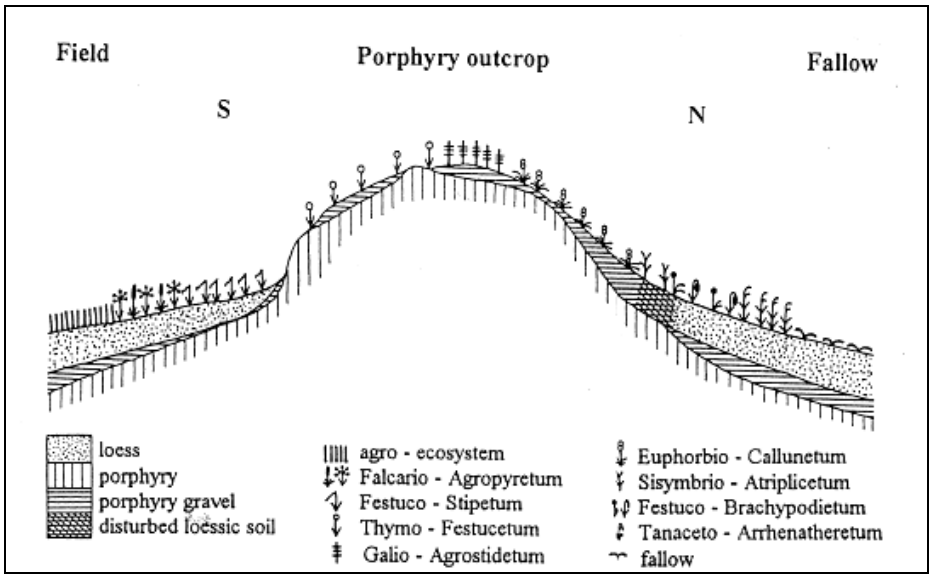

Figure 2: Characteristic vegetation patterns on the porphyry outcrops (Figure from Mahn & Partzsch, 1996)

Xerothermic dry grassland communities exist on the porphyry outcrops (n ~ 150) which are patchily embedded in the agricultural landscape (Figure 4). Their biological diversity is high and a variety of rare species from a number of different taxa is found here. Nevertheless, the habitats and the surrounding environmental conditions pose several challenges to plant populations that exist here. Species and individuals have to cope with resource limitation (e.g. water and nutrients), disturbance, species interactions (e.g. competition, mycorrhization) and population dynamics (e.g. dormancy or meta-population processes) (Harper, 1977; Crawley, 1997). Moreover, within the study region the grassland patches are mainly small, rarely exceeding the size of 500 m² (median size = 0.14 ha, mean size = 0.4 ha, Figure 3). Ecologically, small habitat size limits population size of occurring species and thus increases impacts of inbreeding and drift such as lower individual fitness and lower evolutionary adaptation potential. Small habitats also experience stronger edge effects, such as nutrient input and disturbance by agricultural practices (Partzsch et al., 2003). Furthermore, habitat isolation limits seed and pollen dispersal between patches, accelerating the random loss of genes and species from small habitats (i.e. drift). However, habitat isolation and small patch size are no recent conditions for the species to cope with, but already exist for a long time (Bliss et al., 1996). Plant species are adapted to these conditions and often have traits that ensure survival, vegetative expansion or diaspore banks rather than traits for long distance dispersal (Jackel and Poschlod, 2000). Nevertheless, environmental conditions are not stable in the long term. Recent

changes in land use practices, climatic conditions or abiotic environmental conditions in the last century represent new challenges for the persistence and survival of plant populations in this area. For example, sheep grazing, the traditional but nowadays unprofitable land use form strongly decreased within the last decades (Richter et al., 2003). As a consequence this increases the effects of habitat isolation. On the one hand habitats get lost because succession takes place in the absence of grazing and thus spatial distance between remaining habitats and populations increases (Bliss et al., 1996). On the other hand, the lack of grazing negatively affects seed dispersal because seeds can be transported from site to site in the fur of grazers (Fischer et al., 1996).

Thus, because of historical habitat isolation and recent effects of habitat loss and change this region provides excellent conditions for studying the consequences of restricted gene dispersal (i.e. pollen and seed dispersal), fragmentation and low population size.

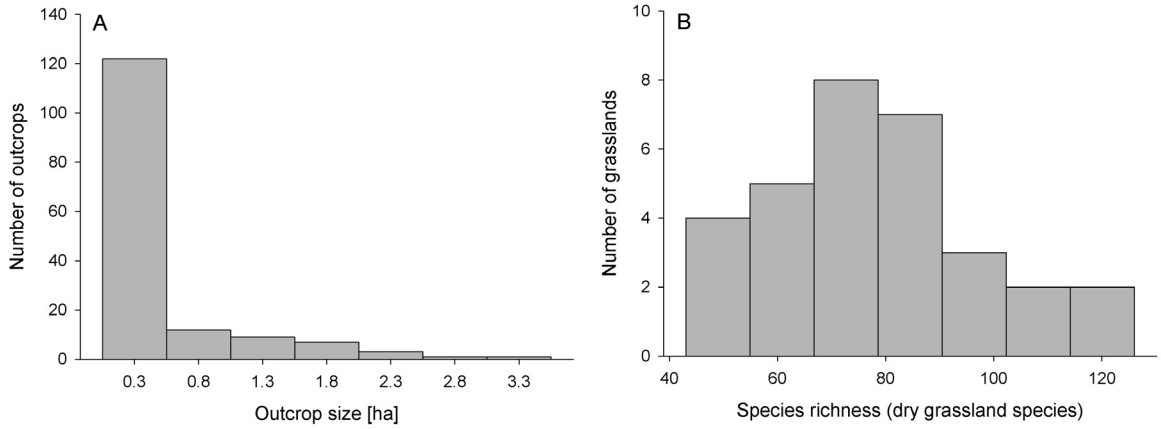

Figure 3: Distribution of patch sizes of all outcrops in the study region (A); species richness of the studied 31 dry grasslands (B).

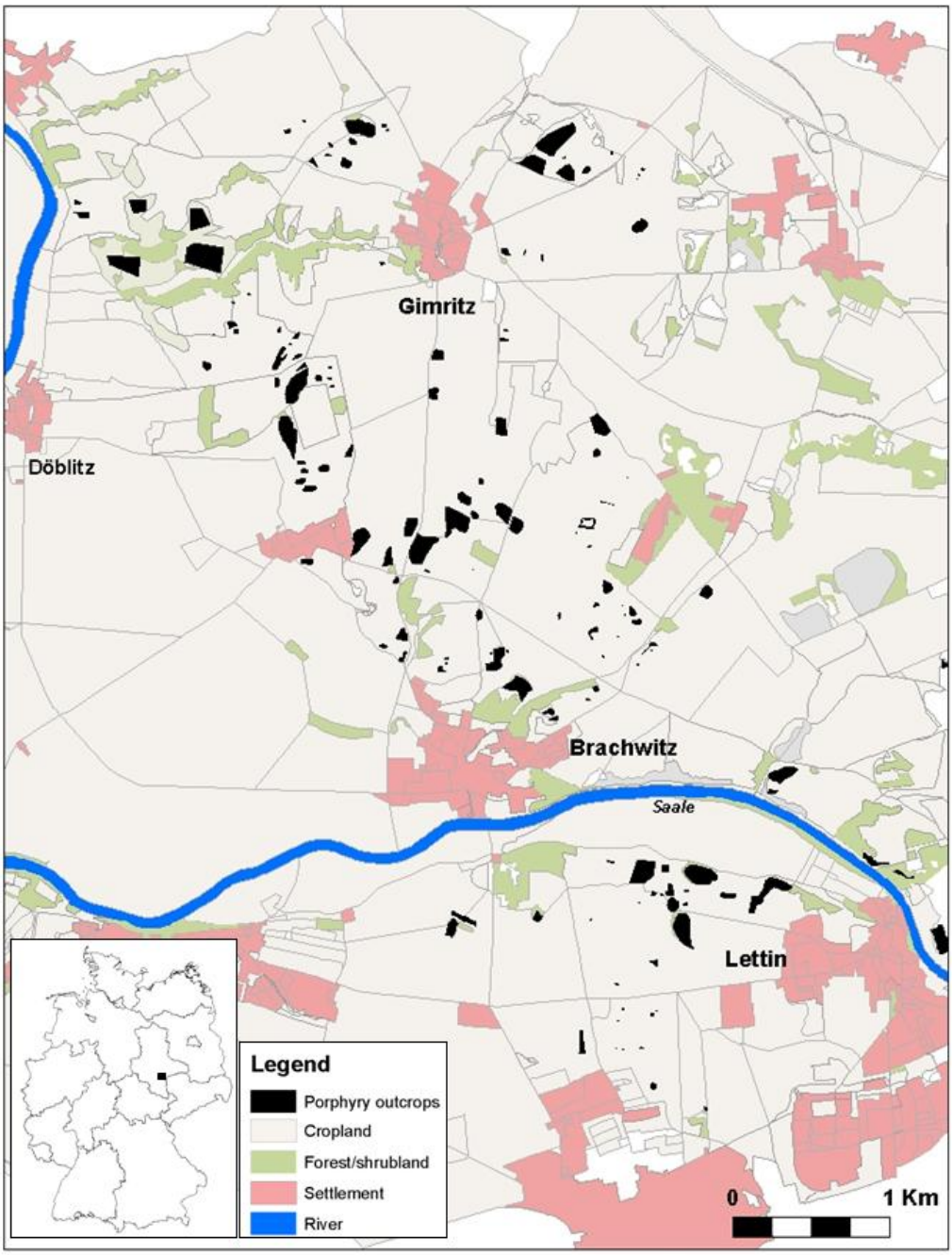

Figure 4: Map of the main study area with ca 150 isolated porphyry outcrops (black). Note that some additional study sites and southern sites from chapter 2 are not shown for clarity (outside of displayed main area).

Aim and structure of this thesis

Much scientific effort in the field of biodiversity research has been put into the study of ecological and economic consequences of biodiversity loss (Díaz et al., 2006; Butchart et al., 2010). As stated above, the drivers of biodiversity loss can affect both, SD of a community and genetic diversity within species. However, these two levels of biodiversity are mostly treated separately, although it is known that GD and SD themselves can be interconnected via direct and indirect mechanisms and processes. Finding GD-SD patterns in natural communities can be strongly dependent on the study species in focus, because species might respond differently to parallel processes according to their life history traits (Aguilar et al., 2008). Moreover, the choice of the study system itself is important for the detection of GD-SD patterns because the processes shaping GD-SD patterns often act on large time scales. Thus, natural and established plant communities are very suitable to study the impacts of parallel processes such as drift and environmental conditions.

This thesis aims to further our understanding of the factors that affect biodiversity at the levels of both, genes and species. By applying a multiple species approach we want to find out if positive GD-SD correlations develop under conditions of long-term habitat isolation (**chapter 1**). Here, we evaluate the impact of parallel processes, i.e. drift and abiotic environmental conditions and discuss the importance of different species life history traits for the GD-SD correlation. As study system we chose species rich dry grasslands in central Germany. These hotspots of biodiversity have a long history of habitat isolation and we expect that parallel processes have contributed to the creation of positive GD-SD correlations.

In chapter 2 and 3 we present two case studies carried out in the same study area as the multiple species study. In both studies we investigate in detail the consequences of habitat isolation, small population size and environmental conditions on genetic diversity and reproductive fitness in two rare plant species, however with different motivations. The first case study (**chapter 2**) on 31 remnant populations of a rare orchid (*Anacamptis morio*) focuses on the impacts of short-term environmental and long-term climatic conditions on population/census sizes. *A. morio* has experienced strong losses during the last few decades, both in the number and sizes of populations and serves now as flagship species for conservation (Böhnert et al., 1986).

In the second case study (**chapter 3**) we analyzed reproductive fitness of remnant populations of *Muscari tenuiflorum,* a species with a long history of habitat isolation. In isolated and small populations genetic reproductive fitness can be reduced because of low genetic diversity and inbreeding depression (Ellstrand and Elam, 1993). However, the detection of relationships between population size, genetic diversity and reproductive fitness may be strongly dependent on the study year because reproductive fitness is often influenced by annually varying environmental conditions. We therefore carried out repeated measurements of reproductive fitness in different study years.

The main objectives of this thesis can thus be summarized to the following questions.

In general, we want to know:

1. How does habitat isolation affect gene flow among plant populations?
2. What is and what affects the relationship between two levels of biodiversity? Do parallel processes create positive GD-SD patterns?

Since annual variation in environmental conditions may affect census sizes and thus reproductive fitness we ask for two species in detail:

3. How do habitat isolation and climate change affect populations of the rare orchid *A. morio* with regard to reproductive fitness and long-term population trends?
4. How consistent are relationships between population size and reproductive fitness across different years?

Study species

Anacamptis morio

- Orchidaceae
- breeding system: XF
- plant size: 10-30 cm
- pollinators: Apidae
- 12 populations studied
- mean PS: 830

Dianthus carthusianorum

- Caryophyllaceae
- XF
- 25-65 cm
- Lepidoptera
- 26 populations
- mean PS: 3760

Spergula morisonii

- Caryophyllaceae
- AFXF
- 5-20 cm
- autogamous
- 17 populations
- mean PS: 2320

Anthericum liliago

- Liliaceae
- AFXF
- 30-60 cm
- Apidae, Syrphidae
- 18 populations
- mean PS: 2930

Scabiosa ochroleuca

- Dipsacaceae
- XF
- 20-60(-80) cm
- Apidae, Syrphidae
- 21 populations
- mean PS: 53

Thymus serpyllum

- Lamiacee
- XF
- 2-10 cm
- Apidae, Lepidoptera
- 20 populations
- mean PS: 218

Carex humilis

- Cyperaceae
- AFXF
- 5-10(-15) cm
- wind pollinated
- 20 populations
- mean PS: 1790

Silene otites

- Caryophyllaceae
- X
- 20-60 cm
- Diptera, Lepidoptera
- 15 populations
- mean PS: 97

Muscari tenuiflorum

- Hyacinthadeae
- XF
- 20-50 cm
- Apidae
- 31 populations
- mean PS: 980

AFXF = mixed mating system, XF = mainly outcrossing, X = obligate outcrossing (taken from biolflor database; www.ufz.de/biolflor)

CHAPTER 1

Connection between species diversity and genetic diversity: an empirical assessment in eight dry grassland species

Gitte Hornemann, Stefan G. Michalski and Walter Durka

ABSTRACT

The relationship between genetic diversity (GD) and species diversity (SD) is still not clearly understood. By now, many theoretical and experimental studies on this essential issue of biodiversity research exist and different hypotheses have been developed that predict either positive or negative GD-SD correlations. Especially processes like drift and selection are believed to indirectly create positive GD-SD correlations by acting similarly and in parallel on both levels. In this study we analyze the impact of parallel processes on genetic diversity of eight dry grassland plant species in central Germany. We used amplified fragment length polymorphism (AFLP) to determine genetic diversity within species. In total 31 isolated grassland patches differing in species richness (range: 43 to 126 dry grassland species per site) and habitat heterogeneity were studied. Drift and selection are expected to be strong determinants of GD and SD in this study system because of long term habitat isolation and restricted gene flow among populations and plant communities. We found high genetic diversity within populations throughout all study species. In three of the eight species we found a positive GD-SD correlation. However, effects of drift and selection are generally low within our study system and probably did not create the observed patterns. Although gene flow appears to be restricted in some cases, species specific responses to habitat isolation are strongly dependent on the species' life history traits, such as breeding system and dispersal strategy. Single species studies may thus not be able to draw general conclusions about the processes acting in such study systems. Therefore, our study highlights the importance for multi species studies.

INTRODUCTION

Genetic diversity (GD) and species diversity (SD) are fundamental levels of biodiversity. There is strong interest in understanding the relationship between these levels, especially in the face of recent climate change and biodiversity loss (Struebig et al., 2011; Wei and Jiang, 2011). However, although it is known that GD and SD themselves can be interconnected, studies on the effects of GD or SD on e.g. productivity, fitness, invasibility or stability traditionally treat both levels independently (Vellend and Geber, 2005). A number of theoretical and empirical attempts have tried to explain the possible connections between multiple biodiversity levels and different processes have been proposed to cause positive but also negative GD-SD correlations.

Most basically, SD may directly depend on GD within species because it provides the raw material for speciation. Furthermore, GD can increase individual fitness and thus decrease the extinction probability of a species (Booth and Grime, 2003). GD may also favor species coexistence because it reduces competition among species ("coexistence hypothesis"; Silvertown et al., 2009). For all these processes a positive GD-SD correlation is expected and support comes from simulation models (Vellend, 2006) as well as from empirical studies (Crutsinger et al., 2006; Johnson et al., 2006; Lankau and Strauss, 2007; Fridley and Grime, 2010). In contrast, a negative GD-SD correlation may develop if populations in species poor communities have high GD because they can reach larger population sizes there ("niche variation hypothesis"; Van Valen, 1965; Nestmann et al., 2011). According to Johnson (1973) this should mainly occur in environments of high predictability.

Additionally to direct effects between both levels also indirect processes acting in parallel can result in GD-SD correlations. These processes are drift, migration and selection which similarly affect GD and SD and subsequently create positive relationships between both levels (Vellend and Geber, 2005).

In small and isolated populations and communities drift causes a loss of both, alleles and species, if this is not counteracted by occasional migration events (MacArthur and Wilson, 1963; Ellstrand and Elam, 1993; Young et al., 1996). Also, environmental heterogeneity can link GD and SD. For example, if varying selective pressures favor different genotypes or species, then the extinction risk of single alleles or species is lower and hence, GD and SD will be maintained over time. Parallel processes do not only affect GD and SD within communities but also have important effects on differentiation patterns. Hence, genetic population differentiation and community distance increase with spatial distance of populations and

habitats respectively (MacArthur and Wilson, 1963). Hence, nearby habitats may share similar species because of frequent species migration at the local scale or because of similar environmental conditions. At the genetic level closely located populations may share more alleles because of frequent gene flow or because selection favors similar genotypes in similar environments (Wright, 1943). Therefore, spatial distance, differences in community composition and genetic differentiation should be strongly related and result in patterns of isolation by distance.

Finding GD-SD correlations created by parallel processes strongly depends on the environmental conditions of the study system. GD-SD correlations should mainly develop if gene flow and drift are at equilibrium. Thus, low migration and high drift will reduce GD and SD at larger spatial distances and environmental differences. In contrast, study systems that are strongly influenced by site or species specific processes that are independent on spatial distance will probably not show strong GD-SD correlations. Moreover, the genetic response to underlying processes like drift or migration is strongly affected by species specific life history traits. For example, individual life span, breeding system, rarity status, population history or dispersal modes influence GD diversity within populations and thus also GD-SD correlations (Frankham et al., 2002; Vellend and Geber, 2005; Puşcaş et al., 2008). Species that are dependent on specialized dispersers or pollinators may show stronger responses to habitat isolation and low gene flow. In contrast, effective wind dispersal of pollen or seeds can counterbalance genetic drift effects despite strong habitat isolation (Berge et al., 1998; Thiel-Egenter et al., 2009). Also, selfing species are expected to show lower response to habitat isolation and low gene exchange among populations (Honnay and Jacquemyn, 2007). Species specific responses to parallel processes must be considered if GD-SD correlations are studied.

Experimental studies on GD-SD correlations often used artificial communities, which are generally set-up with a single focal species of with a low number of study species (Booth and Grime, 2003; Lankau and Strauss, 2007; Silvertown et al., 2009; Fridley and Grime, 2010; Nestmann et al., 2011). However, such approaches exhibit some drawbacks. Experimental communities hardly reflect GD and SD within natural habitats where, for example, competition between species and genotypes is probably much stronger. Moreover, most experimental studies are performed within short time spans. However, processes shaping GD and SD are long-term processes and many generations have to pass to create abovementioned GD-SD correlations

(Slatkin, 1987). Thus, studies analyzing GD in a set of different species in a natural environment can provide more insights into underlying processes.

Here, we analyze the interplay between GD and SD in eight plant species of dry grasslands occurring on isolated habitat islands within the agricultural matrix. At a small spatial scale these habitats share similar but still varying environmental conditions. Thus, we expect that both, restricted gene flow among isolated habitats and local adaptation may have shaped GD-SD patterns. By applying a multi species approach we examined whether GD-SD patterns, if existent, are of general nature or if and how they differ between species. By studying eight species in parallel, we want to answer:

1. How do drift and environmental conditions affect SD and GD in the study system?
2. Is there higher GD in species rich habitats?
3. Are there species specific patterns that can be related to species traits?
4. How do spatial distance and environmental conditions affect population differentiation?

METHODS

Sites and sampling

We studied 31 xerothermic dry grassland sites located in central Germany (Figure 6, Table 2). The grasslands are situated on isolated porphyry outcrops within the agricultural landscape. The majority of the grasslands is smaller than one hectare and characterized by thin soil layers, nutrient poorness and low water availability (Bliss et al., 1996). The grasslands established on porphyric hills that are natural open habitats that lack forests due to natural or anthropogenic factors. Throughout the last centuries the semi-dry grasslands underwent occasional grazing or cutting and harbor xerothermic communities with many specialist plant species which are nowadays restricted to these habitats (Bliss et al., 1996). Plant communities are extra-zonal continental dry and semi-dry grasslands and atlantic/subatlantic dwarf shrub communities (Mahn and Partzsch, 1996; Partzsch et al., 2003; Wesche et al., 2005). Here, many plant species are long lived, stress tolerant and primarily adapted to persistence than to dispersal (Jackel and Poschlod, 2000). Seedling establishment is often prevented by summer droughts and dispersal.

For each site presence of all vascular plant species was recorded. Because the sites are embedded in agricultural fields, ruderal species occurred locally and may obliterate species

richness patterns. We therefore restricted the analysis to 218 dry grassland species (habitat type T6, i.e. poor grasslands including xerothermic and semi-dry grasslands of (Haeupler, 2002), www.ufz.de/biolflor), thus excluding generalist species and arable weeds. For each site we calculated species richness, i.e. the number of dry grassland species, which ranged from 43 to 126. Habitat area ranged from 0.06 to 2.37 ha (mean = 0.71 ha).

For each site we calculated a connectivity index by measuring edge to edge distances between the study site and all known grasslands in the region (n = 152) within a radius of 1 km using the equation:

$CI_i = \sum \exp(-\alpha\, d_{ij}) A^b_j$,

where A_j is the size of the neighboring grassland j and d_{ij} is the shortest distance (in km) to the neighboring grassland (Hanski, 1994). We chose $\alpha = 2$ for the effect of distance to migration ($1/\alpha$ is the average migration distance). For the scaling parameter b, we chose b = 0.5 (Moilanen and Nieminen, 2002). The connectivity index was then regressed against species richness.

We chose eight typical xerothermic grassland species that are restricted to dry grasslands and did not occur in the agricultural matrix or field margins or meadows: *Anthericum liliago* L., *Carex humilis* Leyss., *Dianthus carthusianorum* L., *Anacamptis morio* (L.) R. M. Bateman, Pridgeon & M.W.Chase, *Scabiosa ochroleuca* L., *Silene otites* (L.) Wibel, *Thymus serpyllum* L. and *Spergula morisonii* Boreau. Hereafter, we will refer to each species by its genus. We assessed population size at each site either by counting all individuals (in *Anacamptis* flowering individuals were counted) or by multiplying counted subplots (5 x 1 m^2) with estimated occupied area (*Anthericum* and *Dianthus*). In the clonal sedge *Carex* we only recorded occupied area [m²] instead because it is hardly possible to distinguish between genets in the field. Not all species were present at each site. In total 143 populations were sampled, with 11 to 26 populations per species and 1 to 7 species per site (mean = 4.6). In each study species we took leaf samples of up to 12 individuals per population, if possible, which were immediately dried or lyophilized.

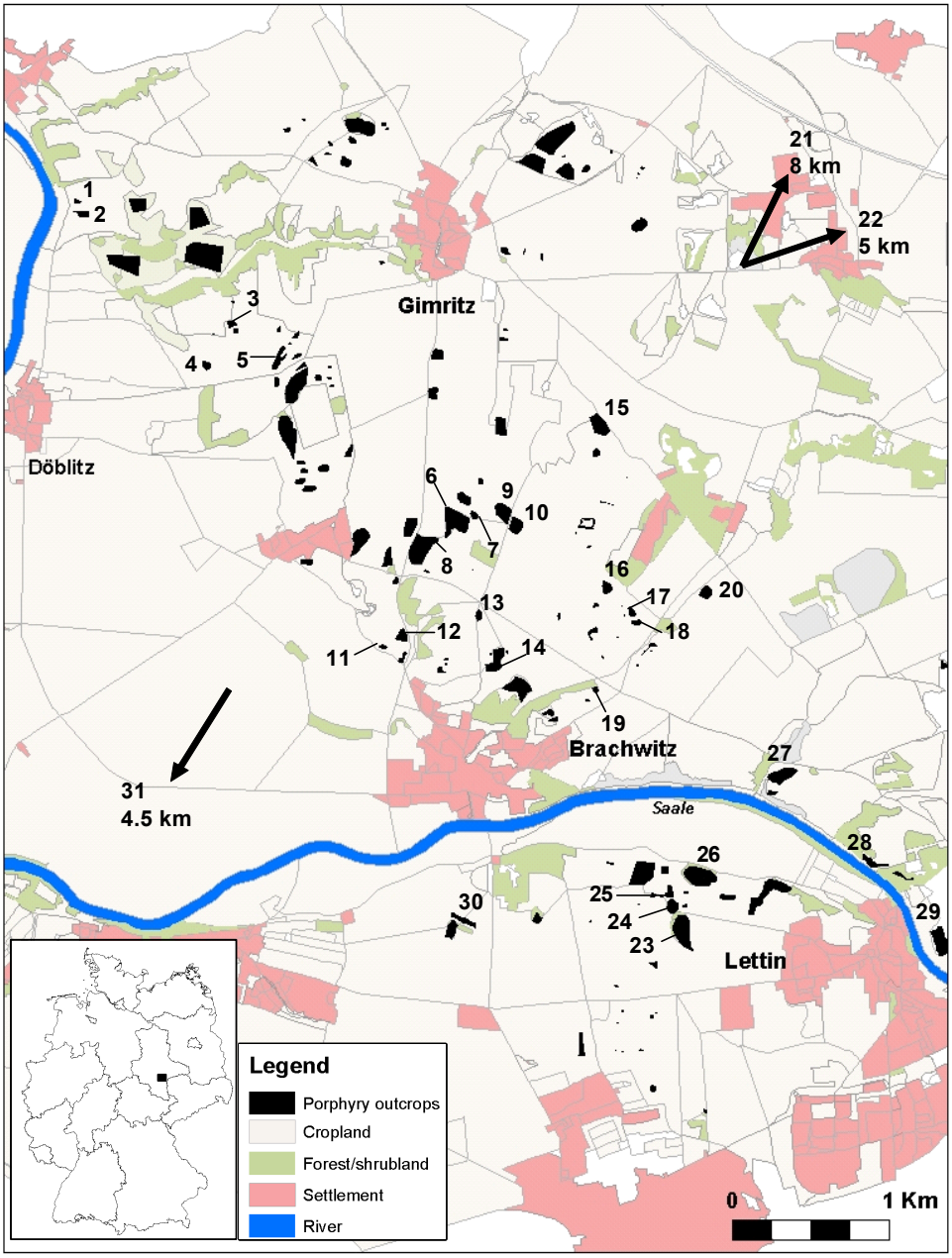

Figure 6: Map of the study area with isolated porphyry outcrops (study sites are numbered). Note that study sites 21, 22 and 31 are not shown for clarity (outside of displayed main area).

Table 2: Sampling sites with details on geographic location, number of sampled species (n), species richness, habitat area [m²], and the three axes of habitat conditions and habitat heterogeneity derived from PCA (in parentheses the direction of indicator values is given)

						Habitat conditions			Habitat heterogeneity		
Site	Lat.	Long.	n	Species richness	Habitat area	axis 1 (+ F, - T)	axis 2 (+ R, + N)	axis 3 (- L, - N)	axis 1 (+ T, - R)	axis 2 (+ F)	axis 3 (- L)
1	51.569	11.834	5	49	1426	-2.181	1.08	-0.35	0.227	0.353	-0.226
2	51.568	11.835	6	68	5773	-3.015	-0.017	0.44	1.819	-2.127	1.509
3	51.562	11.849	7	67	3055	-1.39	-0.058	-0.463	-0.288	1.277	0.396
4	51.560	11.847	3	43	1862	3.185	-0.717	-0.211	-1.617	0.71	0.337
5	51.556	11.854	6	92	21636	0.492	-0.579	0.368	-0.906	-0.174	-0.49
6	51.551	11.869	5	77	18299	0.705	-0.305	-0.196	-1.598	0.466	-0.269
7	51.551	11.871	2	44	1256	2.185	1.547	-1.015	-1.481	-0.465	-1.894
8	51.549	11.866	8	86	20485	-0.739	-1.382	0.314	0.357	-1.575	0.637
9	51.551	11.874	6	69	6505	-0.169	-0.517	-0.559	-1.331	0.669	0.249
10	51.551	11.875	3	59	4470	-1.862	-1.304	-1.03	-1.516	-0.099	0.174
11	51.544	11.862	6	64	2962	-0.163	0.003	-0.693	-0.205	-1.543	0.72
12	51.544	11.864	6	68	5553	1.364	-0.771	-0.094	-0.372	-0.145	-0.757
13	51.545	11.872	4	60	1320	1.891	0.184	0.016	-1.022	-0.953	-0.27
14	51.543	11.873	7	93	8847	1.829	0.62	0.218	-0.262	-0.961	-0.067
15	51.556	11.883	5	84	553	0.06	0.849	-0.47	0.634	-0.947	0.249
16	51.547	11.883	6	86	4264	0.94	0.847	0.559	1.195	-1.344	0.664
17	51.546	11.886	5	60	2058	-0.331	0.02	-1.579	-0.925	0.53	1.455
18	51.545	11.886	4	61	1434	0.181	0.827	-0.883	-0.247	1.015	1.511
19	51.540	11.882	3	50	1096	-1.139	-0.214	-0.313	0.508	1.906	0.179
20	51.547	11.892	5	90	5104	-0.523	0.576	0.248	0.503	0.771	0.707
21	51.633	11.924	3	67	11349	2.737	-0.139	-1.159	-2.039	-1.441	-1.432
22	51.587	11.940	4	68	22998	-2.218	-2.023	-0.677	0.506	-0.04	1.032
23	51.528	11.890	6	124	20414	1.31	-0.641	1.104	-0.178	0.955	-0.89
24	51.529	11.889	6	106	2827	-0.695	0.283	1.396	0.491	0.842	-0.432
25	51.530	11.889	4	79	706	-0.674	-0.345	0.509	-0.497	0.822	-0.948
26	51.529	11.894	3	126	23764	1.399	-1.085	1.656	0.377	-0.645	-1.296
27	51.536	11.898	5	78	2268	1.701	-1.326	1.339	0.412	-1.096	-0.52
28	51.531	11.908	5	90	4920	-1.804	-1.451	-0.447	-0.731	-0.428	-0.448
29	51.526	11.914	5	86	4300	-1.111	-0.02	0.328	-0.192	0.972	-1.091
30	51.528	11.870	4	99	2640	-1.854	1.835	1.829	4.095	1.292	-0.407
31	51.495	11.815	1	79	20106	-1.246	1.904	1.756	3.647	0.433	-2.395

Table 3: Grassland species used in this study in central Germany with information on sampling, abundance and life history

Species	Family	Abundance in study region[b]	n	Breeding system	Life span	Seed weight [mg]	Pollination
Anacamptis morio (L.) R.M.Bateman, Pridgeon & M.W.Chase	Orchidaceae	20	11	XF	p	< 0.01	Apidae
Anthericum liliago L.	Liliaceae	18	18	AFXF	p	5.3	Apidae, Syrphidae
Carex humilis Leyss.	Cyperaceae	20	20	AFXF	p	2.05	wind
Dianthus carthusianorum L.	Caryophyllaceae	43	26	XF	p	1.03	Lepidoptera
Scabiosa ochroleuca L.	Dipsacaceae	24	21	XF	p	1.4	Apidae, Syrphidae
Silene otites (L.) Wibel	Caryophyllaceae	27	15	X	p	0.19	Diptera, Lepidoptera
Spergula morisonii Boreau	Caryophyllaceae	18	18	AFXF	a	0.2	autogamy
Thymus serpyllum L.	Lamiaceae	26	20	XF	p	0.11	Apidae, Lepidoptera

n = number of studied populations, AFXF = mixed mating system, XF = predominantly outcrossing, X = obligate outcrossing (taken from biolflor database; www.ufz.de/biolflor), p = perennial, a = annual

[b] data provided by D. Frank

Abiotic habitat conditions

We calculated mean Ellenberg's indicator values (Ellenberg et al., 1992) for light, moisture, temperature, nitrogen and soil reaction (pH), based on the dry grassland species lists of each site. For the assessment of habitat conditions we used mean indicator values per site and performed a principal component analysis (PCA) in R (function `pca` from package pcaMethods (Stacklies et al., 2007). We extracted the scores of the first three axes which accounted for 85.8 % (48.2 % + 20.2 % + 17.4 %) of variation. The first axis of habitat conditions represents soil moisture (+) and temperature (-), the second axis represents soil reaction (+) and nitrogen (+) and the third axis represents light (-) and nitrogen (-). Similarly we assessed habitat heterogeneity with a PCA based on the coefficients of variation of indicator values per site. The first three PCA axes accounted for 80.7% (35.7 % + 24.6 % + 20.4 %) of variation. The first axis of habitat heterogeneity represents variation in temperature (+) and soil reaction (-). The second axis represents variation in moisture (+) and the third axis variation in light (-). Habitat conditions and habitat heterogeneity were not correlated ($p > 0.05$), except for the first axis of habitat heterogeneity which was significantly correlated with all heterogeneity axes of habitat conditions ($p < 0.02$). Therefore we excluded this first axis of habitat heterogeneity from further analyses.

We computed a community distance (CD) matrix based on the presence/absence of dry grassland species as Jaccard dissimilarity using the `vegdist` function in the vegan package (Oksanen et al., 2011). CD was strongly correlated environmental distance based on indicator values ($r = 0.257$, Mantel $p = 0.002$). Thus, CD is both a measure of the dissimilarity of plant community composition and of abiotic site conditions and is called community distance (CD) herafter.

AFLP analysis

DNA was extracted with the DNeasy 96 plant kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. AFLP analyses followed the AFLP plant mapping protocol (Applied Biosystems, Foster City, CA) with minor modifications. Restriction-ligation samples and pre-amplification products were diluted 1:5 and 1:10, respectively. After initial primer screening, four primer combinations were choosen for each species (Table S1). Fragment analysis was performed on an ABI 3130 genetic analyzer (Applied Biosystems) with GeneScan

600 LIZ as internal size standard. Scoring of fragments was done manually in GeneMapper (version 3.7) and resulted in 85 to 164 polymorphic bands across the six species (Table S1). Individuals with *unsatisfactory* banding patterns were excluded from further analyses. Genotyping error rates were assessed by replicate analyses of x to y samples and ranged from 2.2 to 4.9% between species (Table S1).

Genetic data analysis

Genetic variation at population level was assessed as percentage polymorphic loci (*PLP*), and as mean expected heterozygosity (H_E) based on allele frequencies calculated with the square-root method and assuming Hardy-Weinberg equilibrium following (Lynch and Milligan, 1994) using AFLP-SURV 1.0 (Vekemans, 2002). Additionally we computed band richness (B_R) standardized to the smallest sample size by a rarefaction approach using aflp-div 1.0 (Coart et al., 2005). To generate a composite measure of genetic variation we performed a PCA on data of H_E, *PLP* and B_R, for each species and used the scores of the first PCA axis which accounted for 80.2 – 97.4% of variation across species.

Genetic population differentiation was determined as overall Wrights F_{ST} (Lynch and Milligan, 1994) and pair wise $F_{ST}/(1-F_{ST})$ (Rousset, 1997) in AFLP-SURV. Significance of overall F_{ST} was evaluated by running 1000 bootstrap replicates. To test for isolation-by-distance we correlated pairwise genetic differentiation against log geographic distances. Significance was tested by a Mantel test within the ecodist package in R (Goslee and Urban, 2007).

AFLP are anonymous markers which comprise both neutral loci and loci potentially under selection. Therefore, to assess the impact of selective processes on genetic diversity we performed an outlier analysis followed by logistic regression for each species. We first screened for outlier loci with exceptionally high F_{ST}-values with a Bayesian method implemented in BAYESCAN 2.01 (Foll and Gaggiotti, 2008). Only AFLP loci with band frequencies between 5-95% were used, i.e. excluding very rare and very abundant bands. A Bayes factor of 10 was used as threshold which is considered to represent strong evidence on Jeffreys' scale (Jeffreys, 1961) in favor of the hypothesis, i.e. selection. Second, we performed logistic regressions between band presence and absence at these outlier loci and environmental variables (species richness, habitat conditions and habitat heterogeneity). Because of multiple testing we applied Bonferroni correction for each species separately.

Statistical analysis

Multiple linear regressions were performed to study the relationship between genetic variation and species richness while simultaneously taking into account effects of population size, habitat area, habitat conditions and habitat heterogeneity, in each of the eight study species. Minimum adequate models were selected using AIC and the `stepAIC`-function in R.

We used multiple regression on distance matrices (MRM, (Lichstein, 2007)) to asses the effect of spatial distance and community distance on genetic population differentiation. We fitted models with matrices of pairwise genetic differentiation, spatial distance and community distance using a Pearson correlation and 1000 permutations as implemented in the `MRM` function in ecodist (Goslee and Urban, 2007). We then partitioned the variation into pure spatial ($R^2_{pure\ space}$), pure community distance ($R^2_{pure\ CD}$) and shared components (R^2_{shared}) as follows:

$R^2_{pure\ space} = R^2_{total} - R^2_{CD}$,

$R^2_{pure\ CD} = R^2_{total} - R^2_{space}$,

$R^2_{shared} = R^2_{space} - R^2_{total} + R^2_{CD}$.

R^2_{total} refers to the full model, including all three matrices.

Population size, habitat area and spatial distances were log transformed prior to analyses. Calculations were performed in R, version 2.13.0 (R Development Core Team, 2011), unless mentioned otherwise.

RESULTS

Site descriptors

Habitat area was positively correlated to species richness ($r = 0.594$, $p < 0.001$). Habitat conditions and habitat heterogeneity were independent of habitat area ($p > 0.05$). Correlation analyses of species richness with habitat conditions showed positive correlation to the third axis of abiotic habitat conditions ($r = 0.698$, $p < 0.001$). Species richness was independent of habitat connectivity ($p > 0.05$).

Table 4: Coefficients of multiple regressions relating genetic diversity to population size, species richness and site conditions. Significance levels: . p < 0.1, * p < 0.05, ** p < 0.01

Variable	*Anthericum*	*Anacamptis*	*Carex*	*Dianthus*	*Spergula*	*Scabiosa*	*Thymus*	*Silene*
	t	t	t	t	t	t	t	t
Population size	1.569.	1.388				-1.268	2.664*	
Species richness	1.474	-2.782*		2.361*	2.164.	2.364*	-1.626	
Habitat area	-2.992*	1.774			-2.224*			0.1957
Habitat conditions 1 (+F ,-T)			1.631				2.499*	2.003.
Habitat conditions 2 (+R, +N)	-2.322*	1.399	-1.433	-2.404*	-2.264*	2.149*	2.519*	
Habitat conditions 3 (-L,-N)			1.954.			-1.558		
Habitat heterogeneity 2 (+F)			4.01**					
Habitat heterogeneity 3 (-L)			3.212**		-1.718		2.577*	
total model								
r^2	0.519	0.570	0.574	0.313	0.447	0.351	0.597	0.272
p	0.038	0.216	0.023	0.013	0.105	0.120	0.016	0.149

Genetic diversity

Generally, GD of the eight study species was high (Table 5; range H_E: 0.150 (*Silene*) to 0.233 (*Anthericum*)) and coefficient of variation of H_E ranged from 0.05 (*Scabiosa*) to 0.505 (*Spergula*). Simple correlation analysis of GD as a function of species richness revealed no significant correlation in any species (p > 0.1, Figure 10). Multiple regression analyses of GD revealed significant models in four of eight species (Table 4). A significant positive effect of population size on GD was found only in *Thymus* (t = 2.664, p = 0.019). Species richness had a positive effect on GD in *Dianthus* (t = 2.361, p = 0.027) and *Spergula* (t = 2.164, p = 0.051) and negatively in *Anacamptis* (t = -2.782, p = 0.032). Area had a significant negative effect on GD in *Anthericum* (t = -2.992, p = 0.010) and *Spergula* (t = -2.224, p = 0.046). The five descriptors of habitat conditions and habitat heterogeneity differently explained GD (Table 4). Only in *Carex* and *Thymus* we found significant effects of habitat heterogeneity on GD.

Table 5: Population level values of gene diversity (H_E) and population size of the eight study species.

Site	*Anacamptis*		*Anthericum*		*Carex*		*Dianthus*		*Scabiosa*		*Silene*		*Spergula*		*Thymus*	
	H_E	PS	H_E	PS	H_E	PS*	H_E	PS	H_E	PS	H_E	PS	H_E	PS	H_E	PS
1			0.239	2322			0.186	597	0.207	179				1100	0.166	20
2			0.183	3279	0.214	1301	0.199	5902	0.211	79				9400	0.156	27
3	0.281	2394			0.224	951	0.179	4044	0.216	9	0.162	6	0.162	4200	0.200	26
4							0.194	318						220	0.195	23
5	0.278	77			0.192	10833	0.213	8766	0.205	12				4000	0.183	935
6					0.213	3797	0.181	6387	0.216	12	0.161	7	0.161	100		
7			0.19	50											0.165	11
8	0.174	49	0.237	4778	0.210	2027	0.195	6522	0.205	26	0.173	20	0.173	8300	0.193	740
9	0.263	73	0.203	4317	0.197	401	0.207	4823	0.217	11				450		
10							0.199	1992						240	0.136	20
11					0.174	254	0.203	413	0.228	5	0.168	12	0.168	3000	0.177	237
12			0.198	494	0.209	459	0.198	163	0.231	14				2940	0.192	459
13			0.159	70					0.207	56				2100	0.177	40
14			0.234	8859	0.199	2452	0.173	7860	0.214	210	0.156	37	0.156	2780	0.198	114
15							0.206	138	0.227	12	0.135	5	0.135	130	0.183	37
16			0.247	7100	0.192	342	0.179	742	0.228	70	0.198	31	0.198		0.188	95
17	0.217	17	0.23	55			0.195	260	0.227	12				12		
18							0.197	766	0.236	46	0.162	85	0.162		0.153	5
19			0.296	696	0.224	105	0.187	526								
20			0.231	1945	0.201	158			0.240	12	0.129	51	0.129	251		
21	0.244	8					0.203	4836	0.228	62						
22	0.260	5000			0.218	2961	0.210	13132							0.140	490
23	0.169	856	0.228	9653	0.222	3896	0.216	24107	0.219	98	0.161	348	0.161			354
24	0.191	58	0.257	3020	0.210	714	0.213	1289			0.149	78	0.149		0.128	52
25	0.179	1292	0.266	462			0.214	2583						170		
26	0.207	103							0.217	111	0.159	241	0.159			
27			0.277	663	0.207	359	0.191	104			0.164	10	0.164		0.117	108
28			0.286	1329	0.164	392	0.203	178			0.140	450	0.140		0.098	900
29					0.230	82	0.212	387	0.238	23	0.149	78	0.149		0.100	24
30			0.234	3668	0.208	880	0.197	851	0.233	55						
31					0.169	3379										
Mean	0.218	829	0.233	2931	0.204	1787	0.198	3757	0.221	53	0.158	97	0.158	2317	0.162	225
Overall F_{ST}	0.163		0.123		0.087		0.081		0.123		0.235		0.403		0.164	

H_E = expected heterozygosity, PS = population size (* occupied area in m²)

Genetic differentiation

Overall F_{ST}-values indicated moderate to very high population differentiation (range F_{ST}: 0.087 (*Carex*) to 0.403 (*Spergula*)). CD was significantly positive correlated with log geographic distance (Figure 7, $r = 0.477$, $p = 0.002$). Simple Mantel tests revealed significant isolation by distance, indicating gene flow - drift equilibrium, in *Anacamptis*, *Dianthus, Scabiosa* and *Thymus*. The IBD patterns of *Anthericum, Silene* and *Spergula* indicate a high influence of drift. Low pairwise F_{ST} independent of geographic distance indicated high gene flow among *Carex* populations (Figure 8).

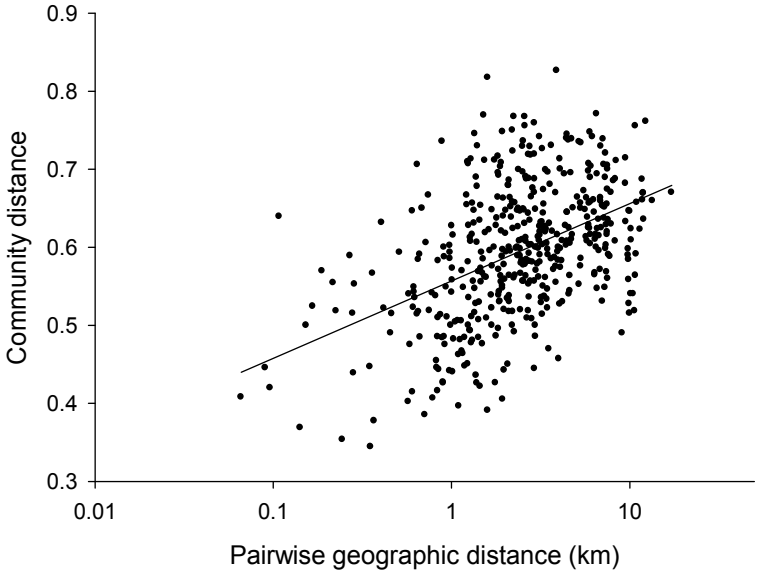

Figure 7: Relationship between pairwise geographic distance and community distance of the 31 study sites.

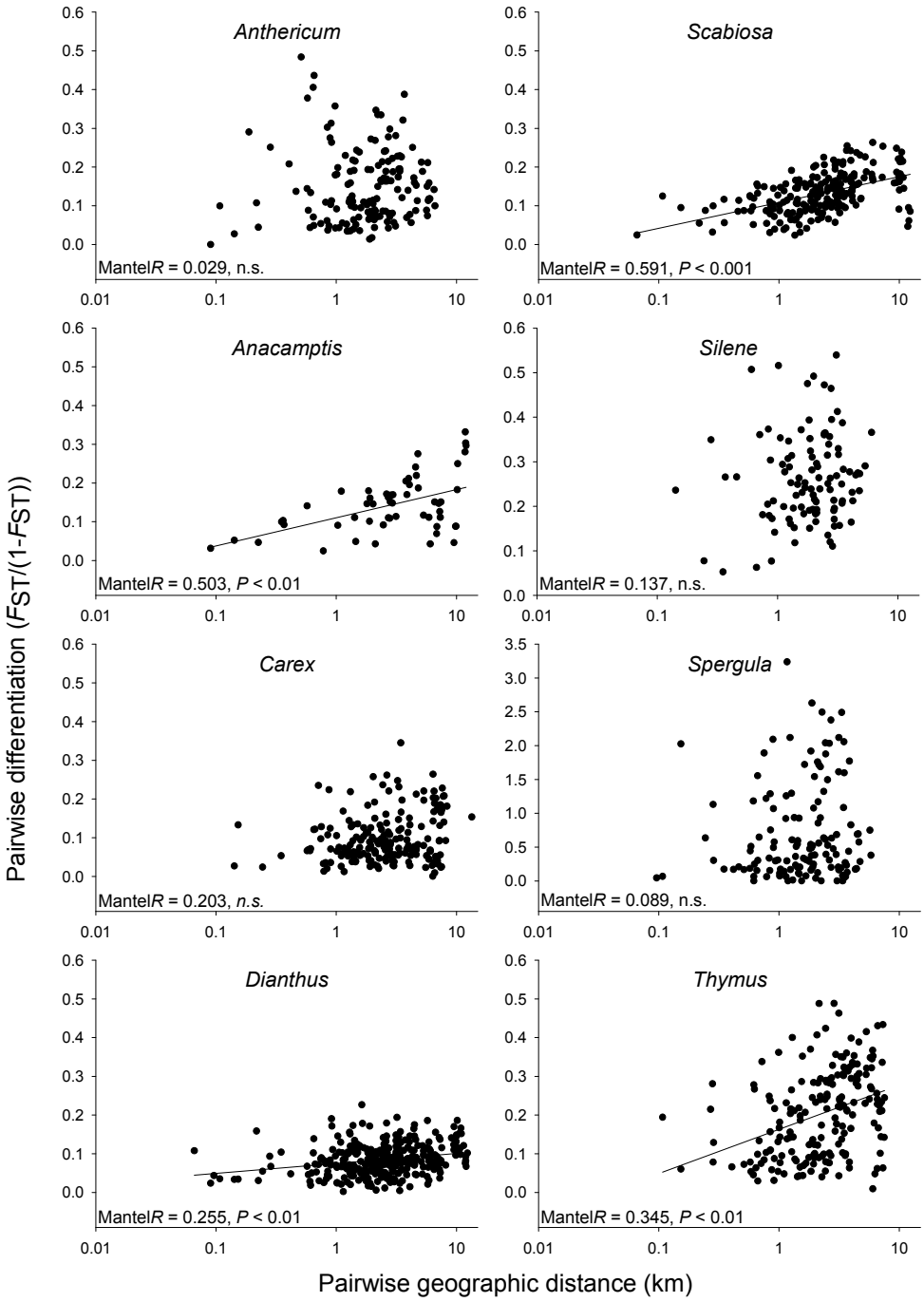

Figure 8: Relationship between logarithmic geographic distance and genetic differentiation ($F_{ST}/(1-F_{ST})$ in the eight study species. Note the different y-axis scale in *Spergula.*

The MRM revealed significant full models, i.e. including spatial and community distance in *Anacamptis, Dianthus, Scabiosa*, *Spergula* and *Thymus*. Generally, the total amount of variance explained by the full models was low and did not exceed 30% (Figure 9). Partitioning the amount of variance into pure and shared components again showed the strong influence of spatial processes on F_{ST} in the four significant MRM models. Only negligible amounts of variation in F_{ST} (0.1 - 2.2 %) was attributed to pure community distance in the significant models, while spatial distance alone explained between 1.6 and 12.3%. Additionally, a relatively high proportion of shared components was apparent due to the strong correlation between the explanatory distance matrices.

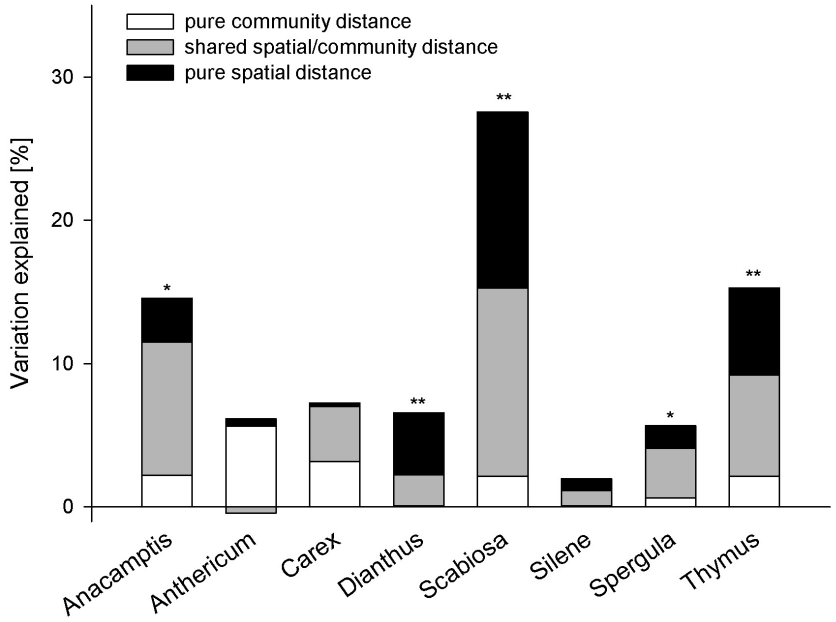

Figure 9: Explained variation in population differentiation (Fst/(1-Fst)) partitioned by MRM into pure spatial distance, pure community distance and shared components. Significance refers to the full models (* $p < 0.05$, ** $p < 0.01$).

Adaptive loci

Out of all 985 screened loci in eight species 77 loci were identified as putatively under selection. Logistic regression of band frequencies at these loci against species richness and habitat descriptors revealed no significant relationships.

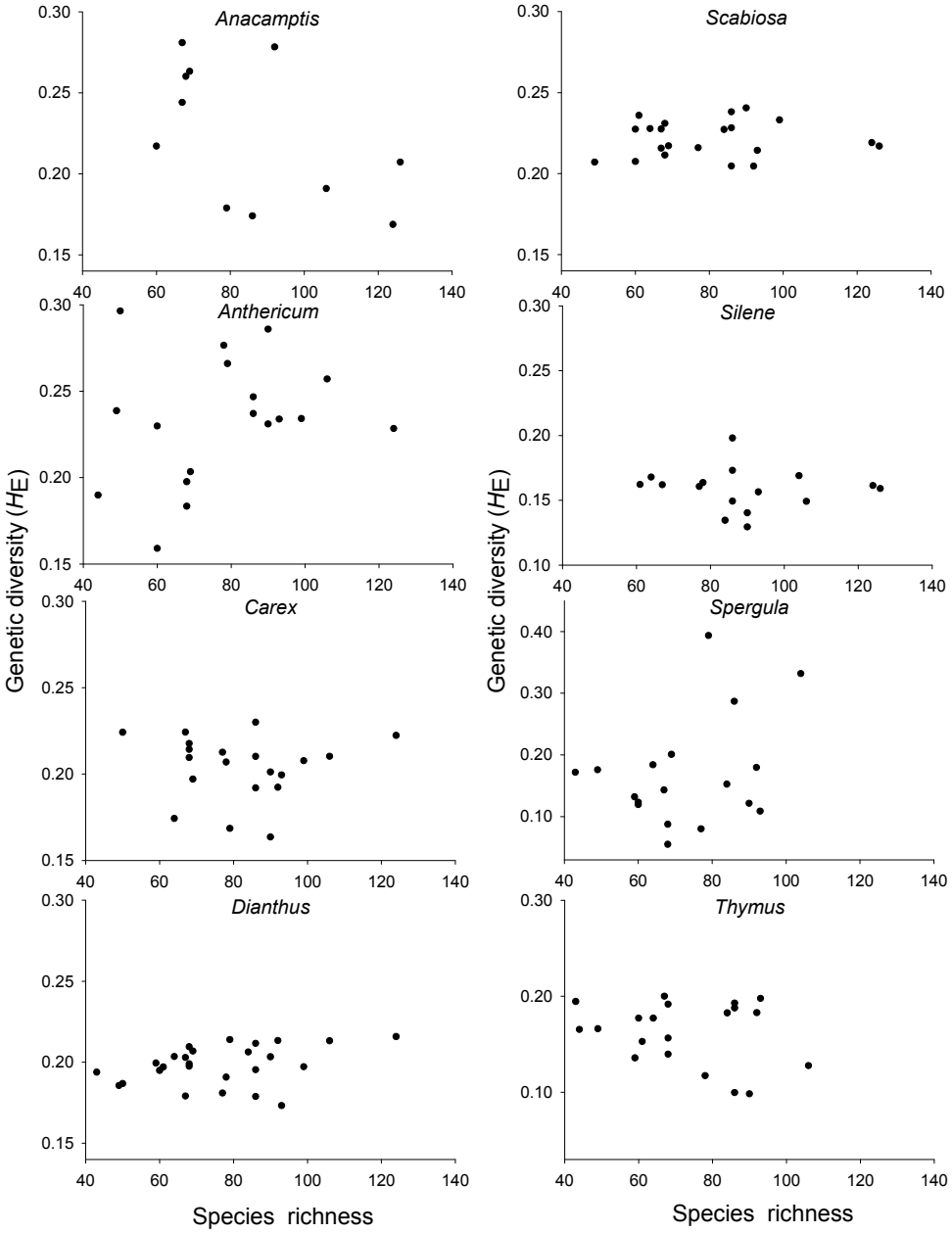

Figure 10: Relationship between species richness and genetic diversity (H_E) in the eight study species. Note the different y-axis scales in *Silene*, *Spergula* and *Thymus* .

DISCUSSION

Semi-natural dry grasslands have repeatedly been used to study metapopulation processes in plants due to their often patchy structure within agriculturally dominated landscapes. Gene flow is often restricted among sites and thus, these isolated habitats are suitable systems to study parallel consequences for both, GD and SD (Wiens, 1976; Picó and Van Groenendael, 2007). Under conditions of restricted dispersal positive GD-SD relationships are expected to develop because of similar effects on both, GD and SD (Vellend, 2004).

1. Impacts of habitat isolation and drift in the study system

On the one hand, habitat connectivity was assumed to promote species dispersal among sites. Species richness should therefore increase with decreasing spatial isolation because of random losses of species over time. However, species richness was independent of habitat connectivity, indicating that habitat isolation did not yet reduce species richness via drift. On the other hand, community composition was probably affected by drift as indicated by the positive relationship between community distance and geographic distance. Thus, *remote* habitats differed more strongly in their plant communities than neighboring habitats. However, increasing differences in environmental conditions with spatial distance might also contribute to those observations.

Moreover, drift was expected to affect GD because of restricted gene flow among isolated populations. Indeed, for some study species, we found evidence for limited gene dispersal between populations as indicated by the isolation by distance patterns in *Anthericum, Silene* and *Spergula*. Remarkably, these processes already become apparent at this small spatial scale (10 km maximum distance among sites). Extreme seed dispersal limitation was also reported previously for *Anthericum* in a population close to our studied populations (Peterson et al., 2002). In their study a strong positive relationship between population size and GD ($r = 0.91$) indicated strong drift effects.

Nevertheless, the populations of the majority of the study species are genetically either well connected, such as in *Carex* or they are at gene flow drift equilibrium as we showed for *Anacamptis*, *Dianthus, Scabiosa* and *Thymus.*

Parallel processes can cause positive GD-SD relationships via effects on population size (Vellend, 2004). Direct positive correlations between GD and population size did not exist in

any of our eight study species. However, the multiple regressions revealed genetic drift effects in *Thymus* in which population size and GD were positively correlated. Also a trend towards higher GD in large populations of *Anthericum* was found. However, in summary our expectations on general strong drift effects in our study system because of long term habitat isolation could thus not be confirmed. A lack of drift combined with high gene flow among populations has also been reported from *Campanula glomerata* in this region and attribute to life history traits such as self-incompatibility, allogamous pollination, high seed numbers and a long lifespan (Bachmann and Hensen, 2007).

2. Community distance effects on genetic differentiation

Differences among communities, i.e. spatial distance and community distance can cause genetic population differentiation. Whereas spatial distance mainly affects gene flow and thus effects of genetic drift (Vekemans and Hardy, 2004), community distance, in contrast, characterizes different environmental conditions to which species may be differently adapted to (Odat et al., 2010). Indeed, spatial distance explained a large amount of genetic differentiation in five of the eight study species, despite the small spatial scale of our study system (mean distance = 3.1 km). Community distance explained a smaller fraction of genetic differentiation throughout all species and a large proportion was attributed to shared components, because spatial distance and community distance were strongly correlated. This means that distant sites were more different in community composition. Nevertheless, in some species (*Anacamptis, Scabiosa, Thymus*) a pure community distance effect accounted for population differentiation and indicated adaptation to local environmental conditions. However, no adaptation to abiotic habitat conditions, habitat heterogeneity or species richness was found, as indicated by the absence of significant correlations between allele frequencies at adaptive loci and environmental parameters. Generally, local adaptation to habitat conditions and thus, population differentiation may be prevented if there is sufficient gene flow between populations (Berge et al., 1998) which is the case in most of our study species. With frequent gene flow among populations selection pressures have to be very strong to cause adaptive population differentiation. Thus, it is not surprising that we find no evidence for adaptive marker loci, at least with regard to the environmental parameters studied here. Moreover, the study species occur under certain restricted and narrow environmental conditions which are often only met in a subpart of the total

habitat. For example, *Thymus* only grows at steep rocky slopes whereas *Carex* prefers deeper soil layers with more dense vegetation. Thus, the study species do and did probably not need to adapt to different conditions because environmental conditions do not vary strongly enough in space and in time.

3. Effects of environmental conditions on GD and SD

We also hypothesized that, additionally to drift effects, varying selective pressures within habitats to act positively on GD and SD (Tilman and Pacala, 1993; Vellend, 2005). However, in our study habitat heterogeneity was not related to SD and also, it was no strong determinant of GD of the eight study species. Some relationships indicate effects of heterogeneity in abiotic site conditions on GD, such as, for example, in *Carex*. Here, GD was on the one hand higher at those sites with higher heterogeneity in soil moisture conditions. On the other hand, in *Carex* we also found that GD is lower at sites heterogeneous in light conditions. These inconsistent patterns together with general low heterogeneity effects throughout all studied species do not allow general conclusions about possible connections between habitat heterogeneity and GD. Thus, for our study system we suppose that habitat heterogeneity is no predictor of both, species richness and GD within species.

Effects of abiotic habitat conditions on GD should mainly arise because of interacting effects with population size because optimal habitat conditions promote optimal growth and reproduction. Here, only in *Thymus*, GD was connected with combined positive effects of habitat conditions and population size.

Instead, habitat area positively affected species richness. Such positive species-area relationships are in accordance with previous patterns within fragmented plant communities and may indicate a sampling effect (Honnay et al., 1999; Bruun, 2000; Krauss et al., 2004). The unexpected negative relationship between area and GD in *Anthericum* and *Spergula* may be related to the age or successional status of the sites. The porphyry outcrops are partly the result of agricultural activities and emerge from the surrounding land surface due to erosion (Mahn and Partzsch, 1996). Thus small outcrops are mostly relatively young and may harbor relatively large proportions of bare soil substrates suited for particular species, like *Spergula* or *Anthericum*, which thus may attain relatively large, stable populations in contrast to larger sites with more closed vegetation.

4. Importance of life history traits for observed patterns

Certain life history traits of our study species may be responsible for the genetic response to habitat isolation, drift or abiotic environmental conditions. Most study species apparently exhibit efficient mechanisms that lower the susceptibility to genetic erosion. Such mechanisms can be long distance pollen and/or seed dispersal or outcrossing breeding systems (Hamrick and Godt, 1996; Aguilar et al., 2008; Ozinga et al., 2009). For example, very high inter-population gene flow and thus low F_{ST}-values were expected and confirmed in the wind pollinated sedge *Carex* (Huh, 2001). Those species being at gene flow drift equilibrium (*Anacamptis, Dianthus, Scabiosa, Thymus*) are predominantly outcrossing, long lived and visited by long-distance pollinators. Moreover, wind mediated seed dispersal (*Anacamptis)* or protandry (*Dianthus*) are additional mechanisms that make species less susceptible to negative genetic consequences of habitat isolation (Loveless and Hamrick, 1984).

In contrast, highest overall F_{ST}-values and lowest GD (H_E) were present in *Silene* and *Spergula,* indicating that - despite a small spatial scale of the study - low interpopulation gene flow may exist. High genetic differentiation among populations is typical for selfers, such as *Spergula* (Hamrick and Godt, 1996). However, explicit breeding system studies are lacking. In contrast, *Silene* is an outcrosser, but generally had very low population sizes (median population size: 37), which can be strongly influenced by stochastic events. In this species, dioecy additionally reduces effective population sizes, minimizes an effective gene exchange between populations and thus contributes to genetic differentiation which is not compensated by the beneficial effect of obligate outcrossing due to dioecy. *Anthericum*, lastly, also is supposed to be highly selfing due a lack of pollinators in many populations (Peterson et al., 2008). Here, the IBD pattern indicated low gene flow among populations. Nevertheless it showed very high GD, which may also be attributed to polyploidy in that species (Soltis and Soltis, 2000).

In summary, the effects of habitat isolation on GD can not be generalized across species. Because of strong differences between species our results stress the importance of multi species studies.

5. Relationship between genetic diversity and species diversity

In three species (*Dianthus, Scabiosa* and *Spergula*) we found a positive GD-SD relationship. However, as discussed above, habitat isolation (i.e. drift) and habitat heterogeneity

did most probably not cause these patterns. Whereas drift might affect GD in *Spergula* (Figure 8) we can exclude this in *Dianthus*, which had throughout very large population sizes, sufficient gene flow among populations and thus showed only moderate differentiation. Furthermore, *Dianthus* occurs frequently at many grasslands in the study region. Interestingly, in *Anacamptis*, a negative GD-SD relationship was found. However, the "niche variation hypotheses" can not explain this relationship because GD was independent of population size.

Similar to patterns within the majority of our study species, Odat et al. (2004) found no connection between GD and SD in the widespread and common *Ranunculus acris* at a comparable spatial scale. Finally, the study design itself might limit the detection of GD-SD patterns. On average we studied 19 (range: 11 – 26) populations per species. First, the number of dry grasslands in the study region is limited. Second, the study species only occur at a subset of all sites which results in the low sample sizes, which reduces the statistical power of the analyses.

CONCLUSION

Random drift at the genetic and species level, i.e. the random loss of alleles or species was found in our study system which suggests parallel effects. The stochastic loss of alleles and extinction of species together with dispersal limitation at pollen and seed level generated isolation by distance patterns at the species level and genetic isolation by distance in some species. However, only weak evidence for correlation of GD with SD was found. Our results suggest that species´ live history traits strongly influence species specific responses to habitat isolation. Detecting effects of habitat isolation on GD-SD relationships strongly depends on the study species chosen. Thus, studying multiple species in parallel is essential to draw general conclusions about processes acting within and among populations and communities.

SUPPLEMENTAL MATERIAL

Table S1: Details on primer combinations used for selective amplification, number of polymorphic loci and error rates for each of the eight study species.

Species	Primer combinations	Poly-morphic loci	Nr of replicate samples	Error Rate [%]
Anacamptis morio	act-cac, aca-ctg, aag-ctgt, agg-ctg	103	22	3.7
Anthericum liliago	act-caac, aca-cag, acc-ctgt, agg-ctg	86	18	4.9
Carex humilis	act-ctg, aca-ctg, acc-cat, agg-ctg	121	34	3.8
Dianthus carthusianorum	act-ctg, aca-ctg, acc-cat, agg-cta	139	30	4
Scabiosa ochroleuca	act-cag, aca-cag, acc-cat, agg-cag	150	19	2
Silene otites	act-ctg, aca-cag, acc-cta, agg-cag	184	35	2.2
Spergula morisonii	act-ctg, aca-cta, acc-ctg, agg-ctg	85	11	3.4
Thymus serpyllum	act-ctg, aca-cta, acc-cta, agg-cag	137	31	4.4

CHAPTER 2

Short-term fitness and long-term population trends in the orchid *Anacamptis morio*

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Plant Ecology (2012), accepted

ABSTRACT

The conservation of endangered species critically depends on the understanding to which degree short-term fitness and long-term trends are affected by intrinsic local conditions and external global dynamics. However, studies combining long-term demographic data with population level analyses of site conditions, GD and reproduction as well as with climatic data are still rare. Here we studied the endangered orchid *Anacamptis morio*, representative for species with a sub-mediterranean distribution. For populations at the northern range edge, we combined long-term monitoring data (1977-2010) with climatic data and analyzed reproductive fitness components, GD and abiotic site conditions. Reproduction was generally low as expected from the deceptive pollination system, and was positively influenced by population size and xerothermic site quality. The majority of populations showed a positive population trend, which was paralleled by an increase in spring temperature and which was positively affected by site quality. High levels of GD were found in the populations which were at gene flow-drift equilibrium. *A. morio* may profit from increasing spring temperatures because of increased reproductive output. Nevertheless, whether climate change results in fitness increase may depend on the maintenance and provision of optimal site quality, i.e. xerothermic and nutrient poor conditions.

INTRODUCTION

The conservation of endangered plant species and declining populations critically depends on the understanding of fitness determinants (Oostermeijer et al., 2003). However, whereas fitness measures at population and individual level may vary among years depending on actual biotic and abiotic environmental conditions (Morris et al., 2008), cumulative fitness across larger time scales eventually determines the long term population trend. Hence, the consequences of site and population characteristics, such as abiotic conditions, population size (Lienert et al., 2002) as well as long term dynamics such as climatic changes (Feehan et al., 2009) need to be taken into account to assess the sustainability of a population (Magnuson, 1990; Oostermeijer et al., 2003). Moreover, the relative contribution of different life-history processes and fitness determinants to long term performance varies across species (Silvertown et al., 1993; Jongejans et al., 2010) and needs to be quantified explicitly to reveal species-specific patterns. Even if in observational studies on endangered species these different aspects are not easy to disentangle due to correlation or multicausality, the potential determinants of fitness have to be addressed to arrive at testable hypotheses.

Abiotic site conditions, such as the soil water relations or nutrients affect plant growth directly or may determine the strength of competitors (Chesson and Huntly, 1989). Thus different habitat types, in particular soil type and water availability can strongly affect reproductive performance, individual fitness and population viability (e.g. Csergö et al., 2011). Also, the spatial relations within and among populations have been shown to be related to fitness (Lienert et al., 2002). Habitat area likely is a proxy for habitat suitability, as it may first be positively correlated to heterogeneity of environmental conditions which may represent a buffer during habitat changes; second habitat area may be inversely related to edge effects, like nutrient input and disturbance (Murcia, 1995). Spatial isolation among populations has been shown to be related to plant fitness (Bizoux et al., 2008) as it affects functional connectivity which may be a proxy for similarity of environmental conditions. Lastly, landscape composition may affect individual fitness due to habitat connectivity or distance to pollinators (Steffan-Dewenter et al., 2001).

Population size has been shown to be a good predictor of plant fitness and population viability (Leimu et al 2006). First, population size first may be itself related to habitat size and

its associated buffering properties mentioned above. Second, population size strongly affects pollination and thus reproductive fitness (Knight et al., 2005). Furthermore, genetic variation often increases with population size which can affect fitness (Ellstrand and Elam, 1993; Leimu et al., 2006). For example, reduced genetic variation entails a higher extinction risk when inbreeding has detrimental effects on fitness (Young et al., 1996). Additionally, genetic variation may reflect the inherent evolutionary potential when environmental conditions change and thus may affect long-term population development (Frankham, 2005). However, as genetic variation often increases with population size, their independent effects on fitness are difficult to disentangle.

Independent of specific local conditions, weather and climate are additional factors acting on reproduction and survival and, hence, on short and long-term population dynamics (Hedhly et al., 2009). Weather conditions during particular time periods, like late frosts or drought may inhibit flowering or fruiting. Moreover, weather conditions can influence the individual resource status or the interactions with pollinators and thus affect reproduction (Jacquemyn et al., 2009). Consequently, varying weather conditions may result in annual fluctuations of reproductive success of individuals or census size of populations (Bernhardt and Edens-Meier, 2010). Climate change, however, operates at larger temporal scales upon plant populations and may affect both phenology and individual fitness components thus leading to altered long term population dynamics (Sparks and Menzel, 2002; Hedhly et al., 2009).

Anacamptis morio (Orchidaceae), representative for species distributions centered in the Mediterranean, has experienced drastic population extinctions within the last century in Central Europe (Jersakova et al., 2002; Jacquemyn et al., 2005; Kull and Hutchings, 2006). In Central Europe, it is now restricted to isolated grassland habitats and serves as a flagship species for conservation (Böhnert et al., 1986). Like many other orchids, *A. morio* is known to be pollen limited which results in low fruit set (Jersakova and Kindlmann, 1998) and may profit from a large display of conspecific or heterospecific flowers (Johnson et al., 2003; Knight et al., 2005). Furthermore, fitness decline in offspring resulting from selfing, i.e. inbreeding depression, suggests an important role of genetic variation for reproduction (Smithson, 2006). Although a number of studies focused on pollination ecology, management requirements and demography in *A. morio,* analyses of reproductive fitness in multiple populations are lacking. Such analyses would provide important indications for the formulation of conservation actions. Additionally,

the impact of genetic variation, population size and local site conditions on reproduction and their importance for long-term population dynamics has not been studied in *A. morio*.

In this study we combine the assessment of reproductive output, genetic variation and site conditions of remnant populations of *A. morio* with an analysis of long-term monitoring data. We want to elucidate a) which abiotic, demographic and genetic population characteristics affect reproductive fitness components in a single year and b) whether and how census sizes across multiple years are influenced by weather and climatic conditions. We furthermore assess c) the long-term trend in population size and its determinants.

MATERIALS AND METHODS

Study species and study sites

Anacamptis morio (L) R. M. Bateman, Pridgeon & Chase (syn. *Orchis morio* L.), the Green-winged Orchid, is a small, perennial, wintergreen geophyte. *A. morio* is food deceptive and mainly visited by early emerging bumblebee queens (Nilsson, 1984). The species is self-compatible but relies on pollinator visits to set fruits (Jersakova and Kindlmann, 1998). Plants produce one inflorescence, with 15–20(–25) flowers (Fay and Rankou, 2010). Fruits contain thousands of wind dispersed dust-like seeds of which only a small proportion is dispersed more than one meter (Jersakova and Malinova, 2007).

A. morio has a wide European distribution centered in the Mediterranean. Across its distribution range it has a broad ecological range and occurs in various types of grasslands and prefers neutral or calcareous soils (Fay and Rankou, 2010). We studied *A. morio* in central Germany where the species has experienced drastic declines in recent decades and is critically endangered (MLRU 1996, Böhnert et al. (1986)). In this area populations are exclusively found in xerothermic grasslands mostly located on isolated porphyry outcrops within the agricultural landscape and most of them undergo conservation management by occasional grazing. The climate of the study area is characterized by low annual rainfall of approximately 460 mm and mean annual temperature of 9.6 °C. Compared to the long term mean, slightly higher than average values were observed for precipitation (633 mm) and temperature (9.9 °C) in the study year 2009.

For a total of 31 populations in the study area long-term monitoring data of the number of flowering *A. morio* plants during peak flowering is available from 1977 onwards, by courtesy of the voluntary working-group for native orchids (Arbeitskreis Heimische Orchideen, AHO Sachsen-Anhalt and AHO Thüringen). The mean number of censuses per population was 21 years (range 9 to 33 years), as not all populations were censused each year. Henceforward, this monitoring data is called the long-term data set.

In 2009, we studied a subset of 19 of these populations for site conditions, genetic variation and reproductive fitness (Table 6, Figure 11). For each site we obtained three descriptors of spatial and environmental site conditions. First, we estimated patch area as proxy for edge effects and habitat heterogeneity. Based on a topographical map (1:25000, Landesvermessungsamt Sachsen-Anhalt) and additional digitization, habitat area (0.15 ha – 131 ha, Tab. 6) was assessed with Arc GIS 10 (ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute). Second, we calculated Hanski´s (1994) connectivity index: $CI_i = \sum_{i \neq j} \exp(-\alpha d_{ij}) A_j^b$, where A_j is the census size 2009 of population j and d_{ij} is the edge to edge distance (km) between populations i and j. We chose $\alpha = 2$ for the effect of distance to migration ($1/\alpha$ is the average migration distance) and $b = 0.5$ for the scaling parameter (Moilanen and Nieminen, 2002). Third, we computed a site quality index summarizing local environmental conditions based on occurrence data of vascular plant species at the respective sites (D. Frank, pers. comm., Hornemann unpubl. data) and the species' mean Ellenberg indicator values for light, soil moisture, temperature, soil mineral nitrogen, soil reaction (pH) and continentality (Ellenberg et al., 1992). Generally, this approach has proven suitable to indicate the prevailing environmental site conditions (Dupré and Diekmann, 1998; Schaffers and Sýkora, 2000). We performed a principal component analysis (PCA) of the mean indicator values. The first PCA axis accounted for 65% of the variation and its scores were used as site quality index (Table 1). The site quality index was negatively correlated to nitrogen and moisture and positively correlated to light, temperature, continentality and soil reaction.

In each population we assessed the total number of flowering individuals in 2009 (census size 2009 hereafter). The number of flowering individuals in a population varies across years and is affected first in the short term by changes in the proportion of adult individuals that actually come into flower and second in the long term by demographic changes in the number of adult individuals. Thus, the census size per year is not an unbiased estimator of population size.

For *A. morio*, both management practices and weather conditions have been shown to affect the proportion of individuals that produce flowers (Silvertown et al., 1994; Wells et al., 1998; Jersakova et al., 2002). Thus, year-to-year variation in census size likely reflects the impact of temporally varying weather conditions rather than changes in population size. However, assuming that variation in the proportion of flowering individuals occurs randomly across years, census size data across multiple years can be used to assess temporal population trend.

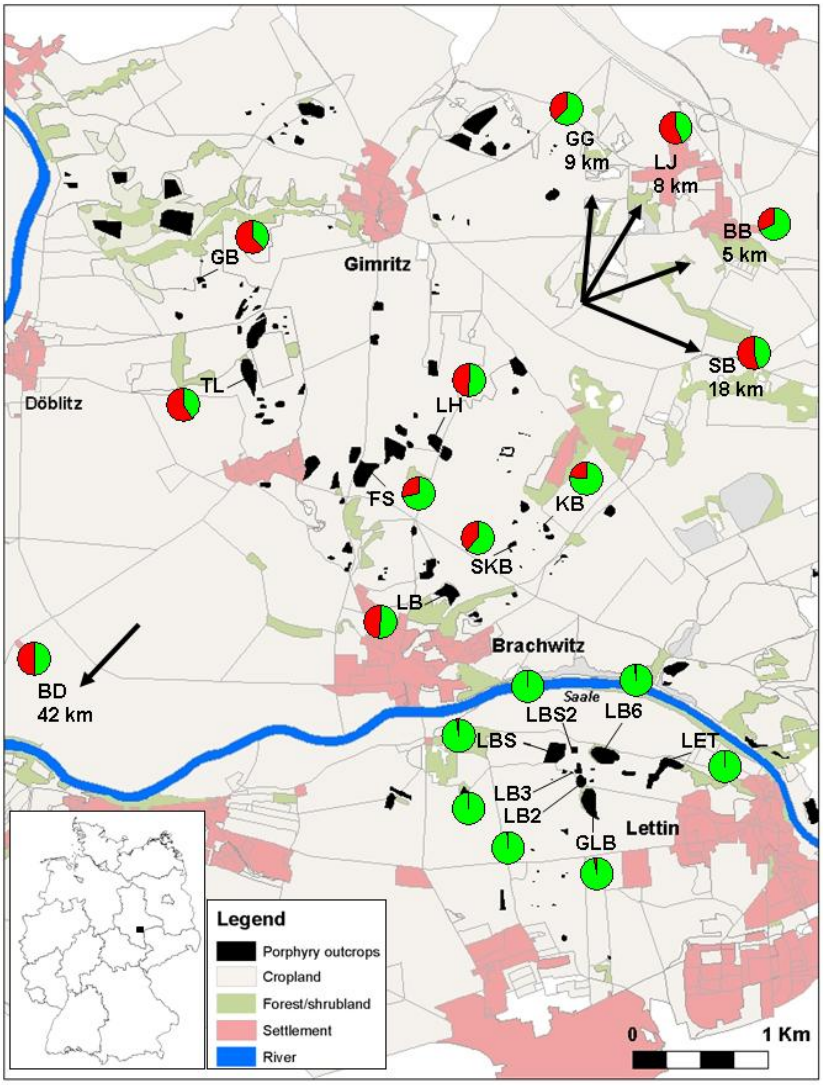

Figure 11: Location of the 19 studied *A. morio* populations. Pie charts denote population level cluster membership coefficients of the Bayesian cluster analysis. For five populations that are located outside of the main study region the direction from (black arrows) and distance (in km) to the main region is given.

Genetic variation and population structure

In each population we arbitrarily selected and marked 30 flowering individuals, if available, and took a leaf sample for genetic analysis. We studied genetic variation with amplified fragment length polymorphisms (AFLP). DNA was extracted using the DNeasy 96 plant kit (Qiagen, Hilden, Germany). AFLP analyses followed the AFLP plant mapping protocol (Applied Biosystems, Foster City, CA) with minor modifications. After screening of 60 primer combinations, four were selected and used for selective amplification with fluorescent labeled primers (ACT/CAC, ACA/CTG, AAG/CTGT, AGG/CTG). Fragment analysis was performed on an ABI 3130 genetic analyzer (Applied Biosystems) with GeneScan 600 LIZ as internal size standard. Scoring of fragments was done manually in GeneMapper (version 3.7) and revealed a total of 103 polymorphic loci in 515 individuals. Based on 22 replicate samples the error rate was 3.7%.

Overall genetic population structure was assessed by Bayesian clustering to test for the existence of spatio-genetic groups (see supplemental information for details). In short, we used STRUCTURE v. 2.3 to determine the most probable number of genetic groups by taking into account samling location (Hubisz et al., 2009). As two spatially structured genetic groups were found (see below) we used, for each population, the proportion of membership to the first group, Q, as a measure of spatio-genetic affiliation in the later analysis of fitness components. For each population we assessed genetic variation as percentage of polymorphic loci (*PLP*) and expected heterozygosity (H_E) following (Lynch and Milligan, 1994) with AFLP-SURV 1.0 (Vekemans, 2002) applying the square root method and assuming Hardy-Weinberg equilibrium. Additionally, we calculated band richness (*Br*), which is the mean number of phenotypes expected at each locus standardized to the smallest sample size (n = 7) by means of a rarefaction method, using aflp-div 1.0 (Coart et al., 2005). We tested for correlation of genetic variation with mean census size (1977-2010). Population differentiation was determined as overall and pairwise F_{ST} in AFLP-SURV. To test for isolation-by-distance we regressed pairwise genetic differentiation $F_{ST}/(1-F_{ST})$ on log geographic distances. Significance was tested by a Mantel test.

Short-term fitness components

In each of the 19 populations, we measured reproductive fitness components in 2009. For all marked individuals we counted (1) the number of flowers per plant. At the time of fruit ripening, we recorded (2) shoot survival, as a part of the shoots withered and (3) the number of

fruits per plant. For surviving shoots, fruit set was calculated as percentage of flowers that turned into fruits. We arbitrarily collected one ripe fruit per plant and determined (4) total seed weight. As a measure of pollination success, we estimated (5) the percentage of seeds containing an embryo. Therefore, the percentage of fully developed seeds was determined by visually analyzing 120 seeds per fruit using a binocular. As an integrative measure of fitness we calculated (6) cumulative fitness as the product of the means of shoot survival, number of flowers, fruit set, seed weight and embryo formation.

We performed standardized multiple regression analyses to explain fitness components and cumulative fitness of populations as a function of demographic, spatial, environmental and genetic predictor variables. Prior to multiple regression analysis, we performed a PCA of the descriptors census size (2009), habitat area, connectivity (*CI*), site quality index, *PLP*, H_E, B_R, and *Q* and discarded highly collinear variables (Figure S3). Minimum adequate models were selected using AIC and the `step`-function in R.

Weather effects on census size

Using the long-term data set, we assessed the impact of weather conditions on census size across years in 31 populations. From data of the local weather station Halle-Kröllwitz (German Weather Service) we extracted the mean monthly precipitation and temperature during four time periods in a year which we hypothesized to affect census size: September - October of the previous year (development of winter leaves), December - February (persistence of winter leaves), April (vegetative growth, bulb regeneration) and May (flowering, fruit production). The log census size was then explained by these weather variables using a linear mixed effect model (`lme`) for repeated measurements. To account for temporal pseudoreplication, the sampling unit (*Year*) was included as random effect. We included the eight weather variables and *Year* as main effects into the model and successively removed non significant terms to obtain a minimal adequate model.

Population trend

Based on the long-term data set we computed the long-term trend in population size (μ) for 31 populations which had been censused in 9 to 33 years (mean 21) in the period between 1977 and 2010. μ, defined as the average change in log-abundance per unit of time was

estimated as the slope of a log-linear regression of log census size against year as implemented by Humbert et al. (2009). We used a statistical model that takes into account both, observational error and environmental stochasticity (EGSS-REML, Humbert et al. 2009). The significance of the population trend is given by the significance of the linear model.

For the subset of 19 populations studied in detail, we performed a multiple regression analysis as described above to explain the population trend μ by taking into account habitat area, site quality index and connectivity (*CI*), assuming these explanatory variables to be constant across years. Census size and other temporally variable predictors were not included as independent variable in this model.

If not stated otherwise, all analyses were performed in R, version 2.13.0 (R Development Core Team, 2011).

Table 2: Sampling sites, census size, genetic variation and reproductive fitness of 19 remnant populations of *A. morio* in central Germany.

Site code	Lat/ Long	Habitat area [ha]	Site quality	Connec-tivity	Mean census size (1977-2010)	Nr of censuses	Census size 2009	μ	n	H_E	*PLP*	B_R	Flowers/ plant	Shoot survival [%]	Fruit set [%]	Seed weight [mg]	Embryo formation [%]	Cumulative Fitness
LBS	51.531/11.887	2.09	-0.681	57.58	1613	22	8375	0.095*	30	0.19	49.5	1.39	11.6	73.3	37.2	11.11	80.1	207014.9
LBS2	51.531/11.888	0.27	-0.243	82.25	10	15	24	0.087*	23	0.16	38.8	1.33	8.8	43.5	26.6	4.22	67.3	21569.16
LB3	51.530/11.889	0.15	1.235	116.43	208	21	1292	0.077	30	0.17	47.6	1.38	9.8	86.7	39.7	8.94	76.1	203705.8
LB6	51.530/11.892	2.07	-0.851	53.57	17	16	103	0.128*	29	0.19	49.5	1.4	9.7	60	50.6	6.54	73.6	91984.75
LB2	51.529/11.890	0.33	-0.487	154.87	18	12	58	0.111	28	0.17	42.7	1.35	8.1	60	36.4	5.21	78.5	65351.86
GLB	51.528/11.891	1.83	0.847	67.35	136	20	856	0.113	28	0.19	54.4	1.35	10.7	80	30.5	5.54	81.6	97922.55
LET	51.529/11.898	3.32	0.825	24.95	11	9	19	-0.029	16	0.14	34	1.3	8	68.8	46.7	5.53	77.2	92918.91
LJ	51.634/11.924	1.39	-2.16	3.82	6	21	8	0.022	7	0.22	57.3	1.57	8.8	37.5	62	2.48	79.6	40253.17
BB	51.586/11.940	3.07	1.929	4.7	3297	27	5012	0.092*	29	0.27	71.8	1.61	9.4	53.3	27.8	6.3	80	43071.83
GB	51.562/11.849	0.31	0.984	9.52	771	26	2394	0.159*	27	0.26	74.8	1.68	10.6	83.3	40.3	5.87	78.1	141348.3
TL	51.555/11.854	2.16	-2.439	11.79	69	27	77	0.034	29	0.26	72.8	1.66	8	40	41.1	5.82	68.9	24367.23
LH	51.551/11.874	0.65	-0.723	15.21	36	31	73	0.046*	28	0.28	76.7	1.67	6.7	66.7	48	6.05	70.9	65276.98
BD	51.308/11.414	100.00	4.329	1	12901	14	20388	0.131	28	0.26	73.8	1.65	9.3	80	31.1	6.15	73.7	86920.29
FS	51.549/11.866	2.05	0.451	15.01	32	25	49	0.041	27	0.25	61.2	1.53	7.5	51.6	52.9	4.29	67.1	47223.03
LB	51.542/11.875	2.78	1.448	16.4	2650	31	3773	0.055*	27	0.26	69.9	1.61	10.2	73.3	40.8	7.08	69	136629.6
KB	51.545/11.886	0.25	0.128	18.75	59	28	17	-0.028	13	0.27	59.2	1.58	8.2	84.6	66	4.19	53.9	94204.24
SKB	51.545/11.882	0.20	-0.819	19.54	47	27	76	0.076	29	0.29	71.8	1.64	8	63.3	37.8	4.56	68.2	43741.97
GG[a]	51.643/11.888	0.24	1.021	3.44	27	25	30	-0.024	28	0.29	76.7	1.65	7.6					
SB	51.520/12.125	1.25	-4.795	2.2	35	32	19	-0.022	16	0.26	64.1	1.63	10.2	42.1	23.2	4.47	63.3	20383.44
mean								0.061		0.23	60.3	1.52	9	63.8	41	5.8	72.62	84660.45

Site quality = PCA scores of axis 1 of Ellenberg indicator values, Connectivity = Hanski's connectivity index, Census size = number of flowering individuals, μ = population trend (asterisks show significant trends); H_E = expected heterozygosity; *PLP* = percentage of polymorphic loci; B_R = band richness; Flowers = Nr of flowers per plant; Shoot survival = percentage individuals that did not wither until time of fruit ripening; Fruit set = fruits/flowers, Seed weight = seed weight per fruit [mg]; Embryo formation = percentage seeds with embryo

[a] no reproductive fitness estimate due to grazing

RESULTS

Genetic variation and population structure

The Bayesian cluster analysis revealed two gene pools and two groups of populations (Figure S1, S2). Group I comprised a number of spatially close populations harboring only one gene pool. Group II consisted of spatially isolated populations and appeared admixed with equal contributions of the two gene pools (Figure 1, Figure S2). Genetic variation (H_E) was higher in group II than in group I (Welch two sample *t*-test: $t = -10.06$, d.f. = 11.93, $p < 0.001$) which may indicate a historical bottleneck in the latter. Overall, H_E and B_R were not significantly correlated to mean census size but a weak positive trend between *PLP* and mean census size was found (p = 0.069). Populations were moderately differentiated with an overall F_{ST} of 0.161 (*SE* 0.06, $p < 0.001$). Population differentiation followed an isolation-by-distance model and increased with geographic distance ($r = 0.458$, Mantel-$p < 0.01$) indicating an equilibrium of genetic drift and gene flow. A Mantel test within the northern group II (excluding population BD) also revealed a positive significant relationship ($r = 0.465$, Mantel-$p < 0.05$).

Short-term fitness

The 19 populations differed strongly in census size, ranging from eight to more than 20.000 flowering individuals in 2009. Population level data on components of reproductive fitness are given in Table 6 and indicate overall low reproductive success and large differences among populations in most fitness components. On average, shoot survival was 64% and fruit set of surviving shoots was 41%. Thus, overall, only 26% of flowers developed into fruits. However, embryo formation was high (73%) and showed low variation among populations.

In the PCA of demographic, spatial, environmental and genetic predictor variables the first axis explained 56% of variation and was strongly loaded by spatial and spatio-genetic structure, (*CI*, *Q*) and by genetic variation (*PLP*, H_E, B_R; Figure S3). As *CI*, *Q* and genetic variation were highly collinear ($R^2 > 0.450$) reflecting the spatio-genetic structuring present (see above), only *CI* was used in the subsequent analyses. Multiple regressions analyzing the combined effect of census size 2009, *CI*, habitat area and site quality on components of reproductive fitness are shown in Table 7. Census size was the most important factor positively affecting several fitness components and cumulative fitness. Strong effects were also found for site quality which

positively affected shoot survival and had a negative effect on number of flowers and seed weight.

To eliminate the effect of the spatio-genetic structuring found, we performed separate analyses for a reduced dataset including only populations of group II. Here, PCA did not show collinearity between *CI* and genetic diversity and hence, these variables were entered as independent effects in the multiple regression analyses. Nevertheless, census size and site quality still had the most prominent effects on several fitness components (Table S5). Additional significant effects were only found for site quality on shoot survival, *CI* on embryo formation and genetic diversity on fruit set.

Table 7: Effects of census size, connectivity (*CI*) and local habitat conditions on components of reproductive fitness and population trend analyzed in 19 *Anacamptis morio* populations. Shown are standardized regression coefficients and significance level of a multiple regression analysis.

	Flowers	Shoot survival	Fruit set	Seed weight	Embryo formation	Cumulative fitness	Population trend (μ)
Census size 2009	0.992***	0.348	-0.432·	0.966***	0.446·	0.778**	---[a]
CI				0.008·	0.007·		0.009
Habitat area		-0.397·				-0.406·	
Site quality	-0.563*	0.567*		-0.209·			0.285*
R^2 (final model)	0.580***	0.565**	0.187·	0.669**	0.281·	0.495**	0.438*

(· $p < 0.1$,* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); [a] census size not included

Weather effects on census size

Several weather variables affected the annual census size in the long-term data set (Table 8). The number of flowering individuals was positively related to higher temperatures in April and to higher temperatures and increased precipitation in the autumn of the previous year and was negatively related to temperatures in May. Weather conditions during winter had no effect. In the analyzed time period (1977-2010), April temperatures showed a linear temporal increase by 0.8 ºC per decade ($r = 0.586$, $p < 0.001$; Figure 12). Thus, when accounting for this temporal trend by including the main effect *Year* into the model, the positive effect of mean April temperatures on census size was not significant anymore, but the effect of May temperature remained and additionally a negative effect of high winter temperature was found (Table 8).

Table 8: Results of the linear mixed-effect models (including and excluding the variable *Year*) for the effect of temperature and precipitation on census size in 31 *Anacamptis morio* populations in 1977-2010.

Year excluded			*Year* included		
Variable	t	p	Variable	t	p
(Intercept)	7.544	<0.001	(Intercept)	-9.956	<0.001
Temp April	3.981	<0.001	Year	10.300	<0.001
Temp May	-3.598	<0.001	Temp May	-4.430	<0.001
Prec Sep-Oct	3.492	0.001	Temp Dec-Feb	-2.144	0.033
Temp Sep-Oct	2.318	0.021			

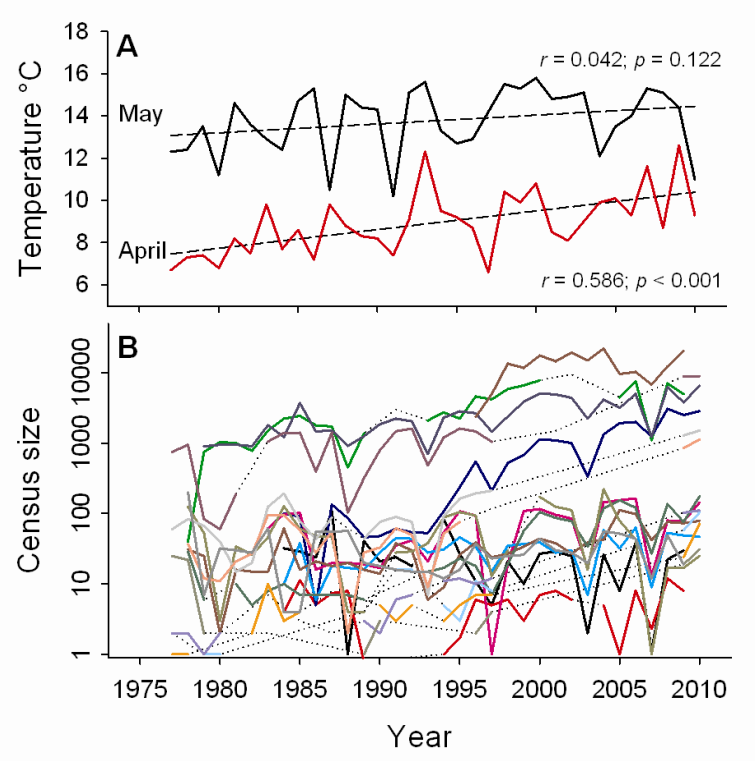

Figure 12: (A) Temporal change 1977 - 2010 of mean temperature in April and May; (B) population sizes of 19 *Anacamptis morio* populations studied in detail (Table 1). Note that another 12 populations mostly with lower population size and non-positive trend are not shown for clarity (cf. Figure 13). Dotted lines are shown for years without census data.

Population trend

In the long-term data set, many populations showed increasing census sizes. Population trend μ was positive for 23 (74 %) of the 31 populations, which were mainly the large populations (Figure 13). Population trend was significantly positive for seven populations and significantly negative for one of the 31 populations. The multiple regression analysis of the effects of local site conditions and genetic variation on μ in the 19 remnant populations revealed a significantly positive effect of site quality (Table 7).

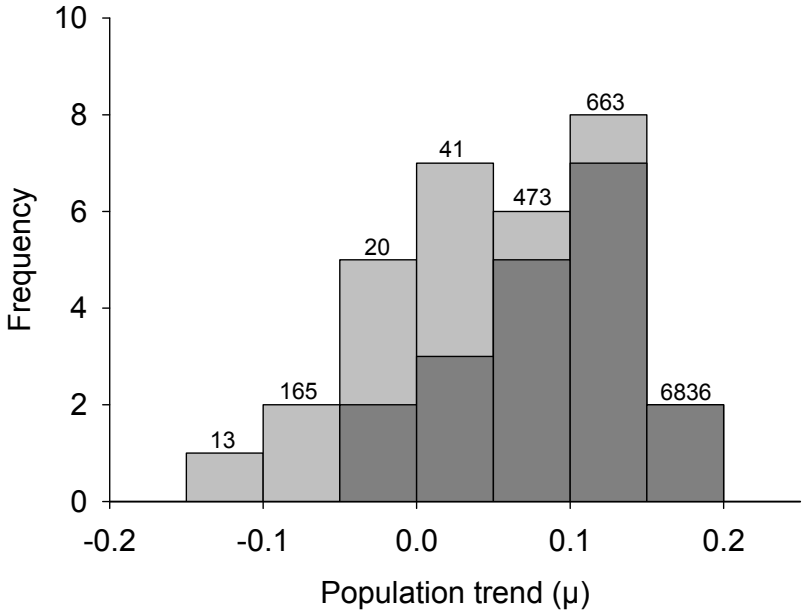

Figure 13: Histogram of population trend (μ) from 1977 to 2010 for 31 *Anacamptis morio* populations. Numbers above bars represent mean census sizes within each category. Dark grey bars represent the subset of 19 populations (Table 1).

DISCUSSION

Effects of site conditions on short term fitness components

Variation among populations in nearly all components of reproductive fitness was most strongly linked to census size and thus conforms to patterns found in many species (Leimu et al., 2006). Such positive relationships with population size can be attributed to different mechanisms. First, large populations and thus large flowering displays may be more attractive to pollinators and can increase pollinator activity (Ågren, 1996). Second, there may be other effects of site conditions (e.g. habitat quality or site heterogeneity) that affect fitness (Leimu, 2010; Lauterbach et al., 2011).

Fruit production is known to be low in deceptive orchids due to pollen limitation. Thus fruit set is usually below 50% whereas nectariferous species generally show much higher rates (Neiland and Wilcock, 1998). Hence, our overall estimate of 26% and 32% fruit set at population and individual level, respectively, fell within the range of reported values in deceptive species and *A. morio* in particular (Neiland and Wilcock, 1998; Jersakova et al., 2006; Smithson, 2006). However, overall fruit set is the product of shoot survival and fruit set per surviving shoot, and these two were affected differently.

First, shoot survival was positively correlated with site quality index which reflects typical dry grassland attributes like low nitrogen and low moisture. Availability of nutrients can strongly influence growth and reproduction in orchids via interspecific competition (Silvertown et al., 1994) and also water stress has been shown to cause early withering (Vallius et al., 2004). Whether shoot mortality of *A. morio* is enhanced on moister and more nitrogen rich sites because of biotic interactions like herbivory or pathogens, remains an open question.

Second, and in contrast to the other fitness measures, fruit set of surviving shoots tended to decrease with census size. This finding fits to an hypothesized influence of deceptive pollination on pollinator behavior and its interaction with population size (Jersakova and Kindlmann, 1998). In general, pollinators learn to avoid the non-rewarding flowers of deceptive species after some visits (Nilsson, 1984). As a consequence, in smaller populations a larger proportion of flowers may have been visited until avoidance takes place as compared to larger populations. This would result in higher fruit set for the smaller populations. However, avoidance behavior is believed to favor long distance pollen dispersal and may foster

outcrossing (Peakall and Beattie, 1996), which in turn can increase seed set and quality for larger populations.

Role of genetic variation and connectivity

Despite spatial isolation and substantial variation in population size, most populations showed high values of genetic variation consistent with other studies of long lived, outcrossing species which show a delayed genetic response to habitat fragmentation and habitat loss (Ewers and Didham, 2006). Still we found indication of genetic drift. First, a group of spatially close populations had reduced diversity, and therefore may have had a common origin in a putatively bottlenecked ancestral population. Second, there was a weak trend towards a positive correlation between genetic variation and mean census size confirming the general pattern (Leimu et al., 2006). Genetic population differentiation in *A. morio* followed an isolation-by-distance pattern confirming that populations are at gene flow - drift equilibrium (Hutchison and Templeton, 1999) and indicating that despite the dust-like seeds, gene flow by seed is spatially limited.

Deceptive orchids generally show low levels of population differentiation compared to rewarding orchids (Scopece et al., 2010), which is attributed to high outcrossing rates in the former. For *A. morio* low differentiation (G_{ST} = 0.064 and 0.055) has been reported in allozyme studies of Italian populations (Scacchi et al., 1990; Rossi et al., 1992). Genetic differentiation in our study (F_{ST} = 0.165) was higher than previous estimates but still in the range of other deceptive species (Scopece et al., 2010). More pronounced differentiation in our study region might be attributed to the location at the northern range margin and putatively stronger spatial isolation of populations.

The populations studied clustered genetically into two groups. Group I (Figure S2) comprised seven, very closely situated populations that showed significant lower genetic diversity than populations of group II. As a consequence, for the complete dataset the effects of genetic diversity and connectivity (*CI*) on fitness components could not be separated. However, using a restricted dataset for which genetic diversity and connectivity (*CI*) were more or less orthogonal, we found a positive effect of *CI* on fruit set and seed weight. Whereas this finding is well expected (cf. Bizoux et al. 2008), the negative effect of genetic diversity on fruit set is counterintuitive and cannot be explained easily. However, due to the limited data, our finding for *CI* and genetic diversity should be treated with caution.

Short-term weather effects on census size

A. morio populations showed strong annual fluctuations in census sizes which could partly be attributed to weather conditions. High temperature in April, one month prior to flowering, had positive effects whereas high temperature during peak flowering in May had negative effects. Warmer April temperatures may enhance photosynthesis rate thus promoting the development of inflorescences. In contrast, high temperature in May, the main flowering month, may result in water stress on the shallow soils and thus may lead to withering of already developed inflorescences. Our results thus confirm earlier reports of high sensitivity of *A. morio* to spring temperatures (Wells et al., 1998). Additionally to flowering and fruit ripening also bulb formation for the nutrient supply in the next season takes place in this sensitive phase. Nevertheless, although optimal weather conditions may be important for individual performance, single years of adverse weather may have little long-term effect as dormant individuals may flower in more favorable years (Jersakova et al., 2002).

Winter temperature has been supposed to affect flowering of *A. morio* because of frost damage to winter leaves (Wells et al., 1998). Mild winters on the other hand may enhance flowering as has been found in *Himantoglossum hircinum*, another winter green orchid that reaches its northern distribution range in central Europe (Pfeifer et al., 2006b). In contrast, we found that higher winter temperature had either no or a negative effect. High winter temperature may be connected with loss of storage carbohydrates due to increased respiration (Bruelheide and Lieberum, 2001).

Long-term effects on population trend

Our evaluation of long-term population trend showed that the majority of populations increased since the late 1970s. As discussed above, although changes in the proportion of vegetative to reproductive plants contribute to variation of census size, this should have minor effects when considering long time periods, allowing to assess changes in population size. The population trend was positive in particular for the large populations. This may be attributed to the fact that the small and decreasing populations are underrepresented because they were already extinct. Moreover, if large population sizes result from positive population trend these two measures are not independent from each other. Still, the positive effect of population size on short term fitness estimates shown above is consistent with the long term trend. Population trend

was also found to increase with site quality. This suggests that nutrient poor and dry conditions, i.e. typical xerothermic grasslands, best fulfill the requirements of *A. morio* both in the short and in the long term. Habitat management should maintain these site conditions to allow for positive population growth. It is known that habitat deterioration is a major reason for decline and extinction of *A. morio* populations. The abandonment of sheep grazing leads to shrub encroachment which is not tolerated by the weak competitor *A. morio* (Silvertown et al., 1994). Also, nutrient input from adjacent agricultural fields or transformation of meadows into arable land leads to habitat deterioration (Kretzschmar et al., 2007), putatively with more severe effects on population sizes.

Climate change was evident as a strong increase of April temperatures, in line with the general pattern of rising spring temperatures in central Europe resulting in advanced onset of flowering (Menzel et al., 2001). In *Ophrys spegodes*, for example, a shift of 6 days per °C rise has been observed within the last four decades (Robbirt et al., 2011). Thus, climate change may drive changes in phenology and subsequently a northward range shift in *A. morio*. However, spring-flowering insect pollinated plants, including *A. morio* are often dependent on a small group of early emerging insects (Nilsson, 1984). Although similar shifts have been observed for insect emergence, earlier onset of flowering may lead to a disruption of plant-pollinator interactions with subsequent consequences for reproduction (Bartomeus et al., 2011).

Implications for conservation

Overall, our results showed that remnant populations of *A. morio* are mostly demographically stable or increasing. They harbour high levels of genetic variation and reproductive fitness is positively affected by nutrient poor and dry habitat conditions. In particular, the findings suggest that climatic change could be beneficial for established populations of *A. morio*. A necessary precondition, however, is the maintenance of typical xerothermic grassland conditions because *A. morio* is intolerant to shade and is outcompeted by larger growing herbs. Climate change may lead to a transformation of semi-dry grasslands - which rely to some extent on management to prevent succession to forest - into naturally open xerothermic grassland. It is thus an open question whether historical management like grazing or mowing is still necessary to prevent succession and shrub encroachment. The dust-like orchid seeds may allow colonization of new sites. However, the genetic isolation by distance pattern

found indicates at least some degree of dispersal limitation. Thus, the likely success of future colonizations will be determined by both, the potential for gene flow (i.e. by seed and pollen dispersal) and the quality of available habitats as a prerequisite for sustainable population sizes and thus, minimized effects of genetic drift.

ACKNOWLEDGEMENTS

We thank the working-groups for native orchids (AHO) of Saxony-Anhalt and Thuringia for providing monitoring data and for help with locating the populations. D. Frank made available species lists of many of the porphyry outcrops. For assistance with laboratory work we thank I. Geier and M. Herrmann. We thank J. Hanspach for help with statistical analyses and A. Kolb for helpful comments. This study was supported by the Federal Ministry of Education and Research (BMBF-FKz 01LC0618C) within the framework of BIOLOG-SUBICON (Successional Change and Biodiversity Conservation). Additionally, kind support was given by Helmholtz Impulse and Networking Fund through Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

SUPPLEMENT

We used STRUCTURE v. 2.3.3 (Falush et al., 2007) to assess population structure. We use the recessive allele option appropriate for dominant data like AFLP. The number of iterations for burnin and analyses MCMC was 50000 each. The number of clusters was run from K = 1 to K = 7 and 10 repeats were analysed per K. The method of Evanno et al. (2005) was used to determine the most probable number of genetic groups based on LnP(D) and ΔK.

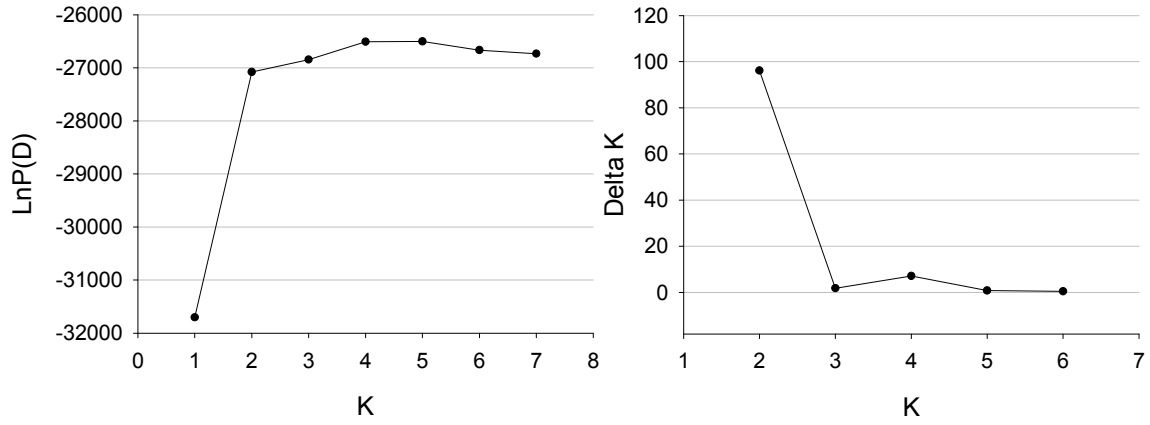

Figure S2: Δ*K* analysis (Evanno et al., 2005) of 491 individuals in 19 populations of *Anacamptis morio*, suggesting *K* = 2 as most probable number of genetic clusters.

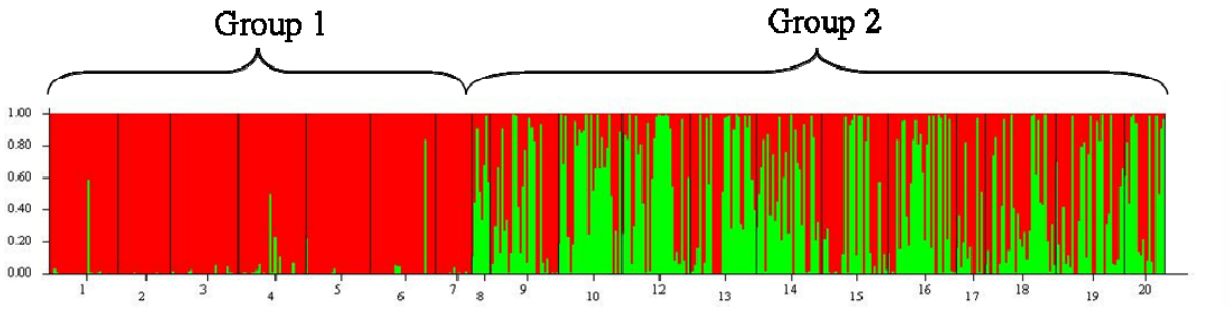

Figure S3: Result of a STRUCTURE analysis at K = 2. Each small bar represents an individual colored according to its membership coefficients in the two clusters.

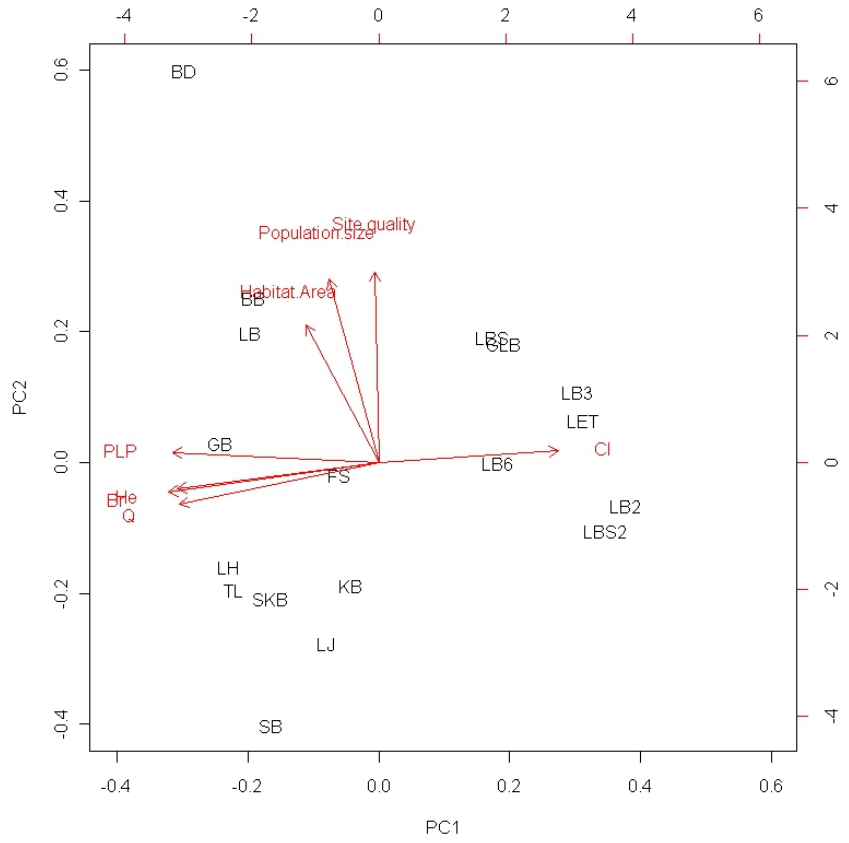

Figure S4: PCA plot showing relationships between local habitat descriptors: census size 2009, area, site quality, *CI* = connectivity index, Q = grouping factor and parameters of genetic variation.

Table S5: Effects of census size, connectivity (*CI*) and local habitat conditions on components of reproductive fitness and population trend analyzed in 11 *Anacamptis morio* populations. In this analysis populations of group I were excluded. Shown are standardized regression coefficients and significance level of a multiple regression analysis.

	Flowers	Shoot survival	Fruit set	Seed weight	Embryo formation	Cumulative fitness	Population trend (μ)
Census size 2009	1.285*		-1.009*	1.446**	0.568·	1.044*	a
Genetic variation	-0.424		-0.523*		-0.469	-0.498	0.346
Habitat area	-0.560	-0.496·		0.265		-0.433	
Site quality	-0.545	0.932**	0.864*	-0.379*			0.295*
CI	-0.283		0.487*	0.598*		0.513	-0.088·
R^2 (final model)	0.737·	0.642*	0.904**	0.871**	0.425	0.658	0.660*

(· $p < 0.1$,* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)
[a] census size not included

CHAPTER 3

Reproductive fitness, population size and genetic variation in *Muscari tenuiflorum* (Hyacinthaceae): the role of temporal variation

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Flora 207 (2012) (in press)

ABSTRACT

In plant populations a positive correlation between population size, genetic variation and fitness components is often found, due to increased pollen limitation or reduced genetic variation and inbreeding depression in smaller populations. However, components of fitness also depend on environmental factors which can vary strongly between years. The dry grassland species *Muscari tenuiflorum* experiences long term habitat isolation and small population sizes. We analyzed seed production of *M. tenuiflorum* in four years and its dependence on population size and genetic variation. Genetic diversity within populations was high (AFLP: $H_E = 0.245$; allozymes: $H_E = 0.348$). An analysis of molecular variance revealed considerable population differentiation (AFLP: 26 %; allozyme: 17 %). An overall pattern of isolation by distance was found, which, however was not present at distances below 20 km indicating stronger effects of genetic drift. Genetic diversity was positively correlated to population size. Self pollination reduced seed set by 24 % indicating inbreeding depression. Reproductive fitness was not correlated to genetic diversity and a positive correlation with population size was present in two of four study years. The absence of a general pattern stresses the importance for multi-year studies. Overall the results show that despite long term habitat isolation *M. tenuiflorum* maintains seed production in many years independent of population size. The long term persistence of populations is thus expected to depend less on intrinsic genetic or demographic properties affecting seed production but on successful plant establishment and persistence which are based on conservation and protection of suitable habitat.

INTRODUCTION

Many plant species do not exist as large continuous populations but rather as isolated patches that are imbedded in a landscape matrix and separated by unsuitable types of vegetation. Patchy habitats often are small in size and are spatially isolated, which is ecologically important because it affects e.g. population sizes, pollinator movement or seed and pollen dispersal (Drury, 1974; Kearns et al., 1998; Steffan-Dewenter and Tscharntke, 1999) and thus may affect seed production and long term population viability.

Small and isolated populations are particularly vulnerable to the effects of genetic drift (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). In the long term genetic drift will reduce genetic diversity which is essential for adaptation to changing environmental conditions (Hughes et al., 2008). In the short term populations may experience higher levels of inbreeding and subsequently reduced individual fitness because inbred plants can have lower seed set, poorer seed quality or reduced offspring survival (Oostermeijer et al., 1995; Kéry et al., 2000; Mix et al., 2006). The effects of genetic drift are even stronger if population sizes fluctuate over time and genetic variation is reduced by bottlenecks (Ellstrand and Elam, 1993; Amos and Harwood, 1998). Isolation additionally leads to increased genetic differentiation of plant populations as effects of drift are not counteracted by gene flow among populations (Aegisdottir et al., 2009; Hensen et al., 2010).

Genetic variation in isolated plant populations has intensively been studied for many plant species (Young et al., 1996; Hensen and Wesche, 2006; Leimu et al., 2006; Aguilar et al., 2008). In general, habitat isolation and small population size are linked to low genetic diversity with potential negative effects on fitness. However, several species traits like breeding system or life span and local or regional characteristics such as rarity, local population history, but also the type of rarity can affect these relationships (Mix et al., 2006). Negative effects of isolation and reduced population size are expected in species that became isolated or rare only recently, but were more common formerly with larger and well connected populations (Ellstrand and Elam, 1993; Aguilar et al., 2008). In contrast, old rare species that are naturally restricted to small and isolated habitats may be less threatened because they are adapted to spatial isolation and small population size (Rabinowitz, 1981; Lutz et al., 2000). Some evidence exists that historically rare and isolated species show no reduction in genetic diversity or fitness with small population size (Brigham, 2003; Aguilar et al., 2008). Mechanisms to counteract negative consequences of

isolation and small population size may include long distance dispersal strategies, permanent seed banks or strategies to cope with genetic threats, e.g. by purging genetic load. Together these factors can buffer species from local extinction (Ozinga et al., 2009).

Seed production in plants is of fundamental importance for local and regional persistence. Seed production is affected by both, environmental factors and population characteristics. However, these effects may differ strongly between years (Hensen and Wesche, 2006; Ågren et al., 2008). Factors that contribute to temporal variation include not only weather conditions and limited resources but also biotic interactions with mutualistic pollinators (e.g. Kenta et al., 2004; Bustamante and Búrquez, 2008) or pathogens. Because of this temporal variation, single observations may lead to biased results, and observations from multiple years may give more insight into underlying processes (Pfeifer et al., 2006a).

Xerothermic dry grasslands represent a type of azonal vegetation in Central Europe that is restricted to spatially isolated regions characterized by low rainfall and therein to limited habitat patches on dry shallow soils and southern exposure. *Muscari tenuiflorum* (Hyacinthaceae) is restricted to these grasslands and thus has a long history of habitat isolation and small population sizes in Central Europe (Herrmann et al., 2006). *M. tenuiflorum* exhibits certain characteristics, like long life span and putatively a high outcrossing rate, which could act as a buffer against loss of genetic variation. Nevertheless, in a one-year study a strong reduction of reproductive fitness in small populations was observed and it was hypothesized to be a consequence of both, pollen limitation and increased inbreeding due to higher selfing rates (Weiss and Mahn, 1996). To further elucidate the observed effects we use repeated assessments of seed production, population genetic approaches, and pollination experiments and asked: (i) What is the level of genetic variation within and among long-term isolated populations of *M. tenuiflorum*? (ii) Is reproductive fitness related to population size and genetic variation and how consistent are these relationships across years? (iii) Do pollinator limitation and inbreeding depression lead to reduced seed set?

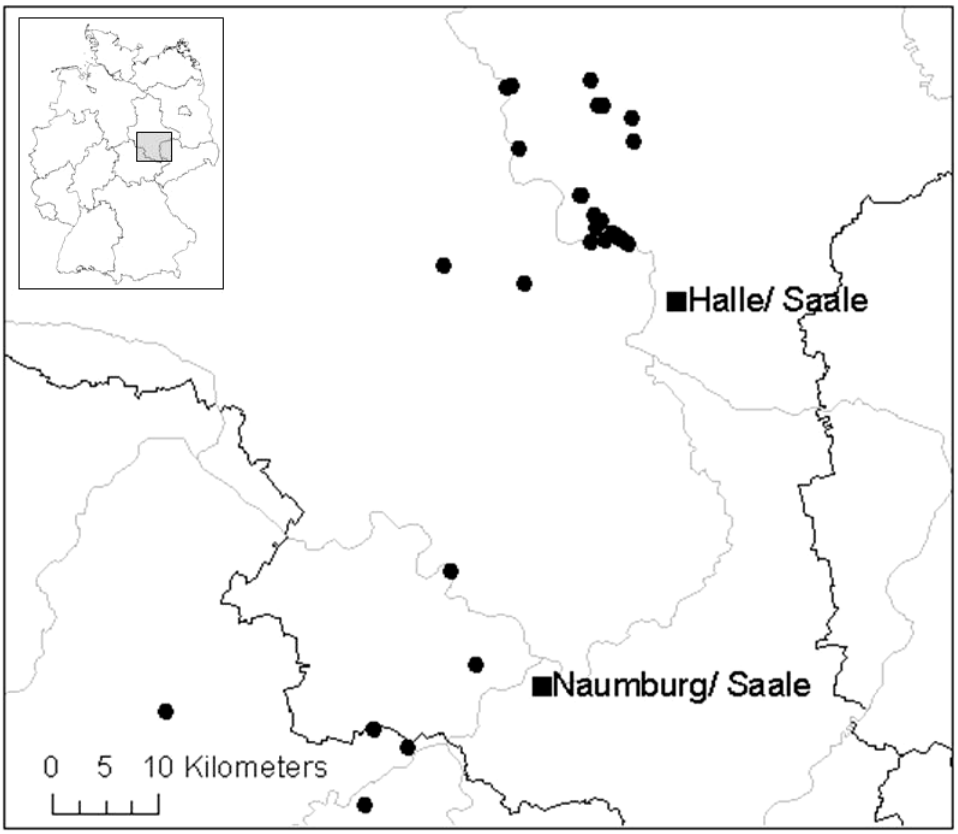

Figure 14: Geographic location of the study sites of *Muscari tenuiflorum* in Central Germany.

MATERIALS AND METHODS

Study species and study sites

Muscari tenuiflorum Tausch. (Hyacinthaceae, 2n = 2x = 18) is a perennial, bulbous herb with exclusively sexual reproduction. The biology of the species has been reviewed previously (Herrmann et al., 2006). Its inflorescences consist of lower fertile and upper sterile flowers. The fertile flowers provide pollen and nectar, and the sterile flowers serve as showy structure to attract pollinators, mainly bumblebees. The species is self-compatible, and seems to be predominantly outcrossed, although a formal estimate of outcrossing rate was not available. The fruit is a capsule with up to six seeds with no adaptations to long distance dispersal. *M. tenuiflorum* reproduces exclusively by seed and does not propagate vegetatively as the bulbs to not produce offset bulbs (Herrmann et al., 2006). It takes at least eight to ten years from seedling stage to maturity and adult plants reach ages far beyond. A seed bank does not exist (Herrmann et al., 2006).

The species' distribution ranges in the submeridional and south-temperate zone (Meusel et al., 1965) from south-eastern Europe to Anatolia, Transcaucasia and the Iranian highlands. The species reaches the north-western edge of its distribution in Germany and occurs at open xerothermic dry grasslands and rarely in dry oak-woodlands on south-facing rocky slopes. In Germany, *M. tenuiflorum* is a red list species and categorized as vulnerable. Here, the species is known to occur at 43 sites (Herrmann et al., 2006), 31 of which were included in the current study (Figure 14). Sites are clustered in two adjacent regions near Halle and Naumburg. Distances between populations range between 100 m and 71 km (23.5 ± 22.0 km, mean ± SD).

Table 9: Summary of study sites and genetic diversity parameters in 31 populations of *M. tenuiflorum.*

				Population size		AFLP				Allozymes			
Site	Name	Latitude / Longitude	Re-gion	Mean (SD)	Years	*N*	H_E	*PLP*	B_R	*N*	H_E	A_R	F_{is}
M01	Dobis	51°36'31.55"/11°46'18.82"	1	1046 (631)	6	12	0.239	52.1	1.42	30	0.349	2.24	0.284**
M02	Gottgau	51°36'31.55"/11°46'18.82"	1	18 (15)	8	4	0.211	35.6	1.36	30	0.199	1.57	0.561***
M03	Schiedsberg	51°38'4.16"/11°55'34.78"	1	8066 (3429)	8	12	0.309	69.2	1.52	31	0.403	2.62	0.09
M04	Schlettau	51°39'59.52"/11°52'13.72"	1	271 (227)	5	12	0.252	56.2	1.43	29	0.438	2.63	-0.004
M05	Krosigk	51°36'53.95"/11°55'47.89"	1	2720 (1545)	6	12	0.287	67.1	1.50	39	0.310	2.29	0.141
M07	Lauchengrund	51°34'5.76"/11°51'20.21"	1	481 (234)	9	12	0.332	74.0	1.56	30	0.386	2.58	0.184*
M08	Ginsterkuppe	51°33'6.94"/11°52'35.66"	1	884 (1217)	9	11	0.303	71.2	1.48	32	0.375	2.42	0.251**
M09	Küsterberg	51°32'48.54"/11°53'13.08"	1	2462 (1995)	10	12	0.262	58.9	1.46	31	0.384	2.72	0.108
M10	Schulberg	51°32'29.09"/11°52'46.14"	1	247 (172)	10	12	0.272	65.8	1.52	30	0.459	2.32	0.245**
M11	Kerbe	51°31'47.32"/11°52'18.24"	1	86 (73)	10	12	0.258	62.3	1.43	30	0.205	1.66	0.435**
M12	Lunzberg 3	51°31'50.95"/11°53'30.86"	1	259 (122)	9	12	0.225	48.6	1.36	26	0.300	1.66	0.093
M13	Lunzberg 2	51°31'48.68"/11°53'27.24"	1	523 (272)	9	12	0.237	52.1	1.41	31	0.464	2.00	0.224*
M15	FM Fels	51°31'51.99"/11°54'50.76"	1	38 (32)	7	12	0.220	52.1	1.37	29	0.326	2.09	-0.047
M16	FM Station	51°31'53.93"/11°54'44.53"	1	74 (103)	7	12	0.173	37.7	1.28	30	0.273	2.12	0.175
M17	FM Hang	51°31'57.80"/11°54'37.26"	1	494 (275)	9	12	0.221	52.1	1.37	24	0.170	1.72	0.14
M18	FM Mitte	51°32'6.53"/11°54'19.60"	1	595 (543)	8	12	0.270	63.7	1.46	37	0.430	2.56	-0.041
M19	Langenbogen	51°29'40.31"/11°46'55.61"	1	800 (469)	4	12	0.301	70.5	1.52	29	0.392	2.29	0.08
M20	Gimritz	51°34'9.65"/11°51'32.66"	1	2183 (1853)	6	12	0.302	72.6	1.55	29	0.456	2.66	0.168*
M21	FM Pfad	51°32'11.69"/11°54'4.54"	1	3800 (2334)	4	12	0.254	59.6	1.44	14	0.348	2.46	0.077
M22	FM Ost	51°31'40.03"/11°55'17.24"	1	498 (323)	5	12	0.216	46.6	1.35	30	0.429	2.3	0.184*
M24	Lämmerberg	51°30'34.72"/11°40'16.02"	1	1800 (1424)	4	12	0.272	62.3	1.46	35	0.415	2.15	0.285***
M25	Pfaffengrund	51°39'34.59"/11°45'23.27"	1	23 (23)	3	8	0.248	59.6	1.44	9	0.252	1.67	0.406
M26	Gerbstedt	51°37'54.45"/11°38'10.13"	1	150	2	-	-	-	-	37	0.333	2.52	0.240**
M29	Rothenburg	51°39'44.35"/11°45'46.63"	1	100	1	12	0.163	34.2	1.25	25	0.338	1.67	0.460**
M32	FM Weide	51°32'9.11"/11°54'8.18"	1	250	1	11	0.272	69.2	1.48	-	-	-	-
M33	Reisdorf	51°6'55.53"/11°34'41.11"	2	200	1	12	0.265	60.3	1.47	29	0.441	2.00	0.128
M34	Bad Sulza	51°6'3.37"/11°37'33.27"	2	200	1	11	0.231	55.5	1.38	10	0.404	2.00	-0.073
M35	Flurstedt	51°3'6.59"/11°34'4.17"	2	300	1	12	0.209	45.9	1.35	29	0.077	1.44	-0.059
M36	Buttstädt	51°7'46.36"/11°17'54.60"	2	1500	1	11	0.238	54.1	1.37	34	0.491	2.33	0.261**
M37	Tote Täler	51°10'13.82"/11°43'4.83"	2	30	1	12	0.110	21.9	1.16	13	0.244	1.65	0.053
M38	Gleina	51°14'58.61"/11°40'58.30"	2	200	1	12	0.189	43.2	1.28	-	-	-	-
mean							0.245	55.81	1.41		0.348	2.15	0.079
SD							0.048	12.68	0.09		0.099	0.38	0.118

Region: north (1), south (2), *Population size*: Number of flowering individuals, *Years*: number of years with census data, *N*: sample size, *PLP*: percentage of polymorphic loci, H_e: expected heterozygosity, B_r: Band richness, A_r: Allelic richness , F_{IS}: inbreeding coefficient.

Sampling

As a measure of population size the number of flowering plants was recorded in 1995, 1998, 2000 and yearly from 2004 to 2008, either by individual counts or by estimations. However, not all populations were included in all analyses. For calculations with mean population size we used the arithmetic mean of all available count data (Table 9). In particularly dry years plants may stay dormant. We are therefore aware, that the number of flowering plants may underestimate the population size. However, such effects are expected to occur simultaneously in all populations as they are located in the same region. Additionally, the populations studied range from a few to many thousand individuals. Thus the differences in census sizes among populations are likely representative for differences in total population sizes.

We used both, dominant amplified fragment length polymorphisms (AFLP) and codominant allozyme markers to characterize genetic variation. For AFLP analyses we randomly collected leaf samples of up to 12 individuals at 30 populations in 2008. Samples were freeze dried immediately after collection. Leaf samples for allozyme analyses were collected from 9 - 39 individuals at 29 populations in 1999. Samples were kept cool and were immediately frozen in the laboratory until analysis.

To quantify components of reproductive fitness we collected, if possible, 20 infructescences per population in July to August of the years 1995 (Weiss and Mahn, 1996), 2000, 2004 and 2007 in 10 to 18 populations. We determined plant height, the number of fruits, number of intact seeds per capsule and the total number of seeds per plant.

As the study was conducted over several years, weather may have had an effect. The 100 year averages for temperature and precipitation during the flowering time (May and June) in the study region are 14.8 °C and 53.4 mm. The respective values in the years of this study, 1995, 2000, 2004 and 2007, were 13.9 °C / 55.7 mm, 17.0 °C / 34.6 mm, 13.9 °C / 85.2 mm and 16.9 °C / 90.1 mm respectively (weather station Leipzig-Schkeuditz, German Weather Service).

Pollination experiment

To examine the effect of spontaneous self-pollination on seed set and the dependence on population size we conducted a pollinator exclusion experiment in 2004 in 15 populations with up to 60 individuals each (in total 530 plants). Before the start of flowering half of the

individuals were bagged with gauze (1 mm mesh size). The other half was marked, left untreated as control with open pollination, but bagged after anthesis to allow collection of all seeds. No manual selfing was conducted as preliminary experiments had shown that hand pollination treatments damaged the flowers. Moreover, because the stigma is placed in between the anthers, spontaneous self pollination does occur in bagged flowers and no hand self pollination is needed. In August we determined the number of intact seeds per capsule and per plant.

Population genetic analyses

We generated AFLPs following (Lachmuth et al., 2010). In short, DNA was extracted from leaf tissue using the DNeasy 96 plant kit (Qiagen, Hilden, Germany) and digested with two restriction enzymes (EcoRI, MseI). After preamplification, we used three selective primer combinations (FAM EcoRI - ACT / MseI-CAG, VIC EcoRI - ACG / MseI - CAC, NED EcoRI - ACC / MseI - CTG). Fragment analysis was performed on an ABI 3130 genetic analyzer with POP7 polymer (Applied Biosystems) and GeneScan 500 LIZ as internal size standard. Only unambiguously scorable polymorphic AFLP bands were manually scored for presence (1) or absence (0) using GeneMapper (version 3.7). An error rate of 3.1 % was estimated from 34 samples replicated from the same DNA extract. Analysis of 345 individuals with three primer combinations provided 146 polymorphic loci in the range of 38 to 493 bp.

We used standard horizontal starch gel electrophoresis to assess genetic variation at allozyme loci. Frozen leaves where extracted with buffer 1 from Soltis et al. (1983). Four loci that had proven to be polymorphic after screening of 14 enzyme systems were analyzed and genotyped according to Wendel and Weeden (1989): ADH (TBE buffer system), GPI (S4 buffer), MDH and PGM (histidin-citrate pH 7 buffer).

For analysis of the outcrossing rate we germinated 168 seeds from 50 open pollinated plants originating from 11 populations. We genotyped them with AFLP and used MLTR (Ritland, 2002) to determine single locus and multilocus outcrossing rates.

For AFLP we calculated within population genetic variation as gene diversity (H_E) and percentage of polymorphic loci (*PLP*) based on allele frequencies which were estimated by the square root method using the inbreeding coefficient derived from the allozyme analysis (F_{IS} = 0.174) using the software AFLP-Surv v. 1.0 (Vekemans, 2002). We computed band richness

(B_R), a rarefaction measure of genetic variation independent of sample size standardized to the smallest sample size with AFLP-Div 1.0 (Coart et al., 2005). Population differentiation was analyzed with *F*-statistics following Lynch and Milligan (1994) with AFLP-Surv.

For allozymes we calculated within population genetic variation as expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}) averaging over loci. Significance of F_{IS} within samples was estimated by 500 permutations. Allelic richness (A_R) was computed using the rarefaction method based on minimum sample size (El Mousadik and Petit, 1996). Population differentiation (F_{ST}) and descriptive parameters were analyzed in FSTAT v. 2.9.3.2 (Goudet, 1995).

As a composite measure of genetic variation we used the scores of the first axis of a PCA of $H_{E\ (AFLP\ +\ allozymes)}$, *PLP*, B_R, A_R and F_{IS} which accounted for 64 % of variation (function pca from package pcaMethods, Stacklies et al., (2007).

For both AFLP and allozyme data population structure was assessed with an analysis of molecular variance (AMOVA) with Arlequin (version 3.1.1.1). In order to test for isolation by distance we correlated pairwise geographic distance against pairwise genetic differentiation and checked for significance using a Mantel test based on 1000 permutations with the EcoDist package (Goslee and Urban, 2007). We used pairwise estimates of Slatkins linearized $F_{ST}/(1-F_{ST})$ and log transformed geographic distances (Rousset, 1997).

Statistical analyses

To analyze if seed set (number of seeds per capsule and seeds per plant) in a given year is dependent on population size of that year, we used linear mixed effect models. To account for the temporal pseudoreplication, the sampling unit (year) was included in the models as a random effect. Simple linear regression was used to assess relationships between log transformed population sizes, genetic variation and components of reproductive fitness.

Multiple linear regressions were used to test the combined effects of population size and genetic variation on components of fitness for each study year separately. As independent variables we used log transformed population size and genetic variation. Dependent variables were seeds per capsule and seeds per plant.

For the analysis of the pollinator exclusion experiment, generalized linear models were used to analyze treatment effects on the number of seeds per capsule and per plant. Treatment

effects on the number of seeds per capsule were tested using an *F* test. The effect on the number of seeds per plant was tested with quasipoisson errors and a log-link function. We adjusted the χ^2 statistics to account for overdispersion employing a corrected *F* test (Crawley, 1993). The factor *Site* was included into the models as interaction term. To analyze if treatment effects were dependent on population size we calculated the log response ratio of seed set for each population, $\ln RR_S = \ln (S_{PE}/ S_{OP})$, with S_{PE} and S_{OP} being mean seed set under pollinator exclusion and open pollination, respectively (Hedges et al., 1999). The $\ln RR_S$ was then regressed against log transformed population size.

If no other program is mentioned statistical tests were performed in R v. 2.7.2. (R Development Core Team, 2008).

RESULTS

Genetic variation within and among populations

Singlelocus and multilocus estimates of outcrossing rate were $t_s = 0.61$ (SD 0.05) and $t_m = 0.81$ (SD 0.05), indicating a mixed mating system with a high proportion of outcrossing and a considerable level of biparental inbreeding ($t_m - t_s = 0.2$). At the population level, estimates of gene diversity of AFLP differed widely among populations and H_E ranged from 0.110 to 0.332, with a mean of 0.245 (Table 9). Percentage polymorphic loci (*PLP*) ranged from 21.9 % to 74.0 %, with a mean of 55.8 %. Genetic differentiation between populations was high (overall $F_{ST} = 0.252 \pm 0.081$ SE). Values for pairwise F_{ST} ranged from 0.016 to 0.561. Hierarchical partitioning by AMOVA showed that 3.45 % of variation was due to differences between the northern and southern region, 26.01 % resided among populations and 70.55 % within populations (Table 10). Mantel tests revealed a significant positive relationship between genetic differentiation and geographic distance in the whole dataset ($r = 0.336$, $p = 0.005$; Figure 15). However, no pattern of isolation by distance was found when the two regions were analyzed separately ($p > 0.3$), indicating a predominant role of genetic drift relative to gene flow.

Allozyme analysis revealed widely differing levels of variation, with allelic richness (A_r) ranging from 1.44 to 2.72 and H_E ranging from 0.077 to 0.491 (Table 9). The inbreeding coefficient (F_{IS}) ranged between -0.077 and 0.561 (mean 0.174). Out of 29 populations, F_{IS} was significantly positive for 13 populations, indicating departure from Hardy-Weinberg equilibrium and a lack of heterozygotes. Genetic differentiation was high (overall $F_{ST} = 0.211 \pm 0.036$ SE),

with pairwise F_{ST} values ranging from -0.031 to 0.784. Similarly, AMOVA revealed 5.47 %, 17.37 % and 77.16 % of variation among regions, among populations within regions and within populations, respectively (Table 10). No pattern of isolation by distance was detected for allozymes ($p > 0.145$).

Patterns of genetic variation were largely consistent between allozyme and AFLP marker systems as revealed by significant correlations (H_E: $r = 0.2$, $p = 0.017$; F_{ST}: $r = 0.443$, $p = 0.003$, Mantel test). All measures of genetic diversity for both genetic marker systems except for F_{IS} were positively correlated to mean population size ($p < 0.05$, Figure 18)

Table 10: Summary of analysis of molecular variance (AMOVA) for the AFLP and allozyme data sets grouped into two regions (north (M01-M32) and south (M33-M38)).
* p < 0.05, ** p < 0.01, *** p < 0.001

	AFLP				Allozymes			
Source of variation	df	Sum of squares	Variance components	Variation [%]	df	Sum of squares	Variance components	Variation [%]
Among groups	1	182	0.81	3.45***	1	24	0.04	5.47***
Among populations within groups	28	2516	6.15	26.01***	27	213	0.13	17.37***
Within populations	327	5455	16.68	70.55***	1595	928	0.58	77.16**
Total	356	8153	23.65		1623	1165	0.75	

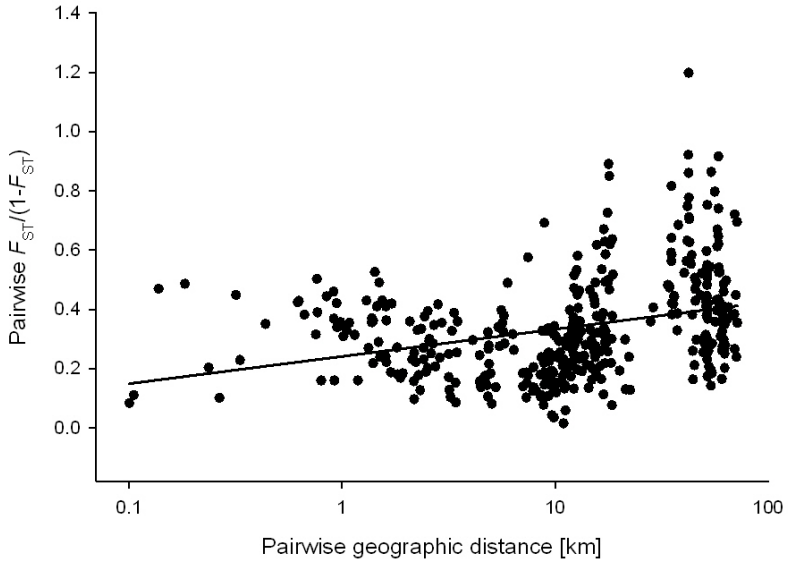

Figure 15: Relationship between pairwise genetic and geographical distances for 30 *M. tenuiflorum* populations (AFLP data); (r = 0.336, Mantel p = 0.005).

Components of reproductive fitness, population size and genetic variation

The pollinator exclusion experiment revealed that even without pollinator visit, considerable seed set was found, indicating self compatibility. At the population level the number of seeds per capsule and seeds per plant differed significantly between treatments (p < 0.001) and sites (p < 0.001) and was reduced under pollinator exclusion. Pollinator exclusion reduced the number of seeds per capsule by 24.6 % (± 13.1 SD) and seeds per plant by 23.7 % (± 14.5 SD) (Figure 16). The reduction of seed set after pollinator exclusion was not significantly correlated to population size or any measure of genetic variation (p > 0.051).

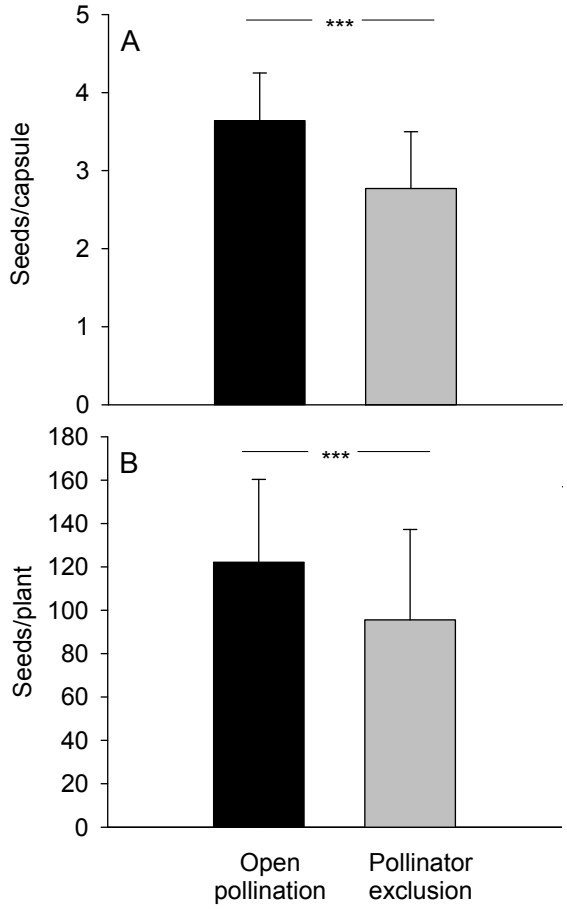

Figure 16: Pollinator exclusion experiment: effects of pollination treatments on the mean number of seeds/capsule (A) and seeds/plant (B) (mean ± SD).

Estimates of components of reproductive fitness varied strongly among years and among populations. Overall, population size had a significant positive effect on the number of seeds per plant ($p < 0.001$) and on the number of seeds per capsule ($p < 0.01$), but such effects differed between the four study years ($p < 0.04$). Separate linear regressions for each of the study years showed that for the number of seeds per plant the relationship was significantly positive in two of four years (1995 and 2000; $p < 0.05$). The number of seeds per capsule was correlated to population size only in 1995 ($p < 0.01$, Figure 17).

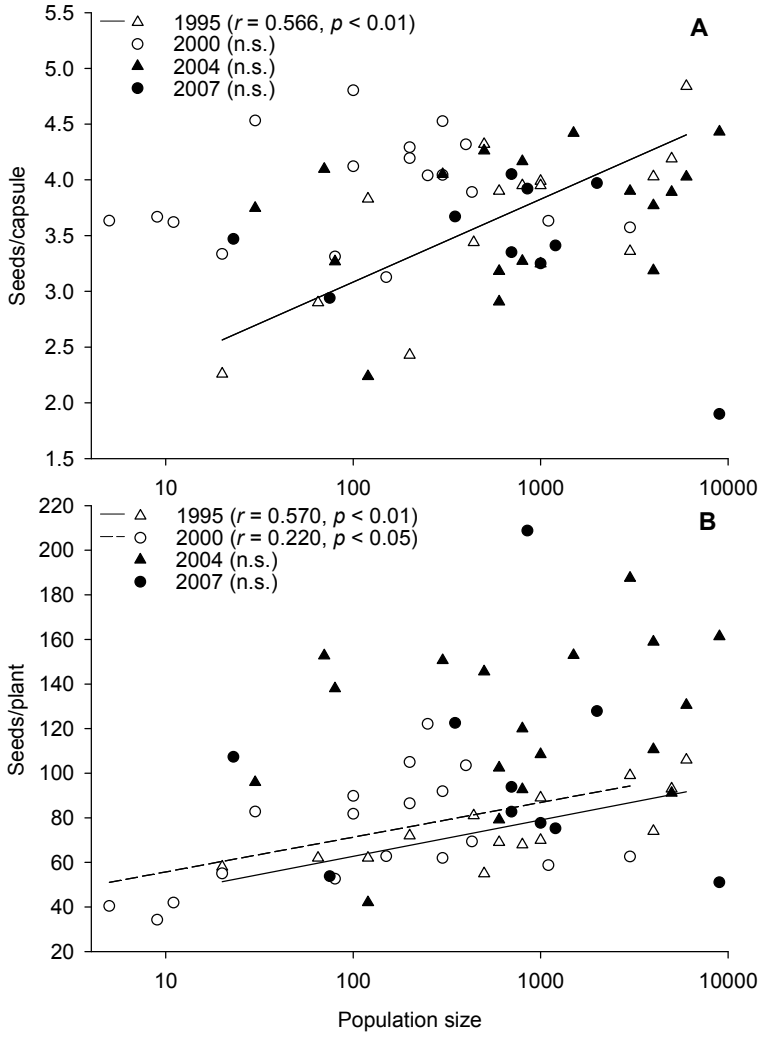

Figure 17: The relationship between population size (number of flowering individuals) and the population mean of the number of seeds per capsule (A) and seeds per plant (B) in four different years. Regression lines are displayed for the significant correlations in 1995 (solid line) and 2000 (dashed line).

A correlation analysis of the population means of the fitness components with the measures of genetic diversity revealed a significant correlation only for plant height with $H_{E\_allozymes}$ ($r = 0.336$, $p = 0.011$) and with F_{IS} ($r = -0.290$, $p = 0.021$).

Multiple regressions on the combined effects of population size and genetic variation on components of fitness for each study year separately revealed no effect of genetic variation. However, population size positively affected the number of seeds per plant in 1995 ($p < 0.01$).

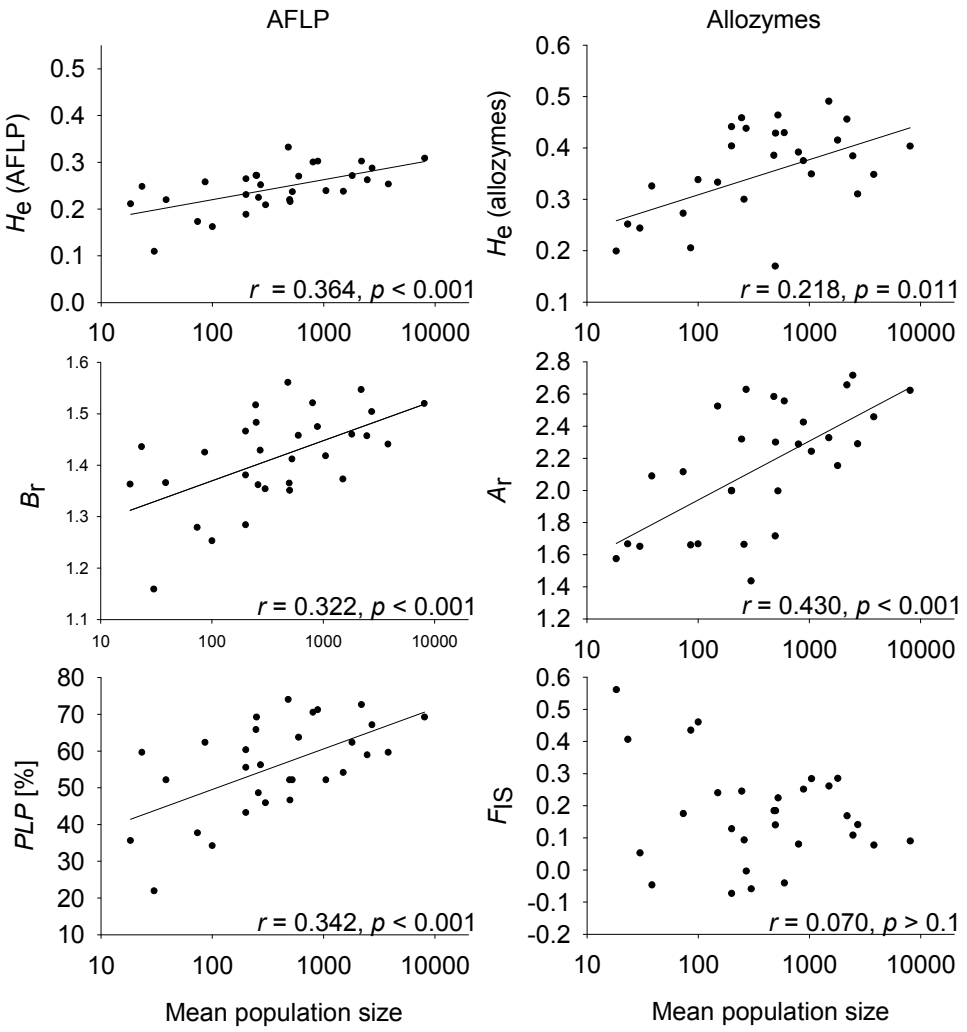

Figure 18: Correlations between mean population size (number of flowering individuals) and measures of genetic variation of AFLP and allozymes.

DISCUSSION

Our study revealed three main findings. First, populations of *M. tenuiflorum* harbor high levels of genetic variation but are affected by genetic drift resulting in population differentiation and reduction of genetic variation in smaller populations. Second, pollinator exclusion leads to a moderate reduction of seed set. Third, seed set was positively correlated to population size in some years, but in two of four years there was no significant relationship. Thus, *M. tenuiflorum* does not generally match the pattern of higher fitness and genetic diversity in large populations

observed in many species (Leimu et al., 2006). Two non mutually exclusive processes may drive a positive correlation between population size and components of reproductive fitness. These processes are pollen limitation because of lower pollinator abundances and inbreeding depression because of higher selfing rates in small populations, both of which may vary temporally.

Seed set

The exclusion experiment revealed that components of reproductive fitness in *M. tenuiflorum* were positively influenced by pollinator visits, although self pollination also results in considerable seed set. Principally, lowered seed set under pollinator exclusion could be attributed to a reduced quantity of pollen reaching the stigmas. Pollen quantity can itself be influenced by species density, pollinator visitation rate or pollinator efficiency (Wilcock and Neiland, 2002; Johnson et al., 2003; Ghazoul, 2006). In large populations higher pollinator activity due to a higher number of flowering plants or larger patch size is expected (Wilcock and Neiland, 2002) and can therefore result in higher seed set in these populations. However, we did not find a general positive relationship between population size and components of reproductive fitness. Also, the abundance of co-flowering species was not related to seed set (unpubl. data). Besides pollen quantity pollen quality has also been shown to affect seed set. Lower reproductive fitness after self pollination, as we found it in this study, has often been attributed to inbreeding depression (Husband and Schemske, 1996). To some extent our results suggest inbreeding effects on plant performance since plant height was positively connected to heterozygosity and negatively to inbreeding coefficients. Nevertheless, our results provide no clear evidence for inbreeding depression in small populations of *M. tenuiflorum*. First, there is no significant relationship between inbreeding coefficients and population sizes despite lower genetic diversity in small populations. Although the highest F_{IS} values were mainly represented in the smallest populations, which may be the result of the long history of inbreeding, there were also very small populations at Hardy-Weinberg equilibrium. The latter may have experienced recent declines and individuals are still representatives of larger and more outcrossing populations. The absence of a connection between population size and F_{IS} has frequently been reported and attributed to a possible selection against homozygotes and an absence of homozygous rare alleles in small populations or Wahlund effects in large populations (Raijmann

et al., 1994; Young et al., 1999; Leimu et al., 2006; Honnay and Jacquemyn, 2007). Second, assuming that inbreeding is responsible for lower seed set in small populations we would expect to find stronger effects of the pollination treatment in small populations than in large ones because of a possible higher relatedness of individuals. However, there was no such effect of population size or genetic variation on the response to the pollination treatments. Probably genetic diversity, which is clearly reduced in small populations, is still high enough to prevent negative effects of inbreeding depression.

Temporal variation

In the repeated seed set measurements we found a positive relationship between population size and seed set. However, both, seed set values and also the relationship between population size and seed set differed greatly between years. Although successful reproduction is essential for population persistence or long term population growth, interannual variation in seed production might be of minor importance in such long lived species as *M. tenuiflorum*. Generally, interannual variation in seed set is very common in long lived plants and single years of low seed production do not critically weaken the long term performance of a population. In contrast, high survival rates of adult plants are more important for population survival in long lived species (Bossuyt and Honnay, 2006). It is known that individuals of *M. tenuiflorum* can get very old (Herrmann et al., 2006), which presents an effective buffer against varying reproductive output among years.

Overall, population size positively affected seed set but still, in two of four study years seed set was independent of population size. Thus, a positive connection between population size and seed set seems to depend on annually changing environmental conditions, which are most likely weather conditions during flowering or fruiting. Both flower development and reproduction in *M. tenuiflorum* are sensitive to weather conditions as the whole inflorescence may wither in bud stage in very dry years (Herrmann et al., 2006). Seed and fruit set in those plants that still flower in dry years are likely to be most strongly affected by water stress. It is well known that drought during seed and fruit maturation leads to seed or fruit abortion (e.g. Lloyd, 1980; Aragon et al., 2008). Fluctuating abiotic conditions are known to cause interannual variation in fruit and/or seed set. In *Rubus chamaemorus*, for example, detrimental late frosts affected flower and fruit development (Ågren, 1988). Delayed flower development after

unfavorable spring conditions can influence fruit set indirectly by an asynchronization of flowering with pollinator activity (Ågren, 1988; Ågren et al., 2008). Thus, possible effects on seed set that are related to population size, such as pollinator activity or herbivory may be concealed by environmental effects. Indeed, precipitation during the flowering period in those study years with no relationship between seed set and population size was very high compared to the long term mean, thus releasing plants from soil water stress. This is consistent with the finding that inbreeding depression is greater under stressful conditions compared to benign environments (Armbruster and Reed, 2005).

The sensitivity of seed set to abiotic conditions also stresses the importance of multi-year studies to elucidate patterns that influence seed set, particularly in systems like these xerothermic dry grasslands that are sensitive to variability of environmental conditions.

Our estimates of components of reproductive fitness were measured in the field, where other environmental conditions like e.g. soil characteristics, management history or interspecific interactions can influence observed patterns. Growing individuals from different sites in the same environment or measuring environmental parameters in situ would surely help to identify environmental effects and to disentangle them from genetic impacts on reproductive fitness.

Genetic diversity within populations

Genetic variation was high in most populations, which is typical for predominantly outcrossing, sexually reproducing and long lived species (Loveless and Hamrick, 1984; Hamrick and Godt, 1989; Nybom, 2004). Such species generally show high genetic variability within and low differentiation among populations (Hamrick and Godt, 1996). However, outcrossing species are especially sensitive to fragmentation and show stronger reductions in genetic diversity and the number of alleles compared to selfing species (Aguilar et al., 2008). Recent population declines and population extinctions in *M. tenuiflorum* may cause such genetic consequences of fragmentation (Dannemann et al., 1999; Frank and Neumann, 1999). In fact, genetic variation was related to population size, but strong reductions were only found at census sizes of less than 200 flowering individuals. Probably, the predominantly outcrossing mating system of the species could maintain high diversity within populations (Loveless and Hamrick, 1984; Nybom, 2004). Additionally, the long individual life span in this geophyte may act as a buffer against the loss of genetic variation (Ellstrand and Elam, 1993). Also, due to cessation of

flowering in very dry years, the variation of census size is not necessarily affecting total population size. This is consistent with the prediction that the genetic response to fragmentation is delayed in long lived species (Lowe et al., 2005; Ewers and Didham, 2006).

We found high genetic differentiation among populations although most genetic variation lies within populations. The absence of isolation by distance at the local scale confirms the low gene flow among populations and a strong influence of genetic drift. Similar patterns have been reported from other naturally isolated and rare species (e.g. Kuss et al., 2008; Aegisdottir et al., 2009). Additionally, colonization of new habitats is unlikely although suitable but unoccupied sites do exist. The heavy seeds can only be dispersed in the fur or hooves of grazers (Herrmann et al., 2006). However, sheep pasturing, in former times the traditional land use practice in former times, strongly declined since 1989 (Herrmann et al., 2006), and the current conservation management focuses on removal of biomass but less on functional connection of habitats through movement of sheep between sites.

In conclusion, our findings show that despite long term habitat isolation and recent declines, *M. tenuiflorum* populations mostly maintain high levels of genetic variation and seed production, the latter being affected by population size only in single years. Slow growth, long lifespan and high outcrossing rate may partly buffer effects of population size. Thus, long term persistence of populations is expected to depend less on intrinsic genetic or demographic properties affecting seed production but on successful plant establishment and persistence which is based on conservation and protection of suitable habitat.

ACKNOWLEDGEMENTS

We thank Katja Wittig for help during fieldwork and I. Geyer and M. Herrmann for assistance in laboratory analyses. This work was supported by the Federal Ministry of Education and Research (BMBF-FKz 01LC0018A) within the framework of BIOLOG-SUBICON (Successional Change and Biodiversity Conservation).

Synthesis

The porphyry hilly landscape of Halle provides excellent conditions for studying natural and long-term habitat isolation and its consequences for plant populations. Moreover, this system allowed us to investigate the processes that shape relationships between levels of biodiversity.

Effects of habitat isolation on genetic diversity within and among populations

The long term habitat isolation of the porphyry outcrops was expected to influence genetic diversity within and among populations of dry grassland plant species. Strong effects of habitat isolation on genetic diversity of several plant species have been reported in earlier studies in this region (Dannemann et al., 1999; Peterson et al., 2008; Hensen et al., 2010) but also low genetic responses to habitat isolation are found (Bachmann and Hensen, 2007). Indeed, we could confirm that gene flow among the studied populations is affected by spatial habitat isolation. Effects were apparent mainly at the inter-population level, i.e. population differentiation. Accordingly, pronounced to high genetic differentiation was present in the studied species (overall F_{ST}: range 0.081 – 0.403) and a positive relationship with geographic distance was found in the majority of species. Hence, gene flow mainly happened between neighboring sites creating positive isolation by distance and confirming strong drift effects at larger scales. Moreover, very low gene exchange – already at very small distances (mean distance = 3 km) – was found between populations of *some* study species. In these, general high pairwise F_{ST}-values were independent of spatial distance.

Low inter population gene flow can lead to reductions of genetic diversity within populations (Young et al., 1996). A reduction of genetic diversity because of genetic drift would be apparent in small populations first (Leimu et al., 2006). Accordingly, under conditions of limited gene dispersal one would expect to find positive relationships between population size and genetic diversity. This relationship was only found in one species (*Muscari*) and little effects appeared within the multi-species study. Moreover, all study species showed high mean genetic diversity (H_E: range 0.160 – 0.245). Both, genetic diversity within species and also the different differentiation patterns of the study species may be attributed to their life history traits as follows (Loveless and Hamrick, 1984). First, the high mean genetic diversity found in nearly all studied species (except *Spergula*) can be linked to their predominantly outcrossing breeding

system. Outcrossing species typically show high genetic diversity within populations (Nybom, 2004). However, reductions in genetic diversity caused by isolation, fragmentation or sudden decreases in population sizes are expected to be more pronounced in outcrossing species (Aguilar et al., 2008), a pattern which we can not confirm with our study species or for our study system. In contrast, selfing species tend to have lower genetic diversity (Hamrick and Godt, 1996). Only one species which is known to show spontaneous self-pollination was included in our analyses (*Spergula*). As expected, here we found lowest genetic diversity, compared to the other species with a higher proportion of allogamous pollination.

Second, the long life span of the study species (except for the annual *Spergula*) favors high genetic diversity, also under conditions of restricted gene flow among populations. Perennial species may suffer less negative genetic consequences of habitat isolation or small population size because less generations pass within a given time span. Thus, the effect of drift is reduced and delayed in perennial species (Hartl and Clark, 1989). Moreover perennial species have a lower extinction risk in dynamic landscapes where environmental conditions change temporarily. A longer life span can compensate for e.g. low reproductive success or limited dispersal (Bossuyt and Honnay, 2006). In annual species fluctuating reproductive output can on the one hand be problematic because recruitment depends on successful reproduction of the previous year. On the other hand, in such species seed production via selfing and persistent seed banks are common, both being insurances against low reproductive output in single years (Thompson and Grime, 1979; Coffin and Lauenroth, 1989).

Third, effective dispersal mechanisms can bridge gene flow barriers, which can be large cereal fields or monocultures between habitat patches, settlements, rivers or roads. An effective mechanism is, for example, wind dispersal of pollen or seeds (Salisbury, 1976; Ozinga et al., 2004; Ghazoul, 2005). In contrast, the dependence on certain dispersal vectors is disadvantageous if habitat isolation or environmental conditions limit the abundance or movement of these vectors. Accordingly, within our study, species with lower dispersal limitation, such as *Anacamptis* (wind dispersed seeds), *Carex* (wind pollinated) and *Dianthus* (abundant, long distance pollinators) had the lowest differentiation indicating high gene exchange between populations. In contrast, the study species with higher dispersal limitation, such as *Muscari* (heavy seeds), *Spergula* (small statured, heavy seeds) and *Silene* (specialized pollinators) showed higher differentiation. However, the spatial isolation of the studied habitats

exists at a rather small scale. Dispersal might sporadically occur by chance independent on inter population distances or dispersal mechanisms. This could mitigate isolation effects.

In summary, in our study system gene flow among plant populations seems to be restricted. This applies for nearly all species studied. However, although species response to limited gene flow among populations strongly depends on their life history traits there is no clear or strong evidence for genetic impoverishment.

GD-SD correlations - effects of parallel processes

Several processes, such as drift and selection, are thought to act in parallel on both, the genetic and the species levels, thus indirectly creating positive GD-SD correlations (Vellend, 2005). In the absence of gene flow (i.e. migration, immigration) genetic drift leads to a reduction of GD and SD, especially in small populations or small habitats respectively (Barrett and Kohn, 1991). Also, selection or environmental heterogeneity can favor GD and SD in similar ways. If varying selective pressures favor different alleles or species in space and time then the extinction risk for single alleles or species is lower (Vellend and Geber, 2005). Thus, heterogeneous habitats should show higher SD and higher GD within species.

Theoretically, our study system of small isolated dry grasslands provided the environmental conditions that favor the impact of drift and habitat heterogeneity on GD and SD (Vellend, 2005). First, spatial isolation limits pollen and seed dispersal between habitats. Second, small patch size restricts population sizes and thus increases the susceptibility to genetic drift. Third, the outcrops represent very heterogeneous habitats. There exist more than 50 different plant communities at the outcrops, indicating a large variety of different environmental/abiotic conditions (Mahn and Partzsch, 1996). Finally, the outcrops, their isolation and the community structures are likely very old (Bliss et al., 1996). Thus, drift and selection should have shaped patterns of biodiversity within the last millennia. However, recent habitat change probably conceals effects of long term habitat isolation.

As expected, the abovementioned environmental conditions of the study system affected gene flow among sites and shaped the genetic structure of the plant populations. Although responses were strongly species specific, gene flow among populations is often restricted and remarkably, this already happens at this rather small spatial scale. Environmental impacts on

GD and SD at have been found at even smaller spatial scales (< 4 km²) before: For example, in *Banksia attenuata* height of dunes positively affected GD and SD (He et al., 2008) and a parallel response to environmental factors of GD in *Daviesia triflora* populations and SD led to positive GD-SD patterns (He and Lamont, 2009).

Nevertheless, we only found little evidence for positive GD-SD correlations created by parallel processes. Also, habitat heterogeneity was no strong determinant of diversity as it had been suggested by other authors (Solbrig and Simpson, 1974; Morishima and Oka, 1979; Bruun, 2000). As mentioned above, the traits of the study species themselves (i.e. their low susceptibility to habitat isolation and low gene flow) might be responsible for the lack of strong GD-SD correlations. The lack of GD-SD correlations in systems where parallel processes are expected to create such correlations has been reported by others previously. For example Odat et al. (2004) studied GD and SD in *Ranunculus acris* populations at a comparable spatial scale. In contrast to our study sites, they studied generally large and continuous grassland habitats. Similar to our study they found no positive GD-SD correlation but detected a positive correlation between genetic distance and variance in communities (difference in species evenness). Different selective forces and restricted gene flow among populations may have contributed to the observed differentiation patterns but still did not result in positive GD-SD correlations.

Other reasons for the lack of GD-SD correlations might be that species within the study system are not at equilibrium conditions. Theoretically this might be true for species that recently established in the area and thus have low GD because of founder events. Also, drift might have reduced GD in some species but not yet SD because the species still persist. A multi species study by Fady and Conord (2009) on Mediterranean tree species also reported differing responses of GD and SD with regard to environmental conditions. In their study climatic effects only affected GD (via effects on population sizes) but not SD. However, a reduction in GD can result in species extinctions later because of fitness declines and lower adaptation potential ("extinction dept") (Gilpin and Soulé, 1986).

From previous studies it appeared that - across different spatial scales – positive correlations between GD and SD can be observed in natural and artificial ecosystems. Often parallel environmental effects contributed to reported patterns although the drivers seem to be very diverse (e.g. local habitat conditions, land use, population size) and not consistent across

species (Fady and Conord, 2009). Since the approaches of previous studies on GD-SD relationships are very different a general conclusion could not be drawn easily from literature. Therefore our multi species approach contributes to the ongoing debate on the effects of parallel processes on GD-SD relationships.

Impact of abiotic environmental conditions and climate change on reproductive fitness

Climate change, agricultural activities and associated habitat changes are major stressors of biodiversity (Salafsky et al., 2008). Plants, as immobile organisms, are particularly sensitive to environmental changes. In particular, habitat specialists often have no possibility to colonize alternative sites, because suitable habitats are absent or too far away. Dry grassland plant species are confronted with this problem. Dry grasslands belong to the most threatened habitats in central Europe and both, their number and sizes are decreasing (Riecken et al., 1994; Poschlod and Schumacher, 1998). Shifts in species ranges and thus in species compositions within communities are projected for the future (Parmesan, 2006). For Germany this has been particularly been predicted for habitat types, with high conservation value such as nutrient poor grasslands with many endangered species. This can have strong effects on population sizes of single species and also on interspecific interactions, because responses are certainly species specific (Pompe et al., 2011). Additional to the direct loss of habitats and populations, remaining populations suffer from negative consequences of habitat isolation and small population size ("extinction dept"; Gilpin and Soule, (1986)).

Using long term monitoring data we were able to study the effects of annually varying weather conditions and climatic changes on the performance of orchid populations in isolated dry grasslands. Additional estimations of genetic diversity and reproductive fitness allowed for a comprehensive view of the processes acting in remnant populations of *Anacamptis morio*. In summary, our results emphasize the importance of maintaining xerothermic site conditions for the short and long term performance of orchid populations. Xerothermic site quality positively affects census sizes and reproduction, both of which can have important influences on the future development of populations, e.g. by affecting genetic diversity, dispersal and colonization of new sites. Compared to pollen dispersal, wind dispersal of seeds probably plays an important role and thus counteracts the random loss of alleles, i.e. drift. However, currently the isolated

orchid populations could preserve high within population diversity. Moreover, population declines that have been reported within the last century may not continue within our study region as long as xerothermic grassland conditions are preserved by continuous site management. Accordingly, the populations that exist today show positive or stable trends.

Additionally to site specific conditions, also long term environmental changes, i.e. climate change, can determine the performance of plant populations. For example, rising spring temperatures increased during the last century in central Europe and this has been connected with shifts in phenology in a number of plant species (Menzel and Fabian, 1999; Menzel et al., 2001). Subsequent consequences for ecological interactions and thus, for reproduction might arise from such changes (Bartomeus et al., 2011). Also, within our study region we observed such an increase of mean April temperatures within the last four decades, whereas temperatures in other months showed no such pattern. At the same time we could show that higher April temperatures are generally connected with higher census sizes of the orchid populations. Thus, it appears that current climate change might positively act on our study species by providing advantageous conditions for plant performance and reproduction (i.e. larger population sizes).

Reproductive fitness, population/census size and the effect of interannual variation

The relationship between reproductive fitness and population size (or census size) is often found to be positive (Leimu et al., 2006), which may have a number of non mutually exclusive causes. Principally, large populations attract more pollinators and thus a higher proportion of flowers is visited leading to increased seed and fruit set (Knight et al., 2005). Moreover, large populations often harbor higher genetic diversity than small populations (Frankham, 1996). Negative effects of inbreeding depression, i.e. reduced reproductive fitness, are more likely in small populations because of a rapid increase in the expression of recessive deleterious alleles (Ellstrand and Elam, 1993). However, these relationships may be influenced by population density and pollinator behavior. For example, in *Lychnis viscaria* large populations attracted more bumblebees but visitation rate and reproductive fitness was higher in sparse populations. Here, probably larger flight distances between individuals increase the duration of flower visits (Mustajärvi et al., 2001).

In addition to population size, a variety of interacting factors can contribute to reproductive fitness, such as resource availability, weather, climate and also pollinator behavior (Wilcock and Neiland, 2002). Such factors also contributed to reproduction in *Muscari* and *Anacamptis*. Nevertheless, reproductive fitness generally increased with population size, thus confirming general patterns found in natural plant populations.

However, the two case studies revealed that both, census sizes and reproductive fitness may vary strongly among years. Most probably, varying environmental conditions cause these fluctuations probably via effects on e.g. water and nutrient availability (Bengtsson, 1993), pollinators (Price et al., 2005), pathogens (Scherm and Yang, 1995) or herbivory (English-Loeb and Karban, 1992). Certainly, these temporary fluctuations do not necessarily severely affect total population sizes in perennial species. Many plant species are adapted to such conditions, for example, by long live spans, seed banks or dormancy (Dalgleish et al., 2010). However, variability in census sizes and fitness can influence the outcome of single-year studies on the relationships between population size and fitness traits. Accordingly in *Muscari* population size and reproductive fitness were positively related in two years, whereas in two other years there was no connection. In *Anacamptis* (one-year study) large populations had higher reproductive fitness. Although, we did not carry out repeated fitness measurements in *Anacamptis*, the observed strong interannual fluctuations in census size may also lead to fluctuating reproductive output.

Final conclusion

This thesis aimed to understand the determinants of genetic and species diversity under conditions of habitat isolation in dry grassland plant species. We put a special focus on studying possible correlations between both levels of biodiversity which are thought to establish under such environmental conditions. Our approach to analyze many species simultaneously allowed us to test the generality of parallel effects on biodiversity levels. However, we found little evidence for positive correlations between genetic and species diversity. In summary, we could show that habitat isolation already restricts gene flow at very small spatial scales. Genetic responses are strongly species specific and determined by the species' life history traits. The majority of the eight study species exhibit traits that make them less susceptible to genetic erosion in the face of long term habitat isolation and small population size. This stresses the importance of including multiple species into studies that aim to analyze environmental impacts on plant populations. The two case studies on *A. morio* and *M. tenuiflorum* added valuable information on reproductive fitness thus showed that both, annual census sizes and reproduction show strong fluctuations among years. Beyond this we could show that also global environmental changes (i.e. climatic warming) may have effects on populations by positively affecting flowering in *A. morio*. However, whether such - at first sight - beneficial effects also result in a better general long term performance of plant populations remains open. According to our results, the performance of populations and species depends on a variety and on the interaction of different factors. These need to be considered to describe the recent status and predict a possible future development of plant populations.

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ACKNOWLEDGEMENTS

First of all I want to thank my supervisor Dr. Walter Durka for his continuous support and encouragement he has provided throughout my time at the UFZ. I am grateful for all his ideas, constructive feedback and for the well-organized research.

I also want to thank Prof. Dr. Birgit Ziegenhagen for giving me the opportunity to submit this thesis at the Philipps-University Marburg. Although we only had a few personal meetings her advice and comments were always very motivating and helpful to me.

I am very grateful to Prof. Dr. Birgit Ziegenhagen (Philipps-University Marburg), Dr. Walter Durka (Helmholtz-Centre for Environmental Research), Prof. Dr. Nina Farwig (Philipps-University Marburg) and Prof. Dr. Diethart Matthies (Philipps-University Marburg) for being my PhD committee.

Thanks to Dr. Stefan Michalski for constructive discussions and for being co-author in two of my articles. His contribution helped a lot to advance the writing process.

For providing vegetation data I thank Dr. Dieter Frank (Landesamt für Umweltschutz), Jens Stolle and the AHOs Sachsen-Anhalt and Thüringen. I thank Stefan Klein (AHO Sachsen-Anhalt) for his help in the orchid study and for helping to locate the populations.

I am very thankful to Ina Geier and Martina Herrmann for their great help in the lab, to Jan Hanspach and Harald Auge for helping with statistical problems and to Klaus Hempel and Alexander Harpke for their support with GIS and maps.

I want to thank all my colleagues at the Department of Community Ecology for the nice and familiar atmosphere. Especially I want to thank Eva and Lena for daily support throughout my PhD time and for never complaining about my frequent visits in their office. Special thanks also to Karin who experienced all of the ups and downs of my work.

Most of all, I would like to thank my husband, Guido, for always being by my side.

Finally, I want to thank my family for the continuous support during the last years and for their patience with my long-term statement to be “almost finished”.

EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit versichere ich, dass ich meine Dissertation mit dem Titel

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selbstständig und ohne unerlaubte Hilfe angefertigt habe. Ich habe mich dabei keiner anderen als der von mir ausdrücklich angegebenen Quellen und Hilfen bedient. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen habe ich als solche kenntlich gemacht.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Ich erkläre weiterhin, dass ich mich zuvor nie um einen Doktorgrad beworben habe.

Halle/Saale, 24.08.2012

Die Seite 121 (Curriculum Vitae) enthält persönliche Daten. Sie ist deshalb nicht Bestandteil der Online-Veröffentlichung.

PUBLICATIONS

Hornemann, G., Michalski, S.G., and Durka, W. (2012) Short-term fitness and long-term population trends in the orchid *Anacamptis morio*. Plant Ecology, DOI: 10.1007/s11258-012-0113-6

Hornemann, G., Weiss, G., and Durka, W. (2012) Year to year variation overrides relationships between reproductive fitness, population size and genetic variation in the rare *Muscari tenuiflorum* (Hyacinthaceae). Flora, vol. 207 (in press)

TALKS AND POSTERS

Hornemann G., Durka W. (2009), Genetic variation, population size and plant fitness in isolated populations of the endangered *Muscari tenuiflorum* (Hyacinthaceae) Conservation Genetics: Integrating Population Genetics and Conservation Biology, Trondheim, Norway, 23-26 May 2009. (poster)

Hornemann G., Durka W. (2009), Genetic variation, population size and plant fitness in isolated populations of the endangered *Muscari tenuiflorum*, 39th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), Bayreuth, Sep 2009. (talk)

Hornemann G., Durka W. (2009), Genetic variation, population size and plant fitness in isolated populations of the endangered *Muscari tenuiflorum* (Hyacinthaceae), Dry grasslands – species interaction and distribution Conference, Halle (Saale), Aug/Sep 2009. (talk)

Hornemann G., Durka W. (2010), Genetic variation, reproductive fitness and long term population development in the endangered *Anacamptis morio*. 23rd Annual Conference of the Plant Population Biology Section of the Ecological Society of Germany, Switzerland and Austria (GfÖ), Nijmegen 13-15 May 2010. (talk)