Testing paradigms in conservation biology: spatio-temporal dimensions of habitat fragmentation in a stenotopic woodland ground beetle

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They paved paradise,
And put up a parking lot,
With a pink hotel, a boutique
And a swinging hot spot.

Don't it always seem to go,
That you don't know what you've got
'till it's gone?
They paved paradise,
And put up a parking lot.

They took all the trees,
Put 'em in a tree museum.
And they charged the people
A dollar and a half just to see 'em.

(Big Yellow Taxi by Joni Mitchell)

Table of Contents

Summary

Habitat fragmentation and changes in land use are currently two major drivers of biodiversity loss around the world by causing habitat loss and reducing connectivity across landscapes. These processes affect not only species diversity, but genetic structure as well. The loss of habitat and the increased isolation prevent gene flow and accelerate genetic drift, causing loss of genetic diversity and facilitating development of genetic differentiation.

The effects of habitat fragmentation and land use changes are usually studied by relating patterns of genetic diversity and differentiation to environmental factors, habitat history, landscape structure, or to a combination thereof. However, these three drivers are rarely addressed simultaneously. In addition, these studies are usually carried out in conservation-driven contexts, and therefore tend to concentrate on hyper-fragmented landscapes and on rare or endangered species. However, how habitat fragmentation and land use affect widespread species in more typical landscapes has not been fully investigated. In this thesis I address these two gaps, and do so in three study regions, allowing for generalization of the results.

I used *Abax parallelepipedus*, a flightless ground beetle with low dispersal power as a model species to test how environmental factors, habitat history, and landscape structure affect genetic diversity and genetic differentiation in three study regions located across Germany. This species seldom leaves wooded habitats, and rarely crosses linear barriers such as roads and railways. It is also known to be susceptible to rapid changes in genetic structure after habitat fragmentation. Nevertheless, *A. parallelepipedus* is widely distributed as it can inhabit a variety of woodland types in which it maintains high population densities. Although all of my study regions represent fairly typical rural landscapes for central Europe, each consisting of a complex matrix of land uses, they differ from one another in terms of environmental factors, habitat history, and landscape structure, and thus can serve as three test cases.

In the first stage of my work, I identified polymorphic microsatellite loci which could potentially be used to study genetic diversity and differentiation in *A. parallelepipedus*. I

then developed PCR and genotyping protocols for two suites of loci, in the end selecting to use the set of 14 fully multiplexed loci for my study. After I had developed the needed study system, I genotyped over 3300 beetles from 142 study sites.

In my investigation of how environmental factors and habitat history affect genetic diversity and genetic differentiation, I found that genetic diversity was being driven by variables that could be related to population sizes rather than by habitat history. I also did not find evidence of an influence of habitat history on the genetic differentiation patterns. Although populations of *A. parallelepipedus* in the past were probably smaller due to deforestation, they apparently remained large enough to prevent rapid genetic drift. Thus, recolonization processes of woodlands planted after the peak of deforestation either occurred without incurring founder effects or bottlenecks, or the effects of thereof have since been erased by gene flow.

As the genetic structure found in my landscapes is driven current processes, rather than historical ones, I carried out a landscape genetics analysis of the genetic differentiation patterns found in each of my study regions, in which I examined the relationship between genetic differentiation and landscape structure. I tested whether I could find patterns of isolation by distance, isolation by resistance, or isolation by barriers in my study regions. Surprisingly, I found no effects of land use or of fragmentation. Based on the importance of population sizes found in my previous study, combined with the beetle's known avoidance of non-wooded areas and its inability to cross roads, I conclude that although there is probably little gene flow across my study regions, large population sizes are preventing the rapid development of genetic differentiation. Models simulating the development of genetic differentiation over time in populations of different starting sizes support this conclusion.

My work highlights the importance of population sizes in determining how patterns of genetic diversity and differentiation will develop across landscapes. While emphasis has been placed in conservation contexts on the deleterious effects of fragmentation on genetic structure, this may be overstated for widespread species in typical landscapes. In such cases, large population sizes may mitigate the development of genetic differentiation and prevent loss of alleles, despite existing barriers and lack of gene flow.

General introduction

Loss of biodiversity is currently of major concern across the globe. In addition to the oft discussed threat to species, diversity at other levels, such as the genetic level and the ecosystem level, is also threatened. This loss leads to, and will continue to lead to, a myriad of irrevocable changes in the way entire ecosystems function, which in addition to the harm caused to nature, also impacts vital ecosystem services essential to human wellbeing (Loreau et al. 2001, Cardinale et al. 2012, Hooper et al. 2012).

Genetic biodiversity consists of two components, genetic diversity and genetic differentiation. Genetic diversity is a measure of the genetic variability within a population (Hughes et al. 2008), and it can be thought of as the genetic equivalent of α-diversity. Genetic differentiation is a measure of dissimilarity between populations, and therefore is a genetic parallel of β-diversity. Changes to both genetic diversity and genetic differentiation stem from changes in allele frequencies in populations, including to the point of complete loss of an allele.

Quite amazingly, the theoretical foundations describing the processes behind changes in the frequencies of alleles were laid down long before the development of molecular biology and the field of modern genetics. According to theory, these changes are controlled by the balance between four proximate causes, namely mutation, selection, migration (also known as gene flow), and stochastic effects (Wright 1931, Frankham et al. 2002). Stochastic effects describe processes which randomly effect allele frequencies, the main being genetic drift, population bottlenecks, and founder effects. Population bottlenecks and founder effects are processes of small populations by definition, and the rate at which genetic drift occurs is also strongly connected to population size, with smaller population sizes leading to more rapid drift (Hartl & Clark 1997, Frankham et al. 2002).

One of the main drivers of the loss of all levels of biodiversity is changes in land use, and therefore understanding the resulting effects on ecological systems and processes is of utmost conservation importance (Sala et al. 2000, Foley et al. 2005, Pereira et al. 2010, Haddad et al. 2015). Land use change can cause two concurrent processes, both of

which severely impact natural systems. The first component is habitat loss in which previously suitable areas are made either less or completely unsuitable for a given species. The second process is isolation, whereby connectivity patterns across the landscape are altered. Both of these processes can affect fundamental biological and physical properties of the landscape and the communities and populations within it. The term habitat fragmentation, or simply fragmentation, can be used to refer to either one of these components, though usually it refers to the combined effects of both together since they almost always occur simultaneously (reviewed among others in Saunders et al. 1991, Harrison & Bruna 1999, Fahrig 2003, Fischer & Lindenmayer 2007).

In addition to the long-studied effects of fragmentation on communities and species (reviewed in Harrison & Bruna 1999, Fahrig 2003, Fischer & Lindenmayer 2007), these processes also strongly affect both genetic diversity and genetic differentiation (Young et al. 1996, Gibbs 2001, Fahrig 2003, Fischer & Lindenmayer 2007). The effects of both fragmentation components can be rapid and dramatic, and must be accounted for in conservation contexts (reviewed among others in Young et al. 1996, Frankham 2005, Keyghobadi 2007). Theoretically, patterns of genetic diversity and genetic differentiation in a landscape are each driven by at least one of the components of fragmentation. Loss of genetic diversity occurs via loss of alleles due to stochastic drift, and therefore is more closely tied with shrinking population sizes due to habitat loss. Development of genetic differentiation occurs when migration between populations is not high enough to counter the developing differences in allele frequencies. Therefore it is more closely related with levels of isolation throughout a landscape.

Assuming that no random extinction and recolonization processes have occurred, in an unchanged landscape, both genetic diversity and differentiation are stable (Figure 1a). However, when a landscape has been altered, the genetics of populations can be affected depending on the size and location of the changes. Loss of small amounts of habitat should not lead to loss of alleles (Figure 1b), but if the lost habitat prevents migration, differentiation will develop, albeit very slowly (Figure 1c). When a large amount of habitat is lost, the concurring loss in population size will lead to loss of alleles (Figure 1d), but differentiation will only develop if migration is also hindered (Figure 1e).

The most extreme scenario is complete isolation (Figure 1f). In the real world however, loss of genetic diversity and development of differentiation almost always co-occur.
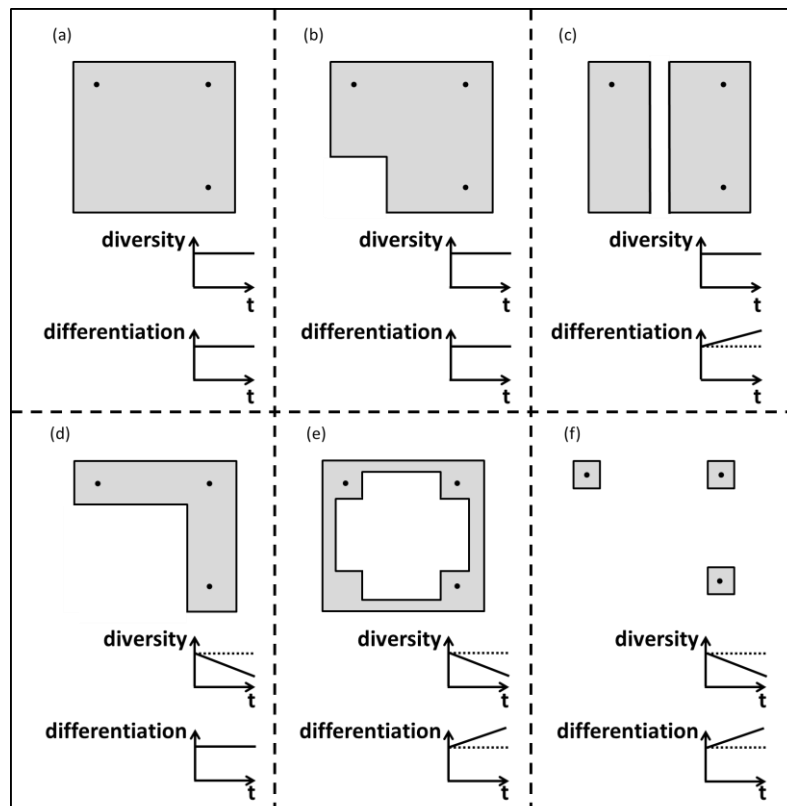
Figure 1: Theoretical effect of land use change on genetic diversity and differentiation. Displayed graphs present expected changes in genetic diversity or differentiation over time. Grey represents remaining habitat, dots represent sampling points. (a) No change in land use. (b) Loss of small amount of habitat which does not affect migration. (c) Loss of small amount of habitat creating two large, isolated areas. (d) Loss of large amount of habitat which does not affect migration. (e) Loss of large amount of habitat hindering migration. (f) Complete isolation.

The ultimate causes of differentiation and levels of diversity are extremely complex, and have been studied in countless species and landscapes. The drivers of these processes usually fall into one of three categories, habitat history, landscape structure, and environmental factors such as temperature and soil conditions (Table 1).

Table 1: Examples of studies for each of the categories of ultimate causes

ultimate cause	examples
habitat history	Desender et al. 2002b, Jacquemyn et al. 2004, Desender et al. 2005a, Hermy & Verheyen 2007 (review), Reisch et al. 2007, Otálora et al. 2011
landscape structure	Funk et al. 2005, Jørgensen et al. 2005, Cushman 2006, Cushman et al. 2006, Sork & Smouse 2006 (review)
environmental factors	Scribner et al. 2001, Brouat et al. 2004, Cena et al. 2006, Alvarez et al. 2009

Habitat history is a crucial factor in past population sizes, as land use in the past may have caused a species to undergo population bottlenecks or even local extinction and recolonization with the co-occurring founder effects. In addition, the migration and gene flow in the past may be different to that of today (Petit & Burel 1998b, Holzhauer et al. 2006). Such changes in the past can be thought of as a form of temporal fragmentation in which the continuity over time instead of over space is disrupted. The length of time for which a habitat has remained unaltered is often referred to as temporal habitat continuity. *Landscape structure* refers to the current mosaic of habitat patches and surrounding matrix, including natural and anthropogenic fragmentation. The size of the habitat patches as well as the structure and permeability of the matrix are crucial factors in determining current population sizes and migration patterns. *Environmental factors* affect habitat suitability and therefore population sizes, while also affecting mobility patterns, for example in poorer conditions individuals may be more likely to migrate in search of resources (carabid examples: Mols 1987, Frampton et al. 1995, Mauremooto et al. 1995, Firle et al. 1998, Fournier & Loreau 2001).

As genetic diversity and differentiation have serious conservation implications, much of the work is done either on areas or on species of conservation concern, such as rare habitats, hyper-fragmented landscapes, and rare or invasive species (recent examples: Barr et al. 2015, Colautti & Lau 2015, Wood et al. 2015, Adrian-Kalchhauser et al. 2016, Goetze et al. 2016, Moracho et al. 2016, Reichel et al. 2016, Tensen et al. 2016, Watts et al. 2016, Yokochi et al. 2016). Unsurprisingly, loss of genetic diversity and development of differentiation are commonly found in such studies. However, how fragmentation affects genetic diversity and genetic differentiation in more widespread species and landscapes, both in terms of spatial distribution and population sizes, is

lacking. Of particular interest are the abundant yet stenotopic species, meaning species with relatively narrow ecological niches. Therefore, these species maintain large population sizes while simultaneously having poor dispersal capabilities as they have clear habitat preferences and avoid entering the matrix. While on the one hand such species should be less affected by habitat loss due to their large population sizes, on the other hand, changes in the matrix should significantly affect their dispersal patterns and therefore gene flow.

One of the particularly interesting habitats in which to examine potential drivers of genetic diversity and differentiation are northwestern European woodlands. These woodlands are often embedded in a complex mosaic of land uses which developed over the course of centuries of anthropogenic influence. Typical land uses include towns, roads, agricultural fields, grasslands, industrial infrastructure, and meadows in addition to woodlands. These woodlands provide a unique opportunity to examine environmental factors together with both temporal and spatial fragmentation in the same landscape, as in addition to their current complex structure, they have complex yet traceable histories of land use and, thus, temporal fragmentation. In these regions, the maximum deforestation is assumed to have occurred approximately 200-400 years ago, coinciding approximately with the development of accurate cartography. Patches which appear as wooded on all maps since the earliest accurate map are referred to as ancient woodlands, in opposition to recent woodlands (Peterken 1993, Rackham 2003, Flinn & Vellend 2005). Additional historical information is available due to the long history of accurate record keeping. While in some cases, such as parts of northwestern Germany or Belgium, the remaining patches of woodlands are extremely small and isolated, in other parts of Europe wooded landscapes contain a variety of habitat patch sizes and levels of fragmentation.

In this thesis I chose to focus on a member of the carabid family. Carabids, otherwise known as ground beetles, have long been intensively studied in a wide variety of fields (Niemelä 1996, Kotze et al. 2011). Due to the species diversity, abundance, and broad geographic and environmental range of the group, combined with their relatively stable taxonomy and ease of collection have caused the carabids to be popular objects of study. As a result, much is known about their biology, ecological requirements,

geographic distribution, and more, especially in Europe and in North America (Kotze et al. 2011). Amongst other fields, they have been often used as a model group in studies of dispersal and distribution (e.g. Dufrêne & Legendre 1997, Antvogel & Bonn 2001) as well as habitat fragmentation (reviewed in Niemelä 2001, Niemelä & Kotze 2009). Due to the abundance of previous studies on carabids, it is possible to precisely select a fitting model species about which much is known. In addition, it is usually possible to compare outcomes to previous studies and to thus understand results in a wider context of closely related species.

In this thesis I close three major study gaps. Firstly, while many studies exist examining the effects of environmental factors, habitat history, and landscape structure on genetic diversity and differentiation, they never addressed all three together due to the involved complexity (Figure 2a). Secondly, in order to be able to further generalize my findings, I used three different landscapes as test cases. While all three landscapes are moderately fragmented rural landscapes, they still differ in terms of landscape structure, landscape history, and more. Studying the same species in several test landscapes allows me gain a holistic view as to the drivers of genetic diversity and differentiation in typical species and landscapes (Figure 2b). Lastly, I have studied a widespread yet stenotopic species, one that is abundant in a large number of forest types in all regions of Germany, in fairly typical, moderately fragmented landscapes. While rare species, hyper-fragmented landscapes, or a combination thereof, have often been studied, non-extreme cases are still under-addressed (Figure 2c).
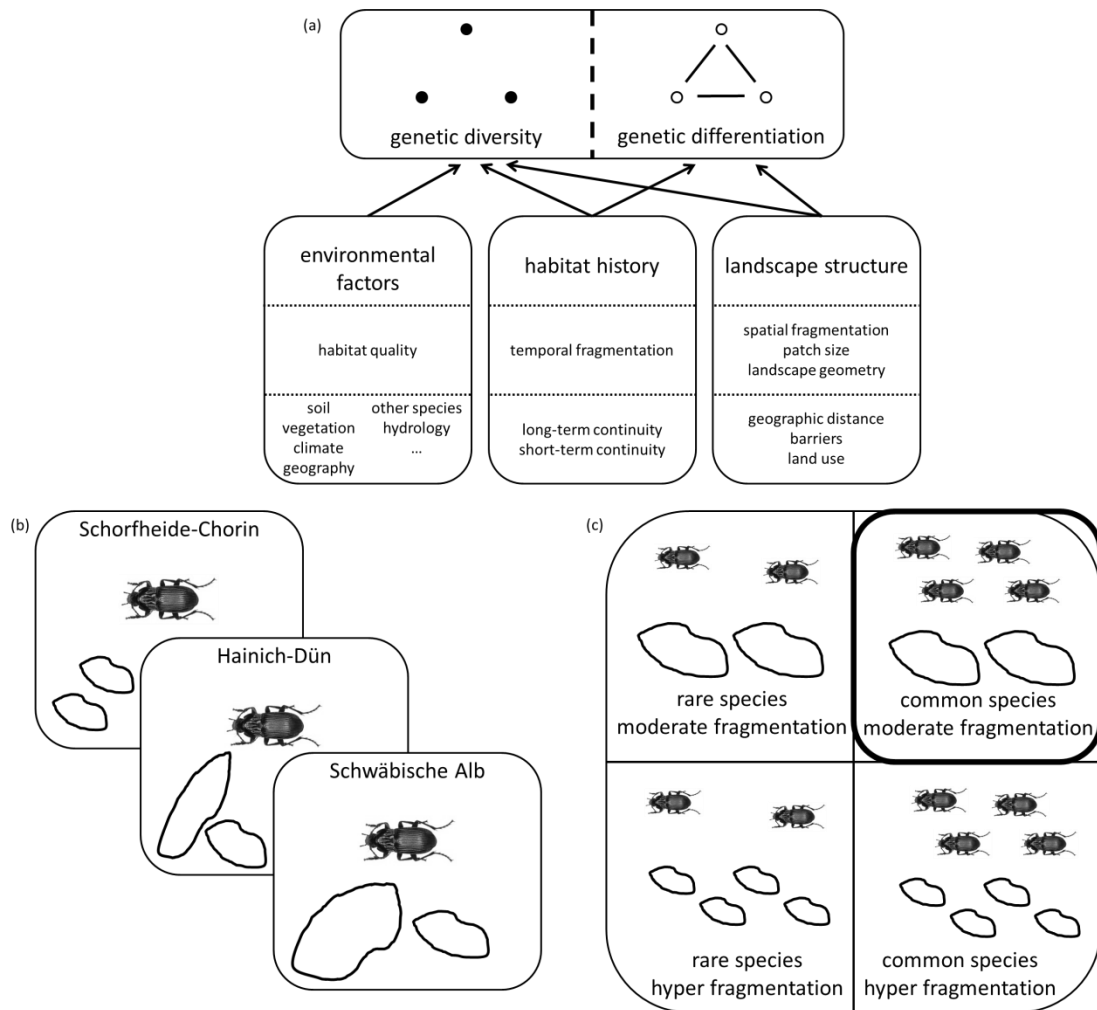
Figure 2: Thesis novelties in (a) the combination of drivers investigated, (b) in several landscapes, and (c) species and landscapes investigated. In (a) the ultimate causes of genetic diversity and genetic differentiation (upper third) are presented together with how these causes are driving changes in allele frequencies (middle third), and examples (bottom third). Arrows connect the ultimate causes with proximate causes they affect. In (c) the number of beetles represents how rare or common a study species is. The number of habitat patches and their sizes represent whether a study area is hyper-fragmented or not.

Hypotheses

In this thesis I examine the effects of environmental factors, habitat history, and landscape structure on genetic diversity and differentiation. All of these drivers have been examined individually or in pairs, but they have not been tested together allowing insight as to the possible interactions between them. In order to generalize the results, such a study needs to be carried out on a number of landscapes, as drivers may be landscape-dependent. I examined these drivers in landscapes which are moderately fragmented and in a widespread, stenotopic species.

Effects of environmental factors

Environmental variables are thought to reflect habitat quality. Less suitable patches for a given species should have a lower carrying capacity and lower population densities and sizes (theory: MacArthur & Wilson 1967, Hodgson et al. 2011; insect examples: Thomas et al. 2001, Kleijn & van Langevelde 2006, Drees et al. 2007, Heisswolf et al. 2009). This should lead in turn to a higher rate of allele loss due to genetic drift (theory and reviews: Wright 1931, Soulé 1976, Frankham 1996, Desender 2005; carabid examples: Keller & Largiadèr 2003b, Desender et al. 2004, Drees et al. 2011).

→ Hypothesis 1: I expect to find environmental factors which are significantly linked to genetic diversity (Figure 3). This hypothesis is addressed in paper 2.
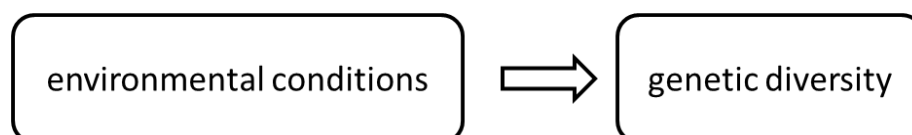

Figure 3: Hypothesis 1. Environmental conditions will affect genetic diversity.

Effects of habitat history

Patches which underwent temporal fragmentation would need to have been recolonized, a process which usually entails loss of alleles due to founder effects and genetic drift during a period of initial low population size. Patches which never

underwent land use changes should maintain the full set of original alleles, in addition to having more time to acquire new ones via migration (Desender et al. 2004, Grapputo et al. 2005, Keyghobadi 2007, Drees et al. 2008, Carter et al. 2010, Drees et al. 2010, Drag & Cizek 2015). Differing habitat histories can also be reflected in the genetic differentiation between populations. If in the past populations were small and isolated, then they had more time for genetic drift to cause them to differentiate, making them more differentiated than more reestablished ones for which they serve as sources (Slatkin 1977, Austerlitz et al. 1997, Tremetsberger et al. 2003, Jacquemyn et al. 2004, Reisch et al. 2007, Vandergast et al. 2009). If the source populations did not differentiate and each recolonization was from a small, yet different subset of sources, the recolonized patches may be more differentiated than the sources (Slatkin 1977, McCauley et al. 1995, Austerlitz et al. 1997, Ingvarsson & Olsson 1997, Vellend 2004, Keyghobadi 2007). In addition, whether recolonization occurred following the propagule or the stepping stone model will also affect whether the past populations or the recolonized ones will be more differentiated (Slatkin 1977).

→ <u>Hypothesis 2</u>: I expect to find a positive relationship between temporal habitat continuity and genetic diversity (Figure 4). This hypothesis is addressed in paper 2.
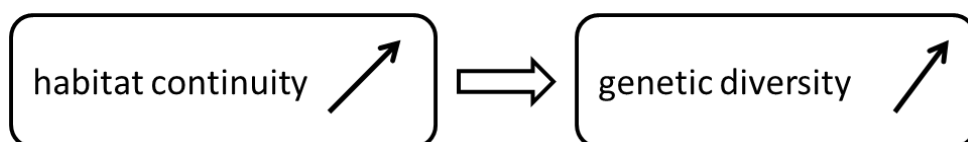
Figure 4: Hypothesis 2. Longer habitat continuity will lead to higher genetic diversity.

→ <u>Hypothesis 3</u>: I expect to find differing patterns of differentiation between the non-temporally fragmented patches and the temporally fragmented ones (Figure 5). This hypothesis is addressed in paper 2.

Figure 5: Hypothesis 3. Populations from ancient and from recent woodlands will have different patterns of genetic differentiation.

Effects of landscape structure

Landscape structure is in essence a description of the spatial habitat fragmentation across a landscape. The fragmentation can be caused both by linear structures as well as by changes in land use which inhibit or prevent migration. The limiting of migration between patches spread throughout the landscape should result in the development of genetic differentiation, while loss of habitat should lead to smaller population sizes and to genetic drift. The severity of these effects is contingent upon the structure of the landscape, including where the lost habitat is located, and the amount of remaining habitat as well as how connected it is. Complete inhibition of migration, usually a result of linear barriers, can lead to the division of a population into two smaller ones, each with a smaller effective population size. This would then lead to loss of alleles and a lowering of genetic diversity (Keller & Largiadèr 2003b, Manel et al. 2003, Cushman 2006, Holderegger & Di Giulio 2010, Oleksa et al. 2015). While conceptually linear barriers and changes in landscape are both types of fragmentation, linear barriers cannot be circumvented and are often completely impermeable. Therefore such barriers can have a strong effect, even if the area which they encompass is not large.

→ Hypothesis 4: I expect to find more genetic differentiation if more linear barriers are found between two populations (Figure 6). This hypothesis is addressed in paper 3.

Figure 6: Hypothesis 4. More linear barriers will lead to more genetic differentiation.

→ Hypothesis 5: I expect to find more genetic differentiation between populations which are separated by more impermeable matrix than between those which are less fragmented (Figure 7). This hypothesis is addressed in paper 3.

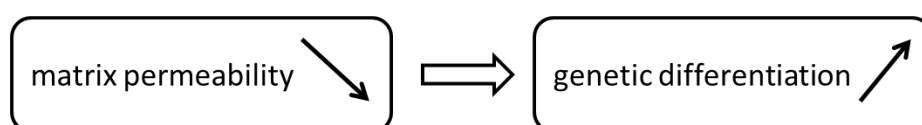Figure 7: Hypothesis 5. Less permeable matrix will lead to more genetic differentiation.

→ <u>Hypothesis 6</u>: I expect to find less genetic diversity in populations surrounded by less permeable matrix than those surrounded by larger amounts of suitable habitat (Figure 8). This hypothesis is addressed in paper 2.
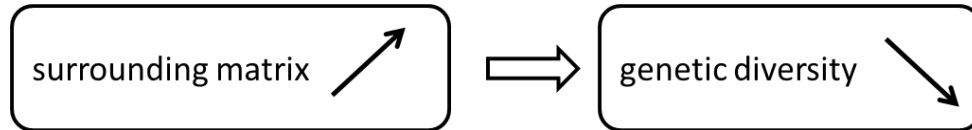

Figure 8: Hypothesis 6. Larger amounts of surrounding less permeable matrix will lead to less genetic diversity.

Research system

Study sites

The Biodiversity Exploratories are a large research platform consisting of three study regions across Germany. The project provides a large scale and long term frame work in which researchers from a wide variety of scientific fields can study the effects of land use, such as forestry and grassland management, on biodiversity on all of its levels. Each study region contains 100 experimental plots, 50 each in the grasslands and in the woodlands. These plots were selected to represent a gradient of land use intensity and methods found in each region (Fischer et al. 2010).

As all of the involved research projects study the same plots, much is known about their climate, soil, flora, fauna, and more. This allows for effective analysis of different levels of biodiversity and environmental conditions (Fischer et al. 2010). Among the plethora of monitored and measured parameters is the invertebrate community, including long term pitfall trapping in each of the plots, which enabled the selection of a species easily found in a vast majority of the plots in all three regions.

The three study regions represent fairly standard rural landscapes for central Europe (Fischer et al. 2010), comprising of a complex mosaic of towns, villages, woodlands, grasslands, agricultural lands, and infrastructure. Despite their superficial similarities, the three Exploratories differ from each other in terms of both environmental conditions as well as land use patterns due to geographical, geological, topographical, and historical differences. Thus, the three regions serve as separate study cases, and comparing the results found in each allows me to generalize my results. This is one of extremely few studies ever carried out in more than one study region, adding greatly to its value.

The *Schorfheide-Chorin* is located in northeast Germany, approximately 80 kilometers northeast of Berlin (Figure 9). Of the three Exploratories, it contains the most woodlands and has the lowest human population density, but it is bisected by a large highway, the largest of the roads contained in any of the study regions. The *Hainich-Dün* is located in the center of the country (Figure 9), and contains one extremely large, consecutive patch of woodland and several additional patches of varying size. The

14

woodlands in this region have a high level of temporal habitat continuity, mostly having not changed since the Middle Ages. The *Schwäbische Alb* is located in southwest Germany (Figure 9), and is the most complex and fragmented of the three landscapes. The size of the woodlands, as well as other land uses such as meadows and agricultural fields, is much smaller than in the other regions. As opposed to the Hainich-Dün, in both the Schorfheide-Chorin and the Schwäbische Alb some of the forests have existed for hundreds of years, while others have been planted more recently. In both cases, the recent woodlands are interspersed between the ancient ones.

Figure 9: Location of study regions (in black) and nearby major cities (black dots) in Germany

The three Exploratories also differ in terms how the roads and their verges are maintained. The road verges in the Schwäbische Alb are the most intensively maintained, consisting of a wide strip of short grass, regardless of road size. In the Hainich-Dün grass strips exist along the verge of larger roads, but are less intensively managed, and often contain a mixture of higher vegetation and grasses. Along smaller roads, such verges are not always maintained at all, and leaf litter can be found directly along the pavement. In the Schorfheide-Chorin verges are much narrower if they exist at all, and the even along the highway the verge is relatively narrow and not intensively managed. On smaller roads, the trees can come up to the road itself. Some of the roads in the Schorfheide-Chorin Exploratory are not paved, and are instead constructed of cobblestone (Table 2).

Table 2: Pictures of a representative federal and state road from each of the study regions

	Schorfheide-Chorin	Hainich-Dün	Schwäbische Alb
federal roads (Bundesstraßen)			
state roads (Landstraßen)			

Study species

Abax parallelepipedus (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae) is a widespread, flightless ground beetle which lives in the litter layer of woodlands across central Europe (Lindroth 1985/86, Loreau 1987, Huber & Baumgarten 2005). Due to its large size of 18-20 mm (Müller-Motzfeld 2006), ease of identification, and ease of finding, *A. parallelepipedus* has been used often in the past as a model organism (e.g. Loreau & Nolf 1993, Petit & Burel 1993, Chaabane et al. 1994, Loreau & Nolf 1994, Chaabane et al. 1996, Charrier et al. 1997, Franceschini et al. 1997, Petit & Burel 1998a, Tischendorf et al. 1998, Keller et al. 2004). Therefore, much is known about its biology, ecology, movement, and distribution patterns across a landscape.

Although *A. parallelepipedus* is restricted to woodlands, it can inhabit most types of wooded areas, ranging from old-growth beech to conifer plantations (Day et al. 1993, Fahy & Gormally 1998, Magura et al. 2000, Lange et al. 2014). In addition to being found in a wide variety of woodlands, the beetle can reach high population densities of approximately 0.2 individuals per square meter (Loreau & Nolf 1993, Loreau 1994, Chaabane et al. 1996, Franceschini et al. 1997, Keller et al. 2004). Additionally, in contrast to other ground beetles species in which population densities can naturally

fluctuate by 2-3 orders of magnitude (den Boer & Van Dijk 1994), the population sizes of *A. parallelepipedus* are relatively stable (Chaabane et al. 1996, Judas et al. 2002, Günther & Assmann 2004). Therefore, changes in allele frequencies caused by historical population sizes can be related to temporal fragmentation rather than to natural population fluctuations.

Like many other carabids, the movement of *A. parallelepipedus* mainly follows random walk patterns (Baars 1979, Loreau & Nolf 1994, Lövei & Sunderland 1996, Charrier et al. 1997, Firle et al. 1998). Even in comparison to its body size, it is fairly immobile (Loreau & Nolf 1994), the average walking distance being only 0.6-2.3 meters per night (reviewed in Brouwers & Newton 2009). Despite the fairly limited dispersal capabilities due to its low mobility and limited habitat preferences, *A. parallelepipedus* still can be a fairly effective recolonizer, provided it can reach the new habitat patch (Day et al. 1993, Magura et al. 2003, Jopp & Reuter 2005, Deuschle & Glück 2008, Brandmayr et al. 2009). Likewise, although the species is known to prefer ancient woodlands in some regions, such as northwestern Germany (Assmann 1999) and Belgium (Desender et al. 2002a), this is probably due to lack of ability to reach newly afforested areas which usually are not connected to ancient woodlands rather than an actual habitat preference. Probably due to the less extreme landscape fragmentation in my study areas, *A. parallelepipedus* could be found in both ancient and in recent woodlands in all three Exploratories.

Another consequence of the species' inability to fly and low mobility, is that *A. parallelepipedus* is unable to effectively cross linear, man-made boundaries such as roads and railways (Mader 1984, Mader et al. 1990, Koivula & Vermeulen 2005). Instances of individuals leaving the forests for such an inhospitable structure are extremely unlikely, and like other flightless forest carabids, beetles reaching the edge of the forest will continue parallel to it rather than crossing into open landscapes (e.g. Niehues et al. 1996). In addition, given the average nightly walking distances, even individuals who attempt to cross such barriers in a straight line will probably be run over before they succeed in reaching the other side. This makes the species vulnerable to influences of such linear barriers on the genetic level, and roads of varying ages and sizes have been shown in Bern, Switzerland to lead to rapid genetic differentiation

(Keller et al. 2004). While some studies have reported finding *A. parallelepipedus* individuals in semi-open habitats (e.g. Magura et al. 2001, Eggers et al. 2010), these studies examine small spatial scales of up to 100 meters in total along the edges of woodlands. While individuals may stray for short distances out of the wooded areas, especially into semi-open landscapes, they will not cross in significant numbers deep into large open structures such as fields, urban areas, or a road with its shoulders (Charrier et al. 1997).

In conclusion, since *A. parallelepipedus* is a widespread species that is nevertheless restricted to woodlands in most parts of central Europe, it makes for a good model species for my questions. It has a clear preference for its habitat while avoiding the surrounding matrix, yet the habitat is easy to define since the species inhabits all types of woodlands. The beetle is found in large number of woodlands across Germany, and has been shown to rapidly develop genetic differentiation in response to fragmentation (Keller et al. 2004). In addition, much is known about its biology, and it is easy to find and identify in the field, making it an easy species with which to work.

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Extended summaries and context of papers

The following three papers address the effects of environmental factors, habitat history, and spatial fragmentation on *Abax parallelepipedus*, a widespread, flightless ground beetle. A synthesis of all of the results together gives an insight as to how historical and contemporary land use can affect the entire genetic structure of a widespread species. These papers are based on the examination of over 3300 individuals of *A. parallelepipedus* from 142 populations.

Paper 1: A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae)

Marcus T, Assmann T, Durka W, Drees C (2013) A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae). Conservation Genetics Resources 5:1151–1156.
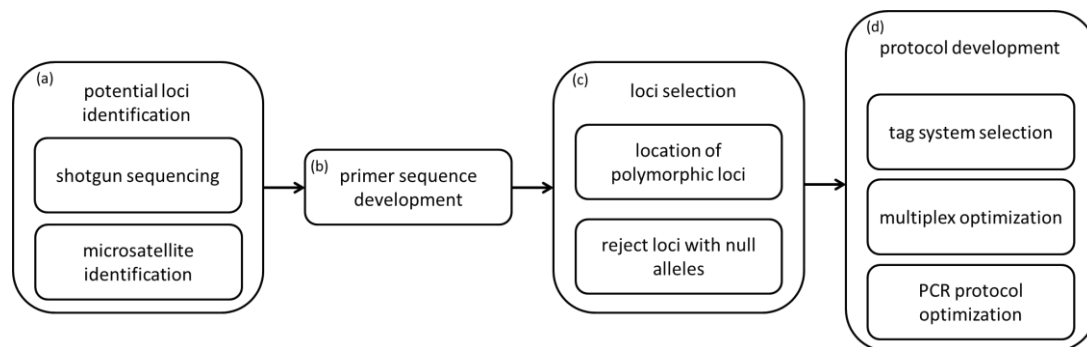
Figure 10: Flow chart of steps involved in primer development

Microsatellite analysis is a well-established method to examine genetic diversity and differentiation, and is nowadays by far the most common method used for these purposes in animal studies (Storfer et al. 2010). This method looks at the variation in number of repeats in microsatellite loci (Queller et al. 1993). A microsatellite locus consists of a sequence of two to six base pairs which is consecutively repeated

19

numerous times. These loci rapidly evolve alleles with differing numbers of repeats, apparently sped up by slippage during DNA replication (Eisen 1999). Alleles can be detected based on PCR fragment length using electrophoresis or automated sequencing machines.

I chose to use microsatellites for two main reasons, their neutrality and their high variability (Selkoe & Toonen 2006). As there is thought to be no strong selective disadvantage against mutations in these non-functional sequences, microsatellites are considered to be a neutral marker. While on the one hand this neutrality allows for the ignoring of selection processes in the analysis of allele frequencies, on the other hand level of microsatellite diversity have been shown to be correlated with genome wide diversity at the population level (Väli et al. 2008) and with fitness (Reed & Frankham 2003) or fitness related traits (Hansson & Westerberg 2002, Chapman et al. 2009). Loci which are under selection due to linkage to nearby adaptive parts of the genome can be identified using statistical methods. Since I am interested in patterns of gene flow and drift, I chose a marker system in which selection does not need to be accounted for.

Microsatellites are also highly variable, as due to their repeating nature, the DNA replication mechanism often slips creating new alleles. There is then no selection against these additional alleles, allowing them to accumulate (Eisen 1999, Schlötterer 2000). Therefore, microsatellites provide the high resolution needed to examine recent changes to genetic structure (Schlötterer 2000, Selkoe & Toonen 2006). As I am interested in the effects of land use, this time scale is the appropriate one for my studies.

One of the major disadvantages to the use of microsatellites is the labor and cost intensive process of developing the needed species-specific primers. Since the studied regions of the genome are quite variable, in most cases primers created for other species, even if they are closely related, do not give satisfactory results. In the case of *Abax parallelepipedus*, primers for five loci had already been published (Keller & Largiadèr 2003a). While these markers served as possible candidates, I needed to ensure that they are polymorphic in my regions. In addition, five markers are too few for a thorough investigation of my study questions.

The first step of primer development is the location of potential microsatellite loci (Figure 10a). Probably due to post-glacial recolonization processes, *A. parallelepipedus* was shown in studies using allozymes to have extremely low levels of genetic diversity, especially north of the Alps (Düring 2001, Desender et al. 2005b). Although microsatellites are more variable than allozymes, I still needed to locate 10-20 polymorphic loci in order to be able to properly analyze genetic patterns across my study regions. With the modern methods of next generation sequencing and automatized DNA sequencers, 10-20 microsatellite loci is the accepted, standard number of loci for most landscape genetics and population genetics studies (Storfer et al. 2010).

The location of microsatellite regions in the genome of the study species, and the development of primers which will attach to the flanking regions of each of the repeating sequences is done based on a library of sequences created by shotgun sequencing. I first extracted DNA from individuals of *A. parallelepipedus* collected in Germany, and sent it for shotgun sequencing in order to obtain a large library of DNA sequences. This library was scanned for fragments containing complete microsatellites consisting of repeating sequences together with their flanking regions.

I identified and developed primers for 49 microsatellites, and additionally redeveloped primers for the loci which had already been published for *A. parallelepipedus* (Keller & Largiadèr 2003a) (Figure 10b). As the flanking regions are usually non-adaptive, there are often minor, regional differences in the flanking regions so I chose to redevelop the primers to ensure better attachment and PCR results. Of 54 tested loci, 20 were polymorphic and contained no null alleles (Figure 10c). Only two of the previously published loci were usable.

In order to save on time and costs, I developed two different protocols to multiplex the usable loci (Figure 10d). Each of these protocols is designed for one of the two methods of attaching the fluorescent tag to the PCR product. This tag is later used by an automated sequencing machine to detect PCR product length. The first set of markers includes all 20 loci and uses the cheaper CAG/M13R method of tagging primers, but requires more PCR reactions and two genotyping runs. In this method, the fluorescent

tag is attached to the PCR products via a universal PCR primer rather than to the species and locus specific primers (Schuelke 2000). The second marker set is a subset of 14 loci and can be amplified and sequenced in one run, but uses more expensive, directly tagged primers (Table 3).

Table 3: Summary of developed marker sets for *Abax parallelepipedus*

	full set of primers	subset of directly labeled primers
number of loci	20	14
tagging method	CAG/M13R	direct labels
number of PCR runs	5	1
number of genotyping runs	2	1

In addition to proving that it is possible to develop a functioning suite of polymorphic microsatellite loci for *A. parallelepipedus*, I also tested if the developed marker sets can be used for other species of the same genus. Since the development of marker sets is an expensive and time-consuming process, marker sets which can be used for several species are particularly valuable. Although due to the high variability in flanking regions, cross-priming does not always work (reviewed in: Rutkowski et al. 2011), six of the developed primers gave high-quality results for three additional *Abax* species, *A. carinatus* (Duftschmid, 1812), *A. ovalis* (Duftschmid, 1812), and *A. parallelus* (Duftschmid, 1812). Further testing is required to determine if the primers may also be suitable for use in additional species, or even in other genera.

In developing a set of working multiplexed primers for *A. parallelepipedus* together with the corresponding protocols, I created a research system which allowed me to genotype the large number of individuals and alleles needed in order to address my research questions. For my analyses, I chose to work with the subset of 14 microsatellite loci, as it contains the best-working of the markers and still has a large enough number of loci to provide robust results while reducing workload and overall costs.

Paper 2: Living in Heterogeneous Woodlands – Are Habitat Continuity or Quality Drivers of Genetic Variability in a Flightless Ground Beetle?
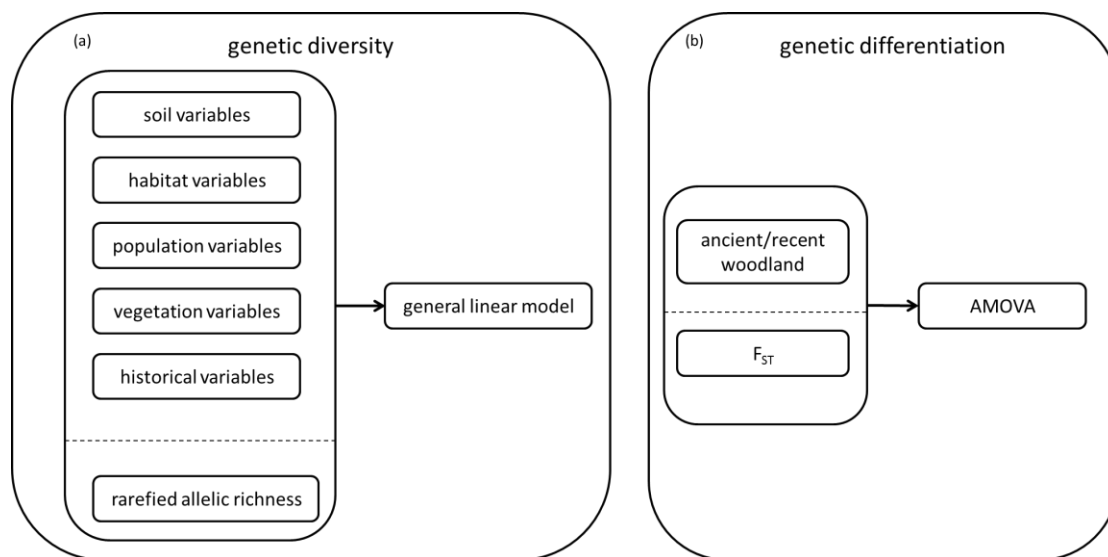
Figure 11: Variables tested and methods used in identifying drivers of (a) genetic diversity and (b) genetic differentiation.

My main object in this study was to examine the relationship of the effects of environmental factors and habitat history on the genetic diversity and differentiation patterns of *Abax parallelepipedus*. I did this by modelling the relationship between rarefied allelic richness and a large suite of variables which are known drivers of genetic diversity. The importance of temporal habitat continuity for the preservation of species diversity has long been recognized (reviewed in Hermy & Verheyen 2007), but it also plays a role in the preservation of genetic diversity (Desender et al. 2002b, Jacquemyn et al. 2004, Desender et al. 2005a, Reisch et al. 2007, Otálora et al. 2011). Therefore, I was interested in finding out what could be the potential drivers behind the varying levels of genetic diversity within each of my study regions.

Both habitat history and environmental factors have been cited as drivers of genetic diversity patterns (e.g. Jacquemyn et al. 2004, Cena et al. 2006, Reisch et al. 2007, Vandepitte et al. 2007, Gaublomme et al. 2013). *Temporal habitat continuity* leads to higher genetic diversity as it both prevents the loss of alleles and facilitates the gain of new ones. Temporal habitat fragmentation should lead to lower genetic diversity in populations as it leads to founder effects and bottlenecks. Habitat stability additionally allows populations more time to acquire additional alleles via migration and even mutation. *Environmental factors* affect genetic diversity via their effects on carrying capacity and population sizes, and therefore the rate of genetic drift. Additionally, many environmental properties of a site, for example soil nutrient ratios, are shaped by land use past and present (e.g. Koerner et al. 1997, von Oheimb et al. 2008). However, few studies analyze potential historical and environmental drivers of genetic diversity together, leaving questions as to their relative importance, as well as whether some drivers may actually be proxies for other unstudied ones, wide open.

The effects of temporal habitat continuity on various levels of diversity is of particular interest in the context of temperate woodlands and forests due to the extent of ongoing cycles of deforestation and afforestation (Flinn & Vellend 2005, Hermy & Verheyen 2007). Due to the long history of intense anthropogenic influence in Europe, primary forests are probably rare, and would in any case be extremely difficult to locate. However, the peak of deforestation and the beginnings of accurate mapping both occurred approximately 200-400 years ago. Therefore, the term 'ancient woodlands', meaning those which appear as forested on all existing maps, has been defined to describe long-term habitat continuity (Peterken 1993, Rackham 2003, Flinn & Vellend 2005). Whether a woodland is an ancient one or a recent one describes the long-term habitat continuity, and carries no information as to the age of the trees themselves. In an ancient woodland, the trees themselves may be relatively young. Stand age is a measure of short-term habitat continuity, as it only provides information about the age of the trees themselves, not about whether the site was forested before the current stand was planted.

In addition to short-term and long-term habitat continuity, I further characterized each of the studied plots using a large suite of variables related to local population sizes

of *A. parallelepipedus*, as well as those related to environmental variables known to affect ground beetles. Of particular interest are those variables related to soil and the number of closed forest species as in addition to characterizing the habitat, they reflect past land use as well. Given the innate inaccuracy of characterizing sites as ancient or recent due to the inherent gaps in map series, use of such variables can be of great value.

Given the difficulties in measuring exact population sizes and densities, and the importance of population size in determining the magnitude and rate of change of allele frequencies, I used three different proxies in my models. The first proxy was related to the amount of woodlands in the surroundings of each plot, while the other two proxies are related to pitfall catches. The use of the number of *A. parallelepipedus* individuals found in killing pitfall traps in 2008 gives a more accurate measure of population sizes. Using the sampling effort required to gather 33 individuals from each plot adds a proxy derived from data from an additional year, from 2011 for the Schwäbische Alb and from 2012 for the Schorfheide-Chorin, preventing bias.

Rarefied allelic richness (El Mousadik & Petit 1996), the average number of alleles per locus after rarefaction to account for differences in sample size due to PCR amplifications or sequencing reactions which did not work, was used to quantify genetic diversity. I chose to use this metric as it the most fitting one to detect recent losses of genetic diversity (Allendorf et al. 2012). Rarefaction was done to 20 individuals, the minimum number of successfully genotyped individuals for any of the single loci in a single population.

I began the modelling process with 27 candidate variables which could be related to genetic diversity patterns in *A. parallelepipedus*. After removing nine variables due to collinearity, I fit a general linear model consisting of the remaining historical and environmental variables and the rarefied allelic richness in each of the studied plots in the Schorfheide-Chorin and in the Schwäbische Alb (Figure 11a). I did not analyze the Hainich-Dün Exploratory in this study as it does not contain plots located in recent woodlands.

In both regions I found no significant effects of parameters connected to habitat history, but rather of those which can be related to population sizes. In the model for the Schorfheide-Chorin, the significant variables were the depth of the litter layer and sampling effort, while in a borderline significant model for the Schwäbische Alb the remaining variable was the percentage of the two kilometers surrounding each plot which was wooded. Deeper litter layers probably have an indirect connection to population sizes of *A. parallelepipedus* since they maintain larger populations of earthworms (Lumbricidae) (Nordström & Rundgren 1974, Phillipson et al. 1976, Cuendet 1984), which are an important food source for the beetles (Thiele 1977, Loreau 1984).

I also examined the effects of long-term temporal habitat continuity on the patterns of genetic differentiation using an AMOVA test (Figure 11b). Past changes in land use may be reflected in genetic differentiation between populations located in temporally fragmented sites and those which are not, due to loss of alleles during the inherent population bottlenecks and founder effects in the fragmented sites. Whether the recolonizing individuals come from a single source or from several may also affect the potential differentiation patterns (Slatkin 1977). In both Exploratories there was no significant genetic differentiation between ancient and recent plots.

The lack of relationship between temporal habitat continuity and either genetic diversity or genetic differentiation in *A. parallelepipedus* is striking, and is most likely a result of the species' dense, stable populations. These results imply that even during the peak of deforestation, the remaining forest patches were able to maintain large enough populations to prevent significant loss of alleles due to genetic drift. The lack of differentiation between ancient and recent woodlands implies the lack of founder effects and bottlenecks during the recolonization processes. This is particularly interesting given the flightlessness and lack of mobility of *A. parallelepipedus*. As in both Exploratories the recent woodlands are scattered in amongst the ancient ones, gene flow may be mitigating any loss of alleles or differentiation which may have existed in the past.

In terms of conservation, this study is quite heartening, since it shows that despite the limited mobility of *A. parallelepipedus*, temporal fragmentation may either have

never caused significant genetic erosion, or it may have been effectively mitigated. For the large number of species which, like *A. parallelepipedus*, are not rare and do not have extremely stringent habitat requirements, the loss of genetic diversity may not be as large a worry as previously thought. Especially in landscapes which are not hyper-fragmented and therefore allow for effective recolonization, the size of patches may actually be a more important factor than their age in determining their importance as reservoirs of genetic diversity. Ensuring that newly reforested areas are connected to older woodlands is of importance in facilitating effective recolonization. Nevertheless, ancient woodlands remain critical for the conservation of genetic diversity in rarer species and in more fragmented habitats, as well as for the conservation of the rare species, some of which are restricted to such habitats.

Paper 3: What you see isn't always what you get: genetic effects of fragmentation in central European rural landscapes

Marcus T, Assmann T, Drees C What you see isn't always what you get: genetic effects of fragmentation in central European rural landscapes.
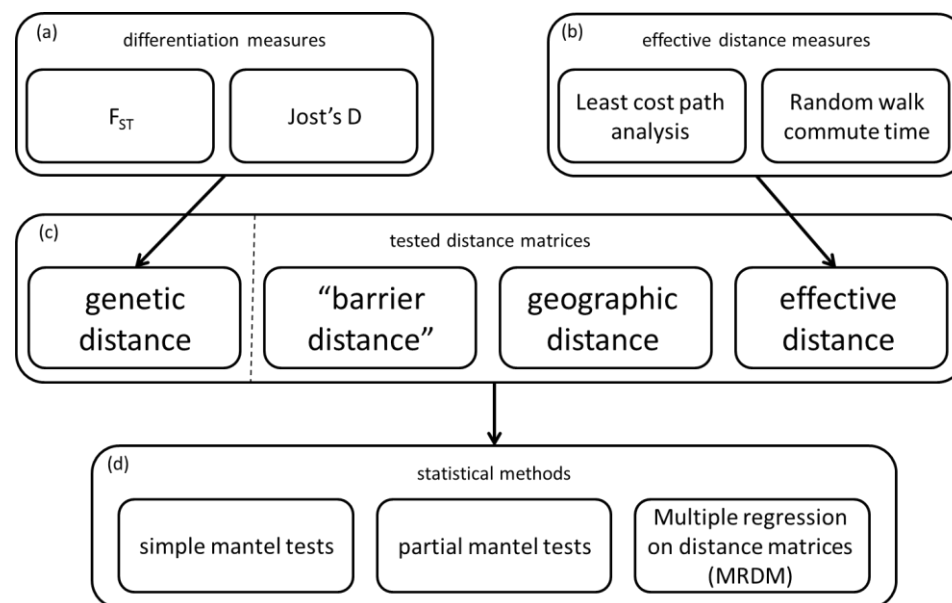
Figure 12: Flow chart of a landscape genetic analysis. Distance measures and methods used to test genetic differentiation across each of the study regions.

Landscape genetics is a new and powerful field, which combines population genetics with landscape ecology, allowing insight into how an entire landscape, as a unit, affects the genetic structure of individuals or of populations (Manel et al. 2003, Holderegger & Wagner 2006, Storfer et al. 2007, Holderegger & Wagner 2008). The methods developed for this field allow for the explicit testing of the relationships between genetic patterns and landscape patterns, mostly those of fragmentation and land use (Storfer et al. 2007). One of the unique advantages provided by landscape genetics, is the ability to examine various types of fragmentation, accounting for barriers as well as land use, as well as the possibility of differing levels of incomplete prevention of migration. This allows for a landscape to be studied in all of its complexity on a large spatial scale.

In this study I carried out a landscape genetics analysis to examine the patterns of genetic differentiation across each of my study regions, allowing me to understand the effects of spatial fragmentation. Due to the limited mobility of *A. parallelepipedus* and its habitat specificity, the transportation infrastructure as well as many of the land uses found in my study regions are expected to severely limit the migration of individuals across the landscape. This fragmentation could lead to significant genetic differentiation across the landscape.

In the field of landscape genetics a pattern of genetic differentiation is also known as an isolation pattern, as the emphasis is placed on the driver isolating the populations and thereby driving the differentiation between them. The three main isolation patterns are isolation by distance, isolation by resistance, and isolation by barrier. An additional possibility is that no differentiation pattern can be found across the landscape.

Isolation by distance is the result of individuals' tendency to mate with those found nearby. As a result, populations which are geographically distant will also be more distant genetically. Isolation by resistance develops when movement of individuals across the landscape is hindered by land use or by environmental features such as soil or elevation. In this case, mating is no longer random across the landscape, but rather affected by ease of movement between populations. Isolation by barrier develops when movement is hindered by linear barriers such as rivers or roads. This differs from isolation by resistance in that such barriers usually cannot be circumvented, and are usually completely impermeable.

Understanding these patterns is a vital tool in conservation contexts, allowing glimpses as to how complex landscapes are affecting migration and gene flow and what fragmenting elements may be driving the development of genetic differentiation. This needs to be studied not only for rare species or hyper-fragmented landscapes, but also for more typical situations, so that a wide array of species and landscapes can be understood, and generalized conclusions can be made. Such generalization also requires the currently sorely lacking reproduction of studies in the same species across several landscapes (Holderegger & Wagner 2008, Segelbacher et al. 2010, Manel & Holderegger 2013, Keller et al. 2015, Richardson et al. 2016). This study aims to close these gaps by

conducting a comprehensive landscape genetics analysis of a fairly widespread yet stenotopic species in three landscapes where the levels of fragmentation are not extreme, and land use patterns are fairly typical for those found in rural regions of central Europe (Fischer et al. 2010).

All three isolation patterns could be presumed as potential drivers of genetic differentiation patterns in *A. parallelepipedus*. *Isolation by distance* could be expected given the size of the Exploratories relative to mobility of the beetles. *Isolation by resistance* could be expected since studies using a variety of methods have shown that it is sensitive to land use, quickly disappearing after clear cutting (Huber & Baumgarten 2005) and not venturing far out of wooded areas (Charrier et al. 1997, Petit & Burel 1998a). Other environmental factors such as soil, altitude, and climate are not expected to be driving diversity patterns, since the values found in the Exploratories are well within the ranges which are hospitable to *A. parallelepipedus*, and indeed the species is found throughout all three study regions. *Isolation by barrier* could be expected as it has been shown that the beetles very rarely cross roads and railways (Mader 1984, Mader et al. 1990, Koivula & Vermeulen 2005). Indeed, a previous study carried out with the species has already shown that roads can lead to rapid genetic differentiation, within a few dozen years (Keller et al. 2004).

In a landscape genetics analysis, isolation patterns are described as distances between spatial points in a landscape (Figure 12c). These landscape distances can then be used to model genetic differentiation, which is in essence the genetic distance sampled at those points. Genetic differentiation can be studied either among individuals or among populations. Although I cannot draw lines objectively delineating populations between my plots, I nevertheless chose to use population based methods since the beetles were trapped in plots rather that scattered across the landscape. The plots are all far apart relative to the movement capabilities of *A. parallelepipedus*, at distances which single individuals could not cross in a single life time.

To calculate the genetic distances, I used two common methods (Figure 12a). The first was the traditional F_{ST} values (Wright 1951, Nei 1972). Although this measure is fairly simple, in cases such as this one with a large number of studied populations and

low levels of diversity, it is considered robust and widely used. I additionally calculated the genetic distances with one of the newer indices, Jost's D (Jost 2008).

Isolation by distance is accounted for as the geographical distance between the plots. I used simple Euclidean distances, or distance as the crow flies. Including geographical distances allowed me not only to test for isolation by distance, but to also statistically control for the effects geographic distance may have on the other distances. For example, plots which are farther apart are probably separated by more roads and railway lines.

To test for isolation by resistance patterns I calculated the effective distance between each pair of plots using two different methods (Figure 12b). Effective distances measure how far apart two points are while accounting for both geographic distance as well as the ease of movement through the landscape. The first method, circuit path analysis, has two main advantages in that it assumes random walk movement, which is the case for *A. parallelepipedus*, and allows for several connecting paths between each pair of points. I included the second method, least cost path analysis, due to its simplicity of interpretation and its well-established use in the field. Both of these methods calculate the distance between each pair of plots based on a resistance surface, in essence a map in which each pixel contains the probability of a beetle to cross it. I assigned these values based on land use and cover, giving land uses more hospitable to *A. parallelepipedus* low resistance values, and very inhospitable land uses having high resistance values.

Isolation by barrier was tested for using an index I developed called 'barrier distance'. Barrier distance was calculated as the minimum cost of crossing roads and railways between two plots, with the cost for crossing larger roads higher than that of crossing smaller ones. This index accounted for railways and the four public road categories in use in Germany.

Since landscape genetics is a relatively new field, best practices have not yet been fully clarified (Segelbacher et al. 2010, Richardson et al. 2016). Therefore, I used three methods to model the effects of the three types of landscape distances on the genetic distances in order to find if there are isolation patterns in each of the Exploratories, and

if so, which (Figure 12d). I used simple Mantel tests to examine the effect of each of the landscape distance on genetic distances separately. I then used partial Mantel tests to allow me to test each type of landscape distance while controlling for the other two. The third method I used was multiple regression of distance matrices (MRDM). Since all of the methods are controversial, using three together allowed me better assess the validity of my results.

Surprisingly, I found no effects of fragmentation in any of the three study regions. In the Schwäbische Alb I found no significant isolation patterns at all, while in the Schorfheide-Chorin I found a significant isolation by distance pattern. A very weak isolation by distance pattern was detected by two out of three methods in the Hainich-Dün as well. As the probability that significant gene flow is occurring across any of the study regions is quite low, I conclude that despite lack of migration, changes in allele frequencies are occurring at an extremely slow rate due to large population sizes. While isolation by resistance and isolation by barrier patterns have not had the time to develop in any of the region, isolation by distance patterns have managed to develop in the Schorfheide-Chorin, as this is the study region with the smallest populations (Marcus et al. 2015). The larger population sizes in the other two study regions may have so far mitigated the development even of isolation by distance, though there may be first signs of it in the Hainich-Dün. An isolation by distance pattern may be developing faster in the Hainich-Dün than in the Schwäbische Alb since it is much larger and so the distances between plots are greater.

The results of my simulations of the development of genetic differentiation in populations of different sizes corroborate the importance of population size. In populations of starting sizes such as those found in my plots, very little differentiation developed, even after 250 years. However, in very small populations, such as those studied in Switzerland (Keller et al. 2004), the development of differentiation was rapid, explaining the seemingly contradictory results between the two studies.

This study highlights the fact that physical fragmentation does not necessarily lead to differentiation. In fact, for many species in landscapes which are not hyper-fragmented, genetic drift is probably an extremely slow process. On the one hand, this

alleviates some conservation concerns, as the loss of alleles due to fragmentation may not be of great concern. On the other hand, landscape genetic analyses, such as the one I carried out, are a common method of detecting fragmentation in the first place, and thus we may be not detecting barriers and fragmenting elements which are preventing migration of individuals.

General discussion

In my work, I tested the effects of environmental factors, habitat history, and landscape structure on the genetic diversity and the genetic differentiation of *Abax parallelepipedus*. Interestingly, I only found effects of environmental factors on genetic diversity. I also found very little genetic differentiation in general across my study regions, although they are complex landscapes which include inhospitable land uses and linear barriers for the studied species. Gaining a better understanding of these results requires a redirection of the emphasis from these traditionally studied ultimate factors back to the more theoretical proximate ones, namely population sizes and gene flow.

My analyses of the genetic diversity and differentiation of *A. parallelepipedus* have shown that the allele frequencies of the studied microsatellite loci likely have remained relatively stable across time and space in all three of my study regions. As selection and mutation are not the major causes of changes in allele frequencies in my study system, this can be the result of stabilizing gene flow or of lack of drift. Stabilization of gene flow would require sufficient migration across the landscapes, while lack of drift would require large effective population sizes. Based on a synthesis of all of my results, I conclude that the main driver of the found genetic stability is lack of drift due to large population sizes.

Of all of my hypotheses, the only one I could confirm was that there are environmental factors which affect genetic diversity (Hypothesis 1) (Table 4). All of the significant variables reflect population sizes, and only in the Schorfheide-Chorin did I find significant effects as opposed to trends. This is probably related to the smaller population sizes in this region due to soil pH affecting prey availability, and explains the larger variance in genetic diversity found in the region relative to the other two. The Schorfheide-Chorin has the lowest soil pH values, falling within the range in which soil pH is expected to limit earthworm populations (Krück et al. 2006), which are an important food source for *A. parallelepipedus* (Thiele 1977, Loreau 1984). The values found in the other two study regions are high enough to not be affecting earthworms. In the Schwäbische Alb the larger population sizes seem to have prevented even small amounts of allele loss.

34

Table 4: Summary of hypotheses and whether they were accepted or rejected.

I did not find significant effects of temporal fragmentation on the genetic diversity (Hypothesis 2) (Table 4). In order for the past fragmentation to have caused loss of alleles, either it must have been severe enough to cause extremely small effective population sizes (e.g. Landergott et al. 2001), or to have persisted for sufficiently long time periods to allow drift to accumulate. Strong fluctuations in population sizes would also encourage the loss of genetic diversity during fragmentation (Drees et al. 2011), but such fluctuations are unknown in my study species (Judas et al. 2002, Günther & Assmann 2004). In the studied case of *A. parallelepipedus* the historical fragmentation was apparently not sufficient in terms of length or severity to cause significant loss of alleles.

I also did not find a significantly different pattern of differentiation in the ancient woodlands relative to the recent ones (Hypothesis 3) (Table 4). Given that in my study regions the recent woodlands are embedded between the ancient ones, recolonization probably can occur from several sources. Therefore I expected that the ancient woodlands would be more differentiated than the recent ones as per the propagule model (Slatkin 1977). In addition, *A. parallelepipedus* is considered to be a relatively effective recolonizer despite its lack of mobility (Magura et al. 2003, Deuschle & Glück 2008, Brandmayr et al. 2009), probably due to its ability to utilize all types of woodlands

(Day et al. 1993, Fahy & Gormally 1998, Magura et al. 2000, Lange et al. 2014). This flexibility allows individuals to cross the various, interspersed woodland types found in all three regions, easing recolonization. Nonetheless, as the allele frequencies appear to have remained stable during peak deforestation, the ancient populations did not become differentiated from another and therefore there are no differences between these populations to be found.

Although there have been several studies showing that *A. parallelepipedus* does not cross roads (Mader 1984, Mader et al. 1990, Koivula & Vermeulen 2005), and previous work has shown that roads can cause significant genetic differentiation in the species (Keller et al. 2004), I did not find isolation by barrier patterns in any of my study regions (Hypothesis 4) (Table 4). This, too, is most likely related to population sizes. The number of individuals successfully crossing roads, especially the larger highways is probably negligible, creating effective fragmentation. However, differentiation between populations on either side of the roads is minimal due to the large population sizes. Since drift in such populations is slow, it may take several hundred generations for significant differentiation to develop. Similarly, although *A. parallelepipedus* is not able to cross non-wooded areas, and all three study regions contain a complex mosaic of land uses, I did not find influences of landscape structure either on differentiation patterns (Hypothesis 5) or on genetic diversity found (Hypothesis 6) (Table 4). This too is probably due to slow changes in allele frequencies due to the large population sizes.

While many cite the rule of thumb that one migrant per generation is enough to prevent genetic drift between populations (e.g. Spieth 1974, Wang 2004), this is quite inaccurate in many natural systems (Wright 1931, Mills & Allendorf 1996). This rule is based on an idealized system, with many assumptions which are violated in my study system, including the island model of migration which is farcical given the size of the study regions relative to the mobility of the species. In this case, the actual amount of migrants needed to prevent genetic differentiation can be significantly higher than one individual per generation (Mills & Allendorf 1996).

While most of the suspected ultimate causes of genetic diversity and differentiation did not show the effects I expected, in some ways it is not surprising given that they all

are drivers via proximate factors, in this case the relevant one being population sizes (Figure 13). Although there are plots with larger populations than others due to environmental factors, populations which underwent temporal fragmentation, and the landscapes contain swaths of unsuitable land uses and are crisscrossed by barriers, population sizes across all three regions remain too large to be significantly affected at the genetic level. Nevertheless, genetic drift occurs also in large populations, albeit at a slow pace, thus changes may be ongoing though are not yet detectable.
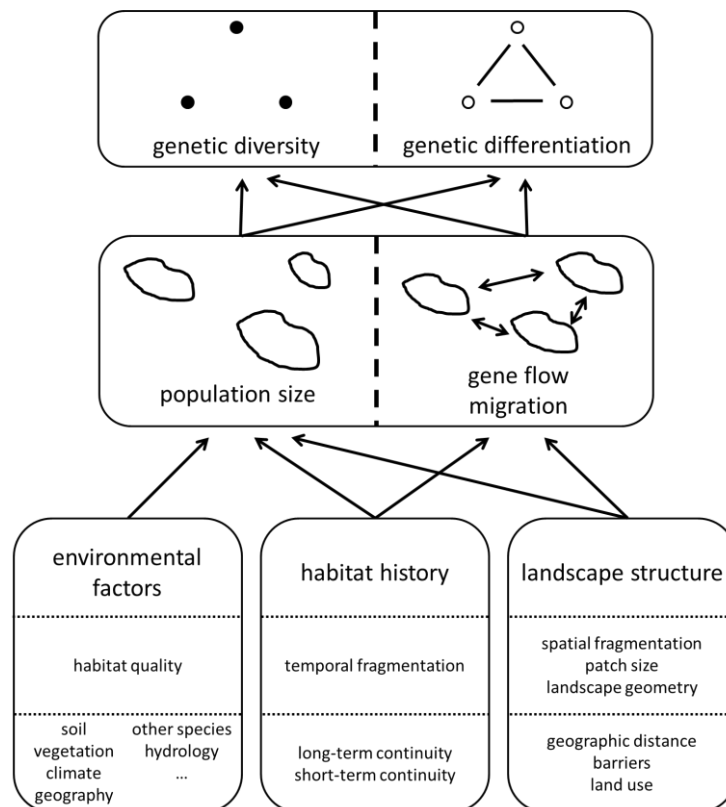
Figure 13: Summary of ultimate and proximate drivers of genetic diversity and differentiation examined in this thesis.

While the lack of diversity and differentiation patterns may also be due to the occurrence of some gene flow, the large population sizes are a vastly more important factor in my study species and regions. Numerous studies of *A. parallelepipedus* have shown that it avoids open habitats, and certainly does not venture into agricultural fields (Charrier et al. 1997). The species tends to avoid roads including the verges, and the few which venture a crossing attempt would probably need several hours to cross the entirety of many roads, getting run over in the process (Mader 1984, Mader et al. 1990,

Koivula & Vermeulen 2005). Therefore, the amount of gene flow across the three study regions is probably not large enough to mitigate differentiation, and there is probably no migration at all across many of the roads and railways.

The comparison of the studies presented in this thesis to two other studies is particularly telling. Wiesner et al. (2014) carried out a small study of a meadow grasshopper in the grasslands of the Hainich-Dün, whereby they also tested a widespread species in one of my study regions. Here too, the moderate fragmentation combined with the naturally high population densities of the study species, prevented the development of genetic differentiation across the landscape. Keller et al. (2004) examined the effects of roads on the genetic differentiation on *A. parallelepipedus*, in a hyper-fragmented area, containing woodland patches of approximately only one hectare. As opposed to me, they found significant differentiation across roads which are newer and smaller than the ones I examined. Despite the high population densities characteristic of *A. parallelepipedus*, in this case the populations in such patches seem to be simply too small to prevent changes in allele frequency due to drift, which caused rapid development of significant genetic differentiation across the roads.

Although there currently appear to be little effects of the spatial fragmentation in my landscapes, it is probable that extremely slow changes in allele frequencies are taking place which over time will lead to significant genetic differentiation, especially across larger roads. If there is no further fragmentation, this process may take hundreds of generations. Although these slow drift processes should lead to the development of genetic differentiation across the landscapes, significant loss of genetic diversity is not to be expected due the large populations.

There is an additional pair of studies which examined the genetic diversity and distribution of fairly common carabids. These studies also highlighted the influence of population sizes. Brouat et al. (2003, 2004) examined the genetic structure of two common members of the *Carabus* genus found in forests in a small, moderately fragmented landscape in the Pyrenees. They found significant isolation by distance and isolation by resistance patterns in the more specialist species. However, they did not test for the two pattern types simultaneously so the relationship between these two

patterns remains unclear. For the more generalist species they did not find significant spatial genetic patterns (Brouat et al. 2003). They also found significant effects of environmental factors which could be related to population sizes and hence to genetic diversity (Brouat et al. 2004). Similar to my results, population sizes were shown here to be of critical importance to genetic structuring. However in this case there is a clear influence of gene flow, as the more abundant species is the one which showed significant effects of landscape. Nevertheless, population sizes were shown to be a significant factor for the levels of both genetic diversity genetic differentiation. The two studied *Carabus* species both usually have smaller populations than *A. parallelepipedus* (Judas et al. 2002, Günther & Assmann 2004), which is probably what enabled the development of isolation patterns found in this study. While Brouat et al. also examined fairly widespread species in a moderately fragmented landscape, the natural smaller populations of their studied species are apparently sufficient to have allowed development of genetic differentiation. Studies examining the relationship between population sizes and the levels of genetic differentiation in natural landscapes would be a fascinating topic for further study.

From a conservation perspective, my results are in many ways quite encouraging. Although fragmentation can have severe, deleterious effects on rare species and in hyper-fragmented landscapes, many species may take centuries to be significantly affected, as long as their population sizes remain large enough. Additionally, ensuring that newly forested areas are connected to other wooded areas, preferably to ancient woodlands, can ensure the preservation of genetic diversity. Ensuring that populations maintain genetic diversity is a critical step in preserving their potential to adapt to future changes and challenges, such as those from climate change (Hughes et al. 2008).

Genetic diversity is a crucial component of the survival of populations and species, allowing long-term and short-term adaptation and evolution in response to changes in their surroundings, as it is a prerequisite for adaptation (Amos & Harwood 1998, Agashe 2009, Engelhardt et al. 2014). Genetic diversity has also been linked to additional, vital processes, such as stabilization of population dynamics (Hughes et al. 2008, Agashe 2009), enhancement of population fitness (Vrijenhoek 1994, Reed & Frankham 2003, Johnson et al. 2006, Gamfeldt & Källström 2007), and resistance to disease and parasites

(Altizer et al. 2003, Díaz et al. 2006, Altermatt & Ebert 2008). Studies have also linked genetic diversity to community level, and even to ecosystem level processes (reviewed in Hughes et al. 2008).

This study also raises practical questions as to the use of genetic methods to examine fragmentation in real-world settings. As this study highlights, lack of differentiation may not always mean that gene flow, and therefore migration, are occurring across the landscape. Rather, due to large population sizes, there may be a significant time lag between physical fragmentation of a landscape and the appearance of significant genetic differentiation (Richmond et al. 2009, Landguth et al. 2010). Therefore, using genetic differentiation alone in making decisions, may lead to inappropriate conservation measures being taken.

Another major conservation message from this study is that fragmentation is a species-specific term (Louy et al. 2007, Holderegger & Di Giulio 2010, Richardson 2012, Whiteley et al. 2014, Richardson et al. 2016). *Abax parallelepipedus* probably does not perceive my study regions as fragmented at all. For a species with such low mobility and high natural population densities, the remaining habitat patches in a moderately fragmented landscape are large enough. Had this study been carried out in the same study regions but on a more mobile species or on one with smaller populations, I would probably have spatial genetic structuring.

The effects of landscape structure on genetic diversity and differentiation have been the subject of intensive study, even more so since the advent of landscape genetics. However, most attempts to generalize results have concentrated on the effects of specific landscape structures, such as roads (Balkenhol & Waits 2009, Holderegger & Di Giulio 2010, Muñoz et al. 2015). While conflicting results have often been noted between studies (Holderegger & Di Giulio 2010), little emphasis is placed on understanding these differences in terms of proximate causes such as population sizes and gene flow.

We tend to address a landscape as fragmented or not, based on the way humans perceive the landscape. How other species perceive that same landscape, however, is dependent on their mobility, population density, body size, and more. For conservation

and practical aspects, it is often convenient to address fragmentation at human scale since that is how we plan our roads and land usage, but this scale may become meaningless when addressing specific species. Baudry and Merriam (1988) encouraged the use of the term "connectedness" to describe the amount of physically connecting elements in a landscape and their properties as perceived by humans, e.g. patch size and distance between elements. They used the term "connectivity" to describe the amount of movement of individuals in a landscape. Use of these more specific terms which differentiate between fragmentation as a structure and fragmentation as a process, would allow for more effective communication, as well as serving as a gentle reminder to incorporate species-specificity into discussions of fragmentation.

In summary, this thesis gives an overarching view into the drivers of genetic diversity and genetic differentiation of a widespread yet stenotopic species in moderately fragmented landscapes. It addresses a wide range of possible ultimate drivers, namely environmental factors, habitat history, and landscape structure in three study regions. Such a comprehensive analysis enables an understanding into the relationship between the proximate drivers, gene flow and drift, and highlights the importance of population sizes in the development of genetic diversity and differentiation.

References

Adrian-Kalchhauser I, Hirsch PE, Behrmann-Godel J, N'Guyen A, Watzlawczyk S, Gertzen S, Borcherding J, Burkhardt-Holm P (2016) The invasive bighead goby *Ponticola kessleri* displays large-scale genetic similarities and small-scale genetic differentiation in relation to shipping patterns. Molecular Ecology 25:1925-1943.

Agashe D (2009) The stabilizing effect of intraspecific genetic variation on population dynamics in novel and ancestral habitats. The American Naturalist 174:255-267.

Allendorf FW, Luikart GH, Aitken SN (2012) Conservation and the Genetics of Populations, 2nd edn. Wiley-Blackwell.

Altermatt F, Ebert D (2008) Genetic diversity of *Daphina magna* populations enhances resistance to parasites. Ecology Letters 11:918-928.

Altizer S, Harvell D, Friedle E (2003) Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology and Evolution 18:589-596.

Alvarez N, Thiel-Egenter C, Tribsch A, Holderegger R, Manel S, Schönswetter P, Taberlet P, Brodbeck S, Gaudeul M, Gielly L, Küpfer P, Mansion G, Negrini R, Paun O, Pellecchia M, Rioux D, Schüpfer F, Van Loo M, Winkler M, Gugerli F, IntraBioDiv C (2009) History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants. Ecology Letters 12:632-640.

Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 353:177-186.

Antvogel H, Bonn A (2001) Environmental parameters and microspatial distribution of insects: a case study of carabids in an alluvial forest. Ecography 24:470-482.

Assmann T (1999) The ground beetle fauna of ancient and recent woodlands in the lowlands of north-west Germany (Coleoptera, Carabidae). Biodiversity and Conservation 8:1499-1517.

Austerlitz F, Jung-Muller B, Godelle B, Gouyon PH (1997) Evolution of coalescence times, genetic diversity and structure during colonization. Theoretical Population Biology 51:148-164.

Baars MA (1979) Patterns of movement of radioactive carabid beetles. Oecologia 44:125-140.

Balkenhol N, Waits LP (2009) Molecular road ecology: exploring the potential of genetics for investigating transportation impacts on wildlife. Molecular Ecology 18:4151-4164.

Barr KR, Kus BE, Preston KL, Howell S, Perkins E, Vandergast AG (2015) Habitat fragmentation in coastal southern California disrupts genetic connectivity in the cactus wren (*Campylorhynchus brunneicapillus*). Molecular Ecology 24:2349-2363.

Baudry J, Merriam HG (1988) Connectivity and connectedness: functional versus structural patterns in landscapes. In: *Proceedings of the 2nd International Seminar of the "International Association for Landscape Ecology"* (ed. Schreiber KF), pp. 23-29, Münster.

Brandmayr P, Pizzolotto R, Colombetta G, Zetto T (2009) In situ extinction of carabid beetles and community changes in a protected suburban forest during the past century: the "Bosco Farneto" near Trieste (Italy). Journal of Insect Conservation 13:231-243.

Brouat C, Chevallier H, Meusnier S, Noblecourt T, Rasplus J-Y (2004) Specialization and habitat: spatial and environmental effects on abundance and genetic diversity of forest generalist and specialist *Carabus* species. Molecular Ecology 13:1815-1826.

Brouat C, Sennedot F, Audiot P, Leblois R, Rasplus J-Y (2003) Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. Molecular Ecology 12:1731-1745.

Brouwers NC, Newton AC (2009) Movement rates of woodland invertebrates: a systematic review of empirical evidence. Insect Conservation and Diversity 2:10-22.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486:59-67.

Carter M, Smith M, Harrison R (2010) Genetic analyses of the Asian longhorned beetle (Coleoptera, Cerambycidae, *Anoplophora glabripennis*), in North America, Europe and Asia. Biological Invasions 12:1165-1182.

References

Cena CJ, Morgan GE, Malette MD, Heath DD (2006) Inbreeding, outbreeding and environmental effects on genetic diversity in 46 walleye (*Sander vitreus*) populations. Molecular Ecology 15:303-320.

Chaabane K, Josnens G, Loreau M (1994) Factors influencing respiration in *Abax ater*. In:Desender K, Dufrêne M, Loreau M, Luff ML, Maelfait J-P (eds) Carabid Beetles: Ecology and Evolution. Kluwer Academic Publishers, Dordrecht, Boston, London. pp. 474.

Chaabane K, Loreau M, Josens G (1996) Individual and population energy budgets of *Abax ater* (Coleoptera, Carabidae). Annales Zoologici Fennici 33:97-108.

Chapman JR, Nakagawa S, Coltman DW, Slate J, Sheldon BC (2009) A quantitative review of heterozygosity-fitness correlations in animal populations. Molecular Ecology 18:2746-2765.

Charrier S, Petit S, Burel F (1997) Movements of *Abax parallelepipedus* (Coleoptera, Carabidae) in woody habitats of a hedgerow network landscape: a radio-tracing study. Agriculture, Ecosystems and Environment 61:133-144.

Colautti RI, Lau JA (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Molecular Ecology 24:1999-2017.

Cuendet G (1984) A comparative study of the earthworm population of four different woodland types in Wytham woods, Oxford. Pedobiologia 26:421-439.

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation 128:231-240.

Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. The American Naturalist 168:486-499.

Day KR, Marshall S, Heaney C (1993) Associations between forest type and invertebrates: ground beetle community patterns in a natural oakwood and juxtaposed conifer plantations. Forestry 66:37-50.

den Boer PJ, Van Dijk TS (1994) Carabid beetles in a changing environment. Wageningen Agricultural University Papers 94:1-30.

Desender K (2005) Theory versus reality: A review on the ecological and population genetic effects of forest fragmentation on wild organisms, with an emphasis on ground beetles. In:Lövei GL, Toft S (eds) Carabidology 2003: Proceedings of the 11th European Carabidologists' Meeting Danish Institute of Agricultural Sciences, Slagelse, Denmark. pp. 49-72.

Desender K, de Bakker D, Versteirt V, de Vos B (2002a) A baseline study on forest ground beetle diversity and assemblages in Flanders (Belgium). In:Szyszko J, den Boer P, Bauer T (eds) How to Protect or What We Know About Carabid Beetles: X European Carabidologist Meeting. Warsaw Agricultural University Press, Warsaw. pp. 237-245.

Desender K, Gaublomme E, Dhuyvetter H, Verdyck P (2005a) Interaction between regional forest history, ecology and conservation genetics of *Carabus problematicus* in Flanders (Belgium). In:Lövei GL, Toft S (eds) Carabidology 2003: Proceedings of the 11th European Carabidologists' Meeting Danish Institute of Agricultural Sciences, Slagelse, Denmark. pp. 73-87.

Desender K, Gaublomme E, Verdyck P, Dhuyvetter H, De Vos B (2004) Ecology and population genetics of *Carabus problematicus* in Flanders, Belgium: is forest history important? In:Honnay O, Bossuyt B, Verheyen K, Hermy M (eds) Forest Biodiversity: Lessons from History for Conservation. CAB International, Wallingford. pp. 117-128.

Desender K, Small E, Gaublomme E, Verdyck P (2005b) Rural-urban gradients and the population genetic structure of woodland ground beetles. Conservation Genetics 6:51-62.

Desender K, Verdyck P, Gaublomme E, Dhuyvetter H, Rasplus J-Y (2002b) Extreme genetic differentiation and isolation by non-distance in *Carabus auronitens* in relation to forest historical ecology in Flanders (Belgium). In:Szyszko J, den Boer PJ, Bauer T (eds) How to Protect or What We Know About Carabid Beetles: X European Carabidologist Meeting. Warsaw Agricultural University Press, Warsaw. pp. 227-235.

Deuschle J, Glück E (2008) Similarity of ground beetle communities of extensively managed orchards depending on management regimes. Bulletin of Insectology 61:73-80.

Díaz S, Fargione J, Chapin FS, III, Tilman D (2006) Biodiversity loss threatens human well-being. PLoS Biology 4:e277.

Drag L, Cizek L (2015) Successful reintroduction of an endangered veteran tree specialist: conservation and genetics of the Great Capricorn beetle (*Cerambyx cerdo*). Conservation Genetics 16:267-276.

References

Drees C, De Vries HH, Härdtle W, Matern A, Persigehl M, Assmann T (2011) Genetic erosion in a stenotopic heathland ground beetle (Coleoptera: Carabidae) - a matter of habitat size? Conservation Genetics 12:105-117.

Drees C, Matern A, von Oheimb G, Reimann T, Assmann T (2010) Multiple glacial refuges of unwinged ground beetles in Europe: molecular data support classical phylogeographic models. In:Habel JC, Assmann T (eds) Relict Species: Phylogeography and Conservation Biology. Springer, Heidelberg. pp. 199-216.

Drees C, Matern A, Rasplus J-Y, Terlutter H, Assmann T, Weber F (2008) Microsatellites and allozymes as the genetic memory of habitat fragmentation and defragmentation in populations of the ground beetle *Carabus auronitens* (Col., Carabidae). Journal of Biogeography 35:1937-1949.

Drees C, Matern A, Vermeulen HJW, Assmann T (2007) The influence of habitat quality on populations: a plea for an amended approach in the conservation of *Agonum ericeti*. Baltic Journal of Coleopterology 7:1-8.

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Düring A (2001) Geographic differentiation of *Abax parallelepipedus* in the GPI locus: restriction of genetic variability to southern glacial refugia. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie 13:101-104.

Eggers B, Matern A, Drees C, Eggers J, Härdtle W, Assmann T (2010) Value of semi-open corridors for simultaneously connecting open and wooded habitats: a case study using ground beetles. Conservation Biology 24:256-266.

Eisen J (1999) Mechanistic basis for microsatellite instability. In:Goldstein DB, Schlötterer C (eds) Microsatellites: Evolution and applications. Oxford University Press, Oxford, United Kingdom. pp. 34-48.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. Theoretical and Applied Genetics 92:832-839.

Engelhardt KAM, Lloyd MW, Neel MC (2014) Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. Biological Conservation 179:6-16.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487-515.

Fahy O, Gormally M (1998) A comparison of plant and carabid beetle communities in an Irish oak woodland with a nearby conifer plantation and clearfelled site. Forest Ecology and Management 110:263-273.

Firle S, Bommarco R, Ekbom B, Natiello M (1998) The influence of movement and resting behaviour on the range of three carabid beetles. Ecology 79:2113-2122.

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. Global Ecology and Biogeography 16:265-280.

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze ED, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11:473-485.

Flinn KM, Vellend M (2005) Recovery of forest plant communities in post-agricultural landscapes. Frontiers in Ecology and the Environment 3:243-250.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK (2005) Global consequences of land use. Science 309:570-574.

Fournier E, Loreau M (2001) Activity and satiation state in *Pterostichus melanarius*: an experiment in different agricultural habitats. Ecological Entomology 26:235-244.

Frampton GK, Çilgi T, Fry GLA, Wratten SD (1995) Effects of grassy banks on the dispersal of some carabid beetles (Coleoptera: Carabidae) on farmland. Biological Conservation 71:347-355.

Franceschini G, Cannavacciuolo M, Burel F (1997) A geostatistical analysis of the spatial distribution of *Abax parallelepipedus* (Coleoptera, Carabidae) in a woodlot. European Journal of Soil Biology 33:117-122.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500-1528.

References

Frankham R (2005) Genetics and extinction. Biological Conservation 126:131-140.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Univ. Press, Cambridge.

Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) in strongly affected by the landscape. Molecular Ecology 14:483-496.

Gamfeldt L, Källström B (2007) Increasing intraspecific diversity increases predictability in population survival in the face of perturbations. Oikos 116:700-705.

Gaublomme E, Maebe K, Van Doninck K, Dhuyvetter H, Li X, Desender K, Hendrickx F (2013) Loss of genetic diversity and increased genetic structuring in response to forest area reduction in a ground dwelling insect: a case study of the flightless carabid beetle *Carabus problematicus* (Coleoptera, Carabidae). Insect Conservation and Diversity 6:473-482.

Gibbs JP (2001) Demography versus habitat fragmentation as determinants of genetic variation in wild populations. Biological Conservation 100:15-20.

Goetze M, Palma-Silva C, Zanella CM, Bered F (2016) East-to-west genetic structure in populations of *Aechmea calyculata* (Bromeliaceae) from the southern Atlantic rainforest of Brazil. Botanical Journal of the Linnean Society 181:477-490.

Grapputo A, Boman S, Lindström L, Lyytinen A, Mappes J (2005) The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. Molecular Ecology 14:4207-4219.

Günther J, Assmann T (2004) Fluctuations of carabid populations inhabiting an ancient woodland (Coleoptera, Carabidae). Pedobiologia 48:159-164.

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD, Lovejoy TE, Sexton JO, Austin MP, Collins CD (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances 1:e1500052.

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. Molecular Ecology 11:2467-2474.

Harrison S, Bruna E (1999) Habitat fragmentation and large-scale conservation: what do we know for sure? Ecography 22:225-232.

Hartl DL, Clark AG (1997) Principles of Population Genetics, 3. Aufl. edn. Sinauer, Sunderland.

Heisswolf A, Reichmann S, Poethke H, Schröder B, Obermaier E (2009) Habitat quality matters for the distribution of an endangered leaf beetle and its egg parasitoid in a fragmented landscape. Journal of Insect Conservation.

Hermy M, Verheyen K (2007) Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. Ecological Research 22:361-371.

Hodgson JA, Moilanen A, Wintle BA, Thomas CD (2011) Habitat area, quality and connectivity: striking the balance for efficient conservation. Journal of Applied Ecology 48:148-152.

Holderegger R, Di Giulio M (2010) The genetic effects of roads: a review of empirical evidence. Basic and Applied Ecology 11:522-531.

Holderegger R, Wagner HH (2006) A brief guide to Landscape Genetics. Landscape Ecology 21:793-796.

Holderegger R, Wagner HH (2008) Landscape Genetics. Bioscience 58:199-207.

Holzhauer S, Ekschmitt K, Sander A-C, Dauber J, Wolters V (2006) Effect of historic landscape change on the genetic structure of the bush-cricket *Metrioptera roeseli*. Landscape Ecology 21:891-899.

Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, O'Connor MI (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105-U129.

Huber C, Baumgarten M (2005) Early effects of forest regeneration with selective and small scale clear-cutting on ground beetles (Coleoptera, Carabidae) in a Norway spruce stand in Southern Bavaria (Höglwald). Biodiversity and Conservation 14:1989-2007.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. Ecology Letters 11:609-623.

Ingvarsson PK, Olsson K (1997) Hierarchical genetic structure and effective population sizes in *Phalacrus substriatus*. Heredity 79:153-161.

References

Jacquemyn H, Honnay O, Galbusera P, Roldán-Ruiz I (2004) Genetic structure of the forest herb *Primula elatior* in a changing landscape. Molecular Ecology 13:211-219.

Johnson MTJ, Lajeunesse MJ, Agrawal AA (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters 9:24-34.

Jopp F, Reuter H (2005) Dispersal of carabid beetles — emergence of distribution patterns. Ecological Modelling 186:389-405.

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Molecular Ecology 14:3219-3234.

Jost L (2008) G_{ST} and its relatives do not measure differentiation. Molecular Ecology 17:4015-4026.

Judas M, Dornieden K, Strothmann U (2002) Distribution patterns of carabid beetle species at the landscape-level. Journal of Biogeography 29:491-508.

Keller D, Holderegger R, van Strien MJ, Bolliger J (2015) How to make landscape genetics beneficial for conservation management? Conservation Genetics 16:503-512.

Keller I, Largiadèr CR (2003a) Five microsatellite DNA markers for the ground beetle *Abax parallelepipedus* (Coleoptera, Carabidae). Molecular Ecology Notes 3:113-114.

Keller I, Largiadèr CR (2003b) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Proceedings of the Royal Society B: Biological Sciences 270:417-423.

Keller I, Nentwig W, Largiadèr CR (2004) Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. Molecular Ecology 13:2983-2994.

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Canadian Journal of Zoology 85:1049-1064.

Kleijn D, van Langevelde F (2006) Interacting effects of landscape context and habitat quality on flower visiting insects in agricultural landscapes. Basic and Applied Ecology 7:201-214.

Koerner W, Dupouey JL, Dambrine E, Benoît M (1997) Influence of past land use on the vegetation and soils of present day forest in the Vosges mountains, France. Journal of Ecology 85:351-358.

Koivula MJ, Vermeulen HJW (2005) Highways and forest fragmentation - effects on carabid beetles (Coleoptera, Carabidae). Landscape Ecology 20:911-926.

Kotze DJ, Brandmayr P, Casale A, Dauffy-Richard E, Dekoninck W, Koivula MJ, Lövei GL, Mossakowski D, Noordijk J, Paarmann W, Pizzolotto R, Saska P, Schwerk A, Serrano J, Szyszko J, Taboada A, Turin H, Venn S, Vermeulen R, Zetto T (2011) Forty years of carabid beetle research in Europe - from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys:55-148.

Krück S, Joschko M, Schultz-Sternberg R, Kroschewski B, Tessmann J (2006) A classification scheme for earthworm populations (Lumbricidae) in cultivated agricultural soils in Brandenburg, Germany. Journal of Plant Nutrition and Soil Science 169:651-660.

Landergott U, Holderegger R, Kozlowski G, Schneller JJ (2001) Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. Heredity 87:344-355.

Landguth EL, Cushman SA, Schwartz MK, McKelvey KS, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. Molecular Ecology 19:4179-4191.

Lange M, Türke M, Pašalić E, Boch S, Hessenmöller D, Müller J, Prati D, Socher SA, Fischer M, Weisser WW, Gossner MM (2014) Effects of forest management on ground-dwelling beetles (Coleoptera; *Carabidae, Staphylinidae*) in Central Europe are mainly mediated by changes in forest structure. Forest Ecology and Management 329:166-176.

Lindroth CH (1985/86) The Carabidae (Coleoptera) of Fennoscandia and Denmark. Brill, Leiden.

Loreau M (1984) Population density and biomass of Carabidae (Coleoptera) in a forest community. Pedobiologia 27:269-278.

Loreau M (1987) Vertical distribution of activity of carabid beetles in a beech forest floor. Pedobiologia 30:173-178.

Loreau M (1994) Ground beetles in a changing environment: determinants of species diversity and community assembly. In:Boyle TJBB, Christopher E.B. (ed) Biodiversity, Temperate Ecosystems, and Global Change. Springer Berlin, Heidelberg. pp. 77-98.

References

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294:804-808.

Loreau M, Nolf CL (1993) Occupation of space by the carabid beetle *Abax ater*. Acta Oecologica 14:247-258.

Loreau M, Nolf CL (1994) Spatial structure and dynamics of a population of *Abax ater*. In:Desender K, Dufrêne M, Loreau M, Luff ML, Maelfait J-P (eds) Carabid Beetles: Ecology and Evolution. Kluwer Academic Publishers, Dordrecht, Boston, London. pp. 476.

Louy D, Habel JC, Schmitt T, Assmann T, Meyer M, Müller P (2007) Strongly diverging population genetic patterns of three skipper species: the role of habitat fragmentation and dispersal ability. Conservation Genetics 8:671-681.

Lövei GL, Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology 41:231-256.

MacArthur RH, Wilson EO (1967) The Theory of Island Biogeography. University Press, Princeton.

Mader HJ (1984) Animal habitat isolation by roads and agricultural fields. Biological Conservation 29:81-96.

Mader HJ, Schell C, Kornacker P (1990) Linear barriers to arthropod movements in the landscape. Biological Conservation 54:209-222.

Magura T, Tóthmérész B, Bordán Z (2000) Effects of nature management practice on carabid assemblages (Coleoptera: Carabidae) in a non-native plantation. Biological Conservation 93:95-102.

Magura T, Tóthmérész B, Elek Z (2003) Diversity and composition of carabids during a forestry cycle. Biodiversity and Conservation 12:73-85.

Magura T, Tóthmérész B, Molnár T (2001) Edge effect on carabid assemblages along forest-grass transects. Web Ecology 2:7-13.

Manel S, Holderegger R (2013) Ten years of landscape genetics. Trends in Ecology and Evolution 28:614-621.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18:189-197.

Marcus T, Boch S, Durka W, Fischer M, Gossner MM, Müller J, Schöning I, Weisser WW, Drees C, Assmann T (2015) Living in heterogeneous woodlands - are habitat continuity or quality drivers of genetic variability in a flightless ground beetle? PloS ONE 10.

Mauremooto JR, Wratten SD, Worner SP, Fry GLA (1995) Permeability of hedgerows to predatory carabid beetles. Agriculture, Ecosystems and Environment 52:141-148.

McCauley DE, Raveill J, Antonovics J (1995) Local founding events as determinants of genetic structure in a plant metapopulation. Heredity 75:630-636.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation-rule in conservation and management. Conservation Biology 10:1509-1518.

Mols PJM (1987) Hunger in relation to searching behaviour, predation and egg production of the carabid beetle *Pterostichus coerulescens* L.: results of simulation. Acta Phytopathologica Entomologica Hungarica 22:187-205.

Moracho E, Moreno G, Jordano P, Hampe A (2016) Unusually limited pollen dispersal and connectivity of Pedunculate oak (*Quercus robur*) refugial populations at the species' southern range margin. Molecular Ecology 25:3319-3331.

Müller-Motzfeld G (2006) Bd. 2: Adephaga I: Carabidae (Laufkäfer), 2 edn. Spektrum Verlag, Heidelberg/Berlin.

Muñoz PT, Torres FP, Megías AG (2015) Effects of roads on insects: a review. Biodiversity and Conservation 24:659-682.

Nei M (1972) Genetic distance between populations. The American Naturalist 106:283-293.

Niehues FJ, Hockmann P, Weber F (1996) Genetics and dynamics of a *Carabus auronitens* metapopulation in the Westphalian Lowlands. Annales Zoologici Fennici 33:85-96.

Niemelä J (1996) From systematics to conservation - carabidologists do it all. Annales Zoologici Fennici 33:1-4.

Niemelä J (2001) Carabid beetles (Coleoptera: Carabidae) and habitat fragmentation: a review. European Journal of Entomology 98:127-132.

Niemelä J, Kotze DJ (2009) Carabid beetle assemblages along urban to rural gradients: a review. Landscape and urban planning 92:65-71.

References

Nordström S, Rundgren S (1974) Environmental factors and lumbricid associations in southern Sweden. Pedobiologia 14:1-27.

Oleksa A, Chybicki IJ, Larsson MC, Svensson GP, Gawroński R (2015) Rural avenues as dispersal corridors for the vulnerable saproxylic beetle *Elater ferrugineus* in a fragmented agricultural landscape. Journal of Insect Conservation 19:567-580.

Otálora MG, Martínez I, Belinchón R, Widmer I, Aragón G, Escudero A, Scheidegger C (2011) Remnants fragments preserve genetic diversity of the old forest lichen *Lobaria pulmonaria* in a fragmented Mediterranean mountain forest. Biodiversity and Conservation 20:1239-1254.

Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fernandez-Manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL, Chini L, Cooper HD, Gilman EL, Guénette S, Hurtt GC, Huntington HP, Mace GM, Oberdorff T, Revenga C, Rodrigues P, Scholes RJ, Sumaila UR, Walpole M (2010) Scenarios for global biodiversity in the 21st century. Science 330:1496-1501.

Peterken GF (1993) Woodland Conservation and Management, 2nd edn. Chapman & Hall, London.

Petit S, Burel F (1993) Movement in *Abax ater* (Col., Carabidae): do forest species survive in hedgerow networks? Vie et Milieu 43:119-124.

Petit S, Burel F (1998a) Connectivity in fragmented populations: *Abax parallelepipedus* in a hedgerow network landscape. Comptes Rendus de l'Academie des Sciences - Série III - Sciences de la vie - Life sciences 321:55-61.

Petit S, Burel F (1998b) Effects of landscape dynamics on the metapopulation of a ground beetle (Coleoptera, Carabide) in a hedgerow network. Agriculture, Ecosystems and Environment 69:243-252.

Phillipson J, Abel R, Steel J, Woodell S (1976) Earthworms and the factors governing their distribution in an English beechwood. Pedobiologia 16:258-285.

Queller DC, Strassmann J E, Hughes CR (1993) Microsatellite and kinship. Trends in Ecology and Evolution 8:285-288.

Rackham O (2003) Ancient Woodland: its history, vegetation and uses in England, 2nd edn. Castlepoint Press, Colvend.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.

Reichel K, Richter F, Eichel L, Kącki Z, Wesche K, Welk E, Neinhuis C, Ritz CM (2016) Genetic diversity in the locally declining *Laserpitium prutenicum* L. and the more common *Selinum carvifolia* (L.) L.: a "silent goodbye"? Conservation Genetics 17:847-860.

Reisch C, Mayer F, Rüther C, Nelle O (2007) Forest history affects genetic diversity - molecular variation of *Dryopteris dilatata* (Dryopteridaceae) in ancient and recent forests. Nordic Journal of Botany 25:366-371.

Richardson JL (2012) Divergent landscape effects on population connectivity in two co-occurring amphibian species. Molecular Ecology 21:4437-4451.

Richardson JL, Brady SP, Wang IJ, Spear SF (2016) Navigating the pitfalls and promise of landscape genetics. Molecular Ecology 25:849-863.

Richmond J, Reid D, Ashton K, Zamudio K (2009) Delayed genetic effects of habitat fragmentation on the ecologically specialized Florida sand skink (*Plestiodon reynoldsi*). Conservation Genetics 10:1281-1297.

Rutkowski R, Szczuka A, Zalewski M, Korczyńska J, Gryziak G (2011) Failure of microsatellite's cross-species amplification in common ground beetle *Pterostichus melanarius* (Illiger). Baltic Journal of Coleopterology 11:17-24.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287:1770-1774.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5:18-32.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365-371.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234.

Scribner KT, Arntzen JW, Cruddace N, Oldham RS, Burke T (2001) Environmental correlates of toad abundance and population genetic diversity. Biological Conservation 98:201-210.

References

Segelbacher G, Cushman SA, Epperson BK, Fortin M-J, Francois O, Hardy OJ, Holderegger R, Taberlet P, Waits LP, Manel S (2010) Applications of landscape genetics in conservation biology: concepts and challenges. Conservation Genetics 11:375-385.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615-629.

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. Theoretical Population Biology 12:253-262.

Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. Landscape Ecology 21:821-836.

Soulé M (1976) Allozyme variation: its determinants in space and time. In:Ayala FJ (ed) Molecular Evolution. Sinauer, Sunderland, Massachusetts. pp. 60-77.

Spieth P (1974) Gene flow and genetic differentiation. Genetics 78:961-965.

Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, Dezzani R, Delmelle E, Vierling L, Waits LP (2007) Putting the "landscape" in landscape genetics. Heredity 98:128-142.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? Molecular Ecology 19:3496-3514.

Tensen L, Groom RJ, van Belkom J, Davies-Mostert HT, Marnewick K, van Vuuren BJ (2016) Genetic diversity and spatial genetic structure of African wild dogs (*Lycaon pictus*) in the Greater Limpopo transfrontier conservation area. Conservation Genetics 17:785-794.

Thiele HU (1977) Carabid Beetles and Their Environment. A Study on Habitat Selection by Adaptions in Physiology and Behaviour. Springer, Berlin. p.^pp. 369.

Thomas JA, Bourn NAD, Clarke RT, Stewart KE, Simcox DJ, Pearman GS, Curtis R, Goodger B (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. Proceedings of the Royal Society B: Biological Sciences 268:1791-1796.

Tischendorf L, Irmler U, Hingst R (1998) A simulation experiment on the potential of hedgerows as movement corridors for forest carabids. Ecological Modelling 106:107-118.

Tremetsberger K, Stuessy TF, Samuel RM, Baeza CM, Fay MF (2003) Genetics of colonization in *Hypochaeris tenuifolia* (Asteraceae, Lactuceae) on Volcán Lonquimay, Chile. Molecular Ecology 12:2649-2659.

Väli U, Einarsson A, Waits L, Ellegren H (2008) To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? Molecular Ecology 17:3808-3817.

Vandepitte K, Jacquemyn H, Roldán-Ruiz I, Honnay O (2007) Landscape genetics of the self-compatible forest herb *Geum urbanum*: effects of habitat age, fragmentation and local environment. Molecular Ecology 16:4171-4179.

Vandergast AG, Lewallen EA, Deas J, Bohonak AJ, Weissman DB, Fisher RN (2009) Loss of genetic connectivity and diversity in urban microreserves in a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus* n. sp. "santa monica"). Journal of Insect Conservation 13:329-345.

Vellend M (2004) Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology 85:3043-3055.

von Oheimb G, Härdtle W, Naumann PS, Westphal C, Assmann T, Meyer H (2008) Long-term effects of historical heathland farming on soil properties of forest ecosystems. Forest Ecology and Management 255:1984-1993.

Vrijenhoek RC (1994) Genetic diversity and fitness in small populations. In:Loeschcke V, Jain SK, Tomiuk J (eds) Conservation Genetics. Birkhäuser Basel, Basel. pp. 37-53.

Wang JL (2004) Application of the one-migrant-per-generation rule to conservation and management. Conservation Biology 18:332-343.

Watts K, Vanhala T, Connolly T, Cottrell J (2016) Striking the right balance between site and landscape-scale conservation actions for a woodland insect within a highly fragmented landscape: a landscape genetics perspective. Biological Conservation 195:146-155.

Whiteley AR, McGarigal K, Schwartz MK (2014) Pronounced differences in genetic structure despite overall ecological similarity for two *Ambystoma* salamanders in the same landscape. Conservation Genetics 15:573-591.

Wiesner KR, Habel JC, Gossner MM, Loxdale HD, Köhler G, Schneider ARR, Tiedemann R, Weisser WW (2014) Effects of habitat structure and land-use intensity on the genetic structure of the grasshopper species *Chorthippus parallelus*. Royal Society open science 1:140133-140133.

References

Wood DA, Halstead BJ, Casazza ML, Hansen EC, Wylie GD, Vandergast AG (2015) Defining population structure and genetic signatures of decline in the giant gartersnake (*Thamnophis gigas*): implications for conserving threatened species within highly altered landscapes. Conservation Genetics 16:1025-1039.

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97-159.

Wright S (1951) The genetical structure of populations. Annual of eugenics 15:323-354.

Yokochi K, Kennington WJ, Bencini R (2016) An endangered arboreal specialist, the western ringtail possum (*Pseudocheirus occidentalis*), shows a greater genetic divergence across a narrow artificial waterway than a major road. PloS ONE 11.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11:413-418.

Full texts of papers

Paper 1: A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae)

* A higher resolution, pdf version of this paper is found on the disk attached to this thesis*

MICROSATELLITE LETTERS

A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae)

Tamar Marcus · Thorsten Assmann · Walter Durka · Claudia Drees

Abstract We report two sets of polymorphic, multi-plexed microsatellite markers for the ground beetle *Abax parallelepipedus*. As the species is flightless, restricted to forests and affected by habitat fragmentation it can serve as a model species for landscape and conservation genetics. A complete set of 20 loci can be amplified in five PCR reactions and sequenced in two rounds, and a subset of 14 loci can be analyzed together in one PCR run and one sequencing round. In a scan of 3,432 individuals from across Germany using the 14 loci subset, we found between three and 14 alleles per locus. After accounting for two loci that are apparently sex-linked, no significant deviations from Hardy–Weinberg equilibrium were found. None of the loci showed evidence for the presence of null alleles. No overall linkage disequilibrium was detected. Some of the loci can also be used to study other *Abax* species.

Keywords *Abax parallelepipedus* · Carabidae · Landscape genetics · Primers

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Introduction

Today's conservation practices mostly account for species diversity, although it is crucial to incorporate measures to conserve genetic diversity as well. In order to do this, we must understand the effects of current and historical landscape structure and land use on genetic parameters. *Abax parallelepipedus* has previously been used in such studies, as its biology and population dynamics are well known, it is strongly restricted to forests, and it has a low dispersal capability as it is flightless. Even recent fragmentation has been shown to have significant effects on the genetic composition of this species (Keller et al. 2004), and current distribution is influenced by habitat continuity (Assmann 1999). *A. parallelepipedus* has also been studied in the context of biological pest control (Kromp 1999). We report a set of 20 multiplexed microsatellite loci as well as a subset of 14 loci which can be amplified and sequenced in a single run. Some of these loci can also be used to study other *Abax* species.

Methods and results

We extracted DNA using the CTAB DNA extraction protocol from *A. parallelepipedus* individuals collected across Germany, and obtained 19,783 DNA sequences from a shot-gun sequencing run on a Roche 454 Genome Sequencer FLX Titanium done by GenoScreen (Lille, France). Primers were designed for 49 microsatellite loci using MSATCOMMANDER (Faircloth 2008). Primers were designed with a GTTT tag to prevent plus-A stutter bands and with either a M13R or a CAG tail. We additionally designed new primers for the five previously published microsatellite loci in *A. parallelepipedus* (Keller

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Table 1 Summary of primer sets. Details for 20 polymorphic microsatellite loci and two multiplex sets developed for A. *parallelepipedus*

Locus	Forward primer 3'–5'	Reverse primer 3'–5'	Repeat type	M13/CAG tag set (n = 24)			Size range	Number of alleles
				Side tagged/color-tag	PCR group	Sequencing group		
apar_2	GCCGCACGATATTAGCGAC	TTGGGAGTAAGTCTGTCCGG	AC	F/VIC-M13R	c	1	185–187	2
apar_4	CCACTGCACGTTCACTACAC	CAGTGAGTCGGGAGTGTC	AG	R/VIC-CAG	d	2	116–118	2
apar_5	CAACAACATTACCGGCGGAG	GCCGAGTCACTTGTTACGTG	AG	F/PET-M13R	e	2	171–175	2
apar_6	AAACATTCTGCGGTGACACC	CTGCTGCCCTCTTGTAAACG	AG	F/PET-M13R	b	1	305–307	2
apar_11	TTCGCCCTCAATCTCACCC	TCGTAGTGATGGCTGTGAGG	AC	F/PET-M13R	b	1	255–257	2
apar_12	GACCGTCGAGTGTAATGACG	CAATCTGCTCCTCAAGTTCAAG	AG	R/VIC-CAG	d	2	150–152	2
apar_14	GACATCTCGACTGCACCTAC	CCCTGTCTTTCCAACATCGC	AG	F/NED-M13R	d	2	138–142	3
apar_16	CGGTACTGTTCACTCTCTTGC	TAGGGTGGTCGGGAAATCAC	AC	F/NED-CAG	a	1	119–123	2
apar_20	ACACTCCACTCAAAGTTGCG	AAACGGTCAACTTTCCACCC	AC	F/NED-M13R	d	2	206–208	2
apar_23	GTGCCTATCGTTCTTTGTCAC	GTTTGCGATATTGTCTCTTGGCGG	AC	F/NED-M13R	d	2	183–185	2
apar_24	GTTTAGACGGTTCATTGCTGCATG	ACAGTTTGGCCTATCGTTACC	ACAT	R/FAM-M13R	a	1	187–191	2
apar_25	GTTTCGTAGCGAAACAGTGCCTTG	ATACTCCGGCCTACTTTGG	AC	R/VIC-M13R	c	1	221–225	3
apar_27	CCTCCTTACCAAGTAACGGG	GTTTGGAAGCGACAGTCAACGTG	AC	F/PET-M13R	e	2	266–270	2
apar_32	TTTACCAACACGCAGGC	GTTTGGACCCACAACACGTTAGCAC	AG	F/FAM-M13R	a	1	115–119	3
apar_34	GTTTGCCATACTAGGTGCTCTGG	ATCTCCGTGAAATCAACGC	AC	R/PET-M13R	b	1	117–125	3
apar_41	CTGATAACAACTGTGAGTGCTG	GTTTCAAACCACCCACCATCGATGG	AAG	F/PET-M13R	b	1	173–182	2
apar_44	GTTTCTTAATGTTCCATGCCGCG	TCTTCTTCGGCAAGCGTTAC	AG	R/PET-M13R	e	2	199–201	2
apar_46	CAGTTCAGTTCATCACGGGC	GTTTGGAACCCAACGCAGAAAAGTC	AAC	F/FAM-CAG	e	2	242–243	2
apar_50	GCTGGACTATTACAGAAGTCTTTTGC	ATGTGGAGGAAGCACGTGTT	CATA	R/NED-CAG	a	1	278–286	2
apar_52	CGGAGGACGTCTCTGCAAA	TCTGGCGTCGTTTGAATGGA	CA	R/FAM-CAG	e	2	182–192	3

1153

Table 1 continued

Locus	Directly labeled primer set (n = 3,432)							GenBank number
	Fluorescent label	Concentration in primer mix (µM)	Size range	Number of alleles	Mean H_O	Mean H_E	F_{IS}	
apar_2	PET	0.75	165–175	6	0.258	0.263	−0.35–1	KF048982
apar_4	–	–	–	–	–	–	–	KF048983
apar_5	FAM	0.5	149–159	6	0.407	0.415	−0.38–0.60	KF048984
apar_6	PET	0.8	286–290	3	0.17	0.186	−0.29–1	KF048985
apar_11	–	–	–	–	–	–	–	KF048986
apar_12	VIC	0.5	122–140	10	0.095	0.102	−0.15–0.66	KF048987
apar_14	NED	0.75	112–124	6	0.365	0.356	−0.59–0.56	KF048988
apar_16	–	–	–	–	–	–	–	KF048989
apar_20	FAM	0.75	180–190	5	0.346	0.344	−0.57–1	KF048990
apar_23	NED	0.5	161–167	4	0.025	0.026	−0.05–1	KF048991
apar_24	–	–	–	–	–	–	–	KF048992
apar_25	NED	0.75	203–209	4	0.427	0.439	−0.32–1	KF048993
apar_27	NED	0.5	244–250	4	0.437	0.441	−0.52–0.53	KF048994
apar_32	FAM	0.5	98–102	3	0.338	0.369	−0.42–0.66	KF048995
apar_34	PET	0.5	104–114	5	0.169	0.168	−0.21–0.66	KF048996
apar_41	–	–	–	–	–	–	–	KF048997
apar_44	VIC	0.5	180–190	6	0.125	0.227	−0.77–1	KF048998
apar_46	FAM	0.5	217–227	5	0.164*	0.344*	−0.11–1	KF048999
apar_50	FAM	0.5	250–294	14	0.153*	0.154*	−0.18–0.64	AJ510195
apar_52	–	–	–	–	–	–	–	AJ510196

In the CAG/M13R tag set 20 loci are amplified in five PCR runs and sequenced in two rounds. In the directly labeled primers set, a subset of 14 loci is amplified and sequenced in one multiplex run. Number of alleles is given for each of the sets as they were tested on different numbers of individuals. Values of observed (H_O) and expected (H_E) heterozygosity are given as the average for all 143 populations while F_{IS} values are given as the range for all of the populations. Values marked with asterisks indicate tests which were preformed only on the females due to apar_44 and apar_46 most probably being sex-linked

Table 2 Trans-species amplification of the directly labeled primer set (Table 1) in other *Abax* species

Species	Population	apar_2	apar_5	apar_6	apar_12	apar_14	apar_20	apar_23
A. carinatus	Boc (n = 23)							
	% working	100	96	100	96	100	100	100
	A	2	3	2	2	4	5	1
	HWE	1	0.0962	–	–	0.0005 (+)	0.0444 (+)	–
	H_O	0.13	0.727	0.043	0.045	0.957	0.652	0
	H_E	0.125	0.627	0.043	0.045	0.602	0.571	0
A. ovalis	Alb_15 (n = 24)							
	% working	96	100	100	96	100	100	100
	A	4	2	2	3	3	3	1
	HWE	0.4323	1	1	0.4194	0 (+)	0.0721	–
	Alb_49 (n = 24)							
	% working	100	100	100	100	100	100	100
	A	1	2	2	2	2	2	1
	HWE	–	0.5494	1	–	0 (+)	0.0144 (+)	–
	HEW_16 (n = 24)							
	% working	92	100	100	100	100	100	100
	A	4	2	2	2	3	4	1
	HWE	0.5195	–	1	1	0.045 (−)	0.0467 (−)	–
	HEW_18 (n = 24)							
	% working	96	100	100	100	100	92	100
	A	3	2	2	2	3	3	1
	HWE	1	1	–	1	0 (+)	0.1372	–
	Sneznik (n = 24)							
	% working	96	100	100	79	100	92	100
	A	4	2	2	1	4	6	2
	HWE	1	0.1241	–	–	0.1881	0.1307	–
	H_O	0.31	0.142	0.108	0.185	0.717	0.44	0.008
	H_E	0.298	0.142	0.103	0.193	0.617	0.457	0.008
A. parallelus	Boc (n = 23)							
	% working	91	100	100	91	100	70	100
	A	2	3	1	2	2	2	1
	HWE	1	1	–	–	0 (+)	0.0952	–
	HEW_16 (n = 24)							
	% working	100	100	100	83	100	100	96
	A	2	3	2	1	2	3	2
	HWE	1	0.0498 (−)	1	–	0 (+)	0.0058 (+)	–
	HEW_18 (n = 24)							
	% working	100	100	100	83	88	100	96
	A	2	3	1	2	2	2	3
	HWE	–	0.0036 (−)	–	–	0 (+)	0.0255 (+)	1
	H_O	0.126	0.268	0.069	0.033	0.984	0.729	0.043
	H_E	0.117	0.364	0.064	0.033	0.511	0.487	0.043

1155

Table 2 continued

Species	Population	apar_25	apar_27	apar_32	apar_34	apar_44	apar_46	apar_50
A. carinatus	Boc (n = 23)							
	% working	91	100	100	100	96	100	91
	A	3	2	2	2	3	2	3
	HWE	0.2229	0.0007 (+)	0.0016 (+)	–	–	0.1775*	1
	H_O	0.667	0.87	0.826	0.043	0.063*	0.235*	0.095
	H_E	0.501	0.51	0.496	0.043	0.063*	0.371*	0.094
A. ovalis	Alb_15 (n = 24)							
	% working	92	100	100	96	92	100	96
	A	4	2	2	4	4	2	2
	HWE	0.0736	0.0022 (+)	0.0068 (+)	0.661	1*	1*	–
	Alb_49 (n = 24)							
	% working	83	100	100	100	100	100	100
	A	3	2	2	2	3	2	1
	HWE	0.5571	0.0185 (+)	0.0002 (+)	1	1*	1*	–
	HEW_16 (n = 24)							
	% working	100	100	100	100	100	100	92
	A	4	3	2	4	3	2	2
	HWE	0.0162 (+)	0.0905	0.0143 (+)	0.515	0.0305 (+)*	1*	1
	HEW_18 (n = 24)							
	% working	92	100	100	88	100	100	100
	A	4	2	2	4	3	2	1
	HWE	0.0059 (+)	0.0066 (+)	0 (+)	0.743	1*	–	–
	Sneznik (n = 24)							
	% working	75	100	92	100	100	100	92
	A	4	2	3	2	3	2	1
	HWE	1	0.0022 (+)	0.0804	0.2007	–	–	–
	H_O	0.624	0.75	0.812	0.439	0.392*	0.246*	0.027
	H_E	0.559	0.484	0.504	0.413	0.329*	0.236*	0.026
A. parallelus	Boc (n = 23)							
	% working	100	100	100	100	96	100	100
	A	3	2	2	3	2	2	2
	HWE	0.4487	0 (+)	0 (+)	1	–	–	–
	HEW_16 (n = 24)							
	% working	96	100	100	100	100	100	100
	A	3	2	2	1	3	2	2
	HWE	0.0168 (−)	0.0008 (+)	0.0158 (+)	–	–	–	1
	HEW_18 (n = 24)							
	% working	83	96	96	100	100	96	100
	A	3	2	2	2	2	2	2
	HWE	0.1425	0.0001 (+)	0.0016 (+)	–	–	0.3056*	–
	H_O	0.469	0.929	0.859	0.043	0.02*	0.229*	0.056
	H_E	0.415	0.509	0.501	0.043	0.02*	0.257*	0.056

For each population we give the percentage of individuals for which a readable result was achieved, the number of alleles found (A), and the p value of the HWE test. Mean values of observed (H_O) and expected (H_E) heterozygosity are given for each species. Values marked with asterisks indicate tests which were preformed only on the females due to apar_44 and apar_46 most probably being sex-linked. Non-HWE populations with a heterozygote excess are marked with (+), populations with a heterozygote deficiency are marked (−)

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56

and Largiader 2003). All primer sets were checked to ensure that they are not replicating the same locus using Geneious v5.4. The 54 loci were tested for polymorphism using fluorescent-labeled M13R or CAG tags (Faircloth 2008). We identified a set of 20 polymorphic loci.

We report two multiplex sets (Table 1). The first contains all 20 loci amplified in five multiplex PCRs using CAG/M13R tagged primers and sequenced in two runs. The second contains a subset of 14 loci which are amplified and sequenced in one run using directly labeled primers.

For all amplifications using CAG/M13R tagged primers, amplification was done in 5 µL reactions containing 2.5 µL of 2× Multiplex PCR kit (Qiagen), 0.06 µM CAG/M13R tailed primer, 0.24 µM of the other primer, 0.25 µM of the fluorescent-labeled M13R (GGAAACAGCTATGACCAT) or CAG (CAGTCGGGCGTCATCA) primer, approximately 30 ng of genomic DNA (0.5 µL), and 1 µL water. We ran a touch-down PCR with the following conditions: 1×15 min at 95 °C, $20 \times [30$ s at 94 °C, 30 s at 60 °C (minus 0.5 °C per cycle), 90 s at 72 °C], $20 \times (30$ s at 94 °C, 30 s at 50 °C, 90 s at 72 °C), 1×10 min at 72 °C. PCR products were diluted 1:100 before sequencing. For amplifications using the directly labeled primers, forward and backward primers were combined in equal amounts into a primer working solution (Table 1). Amplification reaction contained 2.5 µL of 2× Multiplex PCR kit (Qiagen), approximately 30 ng of genomic DNA (0.5 µL), 0.5 µL primer mix, and 1.5 µL of water. The amplification conditions remained unchanged. PCR products were diluted 1:20 before sequencing. Fragment size was scanned using either an ABI 3130xl or an ABI 3730 Genetic analyzer (Applied Biosystems). Genotypes were scored automatically by GeneMapper 3.7 and checked manually. Hardy–Weinberg equilibrium (HWE) was tested using Genepop 4.2, linkage disequilibrium (LD) was checked using FSTAT 2.9.3.2, and suspected presence of null alleles was checked using Micro-Checker 2.2.3. We used pop100gene 1.1.03 to find mean observed heterozygosity (H_O) and expected heterozygosity (H_E) values, numbers of alleles per locus (A), and range of allele size per locus. F_{IS} values were calculated with FSTAT 2.9.3.2.

In a scan of 3,432 individuals from 147 populations across Germany using the subset of the 14 directly labeled primers, allele size ranged from 98 to 294 bp and between 3 and 14 alleles were detected across loci (mean: 2.17). Presence of null alleles was indicated in 12 out of 1,716 tested possibilities and deviations from HWE were detected in 72 out of the 1,507 tested combinations. Two loci, apar_44 and apar_46, apparently are sex-linked as testing only the female individuals greatly reduced the number of populations deviating from HWE. For locus apar_44, out of 81 testable populations the number that deviated from HWE was reduced from 46 to 5, and for apar_46, out of 131 testable populations the reduction was from 78 to 2. No significant linkage disequilibrium was found.

Trans-species amplification was tested using the directly labeled multiplex set in *Abax carinatus* (Duftschmid, 1812), *A. parallelus* (Duftschmid, 1812), and *A. ovalis* (Duftschmid, 1812) sampled in Germany and Slovenia (Table 2). The loci apar_2, apar_6, apar_12, apar_23, apar_46, and apar_50 all gave readable results in more than 90 % of the individuals, with no deviations from HWE for any of the tested populations. The primers can probably be used, not only with the three tested species, but with other *Abax* species as well.

References

Assmann T (1999) The ground beetle fauna of ancient and recent woodlands in the lowlands of north-west Germany (Coleoptera, Carabidae). Biodivers Conserv 8:1499–1517

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Keller I, Largiader CR (2003) Five microsatellite DNA markers for the ground beetle *Abax parallelepipedus* (Coleoptera, Carabidae). Mol Ecol Notes 3:113–114

Keller I, Nentwig W, Largiader CR (2004) Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. Mol Ecol 13:2983–2994

Kromp B (1999) Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agric Ecosyst Environ 74:187–228

Paper 2: Living in Heterogeneous Woodlands – Are Habitat Continuity or Quality Drivers of Genetic Variability in a Flightless Ground Beetle?

* Supplementary material and a higher resolution, pdf version of this paper are found on the disk attached to this thesis*

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Living in Heterogeneous Woodlands – Are Habitat Continuity or Quality Drivers of Genetic Variability in a Flightless Ground Beetle?

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Abstract

Although genetic diversity is one of the key components of biodiversity, its drivers are still not fully understood. While it is known that genetic diversity is affected both by environmental parameters as well as habitat history, these factors are not often tested together. Therefore, we analyzed 14 microsatellite loci in *Abax parallelepipedus*, a flightless, forest dwelling ground beetle, from 88 plots in two study regions in Germany. We modeled the effects of historical and environmental variables on allelic richness, and found for one of the regions, the Schorfheide-Chorin, a significant effect of the depth of the litter layer, which is a main component of habitat quality, and of the sampling effort, which serves as an inverse proxy for local population size. For the other region, the Schwäbische Alb, none of the potential drivers showed a significant effect on allelic richness. We conclude that the genetic diversity in our study species is being driven by current local population sizes via environmental variables and not by historical processes in the studied regions. This is also supported by lack of genetic differentiation between local populations sampled from ancient and from recent woodlands. We suggest that the potential effects of former fragmentation and recolonization processes have been mitigated by the large and stable local populations of *Abax parallelepipedus* in combination with the proximity of the ancient and recent woodlands in the studied landscapes.

59

Introduction

Today, one of the main goals of nature conservation is to maintain biodiversity. This is becoming ever more crucial given the current biodiversity crisis, which is being fuelled by the rapid changes in climate and in land use. Genetic diversity is an essential component of biodiversity, and while much work has been done on species diversity [1–3], fewer studies focus on the genetic level and its drivers. Genetic diversity is considered crucial for species' survival [4–6] as it serves as the basis for short-term and long-term processes of evolution and adaptation, allowing species to cope with changes such as those in climate and land use [7,8]. In addition, genetic diversity has been shown to enhance population fitness [9,10], to enhance resistance to parasites [11], and to stabilize population dynamics [4,5].

Previous studies have uncovered a complex network of factors that drive genetic structure, in the sense of genetic diversity together with genetic differentiation, including landscape parameters [12,13], population history and size [13,14], habitat history [15,16], and environmental drivers such as temperature [14]. These factors affect genetic structure either directly via selection, or more often indirectly via population sizes and gene flow, and can cause changes both in genetic diversity within groups of individuals, and in genetic differentiation between such groups. Variations in the landscape, in population size and structure, and in the environment can be thought of in essence as changes in habitat stability and in habitat suitability across time and space. More suitable habitats usually have larger populations which are less affected by genetic drift. Populations in more stable habitats have experienced less founder effects and bottlenecks, and have also had more time to accumulate alleles due to migration and possibly to mutation. In addition, large scale genetic patterns may exist as a result of long-term processes such as post-glacial recolonization [17].

A common study focus is the effect of habitat continuity and other historical factors on genetic structure, especially in the context of ancient and recent woodlands in Central and Western Europe or in North America (e.g. [13,16,18,19]). In these regions, ancient woodlands are defined as areas that have been wooded continuously since the earliest accurate, comprehensive maps of the area are available, in the case of Europe usually around 200–400 years ago. This is approximately the same time frame when peak fragmentation of the forests is thought to have occurred [20]. Although Central Europe is naturally covered by woodlands, most of the contemporary ones are consequences of afforestation or of natural succession that occurred on cleared or managed sites that were then subsequently abandoned. These changes can often be identified using chronological sequences of historical maps, and such woodlands are known as recent woodlands [21,22].

While studying ancient and recent woodlands sheds light on long-term historical processes, an additional way to look at habitat continuity in forests is to examine stand age, which reflects a short-term definition of site history. Stand age refers to the age of the current trees, regardless of which habitat was there previously. Studies of the effects of habitat continuity on genetic diversity tend to find higher diversity in the ancient woodlands than recent ones [16,23–25], and older stands tend to be more genetically diverse than younger stands [15,26]. In either case, the higher genetic diversity in the longer-term habitats is explained by the habitat stability and the greater resulting suitability for many woodland species.

Woodlands that were clear cut and then replanted immediately may still be considered ancient woodlands, although clear cutting can strongly alter biotic and abiotic properties of a woodland such as light availability, microclimate, and habitat structure (e.g. [27,28]). The litter and soil layers, which serve as the main habitat for many ground beetles and their prey, are also sharply affected by clear cuts. These changes can include amongst others, changes in layer thicknesses, chemical composition, and structure (e.g. [29–32]).

60

Although both environmental (e.g. [14,33,34]) and historical variables have been shown to affect genetic diversity (e.g. [16,18,35]), few studies examine their effects jointly. This is especially surprising as many environmental variables, especially those connected to soil, may have complex relationships with genetic diversity as they reflect the history of a site as well as affecting current population sizes [30,36]. Additionally, most studies have concentrated on rare species or on very fragmented habitats where, unsurprisingly due to the higher probability of stochastic effects, strong genetic effects have been found (e.g. [18,19,23,24]). Much less is known about the drivers of genetic diversity in more common species in woodland habitats that contain patches of varying ages and sizes, although this is a widespread landscape structure both in Europe and in parts of North America. Therefore, it is not known if habitat history is expected to shape the genetic structure of typical woodland species in these landscapes.

Abax parallelepipedus (Piller & Mitterpacher, 1783), a flightless, forest-dwelling ground beetle [37], is a widespread species in Central European woodlands and is an interesting test case in the context of the study of genetic diversity drivers (S2 Fig). On the one hand it is known that fragmentation can cause extremely rapid, significant changes in the genetic structure of this species [38] and it is both flightless as well as restricted to wooded habitats. On the other hand it can reach high, stable population densities of approximately 0.23 individuals/m^2 [39] during the main activity period even in small habitat patches [38,40], which is expected to stabilize genetic structure.

We examined the possible effects of both historical and current parameters on genetic diversity in *Abax parallelepipedus*. The study was carried out in two regions in Germany, in the Schorfheide-Chorin and in the Schwäbische Alb, which both have a mosaic of varied land uses and are fragmented but not overly so. Our study sites, part of the Biodiversity Exploratories research platform, represent a wide range of the environmental conditions and land uses present in each region [41]. We analyzed a set of 14 microsatellite loci in 24 individuals each from 88 plots that are located in a mosaic of ancient and recent woodlands with varying stand ages in both regions. We addressed the following main questions: (i) What are the drivers of genetic diversity in *Abax parallelepipedus*? (ii) Is there significant genetic differentiation between local populations found in ancient and recent woodlands? (iii) Does land use intensity affect genetic diversity due to expected changes in local population sizes?

Methods

Study area and plot selection

In the springs and summers of 2011–2012 we sampled *Abax parallelepipedus* from the Schwäbische Alb (southwestern Germany; n = 46) and the Schorfheide-Chorin (northeastern Germany; n = 42) in the 100 m x 100 m forest plots of the "Biodiversity Exploratories" (Fig 1A). The forest plots in each region represent the forest types commonly found in the regions, and include both unmanaged forests and age class forests. Age class forests result from clear cuts, usually small scale ones, or from shelterwood logging in which trees are removed in two rounds. In the first cut of shelterwood logging, most of the stand is cleared leaving some trees standing to shelter seedlings. These remaining trees are then cut in a second round after the young trees have created a canopy layer. Stands of European beech (*Fagus sylvatica*), pedunculated and sessile oak (*Quercus robur* and *Q. petraea*), and Scots pine (*Pinus sylvestris*) are found in the Schorfheide-Chorin, while the Schwäbische Alb is dominated by stands of European beech and Norway spruce (*Picea abies*). Some of the stands are monodominant while others are mixed stands.

The plots were selected in a two stage process ensuring that the plots represent the gradient of forestry management practices, their intensities, and soil characteristics for the most
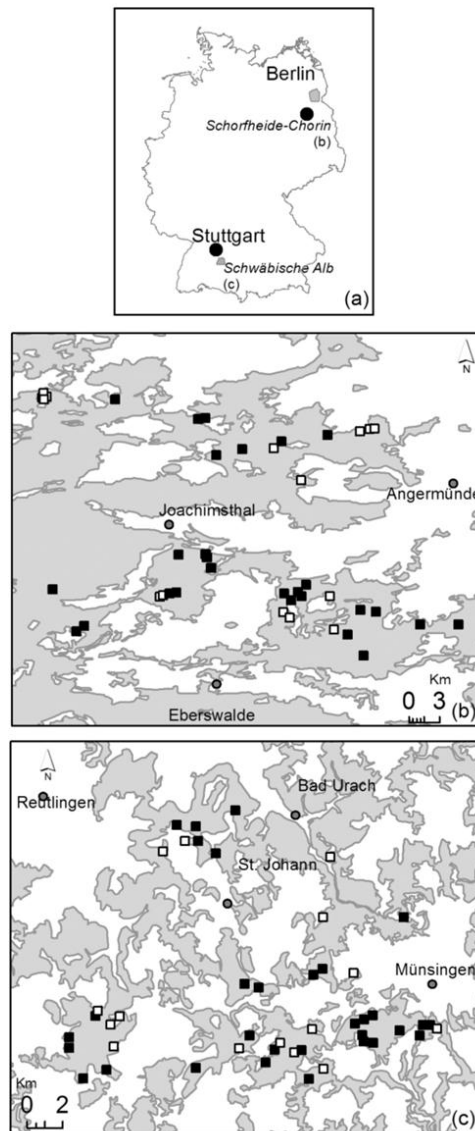
Fig 1. Map of research sites. (a) Location of study regions in Germany. Distribution of plots in woodlands (grey areas) in the Schorfheide-Chorin (b) and in the Schwäbische Alb (c). Woodlands defined as per the Corine Land Cover 2006 dataset [42]. Open boxes are plots located in recent woodlands, closed boxes are

located in ancient woodlands. Small dots indicate named towns and villages. Note that the scales of the maps are different. All maps were created using ArcGIS ver. 10.1 [43].

common soil types in the given region (see S2 Table and [41]). First, 500 potential plots that reflect the common forest types were selected for each region. Then 50 plots were selected in each region from the pools using stratified random sampling. The plots are randomly distributed within the regions (Fig 1B and 1C, for plot numbers see S1 Fig), are located at least 200 m from another plot and at least 100 m from the nearest forest edge. The large number of plots per region allow for a thorough representation of environmental parameters found in each region. For more details on plot selection, see Fischer et al. [41].

Study species

The flightless ground beetle *Abax parallelepipedus* is strictly limited to forests, and inhabits the litter layer [37,44,45]. The species is known to have large, stable populations [39,46,47] and is known to prefer ancient woodlands in some regions, such as the lowlands of northwestern Germany and Belgium [48,49]. The species is considered a forest generalist and can be found in large numbers in both conifer and broadleaf forests of varying ages, including conifer plantations [50–54]. Its dispersal power was found to be low, moving on average between 0.6 m and 2.3 m per night (reviewed in [55]).

Sample collection and microsatellite genotyping

We collected beetles by using ten live pitfall traps per plot baited with red wine on cellulose during the spring and summer of 2011 (Schwäbische Alb) and of 2012 (Schorfheide-Chorin). In all plots, the traps were placed in a straight line, 10 m apart along the plot border to ensure equal sampling area. We gathered all of the *Abax parallelepipedus* individuals we found in the traps approximately once a week and rebaited the traps until we had caught 33 individuals in the plot. We pooled the beetles trapped in all the traps of a plot each collection round and froze them at-80°C. Field work permits were issued by the responsible state environmental offices of Baden-Württemberg and of Brandenburg (according to §72 BbgNatSchG).

We extracted DNA using the CTAB extraction protocol [56] from three legs from each of 24 randomly selected beetles for each plot. We genotyped 14 polymorphic microsatellite loci using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For PCR, sequencing protocols, and information about the loci see [57].

Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using GENEPOP 4.2 [58] and no significant deviation was found (percentage of local populations not in HWE: Schwäbische Alb = 5.288%, Schorfheide-Chorin = 4.240%). Suspected presence of null alleles was tested using MICRO-CHECKER 2.2.3 [59] and no null alleles were found. Linkage disequilibrium (LD) was checked using FSTAT 2.9.3.2 [60,61]. No significant LD was found. Allelic richness, the rarefied numbers of alleles as a measure of genetic diversity, was calculated for each plot using FSTAT 2.9.3.2. The rarefaction was done per local population, with a minimum sample size of 20 individuals, to account for isolated instances of ineffective PCR reactions.

Overall F_{ST} values among local populations, a measure of genetic differentiation, were calculated for each region using Arlequin 3.5.1.3 [62]. Private alleles, meaning those found in only one local population, and unique alleles, meaning those found in a specific group of plots either by region, by ancient or recent woodlands, or by population density, were counted and tallied. The grouping by local population density was done by grouping plots into percentiles based on the number of individuals caught in the 2008 killing traps (see S1 Text). Genetic clustering was

tested using the algorithm developed by Pritchard [63] as implemented in STRUCTURE 2.3.4 for each region separately, to ensure that no underlying clustering is affecting the results. In this analysis we used the admixture model with no use of previous information about sampling location. Burnin length was 20,000 and there were 100,000 MCMC repeats after burnin. Number of clusters was run from K = 1 to K = the number of plots+1 for the Schorfheide-Chorin (K = 43), and for the Schwäbische Alb (K = 47). For the Schwäbische Alb for higher values of K, the runtime was insufficient to find proper solutions, and therefore a second run of K = 1 to K = 30 was analyzed (S3B Fig). We used CLUMPAK [64] and HARVESTER [65] to find the most likely K using the Evanno method [66] and to visualize the results.

Plot characterization

We characterized each plot in terms of variables related to the litter layer, which serves as the beetle's habitat, in terms of variables that can be related to the land use history of the plot, in terms of variables that can be related to local population size, and also in terms of variables related to soil, vegetation, climate, geography, and forest management. To characterize our plots in terms of general parameters known to affect ground beetles and therefore *Abax parallelepipedus* (for general overview see [67], with specific references listed for each variable), we used longitude [68], latitude [69], elevation [70,71], mean annual temperature [71,72], mean annual precipitation [71], forest management type [53], main tree species [53], number of vascular plant species [51,73], soil type [74], soil pH [75], and the Forest Management Intensity index (FORMI) ([53], defined in [76]). Depth of the litter layer and ground cover of litter, of deadwood, and of trees (see [77,78]) were included to characterize the habitat of the beetle and thus local population sizes. Land use history was characterized by defining each plot as an ancient or a recent woodland (see below), by stand age, and by the percentage of closed forest species. The depth of the O_e soil layer, as well as the C/N ratio of the O_i, the O_e, and the A soil layers and the carbon content of the A layer were also included as they are known to reflect historical land use [32,36]. As local population sizes could not be determined directly, we used three proxies as estimates. The first proxy is the percentage of forested landscape in the two kilometers surrounding each plot, the second is the sampling effort needed to collect 33 individuals in 2011 or 2012, and third is the number of *Abax parallelepipedus* trapped in killing traps in 2008 (details found in S1 Text).

Note that sampling effort is expected to be negatively correlated to local population density as the less dense a local population is, the longer it should take to collect 33 individuals. More details on all the variables and on their collecting methods can be found in S2 Table and in S1 Text.

Ancient woodlands are defined as areas that appear as covered by trees over the complete time series of existing sufficiently accurate maps [20,79]. For the Schwäbische Alb, plots were defined as either *ancient* or *recent* based on eight maps dating from 1820 and onwards (S1 Table) ($n_{ancient}$ = 31, n_{recent} = 15). For the Schorfheide-Chorin, plots were defined as either *ancient* or *recent* based on four maps dating from 1767 and onwards (S1 Table) ($n_{ancient}$ = 26, n_{recent} = 16). Any plot that appears as non-wooded on at least one map was defined as recent woodland. All others were defined as ancient woodlands. Stand ages were taken from the latest forestry inventory available [80]. In plots with trees of more than one age class, stand ages were defined as the age of the older age class (S2 Table).

Statistical analyses

We modeled the relationship between plot characteristics, including measures of habitat continuity as well as environmental parameters, and allelic richness. We started with 27 predictor

variables (S2 Table). We first tested for collinearity between the predictor variables and removed the smallest number of variables possible while eliminating all instances where Spearman's rho > |0.7| [81], leaving us with 18 predictors (S3 Table). When we could not choose which variable to eliminate based on maximizing the number of remaining variables, we chose to retain the one more correlated with allelic richness. We created a general linear model for each region using allelic richness as a response variable. We modeled the regions separately as the means and variances of allelic richness are different due to the environmental conditions and history of the regions. The models were reduced using a backwards step reduction process based on AICc scores. The models with the lowest AICc scores, and the smallest numbers of predictors in the case of $\Delta AICc < 2$ between two models, were selected (see [82]). The residuals were checked to ensure that they are normally distributed and the residuals were plotted against the fitted values to investigate homogeneity of variance. As stand age and the FORMI index are significantly correlated (Spearman Rank Correlation: rho = -0.700, p<0.001, S3 Table) and we were interested in testing both of these parameters, we ran these models twice, once using stand age and once using FORMI as a possible explanatory variable.

We tested for spatial autocorrelation using Moran's I both of the allelic richness values themselves for each region using the APE package [83] and of the residuals of the model using the ncf package [84] and corrected using Bonferroni's correction for multiple testing. We examined the effects of long-term habitat continuity on genetic differentiation using two methods. We first ran an AMOVA in Arlequin 3.5.1.3 [62] for each region separately, grouping the plots by whether they are located in an ancient or in a recent woodland. We then tested the effects of stand age, of location in ancient or in recent woodlands, and of the interaction between them on genetic differentiation using GESTE 2.0 [85]. This program uses hierarchical Bayesian methods to find population-specific F_{ST} values, which are then modeled with the historical variables we provided in a generalized linear model. GESTE was run using default parameters. If not otherwise stated, all statistical analyses were done using R 3.0.0 [86].

Results

We analyzed 2112 individuals from 88 local populations and found 71 alleles across the 14 loci. Numbers of alleles (A) per locus ranged from 3–13 alleles with a mean of 5.1 (Table 1). All loci were polymorphic. For local level population genetics statistics see S4 Table. Mean allelic richness across all local populations was 1.96 alleles per locus. The allelic richness was not spatially autocorrelated in either region (Moran's I: Schwäbische Alb p = 0.439, Schorfheide-Chorin p = 0.535). The sampling effort in the Schorfheide-Chorin in number of days needed to collect 33 individuals (range = 8–80 days, mean = 46±20 days) was higher than that in the Schwäbische Alb (range = 5–27 days, mean = 16±7 days) (Wilcoxon rank sum test: W = 200.5, p<0.001). No evidence of spatial genetic clustering was found for either region as two and three gene pools were identified for the Schorfheide-Chorin and for the Schwäbische Alb respectively, which however, were largely admixed within individuals and mixed across most of the populations (Schwäbische Alb: S3 Fig; Schorfheide-Chorin: S4 Fig; for distribution of individuals belonging to each cluster see S1 Fig; plot numbers in S3 and S4 Figs refer to map found in S1 Fig).

There were 24 (34%) alleles that occurred only in the Schwäbische Alb, and four (6%) only in the Schorfheide-Chorin (Table 1). When the local populations from ancient woodlands and the recent woodlands were pooled regardless of region, 12 alleles (17%) were found only in ancient woodlands, and eight alleles (11%) occurred exclusively in recent woodlands. We found 6 (8%) private alleles, meaning alleles found only in one local population, both for ancient woodlands and for recent woodlands. For all allele frequencies, see S4 Table. When

Genetic Variability of a Flightless Ground Beetle

Table 1. Distribution of alleles in recent and ancient woodlands of two regions in Germany. In total 72 alleles were found.

Region	Group	Total number of alleles* (range per plot)	Unique alleles**	Private alleles***
Schwäbische Alb	All	68 (30–38)	24	9
	Ancient woodlands (n = 31)	62 (30–37)	9	5
	Recent woodlands (n = 15)	56 (30–38)	5	4
Schorfheide-Chorin	All	47 (18–30)	4	3
	Ancient woodlands (n = 27)	40 (18–30)	1	1
	Recent woodlands (n = 15)	40 (19–29)	3	2

*not rarefied
**found in one region only
***found only in a single plot.

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plots were grouped based on the number of *Abax parallelepipedus* individuals found in the 2008 killing traps, the highest number of private alleles was found in the plots with the lowest local population density (for details by region see Table 2).

In order to understand the drivers of genetic diversity, we modeled the effects of habitat continuity and stand age together with an additional 16 habitat, soil, vegetation, and local population variables on allelic richness (S2 Table). For the Schwäbische Alb, the only variable to remain in the model was the percentage of the two kilometers surrounding each plot that is forested which had a negative effect (Table 3), though the effect on allelic richness was borderline significant. In the Schorfheide-Chorin region, genetic diversity was positively affected by the depth of the litter layer ($p = 0.018$) and by the sampling effort ($p = 0.009$) (Table 3). We found no effects of land use history in any of our models, neither of stand age nor of habitat continuity. As stand age fell out of the models in the initial reduction step, replacing stand age with land use intensity (FORMI index) gave the same results as shown in Table 3. The residuals of the models showed no spatial autocorrelation (S5 Fig).

The level of overall differentiation in the study species was low, but was lower by an order of magnitude in the Schwäbische Alb (Schwäbische Alb: $F_{ST} = 0.005$, $p = 0.002$; Schorfheide-Chorin: $F_{ST} = 0.047$, $p < 0.001$). The AMOVA grouping local populations collected from ancient or from recent woodland showed no significant differentiation for either region (Table 4), although local populations within groups were significantly differentiated. Modelling the relationship between historical variables and population-specific F_{ST} values also did not find any significant effects, as for both regions the model with the highest posterior probability was that which contained only a constant.

Discussion

We found no relationship between either long-term or short-term habitat continuity and genetic diversity in the 88 local populations of *Abax parallelepipedus* from the Schwäbische Alb

Table 2. Number of private alleles in classes of local population density.

Region	0-20th percentile	21st-40th percentile	41st-60th percentile	61st-80th percentile	81st-100th percentile
Schwäbische Alb	7	2	1	0	2
Schorfheide-Chorin	2	1	3	2	1
Both regions	5	1	3	1	2

Categories are based on number of *Abax parallelepipedus* individuals found previously using killing traps in 2008 (see S1 Text).

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Table 3. Variables that remained in the general linear model for each region.

Region	Variable or model information	Estimate±SE	t-statistic	p-value
Schwäbische Alb	Percentage of surrounding landscape (2 km radius) that is forested	-0.377±0.195	-1.938	0.059
	Initial/final AICc: -9.899/-59.299			
	Adjusted R^2 = 0.056			
	$F_{(1,44)}$ = 3.756, p = 0.059			
Schorfheide-Chorin	Depth of O_l layer	0.087±0.035	2.464	0.018
	Sampling effort	0.004±0.002	2.732	0.009
	Initial/final AICc: 59.258/-11.251			
	Adjusted R^2 = 0.253			
	$F_{(2,39)}$ = 7.949, p = 0.001			

Shown for each *variable* are the model estimates ± SE, t-values, and the p-value of the t-statistic. For each *model* initial and final AICc scores and adjusted R^2 values are presented. See S6 and S7 Figs for genetic diversity plotted against each of the remaining variables as well as against the proxies of local population size for each of the two regions.

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and from the Schorfheide-Chorin. For the Schwäbische Alb we did not find a significant effect of any of the 18 variables we tested on genetic diversity. For the Schorfheide-Chorin however, we found a significant effect of both the depth of the litter layer and required sampling effort, which serves as a proxy for local population density. We furthermore found no significant differentiation between local populations from ancient and recent woodlands in either region.

Landscape history

Based both on the lack of differentiation between the local populations from ancient woodlands sites as well as on the similar levels of genetic diversity of those from the ancient and the recent woodlands, we can conclude that genetic structure (in the sense of a combination of genetic diversity and genetic differentiation) of *Abax parallelepipedus* sampled in the studied regions does not reflect historical processes. This strongly suggests the maintenance of relatively stable, large populations during the peak of fragmentation, as well as lack of founder effects and bottlenecks during recolonization processes. It is also possible that drift effects which may have occurred, have been mitigated by high levels of subsequent gene flow and recolonization from several source sites [87].

We found a similar lack of effect of stand age, which implies effective recolonization of woodlands after clear cuts, although clear cut areas prior to tree regrowth should not be a

Table 4. Results of AMOVA comparing the genetic differentiation between local populations found in ancient and recent woodlands for each region.

	Schwäbische Alb				Schorfheide-Chorin			
	Sum of Squares	Variance component (p-value)	Percentage variation	Range of degrees of freedom	Sum of Squares	Variance component (p-value)	Percentage variation	Range of degrees of freedom
Among groups	1.322	-0.001 (p = 0.874)	-0.062	1	2.502	<0.001 (p = 0.336)	0.009	1
Among local populations within groups	109.428	0.011 (p<0.001)	0.547	44	97.441	0.036 (p<0.001)	4.673	40
Within local populations	4167.022	1.979 (p = 0.001)	99.515	2098–2110	1420.627	0.741 (p<0.001)	95.318	1902–1920
Total	4277.826	1.989			1520.570	0.778		

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suitable habitat for our study species. *Abax parallelepipedus* is relatively immobile for a ground beetle of its size [88], nevertheless it still can move efficiently into newly planted woodlands from adjacent ones, especially as it is not restricted to a specific type of forest [40,50,89,90]. This mobility and forest generalist habitat requirements also explain the lack of effect of land use intensity. As forest management in Germany today generally consists of very small scale clear-cuts or of shelterwood forestry, the remaining, neighboring forests or the protective layer of trees can apparently sustain large enough populations of *A. parallelepipedus* to maintain genetic diversity.

Our results highlight, that although it is commonly accepted that ancient woodlands [20] and less fragmented landscapes are of greater conservation value due to their greater genetic diversity, this may not be true in all cases. Woodlands must be evaluated in the context of the overall structure of the surrounding landscape, and not on the basis of their site-based characteristics alone. In our study sites, the proximity of the recent woodlands to the ancient ones, combined with the relatively large fragment size of the ancient woodlands seems to have completely mitigated the effects of long-term and short-term changes in land use. Vandepitte et al. [13] found similar results in a study of the genetic structure of the herb *Geum urbanum* L., a species that also disperses fairly rapidly into new woodlands [91]. This study also took place in an area that contains ancient and recent woodlands in close proximity, so here too gene flow may be mitigating any historical effects at the genetic level.

Most other studies that found an effect of habitat continuity on genetic diversity, including the studies of *Carabus problematicus* Herbst and of *C. auronitens* Fabricius, sampled extremely fragmented woodlands (e.g. [23,24,25,92]). In these cases, the genetic traces of the fragmentation and recolonization processes would likely be stronger, as fewer individuals can migrate to the newly wooded patches leading greater, unmitigated founder effects and genetic erosion that persist due to low gene flow.

Population size

Another factor contributing to the stability of the genetic diversity and lack of genetic differentiation is the stability of the population sizes of *Abax parallelepipedus* [39,46]. As a result, the likelihood of populations undergoing changes in their genetic structure due to random effects caused by sudden drops in population size is lower (e.g. [93]). In a linear model we found a significant negative relationship between sampling effort and the log-transformed number of individuals collected in pitfall traps during 2008 (Fig 2; Schwäbische Alb: closed circles, solid line; Schorfheide-Chorin: open circles, dashed line; methods found in S1 Text). This not only justifies our use of sampling effort as a proxy for local population size, but also shows the stability of the local population sizes between the years as the pitfall trapping and our collecting were not carried out in the same years.

In the Schorfheide-Chorin we found significant, positive effects of the depth of the litter layer and of the sampling effort on genetic diversity. We interpret these results as representing effects of local population density on genetic diversity as sampling effort directly reflects local population density, while the depth of the litter layer reflects prey availability. Deeper litter layers contain more earthworms (Lumbricidae) [94–96] and thus can support larger local populations of *Abax parallelepipedus*, as they serve as a main food source for the beetles [67,97]. This highlights the importance of population size in preventing loss of alleles and preventing differentiation due to drift. We found a non-significant negative correlation between allelic richness and percentage of forest cover of the two kilometers surrounding each plot for *Abax parallelepipedus* in the Schwäbische Alb. The non-significance of this driver in the Schwäbische Alb is probably due to the extremely low variation in allelic richness found there.
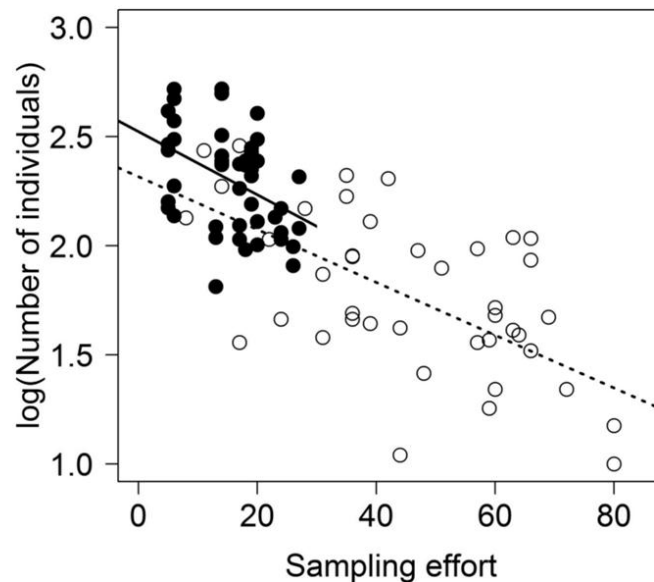
Fig 2. Comparison of sampling effort and number of individuals caught in previous pitfall trapping. Comparison of number of days required to catch 33 individuals of *Abax parallelepipedus* in our live traps and number of individuals caught in two pitfall traps per plot from April to October 2008 (S1 Text for details). Linear model shows a significant negative relationship in both regions (closed circles, solid line—Schwäbische Alb: estimate = -0.014±0.005, p = 0.003; open circles, dashed line—Schorfheide-Chorin: estimate = -0.012± 0.003, p<0.001).

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At first, the direction of one of our results, namely the positive effect of sampling effort in the Schorfheide-Chorin, seems to contradict general population genetics theory in that lower local population densities seem to lead to higher genetic diversity. This may be due to the longer period of time required to gather 33 individuals in plots with lower local population densities. The longer trapping times potentially enabled individuals from more distant interaction groups (sensu [98,99]), thus with additional alleles to be sampled. An interaction group is a micro-scale, sub-plot level population structure that describes a group of individuals that live in an area small enough to ensure that they are likely to meet for reproductive purposes. As most individuals will reproduce with a member of their interaction group, members of an interaction group tend to be more similar genetically to each other than to individuals from farther away. The longer our traps were open, the better our chances to catch individuals from more distant interaction groups that likely have a slightly different genetic makeup resulting in an overall higher genetic diversity value. This hypothesis is corroborated by the fact that the plots with the lowest local population densities have the highest number of private alleles.

The generally low level of allelic richness, as well as the higher variability in the Schorfheide-Chorin may be explained by the lower local population sizes and densities relative to the Schwäbische Alb. We attribute this to the higher soil acidity of the Schorfheide-Chorin, which is known to lead to smaller earthworm populations [100], which serve as a main food source

[67,97] for the beetle. This relationship between soil pH, prey, and population sizes of *Abax parallelepipedus* has been reported previously by Jukes et al. [69] and Magura et al. [75]. The sampling effort needed to trap 33 individuals in the Schorfheide-Chorin was higher than in the Schwäbische Alb, there were less individuals of *A. parallelepipedus*, and there was a significant correlation between sampling effort and pH in the Schorfheide-Chorin (linear model: estimate = -0.004±0.002, p = 0.045). These local populations with lower density are more susceptible to genetic drift and other stochastic processes, leading to the loss of alleles.

Conclusions

We found a weak genetic structure in *Abax parallelepipedus*, a common forest species in a moderately fragmented landscape that is mainly driven by current rather than historical parameters. Under such conditions, sufficiently large population sizes and gene flow have so far either prevented or mitigated genetic effects of historical and current fragmentation. Although we found no effect of long-term or short-term habitat continuity on genetic diversity or differentiation, we do not question the conservation value of ancient woodlands and of old stands. While these properties may not be important drivers of genetic diversity in our study species and regions, history is an important driver in more fragmented regions such as Flanders [24,92] and in rarer species such as the lichen *Lobaria pulmonaria* [25,101]. Ancient woodlands and old stands also have importance for conserving species diversity as well as species that are restricted to these habitats [48,102]. In addition, our results reflect the importance of micro-scale population structures such as interaction groups, and highlight the need to account for such structures while examining historical as well as current drivers of genetic population structure. Finally, we emphasize the importance of a landscape approach to conservation, and the importance of ensuring proximity of ancient and more recent woodlands in order to allow both species and individuals with different alleles to effectively colonize new sites.

Supporting Information

S1 Fig. Map of plot numbers and of clustered individuals. (a) Schorfheide-Chorin, (b) Schwäbische Alb. Individuals in the Schorfheide-Chorin were assigned to the cluster to which they had larger than a 50% chance of belonging as per STRUCTURE. The pie charts present the number of individuals sampled from each local population belonging to each of the clusters. Replacing the number of individuals belonging to each cluster with the likelihood of belonging to each cluster gives a similar pattern. Grey areas are forested (see legend of Fig 1). Squares indicate named towns and villages. Note that the scales of the maps are different. All maps were created using ArcGIS ver. 10.1 [43].
(TIF)

S2 Fig. Photograph of *Abax parallelepipedus*.
(TIF)

S3 Fig. Results of STRUCTURE analysis–Schwäbische Alb. (a) ΔK/K plot, (b) mean likelihood and variance for each K–for K = 1 to K = 47, (c) mean likelihood and variance for each K–for K = 1 to K = 30, (d) membership probability of individuals for K = 3.
(TIF)

S4 Fig. Results of STRUCTURE analysis–Schorfheide-Chorin. (a) ΔK/K plot, (b) mean likelihood and variance for each K–for K = 1 to K = 43, (c) membership probability of individuals for K = 2.
(TIF)

S5 Fig. Correlograms of model residuals. Empty circles indicate non-significant values. (TIF)

S6 Fig. Rarefied allelic richness and important variables–Schwäbische Alb. Relationship between rarefied allelic richness and proxies of population size and depth of the litter region for the Schwäbische Alb. Results are similar to those of the reported models (Spearman Rank Correlation; forested surrounding landscape: rho = -0.169, p = 0.261, sampling effort: rho = 0.088, p = 0.562, individuals from killing traps: rho = -0.286, p = 0.054, depth of litter layer: rho = 0.012, p = 0.935). (TIF)

S7 Fig. Rarefied allelic richness and important variables–Schorfheide-Chorin. Relationship between rarefied allelic richness and proxies of population size and depth of the litter region for the Schorfheide-Chorin. Results are similar to those of the reported models (Spearman Rank Correlation; forested surrounding landscape: rho = 0.048, p = 0.761, sampling effort: rho = 0.397, p = 0.009, individuals from killing traps: rho = -0.212, p = 0.177, depth of litter layer: rho = 0.421, p = 0.005). (TIF)

S1 Table. Maps used to define woodlands as recent or ancient. (PDF)

S2 Table. Full list of variables used to characterize plots. (PDF)

S3 Table. Collinearity matrix of continuous predictor variables for variable selection (Spearman's rho values). Values greater than |0.7| are marked in bold. (PDF)

S4 Table. Allele frequencies and related indices. (PDF)

S1 Text. Supplementary methods. (DOCX)

Genetic Variability of a Flightless Ground Beetle

Author Contributions

Conceived and designed the experiments: TM MF WWW CD TA. Performed the experiments: TM SB MMG JM IS. Analyzed the data: TM SB CD TA. Contributed reagents/materials/analysis tools: TM SB WD CD TA. Wrote the paper: TM CD TA.

References

1. Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, et al. (2000) Global biodiversity scenarios for the year 2100. Science 287: 1770–1774. PMID: 10710299

2. van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11: 296–310. PMID: 18047587

3. Gibson L, Lee TM, Koh LP, Brook BW, Gardner TA, Barlow J, et al. (2011) Primary forests are irreplaceable for sustaining tropical biodiversity. Nature 478: 378–381. doi: 10.1038/nature10425 PMID: 21918513

4. Agashe D (2009) The stabilizing effect of intraspecific genetic variation on population dynamics in novel and ancestral habitats. Am Nat 174: 255–267. doi: 10.1086/600085 PMID: 19519220

5. Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. Ecol Lett 11: 609–623. doi: 10.1111/j.1461-0248.2008.01179.x PMID: 18400018

6. Engelhardt KAM, Lloyd MW, Neel MC (2014) Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. Biol Conserv 179: 6–16.

7. Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. Philos Trans R Soc Lond B Biol Sci 353: 177–186. PMID: 9533122

8. Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc Natl Acad Sci USA 102: 2826–2831. PMID: 15710890

9. Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17: 230–237.

10. Altizer S, Harvell D, Friedle E (2003) Rapid evolutionary dynamics and disease threats to biodiversity. Trends Ecol Evol 18: 589–596.

11. Altermatt F, Ebert D (2008) Genetic diversity of *Daphina magna* populations enhances resistance to parasites. Ecol Lett 11: 918–928. doi: 10.1111/j.1461-0248.2008.01203.x PMID: 18479453

12. Gaublomme E, Maebe K, Van Doninck K, Dhuyvetter H, Li X, Desender K, et al. (2013) Loss of genetic diversity and increased genetic structuring in response to forest area reduction in a ground dwelling insect: a case study of the flightless carabid beetle *Carabus problematicus* (Coleoptera, Carabidae). Insect Conserv Divers 6: 473–482.

13. Vandepitte K, Jacquemyn H, Roldán-Ruiz I, Honnay O (2007) Landscape genetics of the self-compatible forest herb *Geum urbanum*: effects of habitat age, fragmentation and local environment. Mol Ecol 16: 4171–4179. PMID: 17784918

14. Cena CJ, Morgan GE, Malette MD, Heath DD (2006) Inbreeding, outbreeding and environmental effects on genetic diversity in 46 walleye (*Sander vitreus*) populations. Mol Ecol 15: 303–320. PMID: 16448402

15. Jacquemyn H, Honnay O, Galbusera P, Roldán-Ruiz I (2004) Genetic structure of the forest herb *Primula elatior* in a changing landscape. Mol Ecol 13: 211–219. PMID: 14653801

16. Reisch C, Mayer F, Rüther C, Nelle O (2007) Forest history affects genetic diversity—molecular variation of *Dryopteris dilatata* (Dryopteridaceae) in ancient and recent forests. Nord J Bot 25: 366–371.

17. Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405: 907–913. PMID: 10879524

18. Vellend M (2004) Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology 85: 3043–3055.

19. Wahbe TR, Ritland C, Bunnell FL, Ritland K (2005) Population genetic structure of tailed frogs (*Ascaphus truei*) in clearcut and old-growth stream habitats in south coastal British Columbia. Can J Zool 83: 1460–1468.

20. Peterken GF (1993) Woodland Conservation and Management. London: Chapman & Hall. 374 p.

21. Pott R, Hüppe J (1991) Die Hudelandschaften Nordwestdeutschlands. Münster: Westfälischen Museum für Naturkunde. 313 p.

22. Desender K (2005) Theory versus reality: a review on the ecological and population genetic effects of forest fragmentation on wild organisms, with an emphasis on ground beetles. In: Lövei GL, Toft S,

editors. Carabidology 2003: Proceedings of the 11th European Carabidologists' Meeting Slagelse, Denmark: Danish Institute of Agricultural Sciences. pp. 49–72.

23. Desender K, Verdyck P, Gaublomme E, Dhuyvetter H, Rasplus JY (2002) Extreme genetic differentiation and isolation by non-distance in *Carabus auronitens* in relation to forest historical ecology in Flanders (Belgium). In: Szyszko J, den Boer PJ, Bauer T, editors. How to protect or what we know about Carabid Beetles: X European Carabidologist Meeting. Warsaw: Warsaw Agricultural University Press. pp. 227–235.

24. Desender K, Gaublomme E, Dhuyvetter H, Verdyck P (2005) Interaction between regional forest history, ecology and conservation genetics of *Carabus problematicus* in Flanders (Belgium). In: Lövei GL, Toft S, editors. Carabidology 2003: Proceedings of the 11th European Carabidologists' Meeting Slagelse, Denmark: Danish Institute of Agricultural Sciences. pp. 73–87.

25. Otálora MG, Martínez I, Belinchón R, Widmer I, Aragón G, Escudero A, et al. (2011) Remnants fragments preserve genetic diversity of the old forest lichen *Lobaria pulmonaria* in a fragmented Mediterranean mountain forest. Biodivers Conserv 20: 1239–1254.

26. Wang Z, An S, Liu H, Feng J, Zhang F, Leng X (2006) Effect of stand age and management regime on genetic diversity of *Thuidium cymbifolium* in western China. Biol Conserv 129: 551–557.

27. Covington WW (1981) Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwoods. Ecology 62: 41–48.

28. Gálhidy L, Mihók B, Hagyó A, Rajkai K, Standovár T (2006) Effects of gap size and associated changes in light and soil moisture on the understorey vegetation of a Hungarian beech forest. Plant Ecol 183: 133–145.

29. Kohlpaintner M, Huber C, Weis W, Göttlein A (2009) Spatial and temporal variability of nitrate concentration in seepage water under a mature Norway spruce [*Picea abies* (L.) Karst] stand before and after clear cut. Plant Soil 314: 285–301.

30. Schmitz MF, Atauri JA, de Pablo CL, de Agar PM, Rescia AJ, Pineda FD (1998) Changes in land use in Northern Spain: effects of forestry management on soil conservation. For Ecol Manage 109: 137–150.

31. Flinn KM, Marks PL (2007) Agricultural legacies in forest environments: tree communities, soil properties, and light availability. Ecol Appl 17: 452–463. PMID: 17489252

32. Koerner W, Dupouey JL, Dambrine E, Benoît M (1997) Influence of past land use on the vegetation and soils of present day forest in the Vosges mountains, France. J Ecol 85: 351–358.

33. Scribner KT, Arntzen JW, Cruddace N, Oldham RS, Burke T (2001) Environmental correlates of toad abundance and population genetic diversity. Biol Conserv 98: 201–210.

34. Brouat C, Chevallier H, Meusnier S, Noblecourt T, Rasplus JY (2004) Specialization and habitat: spatial and environmental effects on abundance and genetic diversity of forest generalist and specialist *Carabus* species. Mol Ecol 13: 1815–1826. PMID: 15189205

35. Scheidegger C, Bilovitz PO, Werth S, Widmer I, Mayrhofer H (2012) Hitchhiking with forests: population genetics of the epiphytic lichen *Lobaria pulmonaria* in primeval and managed forests in southeastern Europe. Ecol Evol 2: 2223–2240. doi: 10.1002/ece3.341 PMID: 23139881

36. von Oheimb G, Härdtle W, Naumann PS, Westphal C, Assmann T, Meyer H (2008) Long-term effects of historical heathland farming on soil properties of forest ecosystems. For Ecol Manage 255: 1984–1993.

37. Lindroth CH (1985/86) The Carabidae (Coleoptera) of Fennoscandia and Denmark. Leiden: Brill. 225 p.

38. Keller I, Nentwig W, Largiadèr CR (2004) Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. Mol Ecol 13: 2983–2994. PMID: 15367114

39. Chaabane K, Loreau M, Josens G (1996) Individual and population energy budgets of *Abax ater* (Coleoptera, Carabidae). Ann Zool Fenn 33: 97–108.

40. Jopp F, Reuter H (2005) Dispersal of carabid beetles—emergence of distribution patterns. Ecol Modell 186: 389–405.

41. Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, et al. (2010) Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic Appl Ecol 11: 473–485.

42. European Environment Agency (2006) Corine Land Cover 2006 dataset. Available from http://www.eea.europa.eu/data-and-maps/data/clc-2006-vector-data-version-3.

43. ESRI (2012) ArcGIS Desktop: Release 10.1. Redlands, CA: Environmental Systems Research Institute.

73

44. Loreau M (1987) Vertical distribution of activity of carabid beetles in a beech forest floor. Pedobiologia 30: 173–178.

45. Huber C, Baumgarten M (2005) Early effects of forest regeneration with selective and small scale clear-cutting on ground beetles (Coleoptera, Carabidae) in a Norway spruce stand in Southern Bavaria (Höglwald). Biodivers Conserv 14: 1989–2007.

46. Günther J, Assmann T (2004) Fluctuations of carabid populations inhabiting an ancient woodland (Coleoptera, Carabidae). Pedobiologia 48: 159–164.

47. Judas M, Dornieden K, Strothmann U (2002) Distribution patterns of carabid beetle species at the landscape-level. J Biogeogr 29: 491–508.

48. Assmann T (1999) The ground beetle fauna of ancient and recent woodlands in the lowlands of north-west Germany (Coleoptera, Carabidae). Biodivers Conserv 8: 1499–1517.

49. Desender K, de Bakker D, Versteirt V, de Vos B (2002) A baseline study on forest ground beetle diversity and assemblages in Flanders (Belgium). In: Szyszko J, den Boer P, Bauer T, editors. How to protect or what we know about Carabid Beetles: X European Carabidologist Meeting. Warsaw: Warsaw Agricultural University Press. pp. 237–245.

50. Day KR, Marshall S, Heaney C (1993) Associations between forest type and invertebrates: ground beetle community patterns in a natural oakwood and juxtaposed conifer plantations. Forestry 66: 37–50.

51. Fahy O, Gormally M (1998) A comparison of plant and carabid beetle communities in an Irish oak woodland with a nearby conifer plantation and clearfelled site. For Ecol Manage 110: 263–273.

52. Butterfield J, Luff ML, Baines M, Eyre MD (1995) Carabid beetle communities as indicators of conservation potential in upland forests. For Ecol Manage 79: 63–77.

53. Magura T, Tóthmérész B, Bordán Z (2000) Effects of nature management practice on carabid assemblages (Coleoptera: Carabidae) in a non-native plantation. Biol Conserv 93: 95–102.

54. Lange M, Türke M, Pašalić E, Boch S, Hessenmöller D, Müller J, et al. (2014) Effects of forest management on ground-dwelling beetles (Coleoptera; Carabidae, Staphylinidae) in Central Europe are mainly mediated by changes in forest structure. For Ecol Manage 329: 166–176.

55. Brouwers NC, Newton AC (2009) Movement rates of woodland invertebrates: a systematic review of empirical evidence. Insect Conserv Divers 2: 10–22.

56. Doyle J (1991) DNA protocols for plants—CTAB total DNA isolation. In: Hewitt GM, Jonston AWB, Young JPW, editors. Molecular Techniques in Taxonomy. Berlin: Springer. pp. 283–285.

57. Marcus T, Assmann T, Durka W, Drees C (2013) A suite of multiplexed microsatellite loci for the ground beetle Abax parallelepipedus (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae). Conserv Genet Resour 5: 1151–1156.

58. Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8: 103–106. doi: 10.1111/j.1471-8286.2007.01931.x PMID: 21585727

59. van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535–538.

60. Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86: 485–486.

61. Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www2.unil.ch/popgen/softwares/fstat.htm.

62. Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564–567. doi: 10.1111/j.1755-0998.2010.02847.x PMID: 21565059

63. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959. PMID: 10835412

64. Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour. doi: 10.1111/1755-0998.12387

65. Dent E, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361.

66. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611–2620. PMID: 15969739

67. Thiele HU (1977) Carabid beetles and their environment. A study on habitat selection by adaptions in physiology and behaviour. Springer, Berlin. pp. 369.

74

68. Oxbrough A, Irwin S, Kelly TC, O'Halloran J (2010) Ground-dwelling invertebrates in reforested conifer plantations. For Ecol Manage 259: 2111–2121.

69. Jukes MR, Peace AJ, Ferris R (2001) Carabid beetle communities associated with coniferous plantations in Britain: the influence of site, ground vegetation and stand structure. For Ecol Manage 148: 271–286.

70. Martínez A, Iturrondobeitia JC, Goldarazena A (2009) Effects of some ecological variables on carabid communities in native and non native forests in the Ibaizabal basin (Basque Country: Spain). Annals of Forest Science 66.

71. Eyre MD, Rushton SP, Luff ML, Telfer MG (2005) Investigating the relationships between the distribution of British ground beetle species (Coleoptera, Carabidae) and temperature, precipitation and altitude. J Biogeogr 32: 973–983.

72. Gillingham PK, Palmer SCF, Huntley B, Kunin WE, Chipperfield JD, Thomas CD (2012) The relative importance of climate and habitat in determining the distributions of species at different spatial scales: a case study with ground beetles in Great Britain. Ecography 35: 831–838.

73. Buse A (1988) Habitat selection and grouping of beetles (Coleoptera). Ecography 11: 241–247.

74. Petit S, Usher MB (1998) Biodiversity in agricultural landscapes: the ground beetle communities of woody uncultivated habitats. Biodivers Conserv 7: 1549–1561.

75. Magura T, Elek Z, Tothmeresz B (2002) Impacts of non-native spruce reforestation on ground beetles. Eur J Soil Biol 38: 291–295.

76. Kahl T, Bauhus J (2014) An index of forest management intensity based on assessment of harvested tree volume, tree species composition and dead wood origin. Nat Conserv 7: 15–27.

77. Magura T, Tóthmérész B, Molnár T (2001) Edge effect on carabid assemblages along forest-grass transects. Web Ecol 2: 7–13.

78. Hawes C, Stewart AJA, Evans HF (2002) The impact of wood ants (*Formica rufa*) on the distribution and abundance of ground beetles (Coleoptera: Carabidae) in a Scots pine plantation. Oecologia 131: 612–619.

79. Rackham O (2003) Ancient Woodland: its history, vegetation and uses in England. Colvend: Castlepoint Press. 624 p.

80. Schall P, Ammer C (2013) How to quantify forest management intensity in Central European forests. Eur J For Res 132: 379–396.

81. Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, et al. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36: 27–46.

82. Scherber C, Eisenhauer N, Weisser WW, Schmid B, Voigt W, Fischer M, et al. (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468: 553–556. doi: 10.1038/nature09492 PMID: 20981010

83. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20: 289–290. PMID: 14734327

84. Bjornstad ON (2013) ncf: Spatial nonparametric covariance functions, available from http://cran.r-project.org. R package version 1.1–5.

85. Foll M, Gaggiotti O (2006) Identifying the environmental factors that determine the genetic structure of populations. Genetics 174: 875–891. PMID: 16951078

86. R Development Core Team (2009) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

87. Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. Theor Popul Biol 12: 253–262. PMID: 601717

88. Loreau M, Nolf CL (1993) Occupation of space by the carabid beetle *Abax ater*. Acta Oecol 14: 247–258.

89. Brandmayr P, Pizzolotto R, Colombetta G, Zetto T (2009) In situ extinction of carabid beetles and community changes in a protected suburban forest during the past century: the "Bosco Farneto" near Trieste (Italy). J Insect Conserv 13: 231–243.

90. Deuschle J, Glück E (2008) Similarity of ground beetle communities of extensively managed orchards depending on management regimes. Bull Insectology 61: 73–80.

91. Hermy M, Honnay O, Firbank L, Grashof-Bokdam C, Lawesson JE (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biol Conserv 91: 9–22.

92. Desender K, Gaublomme E, Verdyck P, Dhuyvetter H, De Vos B (2004) Ecology and population genetics of *Carabus problematicus* in Flanders, Belgium: is forest history important? In: Honnay O,

75

Bossuyt B, Verheyen K, Hermy M, editors. Forest Biodiversity: lessons from History for Conservation. Wallingford: CAB International. pp. 117–128.

93. Keller I, Largiadèr CR (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Philos Trans R Soc Lond B Biol Sci 270: 417–423.

94. Phillipson J, Abel R, Steel J, Woodell S (1976) Earthworms and the factors governing their distribution in an English beechwood. Pedobiologia 16: 258–285.

95. Cuendet G (1984) A comparative study of the earthworm population of four different woodland types in Wytham woods, Oxford. Pedobiologia 26: 421–439.

96. Nordström S, Rundgren S (1974) Environmental factors and lumbricid associations in southern Sweden. Pedobiologia 14: 1–27.

97. Loreau M (1984) Population density and biomass of Carabidae (Coleoptera) in a forest community. Pedobiologia 27: 269–278.

98. Franceschini G, Cannavacciuolo M, Burel F (1997) A geostatistical analysis of the spatial distribution of Abax parallelepipedus (Coleoptera, Carabidae) in a woodlot. Eur J Soil Biol 33: 117–122.

99. den Boer PJ (1981) On the survival of populations in a heterogeneous and variable environment. Oecologia 50: 39–53.

100. Krück S, Joschko M, Schultz-Sternberg R, Kroschewski B, Tessmann J (2006) A classification scheme for earthworm populations (Lumbricidae) in cultivated agricultural soils in Brandenburg, Germany. J Plant Nutr Soil Sci 169: 651–660.

101. Jüriado I, Liira J, Csencsics D, Widmer I, Adolf C, Kohv K, et al. (2011) Dispersal ecology of the endangered woodland lichen Lobaria pulmonaria in managed hemiboreal forest landscape. Biodivers Conserv 20: 1803–1819.

102. Hermy M, Verheyen K (2007) Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. Ecol Res 22: 361–371.

76

Paper 3: What you see isn't always what you get: genetic effects of fragmentation in central European rural landscapes

Marcus T, Assmann T, Drees C What you see isn't always what you get: genetic effects of fragmentation in central European rural landscapes.

* Supplementary material is found on the disk attached to this thesis*

Abstract

Context: Landscape genetics attempts to understand the drivers of genetic structure, such as geographic distance, movement barriers, and different land use types, in a given species. However, most studies focus on rare species or on hyper-fragmented habitats leading to a lack of knowledge as to how widespread species may be affected by changes such as fragmentation in landscape structure.

Objectives: We examine the drivers of genetic structure in a widespread, forest-dwelling and flightless ground beetle in rural landscapes typical for central Europe.

Methods: We used microsatellite markers to genotype individuals of our study species in three large study regions across Germany. The genetic differentiation patterns were related to landscape patterns, such as geographical distance, land use, and potential barriers. We modeled the expected development of F_{ST} values over the course of 250 years.

Results: In the Schwäbische Alb we found no significant isolation patterns, while in the Schorfheide-Chorin we found significant isolation by distance. In the Hainich-Dün we found a very weak isolation by distance pattern. The EASYPOP models of F_{ST} development over time support our findings of little differentiation after 250 years, given the large populations found in our study regions.

Conclusions: We did not find the expected genetic traces of the physical fragmentation due to roads, railways, and land use. Large effective population sizes probably prevent differentiation between the populations. We reiterate the need for careful interpretation of genetic structure while making conservation decisions as current levels of differentiation may not accurately reflect ongoing gene flow.

Introduction

Landscape modification and the oft resulting habitat fragmentation, in the sense of a process encompassing habitat loss and habitat degradation across a landscape, present major threats to biodiversity around the globe (Fahrig 2003, Foley et al. 2005, Haddad et al. 2015, Sala et al. 2000). The effects of fragmentation have long been discussed at the species and community levels, but it also has major effects at the genetic level of biodiversity (Fahrig 2003, Fischer and Lindenmayer 2007). Fragmentation often causes a reduction in migration and in effective population sizes, thereby reducing gene flow while simultaneously heightening the risk of genetic drift in the populations. The result is often a loss of genetic diversity and an increase in genetic differentiation possibly reducing population fitness (Reed and Frankham 2003, Young et al. 1996) and ability to adapt (Ellstrand and Elam 1993, Young et al. 1996).

Landscape genetics is a fast-growing field of study which aims to understand how geographical and environmental parameters affect genetic patterns across a landscape (see Holderegger and Wagner 2006, Holderegger and Wagner 2008, Manel et al. 2003, Storfer et al. 2007). One of the major contributions of the field is a deeper and more applied understanding of how fragmentation affects genetic structure at the landscape level (Storfer et al. 2010, van Strien et al. 2014). Fragmenting elements in a landscape can be classified either as patches or as linear structures. While patches of unsuitable or less-suitable habitat can usually be circumvented or traversed, either by individuals or by the population as a whole, gene flow is nevertheless inhibited. In studies this has traditionally been accounted for by using measures of effective distances in place of Euclidean distances such as least cost paths (Adriaensen et al. 2003), and more recently, by using models based on circuit theory (McRae 2006). Linear fragmenting elements, such as roads, railways, or rivers, cannot be circumvented and must instead be crossed. Such barriers are addressed by "isolation by barrier" models (e.g. Cushman et al. 2006).

Central European woodlands have been subject to land use modification and fragmentation for hundreds of years (Vos and Meekes 1999). Today, woodlands are mostly found embedded in a complex matrix which includes, among others, agricultural lands, meadows and pastures, villages, small to mid-sized towns, and natural and man-

made waterbodies. Depending on species and on landscape feature, these other types of land cover hamper to completely prevent the movement of woodland species. This forces individuals to either migrate via less-suitable habitats or to take more circuitous paths between forest patches lessening migration and gene flow across the landscape. Two additional major, anthropogenic features in modern European landscapes are train tracks and roadways, which for many woodland species, especially smaller ones, constitute complete barriers to migration (reviewed in Forman and Alexander 1998, Mader et al. 1990, Trombulak and Frissell 2000).

Due to the immediacy of the conservation concerns, studies of genetic diversity and differentiation often focus on either rare or endangered species or on severely fragmented habitats (See: Holderegger and Di Giulio 2010, recent examples: Barr et al. 2015, Watts et al. 2016, Wood et al. 2015, Yokochi et al. 2016). They tend to examine vertebrate or plant species (reviewed in: Holderegger and Di Giulio 2010, Storfer et al. 2010), probably due to a combination of the general tendency of conservation efforts to concentrate on these groups together with ease of identification. These situations however, do not represent the more common, rural landscapes and deal with endangered instead of widespread species, so it is not clear what impact fragmentation may have in more typical circumstances. In order to fully understand the impacts of fragmentation and assess its conservation urgency, it is vital to study how the majority of landscapes and of species respond.

To address these gaps, we examined the landscape-level genetic patterns of a widespread, flightless, stenotopic forest-dwelling ground beetle. We studied *Abax parallelepipedus* (Piller & Mitterpacher, 1783) (Lindroth 1985/86) in rural landscapes across Germany to see if we could find patterns of genetic differentiation, and if so, could they be related to habitat fragmentation. The species is known to react rapidly at the genetic level to roads in severely fragmented landscapes (Keller et al. 2004), and genetic patterns in the studied regions are thought to be driven by current drivers rather than by historical land-use (Marcus et al. 2015).

It has been widely emphasized that studies which examine multiple landscapes thereby allowing generalization are sorely lacking (e.g. Keller et al. 2015, Richardson et

al. 2016, Segelbacher et al. 2010). Therefore we studied three landscapes in Germany which differ in terms of many environmental variables (Fischer et al. 2010). Our study regions are all rural landscapes fairly representative of those found in central Europe. These landscapes each consist of a mosaic of settlements and towns, agricultural lands, meadows and pastures, roads and railways, forests, and protected lands. As all three are quite different from each other in terms of soils, altitude, climate, and both current as well as historical land use (Fischer et al. 2010), they can be treated as three test cases.

We hypothesize that the genetic structure in each region would be driven by one or a combination of the following processes:

(a) Isolation by distance – The studied beetle species is flightless and moves on average between 0.6 m and 2.3 m per night (reviewed in Brouwers and Newton 2009), and our study regions are quite large (Fischer et al. 2010), which may create a classical isolation by distance pattern (Wright 1943).

(b) Isolation by resistance – Given that *A. parallelepipedus* individuals are unlikely to successfully leave the forest in the studied regions (Charrier et al. 1997, Huber and Baumgarten 2005, Petit and Burel 1998b), effects of land-use on genetic structure are to be expected. We will use the term "isolation by resistance", to refer to cost distances based both on low cost paths and on circuit theory as they both in essence examine the same thing, namely the effects of current land-use on mobility, gene flow, and thus genetic structure.

(c) Isolation by barrier – Roads are a common type of barrier known to cause genetic differentiation in a wide variety of species (reviewed in Holderegger and Di Giulio 2010), including ground beetles (Keller and Largiadèr 2003). Our study species is known to avoid crossing train lines and roads of any size (Koivula and Vermeulen 2005, Mader 1984, Mader et al. 1990) and it has already been shown that this can lead to significant genetic differentiation (Keller et al. 2004). Therefore, it is likely that the transportation networks in the study regions constitute absolute barriers for *A. parallelepipedus*. This may especially be true for the Schorfheide-Chorin study region which is transected by a major four to six lane highway.

We analyzed 3342 individuals of *A. parallelepipedus* from 142 plots in three regions, and related the found genetic differentiation patterns based on 14 polymorphic microsatellite loci to the three hypothesized drivers.

Methods

Study area and plot selection

In the springs and summers of 2011-2012 we sampled *Abax parallelepipedus* (Piller and Mitterpacher, 1783) from the Schorfheide-Chorin (northeastern Germany; n=45; ~1300 km^2), the Hainich-Dün (central Germany, n=47; ~1300 km^2), and the Schwäbische Alb (southwestern Germany; n=50; ~420 km^2) in the 100 m x 100 m forest plots of the "Biodiversity Exploratories" (Figure 1). The forest plots in each Exploratory represent the forest types commonly found in the region, both in terms of species and in terms of forest management. The studied stands in the Schorfheide-Chorin are dominated by stands of European beech (*Fagus sylvatica*), pedunculated and sessile oak (*Quercus robur* and *Q. petrea*), and Scots pine (*Pinus sylvestris*), while the Schwäbische Alb and the Hainich-Dün stands are dominated by stands of European beech and Norway spruce (*Picea abies*).
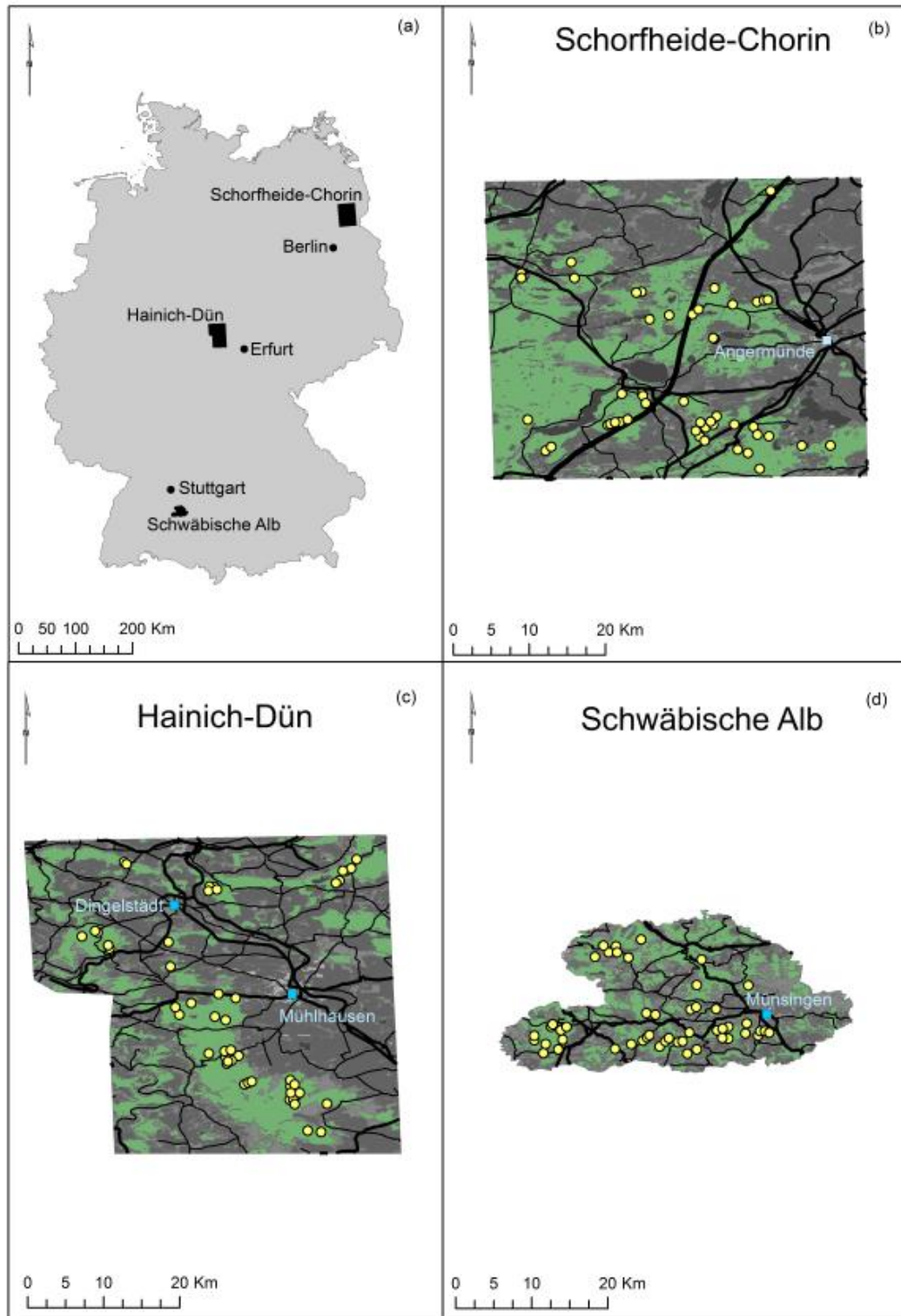
Figure 1: (a) Location of study regions in Germany. (b-d) Resistance maps of the study regions. Landscape elements with no resistance are marked in green. Resistant elements are marked in grey, the darker the grey, the higher the resistance. Black lines mark roads and railway lines, the thicker the line, the greater the barrier. Yellow circles mark plots, blue squares mark the named towns. Note that (b-d) are all drawn to the same scale.

The plots were selected in a two stage process ensuring that the plots represent the gradient of forestry management practices, forest management intensities, and soil characteristics for the most common soil types in the given region. First, 500 potential plots that reflect the common forest types were selected for each region. Then 50 plots were selected in each region from the pools using stratified random sampling. The plots are randomly distributed within the regions, and are located at least 200 m from another plot and at least 100 m from the nearest forest edge. For more details on plot selection see Fischer et al. (2010).

Study species

The flightless ground beetle *A. parallelepipedus* is strictly limited to forests in our study regions (Huber and Baumgarten 2005, Lindroth 1985/86, Loreau 1987). The species is known to have large, stable populations (Chaabane et al. 1996, Günther and Assmann 2004, Judas et al. 2002), and is considered to be a forest generalist as it can be found in large numbers in both conifer and broadleaf forests of varying ages, including conifer plantations (Day et al. 1993, Fahy and Gormally 1998, Lange et al. 2014, Magura et al. 2000). Its dispersal power was found to be low, moving on average between 0.6 and 2.3 meters per night (reviewed in Brouwers and Newton 2009).

Sample collection and microsatellite genotyping

We collected beetles by using ten live pitfall traps per plot which were baited with red wine on cellulose during the spring and summer of 2011 (Schwäbische Alb, Hainich-Dün) and of 2012 (Schorfheide-Chorin). In all plots the traps were placed 10 m apart in a straight line along the plot border to ensure equal sampling area. We gathered all of the *A. parallelepipedus* individuals we found in the traps approximately once a week, and rebaited the traps until we had caught 33 individuals in the plot. We pooled the beetles trapped in all the traps of a plot each collection round and froze them at -80°C.

We extracted DNA using the CTAB extraction protocol (Doyle 1991) from three legs from each of 24 randomly selected beetles for each plot. We genotyped 14 polymorphic microsatellite loci using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City,

CA, USA). For PCR and sequencing protocols and information about the loci properties see Marcus et al. (2013).

Standard genetic tests

Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using Genepop 4.2 (Rousset 2008) and no significant deviation was found (percentage of local populations not in HWE: Schwäbische Alb=5.3%, Hainich-Dün=4.3%, Schorfheide-Chorin=4.2%). Suspected presence of null alleles was tested using Micro-Checker 2.2.3 (van Oosterhout et al. 2004) and no null alleles were found. Linkage disequilibrium (LD) was checked using FSTAT 2.9.3.2 (Goudet 1995, 2001), and none was found. Overall F_{ST} values for each region were also calculated using FSTAT 2.9.3.2.

Genetic and landscape distances

Unless otherwise noted, all calculations and analyses were carried out using the software package R 3.0.0 (R Development Core Team 2009).

We calculated pairwise F_{ST} values (Nei 1973, Wright 1969) as well as Jost's D values (Jost 2008) for each pair of plots in each of the three regions using the PopGenReport package (Gruber and Adamack 2015) in R. These measures of genetic distance were compared to three different types of landscape distance matrices: (1) geographic distance as per Euclidean distance, (2) effective distance calculated either as a least cost path based on Dijkstra's algorithm (Dijkstra 1959) or as a commute distance (Chandra et al. 1997, Fouss et al. 2007), (3) barrier distance based on the cost of crossing railway lines and roads between each pair of plots. Geographic distances were calculated using the fossil package (Vavrek 2011) in R, while the both types of effective distances were calculated using the gdistance package (van Etten 2014).

To create the transition matrices (van Etten 2014) required to calculate the effective distances, we assigned resistance values to each of the land use types in the digital landscape model ("Digitales Landschaftsmodell" scale: 1:25000) maps in ArcGIS ver. 10.1 (ESRI 2012) (for assigned values see Table S1). The layers were clipped to include our study areas only, rasterized in ArcGIS at a resolution of 10 m x 10 m, and then

aggregated to a resolution of 100 m x 100 m in R using the raster package (Hijmans 2014). During the aggregation we ensured that hedgerows continued to be represented. Barrier distances were calculated as the least cost of crossing barriers between each pair of plots, with costs being assigned based on standard German road classification of roads into highways ("Autobahnen"), federal roads ("Bundesstraßen"), state roads ("Landstraßen"), and county roads ("Kreisstraßen"). The network of roads was based on the "Digitales Landschaftsmodell" maps, with visual verification using Google Earth (Google Earth ver. 7.1.2.2041 2013) (for assigned values see Table S2).

Statistical analyses

As there is still much debate as to which method is the best for comparing distance matrices (Segelbacher et al. 2010, Richardson et al. 2016), we used three methods in common use in landscape genetics analyses. We compared the assembled distance matrices using: (1) simple Mantel tests (Mantel 1967), (2) partial Mantel tests (Smouse et al. 1986), and (3) multiple regressions on distance matrices (MRDM) (Legendre et al. 1994, Lichstein 2007). These were all run using the ecodist package (Goslee and Urban 2007) in R. In all cases 10,000 permutations were run (Goslee and Urban 2007, Jackson and Somers 1989). In order to ensure that our results are not an artifact of the resistance values we assigned, we always tested a range of resistance values with the minimum resistance value always being 0.001, while the maximum ranged from 0.999 to 99 with the interim values scaled in between. In all cases, the two types of effective distances were tested separately.

The standard Mantel tests were run using Spearman's rank correlations. We then used ranked partial Mantel tests (Smouse et al. 1986) to test the correlation between genetic distances and each type landscape distance while controlling for the other types of landscape distances. This is especially relevant for this study, as we found correlations of greater than $|0.7|$ and $p<0.001$ using standard correlation tests between the two types of effective distances and between geographic distance and barrier distance in the Schwäbische Alb and in the Hainich-Dün (Table S3). As partial Mantel tests are susceptible to inflated Type I errors, we used a significance threshold of $p=0.001$ rather than the standard threshold of $p=0.05$ (Diniz-Filho et al. 2013, Oden and Sokal 1992).

Modelling of F_{ST} development

To better understand the effects of initial population size on development of F_{ST} values between fragmented populations of *A. parallelepipedus* over time, we modelled it using EASYPOP 2.0.1 (Balloux 2001). We modelled for six different starting population sizes, 600, 1000, 3000, 6000, 60,000, and 600,000 individuals, representing the range from approximately the smallest patch sizes in which genetic differentiation has been studied in our species (outskirts of Bern, Keller et al. (2004)) to somewhat larger than those expected in the largest forest patches in our study sites. The population sizes are based on the conservative estimate of 1000 individuals per hectare (Franceschini et al. 1997, Keller et al. 2004, Loreau and Nolf 1993).

In each case we had five populations and 10 repeats. As we assume the maximum deforestation in our regions was approximately 250 years ago and as *A. parallelepipedus* has a generation time of one year, we modelled the F_{ST} values for a period of 250 generations assuming a mutation rate of 0.0005 (Estoup and Angers 1998, Waples and Gaggiotti 2006) and no migration between the populations (See Appendix 1 for a full list of model settings). For sake of computability, we randomly selected 100 individuals from each of the five populations to calculate the overall F_{ST} values (Weir and Cockerham 1984) between them using FSTAT 2.9.3.2 (Goudet 1995, 2001) for each generation and each run.

Results

We analyzed 3342 individuals from 142 local populations of *Abax parallelepipedus* and found 81 alleles across the 14 loci. Numbers of alleles per locus ranged from 3-14 alleles with a mean of 5.788. All loci were polymorphic.

The level of overall differentiation in the study species was low, but was higher by an order of magnitude in the Schorfheide-Chorin than in the other study regions (Schwäbische Alb: F_{ST}=0.005, SE=0.001, 95% CI [0.002, 0.007]; Hainich-Dün: F_{ST}=0.003, SE=0.002, 95% CI [0.000, 0.005]; Schorfheide-Chorin: F_{ST}=0.055, SE=0.006, 95% CI [0.040, 0.062]). As F_{ST} and Jost's D values were in all three cases highly and significantly

correlated (Schwäbische Alb: rho=0.985, p<0.001; Hainich-Dün: rho=0.998, p<0.001; Schorfheide-Chorin: rho=0.982, p<0.001), we in all cases report the results of analyses run using F_{ST} values.

The standard Mantel tests revealed significant correlations between genetic distance and both geographic distance and barrier distance in the Hainich-Dün and in the Schorfheide-Chorin. None of the Mantel tests were significant in the Schwäbische Alb (Table 1). Using partial Mantel tests the only significant pattern found was one of isolation by distance in the Schorfheide-Chorin (Table 2). We also ran all of the tests without one plot which was geographically distant and isolated from the others to ensure it is not skewing the results, and saw no differences. In the MRDM tests we found a significant pattern of isolation by distance both for the Hainich-Dün and for the Schorfheide-Chorin. No significant isolation pattern was found for the Schwäbische Alb (Table 3). The different resistance values had no effect on the results in any of the cases, so we conclude our assigned resistance values are not biasing the results (Tables 1-3).

Table 1: Results of simple Mantel tests for all three study regions. Cost and commute refer to distances calculated using least cost path and commute distance functions respectively. Geographic refers to Euclidean distances, and barrier refers to barrier distances calculated based on the cost of crossing roads and railway lines. Significant results (p<0.05) are marked in bold.

maximum resistance cost	tested distance	Schwäbische Alb		Hainich-Dün		Schorfheide-Chorin	
		mantel r	p-value	mantel r	p-value	mantel r	p-value
1	cost	0.088	0.083	0.018	0.402	0.094	0.09
	commute	0.072	0.137	0.083	0.108	0.064	0.211
5	cost	0.088	0.075	0.018	0.388	0.09	0.108
	commute	0.071	0.14	0.082	0.114	0.063	0.213
10	cost	0.088	0.084	0.018	0.391	0.09	0.102
	commute	0.072	0.134	0.082	0.11	0.063	0.214
25	cost	0.088	0.08	0.018	0.395	0.09	0.103
	commute	0.072	0.13	0.082	0.108	0.063	0.223
50	cost	0.088	0.078	0.018	0.389	0.09	0.102
	commute	0.071	0.133	0.082	0.11	0.063	0.221
100	cost	0.088	0.078	0.018	0.407	0.09	0.103
	commute	0.071	0.134	0.081	0.113	0.063	0.211
--	geographic	0.032	0.21	**0.156**	**0.001**	**0.36**	**p<0.001**
--	barrier	0.027	0.219	**0.121**	**0.004**	**0.177**	**p<0.001**

Table 2: Results of the partial Mantel tests for all three study regions. Tested distances are marked in bold, while the others are the controlled for distances. Cost and commute refer to distances calculated using least cost path and commute distance functions respectively. Geographic refers to Euclidean distances, and barrier refers to barrier distances calculated based on the cost of crossing roads and railway lines. Significant results (p<0.05) are marked in bold.

maximum resistance cost		Schwäbische Alb		Hainich-Dün		Schorfheide-Chorin	
		mantel r	p-value	mantel r	p-value	mantel r	p-value
1	**cost**, barrier, geographic	0.09	0.072	0.003	0.478	0.019	0.399
	cost, barrier, **geographic**	0.023	0.291	0.111	0.019	**0.322**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.558	-0.053	0.848	-0.076	0.955
	commute, barrier, geographic	0.078	0.111	0.069	0.158	0.032	0.343
	commute, barrier, **geographic**	0.024	0.285	0.103	0.03	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.519	-0.046	0.806	-0.078	0.958
5	**cost**, barrier, geographic	0.09	0.072	0.003	0.483	0.021	0.388
	cost, barrier, **geographic**	0.023	0.289	0.111	0.022	**0.323**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.553	-0.053	0.841	-0.077	0.952
	commute, barrier, geographic	0.078	0.107	0.069	0.159	0.032	0.352
	commute, barrier, **geographic**	0.024	0.283	0.103	0.029	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.524	-0.047	0.815	-0.078	0.962
10	**cost**, barrier, geographic	0.09	0.069	0.003	0.474	0.021	0.382
	cost, barrier, **geographic**	0.023	0.289	0.111	0.019	**0.323**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.559	-0.052	0.842	-0.077	0.952
	commute, barrier, geographic	0.078	0.115	0.068	0.154	0.032	0.357
	commute, barrier, **geographic**	0.024	0.285	0.104	0.028	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.526	-0.047	0.816	-0.078	0.961
25	**cost**, barrier, geographic	0.09	0.071	0.003	0.485	0.021	0.376
	cost, barrier, **geographic**	0.023	0.294	0.111	0.02	**0.323**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.557	-0.053	0.847	-0.077	0.954
	commute, barrier, geographic	0.078	0.109	0.068	0.157	0.032	0.354
	commute, barrier, **geographic**	0.024	0.279	0.103	0.028	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.524	-0.047	0.814	-0.078	0.961
50	**cost**, barrier, geographic	0.09	0.074	0.003	0.48	0.021	0.384
	cost, barrier, **geographic**	0.023	0.284	0.111	0.022	**0.323**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.554	-0.053	0.847	-0.077	0.955
	commute, barrier, geographic	0.077	0.121	0.068	0.154	0.032	0.351
	commute, barrier, **geographic**	0.024	0.288	0.103	0.03	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.521	-0.047	0.82	-0.078	0.961
100	**cost**, barrier, geographic	0.09	0.075	0.003	0.48	0.021	0.377
	cost, barrier, **geographic**	0.023	0.295	0.111	0.022	**0.323**	**p<0.001**
	cost, **barrier**, geographic	-0.005	0.55	-0.053	0.846	-0.077	0.954
	commute, barrier, geographic	0.078	0.113	0.068	0.156	0.032	0.346
	commute, barrier, **geographic**	0.024	0.281	0.104	0.029	**0.325**	**p<0.001**
	commute, **barrier**, geographic	-0.002	0.519	-0.047	0.823	-0.078	0.963

Table 3. Results of the MRDM analyses for all three study regions. Cost and commute refer to distances calculated using least cost path and commute distance functions respectively. Geographic refers to Euclidean distances. Significant results (p<0.001) are marked in bold.

maximum cost	tested effective distance	**Schwäbische Alb** remaining distance	regression coefficient	p	r^2	p	F	p
1	cost	cost	0.088	0.15	0.008	0.15	9.08	0.15
	commute	commute	0.072	0.266	0.005	0.266	6.14	0.266
5	cost	cost	0.088	0.158	0.008	0.158	9.10	0.158
	commute	commute	0.071	0.266	0.005	0.266	6.02	0.266
10	cost	cost	0.088	0.159	0.008	0.159	9.08	0.159
	commute	commute	0.072	0.262	0.005	0.262	6.07	0.262
25	cost	cost	0.088	0.147	0.008	0.147	9.10	0.147
	commute	commute	0.072	0.277	0.005	0.277	6.05	0.277
50	cost	cost	0.088	0.154	0.008	0.154	9.10	0.154
	commute	commute	0.071	0.274	0.005	0.274	5.92	0.274
100	cost	cost	0.088	0.159	0.008	0.159	9.10	0.159
	commute	commute	0.071	0.267	0.005	0.267	6.02	0.267

maximum cost	tested effective distance	**Hainich-Dün** remaining distance	regression coefficient	p	r^2	p	F	p
1	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
5	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
10	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**p<0.001**	0.024	**p<0.001**	25.68	**p<0.001**
25	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**p<0.001**	0.024	**p<0.001**	25.68	**p<0.001**
50	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
100	cost	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**
	commute	geographic	0.156	**0.001**	0.024	**0.001**	25.68	**0.001**

maximum cost	tested effective distance	**Schorfheide-Chorin** remaining distance	regression coefficient	p	r^2	p	F	p
1	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.13	**p<0.001**	140.12	**p<0.001**
5	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
10	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
25	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
50	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
100	cost	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**
	commute	geographic	0.36	**p<0.001**	0.129	**p<0.001**	140.12	**p<0.001**

Modelling the development of F_{ST} values over time showed the expected effects of population size, with very small populations quickly developing large F_{ST} values, while large populations maintain low F_{ST} values even after 250 years (Figure 2). The F_{ST} values we found for our study regions correspond, as expected, to those found for fairly large populations.
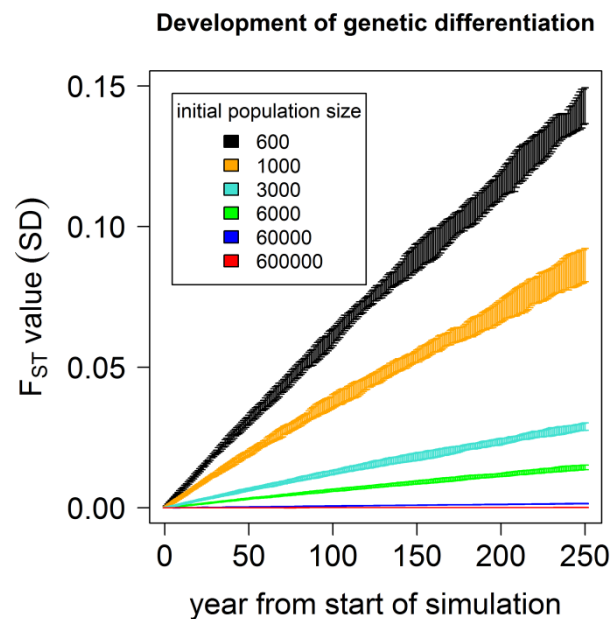

Figure 2: Results of EASYPOP models with standard deviations for each F_{ST} value

Discussion

Given the limited dispersal abilities of *Abax parallelepipedus*, its habitat specificity, and the size and complexity of the land use in the three studies regions, we expected to find significant traces of land use and landscape structure in the genetic differentiation patterns. Surprisingly, we only found isolation by distance patterns, and even that not in all of our regions. These results are surprising in light of the significant isolation by barrier pattern previously found with the same species in Switzerland (Keller et al. 2004).

Using three different methods, individual Mantel tests for each compared distance pair, partial Mantel tests, and MRDM we found slightly differing results, which are probably the result of the differing strengths and weaknesses of the tests themselves.

For the Schwäbische Alb we found no evidence of isolation by distance, resistance, or barrier with any of the statistical methods. We therefore conclude that the little differentiation which exists between the sampled plots is either not driven by any of the tested processes, or is developing at such a slow rate that it is not yet detectable. We also conclude that an isolation by distance pattern exists in the Schorfheide-Chorin, as confirmed by equivocal results in all three methods. The isolation by barrier patterns found by the simple Mantel tests only in the Hainich-Dün and in the Schorfheide-Chorin are probably artifacts as they were not found with any of the other, more reliable tests (Balkenhol et al. 2009, Cushman and Landguth 2010).

As to whether or not there is isolation by distance in the Hainich-Dün, we conclude that there probably is an extremely weak pattern, which was detected by the MRDM and the simple Mantel tests, but not by the partial Mantel tests at the significance threshold which we used. The p-values of the partial Mantel tests where the tested factor was geographic distance, ranged from 0.019-0.03 (table 4), falling between the "traditional" significance threshold of 0.05 and our threshold of 0.001. In any case, the pattern's significance is not of importance due to its small explanatory power (in all cases Mantel's $r^2 \approx 0.1$).

Table 4: Summary of all isolation patterns found for each of the three study regions

region	isolation pattern	Mantel test	partial Mantel test	MRDM
Schwäbische Alb	Isolation by distance	--	--	--
	Isolation by effective distance	--	--	--
	Isolation by barrier	--	--	--
Hainich-Dün	Isolation by distance	+	--	+
	Isolation by effective distance	--	--	--
	Isolation by barrier	+	--	--
Schorfheide-Chorin	Isolation by distance	+	+	+
	Isolation by effective distance	--	--	--
	Isolation by barrier	+	--	--

While it would be easy to claim that the surprising lack of isolation patterns and of genetic differentiation is the result of ongoing gene flow, it is probably limited across the studied landscapes. *Abax parallelepipedus* rarely crosses smaller state and county roads (Mader 1984, Mader et al. 1990), never mind the larger regional highways and federal roads (Koivula and Vermeulen 2005) that crisscross all three study regions. It also would

not be able to cross the urban areas, agricultural fields, pastures, and meadows commonly found in all three areas (Charrier et al. 1997, Huber and Baumgarten 2005, Petit and Burel 1998b). Therefore, our surprising results are rather a result of large effective population sizes which lead to changes in the genetic structure occurring at a rate so slow that it is currently unperceivable (Holderegger and Di Giulio 2010, Jackson and Fahrig 2016, Marsh et al. 2008, Richardson et al. 2016, Weckworth et al. 2013). Although there exists a rule of thumb stating that one migrant per generation is enough to mitigate genetic differentiation, this rule is often not applicable in natural landscapes where more individuals per generation are needed (Mills and Allendorf 1996, Wright 1931).

The plots we studied are located in relatively large forests, and the species can reach high, stable population densities of approximately 0.2 individuals/m^2 (Chaabane et al. 1996, Franceschini et al. 1997, Loreau 1994, Loreau and Nolf 1993). These would limit changes in the genetic structure even if no gene flow is taking place. This is supported by the results of the EASYPOP models, whereby even after 250 years of complete fragmentation, populations which are of sizes comparable to those found in our study regions have not yet developed strong patterns of differentiation. Population sizes typical to those in hyper-fragmented landscapes such as the one studied in Switzerland, however, quickly develop strong patterns of differentiation (Keller et al. 2004), explaining the contradictory results of two seemingly similar studies on the genetic structure of *A. parallelepipedus*. Stabilization of genetic structure by large population sizes would also explain why we found significant isolation by distance specifically in the Schorfheide-Chorin, as this region has the smallest population sizes of the three study areas (Marcus et al. 2015). In addition, a study of a grasshopper carried out in the grasslands of the Hainich-Dün also found a surprising lack of differentiation which was also attributed to large effective population sizes (Wiesner et al. 2014).

While at the moment the genetic effects of negligible gene flow on *A. parallelepipedus* in our study regions seem to be mitigated by large population sizes, differentiation is of course a long, ongoing process (see: Landguth et al. 2010, Petit and Burel 1998a). Although the traces of fragmentation cannot yet be seen at the genetic

level, they are most likely developing and growing with the passing generations in a process that has a considerable time lag until it can be detected.

Confounding of landscape fragmentation as perceived by the human eye and effective population size is a known pitfall in landscape genetics (e.g. Richardson et al. 2016). Most studies examine timescales in which mutation is not relevant and selection is excluded by design, so they in essence examine the balance between gene flow and differentiation caused by drift and other stochastic effects. In such a system, rapid development of genetic differentiation is usually contingent upon strong stochastic effects. Therefore if effective population sizes are not small, differentiation will develop slowly, even if there is complete lack of gene flow.

Since many studies concentrate on extremely fragmented landscapes or on rare and endangered species, they are testing scenarios with small effective populations, and therefore do indeed find significant differentiation in relatively short time spans. This is probably one of the causes which leads to the emphasis placed on the dangers of habitat fragmentation to the genetic structure of species and populations. However, in cases where the landscape is less fragmented or the studied species is not very rare, the effects of fragmentation may be difficult to detect even after relatively long time periods. As genetic structure is often used in conservation to identify lack of gene flow and to make practical decisions (Spear et al. 2010, Storfer et al. 2007, van Strien et al. 2014), it is critical to remember that lack of differentiation and structure is not always a clear sign of lack of gene flow.

Recent papers and reviews have highlighted the need to generalize the results generated since the advent of landscape genetics as a field of study (e.g. Jackson and Fahrig 2016, Richardson et al. 2016). We here highlight the need to return to basic theory while interpreting results as well as expanding study areas and species beyond model organisms, mammals, endangered or rare species, and highly fragmented landscapes in order to gain a fuller picture of how landscape structure and land use can affect genetic structure. In many of these cases, negligible gene flow may be effectively mitigated by large population sizes, and in a conservation context this has vast implications. Firstly, the current emphasis placed on the dangers of fragmentation to

genetic structure may be over-stated as genetic differentiation is a gradual process for many species. Secondly, our current methods of using genetic differentiation to detect fragmentation may lead to erroneous conclusions if results are not carefully interpreted, as existing fragmentation may not yet be detectable in the genetic structure. We therefore need to interpret results not only in terms of generation times and marker systems, but also in light of population sizes.

References

Adriaensen F, Chardon JP, De Blust G, Swinnen E, Villalba S, Gulinck H, Matthysen E (2003) The application of 'least-cost' modelling as a functional landscape model. Landsc Urban Plann 64(4):233-247

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. Ecography 32(5):818-830

Balloux F (2001) EASYPOP (Version 1.7): A computer program for population genetics simulations. J Hered 92(3):301-302

Barr KR, Kus BE, Preston KL, Howell S, Perkins E, Vandergast AG (2015) Habitat fragmentation in coastal southern California disrupts genetic connectivity in the cactus wren (*Campylorhynchus brunneicapillus*). Mol Ecol 24(10):2349-2363

Brouwers NC, Newton AC (2009) Movement rates of woodland invertebrates: a systematic review of empirical evidence. Insect Conserv Divers 2(1):10-22

Chaabane K, Loreau M, Josens G (1996) Individual and population energy budgets of *Abax ater* (Coleoptera, Carabidae). Ann Zool Fenn 33:97-108

Chandra AK, Raghavan P, Ruzzo WL, Smolensky R, Tiwari P (1997) The electrical resistance of a graph captures its commute and cover times. Comput Complex 6(4):312-340

Charrier S, Petit S, Burel F (1997) Movements of *Abax parallelepipedus* (Coleoptera, Carabidae) in woody habitats of a hedgerow network landscape: a radio-tracing study. Agric Ecosyst Environ 61:133-144

Cushman SA, Landguth EL (2010) Spurious correlations and inference in landscape genetics. Mol Ecol 19(17):3592-3602

Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. Am Nat 168(4):486-499

Day KR, Marshall S, Heaney C (1993) Associations between forest type and invertebrates: ground beetle community patterns in a natural oakwood and juxtaposed conifer plantations. Forestry 66(1):37-50

Dijkstra EW (1959) A note on two problems in connexion with graphs. Numer Math 1(1):269-271

Diniz-Filho JAF, Soares TN, Lima JS, Dobrovolski R, Landeiro VL, de Campos Telles MP, Rangel TF, Bini LM (2013) Mantel test in population genetics. Genet Mol Biol 36(4):475-485

Doyle J (1991) DNA protocols for plants - CTAB total DNA isolation. In: Hewitt GM, Jonston AWB, Young JPW (eds), Molecular Techniques in Taxonomy, NATO ASI Series. Springer, Berlin, pp. 283-285

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24(1):217-242

ESRI (2012) ArcGIS Desktop: Release 10.1. Environmental Systems Research Institute, Redlands, CA

Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: Carvalho GR (ed), Advances in Molecular Ecology. IOS Press, Amsterdam, pp. 55-86

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34:487-515

Fahy O, Gormally M (1998) A comparison of plant and carabid beetle communities in an Irish oak woodland with a nearby conifer plantation and clearfelled site. For Ecol Manag 110(1-3):263-273

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. Glob Ecol Biogeogr 16(3):265-280

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze ED, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic Appl Ecol 11(6):473-485

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK (2005) Global consequences of land use. Science 309(5734):570-574

Forman RTT, Alexander LE (1998) Roads and their major ecological effects. Annu Rev Ecol Syst 29:207-231

Fouss F, Pirotte A, Renders J-M, Saerens M (2007) Random-walk computation of similarities between nodes of a graph with application to collaborative recommendation. IEEE Trans Knowl Data Eng 19(3):355-369

Franceschini G, Cannavacciuolo M, Burel F (1997) A geostatistical analysis of the spatial distribution of *Abax parallelepipedus* (Coleoptera, Carabidae) in a woodlot. Eur J Soil Biol 33(3):117-122

Google Earth ver. 7.1.2.2041 (2013) available from https://www.google.com/earth/

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw 22(7):1-19

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86(6):485-486

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www2.unil.ch/popgen/softwares/fstat.htm

Gruber B, Adamack AT (2015) landgenreport: a new R function to simplify landscape genetic analysis using resistance surface layers. Mol Ecol Resour 15(5):1172-1178

Günther J, Assmann T (2004) Fluctuations of carabid populations inhabiting an ancient woodland (Coleoptera, Carabidae). Pedobiologia 48(2):159-164

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD, Lovejoy TE, Sexton JO, Austin MP, Collins CD (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. Sci Adv 1(2):e1500052

Hijmans RJ (2014) raster: Geographic data analysis and modeling, available from http://CRAN.R-project.org/package=raster

Holderegger R, Di Giulio M (2010) The genetic effects of roads: a review of empirical evidence. Basic Appl Ecol 11:522-531

Holderegger R, Wagner HH (2006) A brief guide to Landscape Genetics. Landsc Ecol 21:793-796

Holderegger R, Wagner HH (2008) Landscape Genetics. Bioscience 58(3):199-207

Huber C, Baumgarten M (2005) Early effects of forest regeneration with selective and small scale clear-cutting on ground beetles (Coleoptera, Carabidae) in a Norway spruce stand in Southern Bavaria (Höglwald). Biodivers Conserv 14(8):1989-2007

Jackson DA, Somers KM (1989) Are probability estimates from the permutation model of Mantel test stable. Can J Zool 67(3):766-769

Jackson ND, Fahrig L (2016) Habitat amount, not habitat configuration, best predicts population genetic structure in fragmented landscapes. Landsc Ecol 31(5):951-968

Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17(18):4015-4026

Judas M, Dornieden K, Strothmann U (2002) Distribution patterns of carabid beetle species at the landscape-level. J Biogeogr 29:491-508

Keller D, Holderegger R, van Strien MJ, Bolliger J (2015) How to make landscape genetics beneficial for conservation management? Conserv Genet 16(3):503-512

Keller I, Largiadèr CR (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Proc R Soc Biol Sci Ser B 270(1513):417-423

Keller I, Nentwig W, Largiadèr CR (2004) Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. Mol Ecol 13:2983-2994

Koivula MJ, Vermeulen HJW (2005) Highways and forest fragmentation - effects on carabid beetles (Coleoptera, Carabidae). Landsc Ecol 20(8):911-926

Landguth EL, Cushman SA, Schwartz MK, McKelvey KS, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. Mol Ecol 19(19):4179-4191

Lange M, Türke M, Pašalić E, Boch S, Hessenmöller D, Müller J, Prati D, Socher SA, Fischer M, Weisser WW, Gossner MM (2014) Effects of forest management on ground-dwelling beetles (Coleoptera; Carabidae, Staphylinidae) in Central Europe are mainly mediated by changes in forest structure. For Ecol Manage 329:166-176

Legendre P, Lapointe FJ, Casgrain P (1994) Modeling brain evolution from behavior - a permutational regression approach. Evolution 48(5):1487-1499

Lichstein JW (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecol 188(2):117-131

Lindroth CH (1985/86) The Carabidae (Coleoptera) of Fennoscandia and Denmark. Brill, Leiden

Loreau M (1987) Vertical distribution of activity of carabid beetles in a beech forest floor. Pedobiologia 30(3):173-178

Loreau M (1994) Ground beetles in a changing environment: determinants of species diversity and community assembly. In: Boyle TJBB, Christopher EB (ed), Biodiversity, Temperate Ecosystems, and Global Change. Springer Berlin, Heidelberg, pp. 77-98

Loreau M, Nolf CL (1993) Occupation of space by the carabid beetle *Abax ater*. Acta Oecol. 14(2):247-258

Mader HJ (1984) Animal habitat isolation by roads and agricultural fields. Biol Conserv 29(1):81-96

Mader HJ, Schell C, Kornacker P (1990) Linear barriers to arthropod movements in the landscape. Biol Conserv 54(3):209-222

Magura T, Tóthmérész B, Bordán Z (2000) Effects of nature management practice on carabid assemblages (Coleoptera: Carabidae) in a non-native plantation. Biol Conserv 93:95-102

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18(4):189-197

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27(2):209-20

Marcus T, Assmann T, Durka W, Drees C (2013) A suite of multiplexed microsatellite loci for the ground beetle *Abax parallelepipedus* (Piller and Mitterpacher, 1783) (Coleoptera, Carabidae). Conserv Genet Resour 5(4):1151-1156

Marcus T, Boch S, Durka W, Fischer M, Gossner MM, Müller J, Schöning I, Weisser WW, Drees C, Assmann T (2015) Living in heterogeneous woodlands - are habitat continuity or quality drivers of genetic variability in a flightless ground beetle. PLoS ONE 10(12): e0144217

Marsh DM, Page RB, Hanlon TJ, Corritone R, Little EC, Seifert DE, Cabe PR (2008) Effects of roads on patterns of genetic differentiation in red-backed salamanders, *Plethodon cinereus*. Conserv Genet 9(3):603-613

McRae BH (2006) Isolation by resistance. Evolution 60(8):1551-1561

Mills LS, Allendorf FW (1996) The one-migrant-per-generation-rule in conservation and management. Conserv Biol 10(6):1509-1518

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A 20:3321-3323

Oden NL, Sokal RR (1992) An investigation of 3-matrix permutation tests. J Classif 9(2):275-290

Petit S, Burel F (1998a) Connectivity in fragmented populations: *Abax parallelepipedus* in a hedgerow network landscape. C R Acad Sci Serie III Sci Vie 321:55-61

Petit S, Burel F (1998b) Effects of landscape dynamics on the metapopulation of a ground beetle (Coleoptera, Carabide) in a hedgerow network. Agric Ecosyst Environ 69(3):243-252

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17(1):230-237

Richardson JL, Brady SP, Wang IJ, Spear SF (2016) Navigating the pitfalls and promise of landscape genetics. Mol Ecol 25(4):849-863

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8(1):103-106

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770-1774

Segelbacher G, Cushman SA, Epperson BK, Fortin, M-J, Francois O, Hardy OJ, Holderegger R, Taberlet P, Waits LP, Manel S (2010) Applications of landscape genetics in conservation biology: concepts and challenges. Conserv Genet 11(2):375-385

Smouse PE, Long JC, Sokal RR (1986) Multiple-regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool 35(4):627-632

Spear SF, Balkenhol N, Fortin M-J, McRae BH, Scribner K (2010) Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. Mol Ecol 19(17):3576-3591

Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, Dezzani R, Delmelle E, Vierling L, Waits LP (2007) Putting the "landscape" in landscape genetics. Heredity 98(3):128-142

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now. Mol Ecol 19(17):3496-3514

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14(1):18-30

van Etten J (2014) gdistance: distances and routes on geographical grids, available from http://CRAN.R-project.org/package=gdistance

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4(3):535-538

van Strien MJ, Keller D, Holderegger R, Ghazoul J, Kienast F, Bolliger J (2014) Landscape genetics as a tool for conservation planning: predicting the effects of landscape change on gene flow. Ecol Appl 24(2):327-339

Vavrek MJ (2011) fossil: Palaeoecological and palaeogeographical analysis tools. Palaeontol Electron 14(1)

Vos W, Meekes H (1999) Trends in European cultural landscape development: perspectives for a sustainable future. Landsc Urban Plann 46(1-3):3-14

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15(6):1419-1439

Watts K, Vanhala T, Connolly T, Cottrell J (2016) Striking the right balance between site and landscape-scale conservation actions for a woodland insect within a highly fragmented landscape: a landscape genetics perspective. Biol Conserv 195:146-155

Weckworth BV, Musiani M, DeCesare NJ, McDevitt AD, Hebblewhite M, Mariani S (2013) Preferred habitat and effective population size drive landscape genetic patterns in an endangered species. Proc R Soc Biol Sci Ser B 280(1769)

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Wiesner KR, Habel JC, Gossner MM, Loxdale HD, Köhler G, Schneider ARR, Tiedermann R, Weisser WW (2014) Effects of habitat structure and land-use intensity on the genetic structure of the grasshopper species *Chorthippus parallelus*. R Soc Open Sci 1(2):140133-140133

Wood DA, Halstead BJ, Casazza ML, Hansen EC, Wylie GD, Vandergast AG (2015) Defining population structure and genetic signatures of decline in the giant gartersnake (*Thamnophis gigas*): implications for conserving threatened species within highly altered landscapes. Conserv Genet 16(5):1025-1039

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97-159

Wright S (1943) Isolation by distance. Genetics 31:39-59

Wright S (1969) Evolution and the genetics of populations Vol. 2: The theory of gene frequencies. University Press, Chicago

Yokochi K, Kennington WJ, Bencini R (2016) An endangered arboreal specialist, the western ringtail possum (*Pseudocheirus occidentalis*), shows a greater genetic divergence across a narrow artificial waterway than a major road. PLoS ONE 11(1)

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11(10):413-418

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