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Doctoral Dissertation

Spatial genetic variation and differentiation within metapopulation systems: drivers and trends to inform conservation of alpine grouse

Submitted by

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Affidavit

I hereby declare that I have authored this dissertation independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature are duly identified and cited, and the precise references included. Any contribution from colleagues is explicitly stated in the authorship statement of the published papers.

I further declare that this dissertation has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree. I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, 01.08.2022

Florian KUNZ (manu propria)

The natural world is fading. [...] If we act now, we can yet put it right.

David Attenborough on biodiversity | A Life on our Planet 2020

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Preface

This dissertation is based on several research projects with the generous support of the government of Styria, the Styrian hunting association, the FVA Wildlife Institute of the Forest Research Institute of Baden-Württemberg FVA and a lot of dedicated local landowners and hunters. Additional financial support was provided by various partners of the specific projects and the open access funding of the University of Natural Resources and Life Sciences, Vienna.

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List of publications

This doctoral thesis is submitted as a cumulative dissertation based on three selected articles published in peer-reviewed journals. The articles are included in full length below in section 6. A full list of SCI publications is included in appendix A, a selected list of transfer of knowledge is included in appendix B.

Paper I

Sittenthaler M, Kunz F, Szymusik A, Grünschachner-Berger V, Krumböck S, Stauffer C, Nopp-Mayr U (2018) **Fine-scale genetic structure in an eastern Alpine black grouse** *Tetrao tetrix* **metapopulation**. Journal of Avian Biology 49:e01681. <u>https://doi.org/10.1111/jav.01681</u>. Published

Contributor Roles

<u>Marica Sittenthaler</u>: Conceptualization; Formal analysis; Methodology; Project administration; Writing – original draft. <u>Kunz Florian</u>: Conceptualization; Formal analysis; Investigation; Validation; Writing – review and editing. <u>Szymusik Aneta</u>: Investigation. <u>Veronika Grünschachner</u>-Berger: Funding acquisition. <u>Susanne Krumböck</u>: Investigation. <u>Christian Stauffer</u>: Supervision; Resources. <u>Ursula Nopp-Mayr</u>: Conceptualization; Supervision.

Paper II

Kunz F, Klinga P, Sittenthaler M, Schebeck M, Stauffer C, Grünschachner-Berger V, Hackländer K, Nopp-Mayr U (2022) Assessment of drivers of spatial genetic variation of a ground-dwelling bird species and its implications for conservation. Ecology and Evolution 12:e8460. https://doi.org/10.1002/ece3.8460. Published.

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Paper III

Kunz F, Kohnen A, Nopp-Mayr U, Coppes J (2021) **Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system**. Conservation Genetics 22:355– 368. <u>https://doi.org/10.1007/s10592-021-01342-5</u>. Published

Contributor Roles

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Abstract

In the current era of rapid biodiversity loss, understanding genetic variation of populations as well as its trends and drivers is fundamental for their long-term preservation and hence integral for sustainable development. Thereby, species of mountainous areas are of special conservation concern given the landscape's high heterogeneity as well as the challenges induced through global change. As such, Eastern Alpine Black Grouse and Black Forest Western Capercaillie are two metapopulation systems in the focus of conservation. While Eastern Alpine Black Grouse are threatened with range contradiction and local extinctions, Black Forest Capercaillie have experienced a dramatic decline in the past centuries. Both metapopulation systems therefore call for an effective design of conservation strategies. Hence, I conducted analyses targeting the genetic diversity and population structure of these systems, finding slight isolation effects for Black Grouse and pronounced genetic differentiation for Capercaillie. Initiated by these results, I studied whether the observed structure for Black Grouse is in some way driven by the underlying landscape. While genetic diversity is generally high, we found spatial genetic variation to be partially driven by effects of isolation by resistance, with the easternmost subpopulation showing signs of increasing isolation. As those analyses are snapshots in time, I further looked into genetic differentiation in Capercaillie over time. I therefore build simulations projecting genetic differentiation driven by migration rates and tested realistic yet hypothetical scenarios. By making use of newly developed approaches combined with wellestablished methods, I was able to make valuable contributions to our general understanding of metapopulation systems and their genetic viability. Furthermore, all studies within this thesis were informed by practitioners' needs and therefore aimed to impact conservation practice and policy.

Kurzfassung

Vor dem Hintergrund der gegenwärtigen Biodiversitätskrise ist eine nachhaltige Entwicklung wichtiger denn je. Dabei ist der langfristige Erhalt von Wildtierpopulationen und damit auch deren genetischer Diversität ein wesentliches Ziel. Gebirge bewohnende Arten sind im Naturschutz von besonderer Bedeutung, da Gebirge einerseits eine hohe Heterogenität an sensiblen Lebensräumen aufweisen und andererseits vom globalen Wandel besonders betroffen sind. Die Steirische Birkhuhn Metapopulation sowie die Auerhuhn Metapopulation im Schwarzwald sind deshalb besonders relevant. Während es in der Steiermark bereits zu Aussterbeereignissen der Randpopulationen gekommen ist, haben die Auerhühner im Schwarzwald einen dramatischen Rückgang der Populationszahlen zu verzeichnen. Diese Arbeit befasst sich daher mit der genetischen Diversität und Populationsstruktur der beiden Metapopulationen. Angestoßen von diesen Ergebnissen wird mittels statistischer und räumlichexpliziter Modelle der Frage nachgegangen, ob die räumliche genetische Variation einer Metapopulation von der zugrundeliegenden Landschaft beeinflusst ist. Für die Steirischen Birkhühner konnten trotz einer generell hoher genetischer Diversität Effekte der Isolation durch Widerstände in der Landschaft gefunden werden. Analysen dieser Art sind meist nur Momentaufnahmen ohne zeitlichen Bezug, weshalb weiterführend untersucht wird, wie sich genetische Differenzierung über große Zeiträume durch Migrationsraten bedingt entwickelt. Hierfür werden Simulationen mit Populationsmodellen erstellt und realistische Szenarien in die Zukunft prognostiziert und miteinander verglichen. Durch die Verwendung neuer Ansätze zusammen mit gut etablierten Methoden erweitert diese Arbeit unser Verständnis von Metapopulationen und deren genetische Prozesse. Alle Studien innerhalb dieser Arbeit wurden dabei zusammen mit der Naturschutzpraxis initiiert und die Ergebnisse fanden entsprechend Eingang in Naturschutzkonzepte und -strategien.

1. Introduction

Human population growth and development in the last decades and century has led to drastic changes in European landscapes (Plieninger et al. 2016), inducing far reaching consequences on natural habitats (Díaz et al. 2019) and consequently wildlife. Especially in highly human-dominated landscapes, biodiversity - defined as diversity of ecosystems, species and genetic diversity within species (Art. 2 of the Convention on Biological Diversity 1992) - is lost at an alarming rate (Cardinale et al. 2012). Within the Convention on Biological Diversity's (CBD) first strategic plan in 2002, goals were set to reduce the loss of biodiversity. However, these goals were not achieved (Convention on Biological Diversity Conference of the Parties 2010) and we are now in an era of rapid and irreversible loss of biological diversity known as the biodiversity crisis. Consequently, most wildlife populations are increasingly threatened and their long-term survival might be at stake (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services 2019). Acknowledging this challenge, conserving biodiversity of wildlife species as well as associated ecosystems have been made priority targets on agendas of all kinds of acting bodies ranging from international treaties to the European Union, national states and even provinces (Secretariat of the Convention on Biological Diversity 2014; Baynham-Herd et al. 2018). As such, the Conference of the Parties of the CBD has adopted twenty targets aimed at preservation of biodiversity, known as the Aichi Biodiversity Targets (Convention on Biological Diversity Conference of the Parties 2010). In addition, the Sustainable Development Goals (SDGs) were developed by the United Nations General Assembly (2015) as part of the 2030 Agenda for Sustainable Development. Conservation of biodiversity can be regarded as the whole foundation of this Agenda, underpinning many of its goals (Secretariat of the Convention on Biological Diversity 2020). Especially SDG 15 'Life on Land' highlights biodiversity, including the protection, restoration, and promotion of sustainable use of terrestrial ecosystems to halt biodiversity loss. However, the world fell short on achieving the Aichi Biodiversity Targets (Secretariat of the Convention on Biological Diversity 2020) and in its most recent progress report towards the SDGs, the United Nations Economic and Social Council (2021) records the urgent need for more actions focusing on preservation of biodiversity.

1.1. The role of conservation genetics

Perseveration of biodiversity calls for an immense body of scientific fundamentals. The CBD recognized scientific research as important pillar of conservation efforts already in 1992 (Art. 12(b)).

Accordingly, life sciences are nowadays acknowledged as integral part within sustainable development and the achievement of the SDGs (International Science Council 2021). Conservation research enables fundamental understandings of wildlife populations and derives specific management objectives for conservation of nature. Thereby, conservation genetics as a scientific field is highly driven by practical challenges and oriented towards providing solutions and management concepts for the studied populations (Holderegger et al. 2019). As conservation genetic studies allow for the understanding of a population's genetic diversity, structure and many more, they can be valuable tools to support preservation of biodiversity.

1.2. Metapopulation systems and burning questions of conservation

Within conservation genetics, a special focus is laid onto metapopulation systems (Frankham et al. 2010). A metapopulation system consists of loosely connected subpopulations with only a certain amount of gene flow in-between. Often induced from habitat loss and fragmentation, these systems suffer from reduced genetic diversity and increased genetic differentiation compared to a single large population of the same size (Allendorf et al. 2013) and consequently are of high conservation concern (Lowe et al. 2005; Brook et al. 2008). Conservation genetic literature is comprehensive and evidence is vast on metapopulation systems eventually experiencing reduced resilience and increased extinction risks, not uncommonly leading to the extinction of the whole system (Frankham et al. 2010; Allendorf et al. 2013).

As such, conservation of metapopulation systems highly depends on scientific fundamentals and results informing conservation management. Practitioners often design management strategies and actions on a spatial scale. Especially for metapopulation systems, a sound understanding of its population structure on a small spatial scale is therefore crucial. Burning questions for scientists and practitioners alike evolve around potentially isolated subpopulations and the drivers of spatial genetic variation within the metapopulation system as a whole. Landscape genetic methods hereby try to link the genetic population structure and variation with the underlying landscape characteristics (Manel and Holderegger 2013). Besides spatial aspects, also temporal aspects might play an important role and conservation genetic science is urging to understand how migration between subpopulations might act on genetic differentiation over several time periods. In order to carry out projections into the future, simulations can be applied simulating the progress of a metapopulation system under certain conditions (Hoban et al. 2012).

1.3. Wildlife in mountainous landscapes

Mountainous regions are of special concern in nature conservation and sustainable development (Gratzer and Keeton 2017). The combination of different topographic and climatic conditions within these areas result in a high diversity of habitats on a small spatial scale (Körner 2007), leading to a high susceptibility. Although progress has been made in the conservation of mountainous regions, environmental degradation is still increasing, which led the United Nations General Assembly to proclaim 2022 the 'International Year of Sustainable Mountain Development' (United Nations General Assembly 2021).

The Alps, as the highest and most extensive mountainous area in Central Europe, have seen dramatic changes in land use practices over the last century, affecting wildlife populations. Agricultural activities like livestock grazing on man-made subalpine grasslands have been widely changing (Körner 2007; Strebel and Bühler 2015), with many alpine pastures either being more intensively utilized or becoming abandoned and overgrown with forests over time (Groier 2010). Forestry also increased in intensity, with commercial timber exploitation reaching unsustainable rates, tree composition changing and tree density and canopy cover rising (Kräuchi et al. 2000; Bebi et al. 2017). Additionally, ongoing climate change is affecting alpine landscapes faster and stronger than lowlands (Scridel et al. 2018; Rumpf et al. 2022). Plant community distributions are responding to the changing topoclimatic conditions (Theurillat and Guisan 2001), resulting in an altitudinal uphill shift of the upper tree-line and a general increase in deciduous shrubs in higher altitudes (Tasser et al. 2007; Gehrig-Fasel et al. 2007; Rumpf et al. 2022). Furthermore, climate change interacts with land use, adding another layer of complexity affecting natural habitats and wildlife populations (Scridel et al. 2018). Besides these processes, anthropogenic impact also increased directly (such as infrastructure like human settlements, skiing areas or agricultural areas) and indirectly (such as disturbances in natural areas increased due to tourism activities, sport activities, etc.) (Ingold 2005; Arlettaz et al. 2007; Zohmann et al. 2014; Immitzer et al. 2014; Coppes et al. 2017, 2020; Tost et al. 2020; Canonne et al. 2021 and references therein).

Given the high diversity of alpine habitats and of species within those habitats, all these changes are expected to pressure alpine biodiversity (Strebel and Bühler 2015). The high heterogeneity of alpine landscapes however impedes easy solutions to preserve biodiversity over larger areas. Instead, wildlife populations must be assessed on a small scale to account for site-specific factors, while at the same time results must be set in a larger context.

1.4. Grouse species in the focus of conservation

Within Central Europe's mountainous areas, European grouse (Galliformes, Tetraoninae) are within the focus of conservation management. Their conservation status are quite different between populations, with many being of high conservation concern due to ongoing declining trends (Storch 2007a; Jahren et al. 2016). Reasons are multifactorial, with the most dominant human-induced factors being habitat loss, degradation, and fragmentation (Storch 2007b; Segelbacher et al. 2008; Kämmerle et al. 2021) as well as increasing disturbances (Patthey et al. 2008; Arlettaz et al. 2015; Coppes et al. 2017; Tost et al. 2020). Given their generally sensitive response to habitat characteristics, grouse species are considered perfect indicator species for their respective habitats (Suter et al. 2002; Storch 2007a). Consequently, grouse species are often priority species in regional, national and international conservation and copious conservation measurements are required by law.

1.5. Black Grouse *Lyrurus tetrix* (protonym *Tetrao tetrix*) in Styria, Austria

The Black Grouse (*Lyrurus tetrix*) is a midsized, ground-dwelling bird, typical for transition zones between boreal forests and open lands. Due to their sensitive habitat requirements, Black Grouse is generally considered to be an indicator species for this ecotone (Storch 2007a) and are therefore especially important in conservation management. Covering a wide Palearctic distribution from Great Britain to Siberia, it currently shows a decreasing population trend (BirdLife International 2016a). While there are still continuous populations in the northern and eastern range of its occurrence (Höglund et al. 2007), especially the populations within Central Europe are of conservation concern. Given the specific habitat requirements of this species, Central European populations suffered from habitat loss and degradation (Storch 2007b) both in the lowlands and at higher altitudes (Ludwig et al. 2009). As a result, most of them declined dramatically or became extinct in the past decades (Larsson et al. 2008; Watson and Moss 2008; Segelbacher et al. 2014; Rutkowski et al. 2018). The remaining populations within Central Europe are of exist within a metapopulation context (Caizergues et al. 2003; Höglund et al. 2007), further questioning their long-term survival (Frankham et al. 2010).

Consequently, the Black Grouse is nowadays in the spotlight of international conservation. Besides resolutions emerging from several global treaties (like the Convention on Biological Diversity and the Convention on the Conservation of European Wildlife and Natural Habitats), Central European populations are specifically targeted by the European Union Birds Directive (2009/147/EC). Being

listed both in Annex I and Annex II part B, special conservation measurements must be taken to ensure their long-term survival (Art. 4 (1)).

As a sedentary species, dispersal mainly occurs in the first year (natal dispersal) and is female-biased. Hens are dispersing on average distances of about 8 km (Caizergues and Ellison 2002; Warren and Baines 2002; Marjakangas and Kiviniemi 2005; Corrales and Höglund 2012) whereas cocks are philopatric. Although in rare events longer distances are travelled in flight, dispersal is generally believed to be ground-based and habitat characteristics are assumed to be key factors for movement.

Within Central Europe, the core areas of the species' distribution lie within the Alps (Klaus et al. 1990; BirdLife International 2016a). There, habitats are characterized by a patchy mixture of open grasslands and woody plants with low canopy closure in the transitional zones between forests and alpine meadows, the upper treeline ecotone (Sachser et al. 2017). As Black Grouse usually avoid areas with higher tree canopy closure found in more dense forests below the treeline (Patthey et al. 2012; Schweiger et al. 2012; Immitzer et al. 2014; Sachser et al. 2017) and high mountain ridges above the vegetation area (Caizergues and Ellison 2002), Black Grouse habitats in the Alps are naturally fragmented. This is of particular importance at the marginal areas of the Alpine distribution, as habitats are naturally smaller and further apart. The easternmost Alpine Black Grouse occurrences are situated in the Austrian province Styria (Figure 1). Styria is mostly covered by mountains ranging from 200 to almost 3000 m a.s.l., with a high proportion of conifer-dominated forests and a prominent portion of alpine meadows and grasslands. Black Grouse subpopulations are separated by several kilometers partially exceeding the species' dispersal capabilities, resulting in a metapopulation system. In the past decades, several extinction events of the easternmost subpopulations have already been documented (Wöss and Zeiler 2003; Nopp-Mayr and Grünschachner-Berger 2011) and further extinction events of the subpopulations situated the fringe of distribution cannot be ruled out. As such, Black Grouse conservation and management in Styria were in need of a comprehensive understanding of this metapopulation system's population structure and whether the subpopulations at the fringe of the species' occurrence are differentiated. Additionally, informed by results of population structure, the system lend itself as well-suited focal system to explore the landscape's effect on the spatial genetic variation.



Figure 1 Study areas. Mountainous areas within Europe (Alps and European uplands) in grey, provided by the European Environment Agency. Light grey lines represent national borders.

1.6. Western Capercaillie *Tetrao urogallus* in the Black Forest, Germany

While the Black Grouse is preferring the tree-line ecotones, its close relative, the Western Capercaillie (*Tetrao urogallus*) is a typical forest grouse inhabiting lower altitudes. As such, it prefers old coniferdominated forests with moderate canopy cover (Braunisch and Suchant 2008; Graf et al. 2009; Zohmann et al. 2014) interrupted by gaps (Braunisch et al. 2014; Hofstetter et al. 2015). Such forests allow for a diverse ground vegetation, with especially berries (e.g. bilberry *Vaccinium myrtillus*) being important for Capercaillie as food resource (Storch 2002; Graf et al. 2009). Akin to the Black Grouse, the Capercaillie is considered as umbrella species for such forests rich in biodiversity due to its sensitivity to habitat characteristics (Suter et al. 2002; Pakkala et al. 2003). Given its popularity within society (in terms of hunting, poetry, nobility and others), it serves as a flagship species for conservation (Mollet et al. 2008; Suchant and Braunisch 2008). Quite similar to the Black Grouse, the Capercaillie shows a Palearctic distribution with main areas in Scandinavia and Siberia. However, European populations are fragmented and most of them are already isolated (Storch 2007b; Coppes et al. 2015) due to habitat loss and degradation (BirdLife International 2016b) and anthropogenic disturbances (Coppes et al. 2017, 2018). Consequently, the general population trend is decreasing (BirdLife International 2016b) with many populations experiencing drastic declines or extinction, including populations in Germany (Coppes et al. 2019), Poland (Rutkowski et al. 2017), the Carpathians (Klinga et al. 2015, 2017) or Spain (Morán-Luis et al. 2014).

Accordingly, European Capercaillie populations are of high conservation concern. The international wildlife conversation legislation framework for the Capercaillie is mostly the same as for the Black Grouse, resulting in the same obligations to preserve Capercaillie populations.

Dispersal capabilities of Capercaillie resemble those of Black Grouse as well, in that dispersal is femalebased and occurs in the first year (natal dispersal). Median dispersal distances are about 2 - 5 km, rarely exceeding 10 km (Storch and Segelbacher 2000; Storch 2007b).

Within Central Europe, Capercaillie populations are mainly restricted to forested mountain ridges. As such, the Black Forest in south-western Germany (Figure 1) once harbored a thriving population of Capercaillie. At the beginning of the 19th century, numbers of lekking males were estimated at about 3800 individuals (Coppes et al. 2019). However, by the midst of the century, that number declined to about 1300 lekking males (Roth and Suchant 1990) and in 1971, when the first Black Forest wide census was done, only 570 lekking males could have been confirmed (Roth 1974). Since then, Capercaillie numbers declined even further to 167 lekking males in 2018 (Coppes et al. 2019), epitomizing a dramatic decline over more than a century. The reasons for this decline are pretty well understood, with the main cause being habitat deterioration (Kämmerle et al. 2020) due to changes in forestry. Once open forests became denser darker during the course of the century, leading to habitat loss and degradation for Capercaillie (Coppes et al. 2019). Additionally, effects of increasing human disturbance (Coppes et al. 2017), climate change (Braunisch et al. 2013) and predation (Kämmerle and Storch 2019) factor in. Consequently, the recent Capercaillie occurrence in the Black Forest is differentiated into four geographically separated subpopulations. A previous study including data from 1999 to 2004 found genetic differentiation between the subpopulations to be weak, yet first indications of a barrier between the northern subpopulation and the other three subpopulations were described (Segelbacher et al. 2008). Whether these signs were indicating a general ongoing trend of increasing differentiation and, if so, whether conservation strategies such as re-establishing of connectivity might be able to counteract such effects were significant questions of recent Capercaillie conservation in the Black Forest.

1.7. Aims and objectives

Regarding all these challenges of grouse conservation and their context within conservation of biodiversity, the overarching aim of this thesis was to apply conservation genetic approaches to derive specific conservation actions and thus to contribute to the long-term preservation of the studied metapopulation systems. Thereby, conservation, population and landscape genetic methods were used. As an important premise in conservation genetic research (Frankham et al. 2010), all studies were based on specific conservation issues informed by practitioner's needs. Accordingly, results were translated into specific conservation actions and communicated to practitioners via reports, talks, magazine articles and more. Thus, this thesis significantly contributes to the preservation of biodiversity as outlined in SDG 15 'Life on Land'.

Furthermore, as the two described focal systems are representative for many populations of conservation concern worldwide suffering from habitat loss and fragmentation, this thesis aims to increase our general understanding of the population genetics, the genetic differentiation and their interactions with the landscape within such small-scale metapopulation systems. Understanding these processes will greatly improve our scientific knowledge and lead to more conservation research done on lesser known yet equally important species.

Therefore, three studies have been conducted to achieve the three following successive objectives.

1.7.1. Objective 1: Assessing the population genetics of a small-scale metapopulation system

The Styrian Black Grouse, as a peripheral and declining metapopulation system, is of high conservation concern. Fundamental knowledge on their genetic variability was necessary to inform conservation strategies, yet genetic studies were lacking. Consequently, within **paper I**, we aimed at analyzing genetic diversity and structure of these birds. We set genetic variability into an international context, answering whether the Styrian occurrences are to be classified as continuous or contiguous (Höglund et al. 2007). Ultimately, we aimed at assessing the population structure on an up to that point unmatched fine spatial scale and look for potential differentiation between clusters of subpopulations.

Our results of **paper I** were able to inform conservation actions. However, at the same time, the question arose whether the observed population structure is in some way driven by the underlying landscape, which we targeted in **paper II**.

1.7.2. Objective 2: Assessing the drivers of spatial genetic variation

After we were able to identify clusters of subpopulations differentiated from each other within the Styrian metapopulation system, the question emerged whether the spatial genetic variation is at least partially driven by the underlying landscape. Within **paper II**, we therefore assessed geographic distances and landscape resistances using correlative ecological niche modelling. Measures of isolation by distance and resistance were calculated and then regressed against the genetic differentiation between subpopulations in a number of ways. Ultimately, the objective was to assess whether the spatial genetic variation was driven by isolation by distance or isolation by resistance, as management strategies would differ depending on the underlying mechanism.

Our results of **paper II** indicated the landscape's effect on genetic differentiation. As both, **paper I** and **paper II** were based on the same genetic dataset originating from a single sample period, the assessment of genetic differentiation could only be a snapshot. Whether genetic differentiation changes over time and how it is affected by migration rates between subpopulations was therefore studied in **paper III**.

1.7.3. Objective 3: Tracking and simulating genetic differentiation over time from past to present to future

Genetic differentiation between subpopulations plays an essential role in conservation as increasing differentiation will ultimately lead to increased extinction risks of metapopulation systems. While **paper II** was able to explain the drivers of genetic differentiation, it could only assess those at one point in time. Accordingly, within **paper III**, we looked at genetic differentiation making use of three different time periods. We aimed at comparing past genetic differentiation with present genetic differentiation. Additionally, we built simulations to project genetic differentiation in future scenarios, driven by different migration rates. We chose Black Forest Capercaillie as focal system for several reasons. Conservation efforts of Capercaillie in the Black Forest are advanced. While the four subpopulations were known, their population structure and genetic differentiation however remained unknown. Additionally, conservation efforts needed to be informed about the effectiveness of reestablishing connectivity as potential management actions. Furthermore, two genetic samplings have been conducted on the Black Forest Capercaillie 15 years apart from each other (subsequently defined as the *historic dataset* and the *recent dataset*). The Black Forest Capercaillie therefore presented itself as an ideal case study where we aimed to 1) compare the genetic differentiation within the

metapopulation system over time and 2) design a simulation to predict genetic differentiation based on future scenarios of migration rates.

2. Methods

The studies were conducted within the whole province of Styria, Austria (**paper I**, **paper II**) as well as the area of the Black Forest in the province of Baden-Württemberg, Germany (**paper III**). Both study sites were comprehensively sampled for Black Grouse samples (in Styria) and Capercaillie samples (in the Black Forest). As both species are of high conservation concern and generally elusive, mostly noninvasive samples (i.e., feces and molted feathers) have been collected. Additionally, tissue samples were available for Styrian Black Grouse (provided by the local hunting organization from birds legally shot during the hunting season).

2.1. Genetic sampling and genotyping

Sampling of feces in both study sites was conducted within a period of five days after snowfall, to ensure high quality of DNA. Samples were stored at -20°C, with tissues being preserved in absolute ethanol.

Extraction of DNA was done using extraction kits by Qiagen and Sigma Aldrich. Adaptions to the manufacturer's protocols to achieve higher yields of DNA are described in **paper I**. Samples were genotyped using nine (for Black Grouse) and twelve (for Capercaillie) short tandem repeat (STR) loci (Segelbacher et al. 2000; Piertney and Höglund 2001; Jacob et al. 2010) and a sexing marker (Kahn et al. 1998). Amplification was done using polymerase chain reaction (PCR), with recipes and cycling conditions described in detail in **paper I** and **paper II**. To account for the reduced quality and quantity of DNA in non-invasive samples (Beja-Pereira et al. 2009), a multiple tubes approach (Navidi et al. 1992; Taberlet et al. 1996) was applied, accepting a consensus genotype only when at least two out of three replicates resulted in the same alleles for heterozygotes and three out of three replicates resulted in the same alleles for heterozygotes and three out of three replicates resulted in the same alleles for heterozygotes and three out of three replicates resulted in the same alleles for heterozygotes. Additional replicates were made in case of ambiguous results. Negative controls were included in each extraction batch and each PCR to check for cross-contamination.

For Styria, 250 samples were processed, resulting in a dataset containing 195 individual genotypes. For the Black Forest, 1278 samples were processed, resulting in 271 individual genotypes.

2.2. Statistical analyses

The following paragraphs provide a brief overview over the methods used within all three studies. Various software and R packages (R Core Team 2019) were applied.

The loci of all datasets were thoughtfully validated by checking for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium (corrected for multiple testing), large allele dropout, null alleles and stuttering as well as their general ability to distinguish individuals. For each metapopulation system, subpopulations were initially distinguished based on topographical and landscape characteristics as well as dispersal capabilities of the respective species. Subsequently, the distinction was evaluated based on various analyses.

Within **paper I**, the genetic diversity and metapopulation structure of the Styrian Black Grouse metapopulation was then assessed on a fine spatial scale. Therefore, standard summary statistics of genetic diversity were calculated. Metapopulation structure was examined using cluster analyses based on Bayesian and frequentist approaches.

Based on **paper I**, in **paper II** population differentiation was assessed in more detail making use of bias corrected measures of population fixation and differentiation and migration rates. In order to disentangle the effect of the landscape on the spatial genetic variation, a correlative ecological niche model (ENM) was calculated based on presence-only data by using machine learning algorithms on species-specific environmental variables of topography, climate and land cover. This was then translated into a resistance surface and measures of distance and resistance between subpopulations were calculated based on the cost distance approach as well as on the circuit theory approach. Maximum likelihood population effect models were then applied, regressing population genetic differentiation and fixation with measures of distance and resistance, to learn whether the spatial genetic variation is driven by effects of isolation by distance or isolation by resistance.

Within **paper III**, the analysis was done in two steps. In a first step, the historic dataset and the recent dataset were compared using corrected measures of genetic fixation and differentiation as well as Bayesian and frequentist clustering approaches. Additionally, migration rates were calculated. In a second step, an individual-based stepwise forward simulation was designed making use of a stage-based transition model. In this model, Capercaillie individuals transitioned from stage 1 (juveniles) into either stage 2 (adult females) or stage 3 (adult males). The models were parameterized making use of published demographic data on stage-specific survival and reproduction and seeded with the genotypes of the sampled individuals from both datasets. Additionally, microsatellite mutation rates and effects of density dependence were included. A simulation from past to present was run to

validate the simulation set-up by comparing the simulated results with the recent dataset. Then, forward simulations were run for 35 years based on the recent dataset incorporating scenarios of different migration rates. Genetic fixation and differentiation were calculated per scenario and compared to draw conclusions.

3. Contributions

In the following chapters, the publications are briefly summarized and the main results and conclusions are presented.

3.1. Paper I: Fine-scale genetic structure in an eastern Alpine black grouse *Tetrao tetrix* metapopulation

In the light of the current biodiversity crisis and the increasing challenges especially in mountainous ecosystems, an understanding of the genetic consequences of habitat fragmentation on wildlife populations is crucial. Especially metapopulation systems are vulnerable to fragmentation, as the reduced connectivity might lead to reduced genetic diversity and increased population structure. Studies focusing on genetic diversity and population structure therefore provide necessary fundamentals to conceive management strategies. While many studies assess population structure on a broad scale, especially fine-scale assessments are needed in conservation practice to design specific management actions. In **paper I**, we focused on the Styrian Black Grouse metapopulation system. As described in chapter 1.5., this metapopulation system is of high conservation concern and up to that point nothing was known about their genetic diversity and population structure. Given Black Grouse are elusive, we established genetic analyses not only on tissue samples from shot birds during the hunting seasons (as those are all males) but also on non-invasive genetic samples (feces and feathers). The sampling was designed to cover the whole study area and altogether 250 samples were accomplished. Samples were classified into eleven a priori defined subpopulations, based on topographical criteria and average dispersal distances.

Overall, this eastern Alpine metapopulation system showed amounts of genetic diversity similar to those of large, continuous populations in Scandinavia (Höglund et al. 2007; Corrales and Höglund 2012) and contiguous populations in northern Scotland and other Alpine countries (Caizergues et al. 2003; Höglund et al. 2011). Besides the high levels of diversity, we found three supported clusters within the metapopulation system: the inneralpine cluster, the southern cluster and the eastern cluster. Additionally, subpopulations of the area Zirbitzkogel were not easily assigned and remained unclear. The inneralpine cluster represented the main occurrences and was probably connected to the rest of the Alpine populations. It was separated from the southern and eastern cluster by the Mur-Mürz-Furche, a major valley with high levels of human settlement, traffic and infrastructure. The southern cluster was probably connected to Black Grouse populations in Carinthia (unpublished data).

We failed to assign subpopulations located at the Zirbitzkogel to either the inneralpine or the southern cluster. Therefore, this subpopulation most probably serves as a stepping stone connecting those two clusters. The eastern cluster however appeared to be the most differentiated. Situated at the fringe of the distribution area, no other subpopulations exist to provide gene flow. Considering past extinction events of the easternmost subpopulations (Wöss and Zeiler 2003; Nopp-Mayr and Grünschachner-Berger 2011), this subpopulation is of special concern and must be monitored with caution.

By analyzing the population genetic makeup on a fine spatial scale, we were able to detect genetic clusters and first warning signs of potential isolation. Based on our results, subpopulations can be distinctly managed, with hunting strategies updated based on the conservation concern of the subpopulation. Additionally, we found indications for a geographical structure in spatial genetic variation. Whether this structure could be attributed to geographical distance alone or effects of fragmentation due to natural or anthropogenic barriers was beyond the scope of this study. However, given the highly human-dominated landscape, we assumed at least some effect of the landscape's resistance to be represented in the genetic variation, for which we performed the study described in **paper II**.

3.2. Paper II: Assessment of drivers of spatial genetic variation of a ground- dwelling bird species and its implications for conservation

Precise knowledge about the metapopulation system's population structure on a fine spatial scale allowed us to look into the drivers of genetic variation. Early on, geneticists assumed the genetic variation to be affected in some way or another by the underlying landscape. As a first concept, isolation by distance (IBD) hereby describes the positive relationship between genetic differentiation and geographic distance (Wright 1943). While commonly observed in panmictic wildlife populations (Sexton et al. 2014), the spatial genetic variation can be affected by additional factory beyond Euclidean distances (Balkenhol et al. 2016). Known as isolation by resistance (IBR, McRae 2006), this concept describes the relationship of spatial genetic variation and a landscape's resistance to movement and hence gene flow (Wagner and Fortin 2013; Wang and Bradburd 2014). Hereby, gene flow can be hindered by intrinsic, species specific factors such as dispersal capabilities and strategies (Lampert et al. 2003; Bech et al. 2009; Corrales and Höglund 2012) and extrinsic factors like landscape topography, vegetation cover and anthropogenic factors like disturbance. It is therefore crucial to have an understanding of the spatial genetic variation's drivers when designing conservation actions

focused on the preservation and reestablishment of connectivity, especially for a ground-based, sedentary species like the Black Grouse.

In order to analyze for drivers of spatial genetic variation, knowledge on the landscape's resistance is necessary. We therefore used a correlative modelling approach based on maximum entropy theory to generate an ENM based on topographical, climatic and land cover variables, including variables on tree height and tree composition, potential positive effects of single tree individuals in subalpine areas and distances to positively and negatively selected land cover types to account for push and pull effects. Our ENM identified habitat patches of Black Grouse with high accuracy (average test AUC = 0.954 based on 20 replicated models using cross-validation to separate training and test data). The ENM was then translated into a resistance model and measures of distance (Euclidean distance, to account for IBD) and resistance (least cost path length LCP and effective resistance, to account for IBR) were extracted between subpopulations and individual presence points. Additionally, we calculated the five indices of genetic differentiation F_{sT} (Weir and Cockerham 1984), G_{sT} (Nei and Chesser 1983), G'_{ST} (Hedrick 2005), G"_{ST} (Meirmans and Hedrick 2011) and D_{Jost} (Jost 2008), as these indices quantify complementary aspects of population structure (Meirmans and Hedrick 2011; Jost et al. 2018). Then, we used several regression frameworks including maximum likelihood population effects models (Clarke et al. 2002) to address whether IBD (Euclidean distances) or IBR (LCP lengths, effective resistances) might explain genetic differentiation on and individual level and on a subpopulation level.



Figure 2 Results of population genetic analyses, ecological niche modeling, and landscape genetic approaches on 195 Styrian Black Grouse individuals. (a) Digital elevation model of the study area Styria, with all 195 individuals, classified in 10 subpopulations (black outline, 5- km buffer around presence points and four clusters (green-, yellow-, orange-, and gray- colored areas of suitable habitat). Least cost were classified into five quantiles of effective resistances. The inset shows the area of the Alps (dark gray) provided by the European Environment Agency and the location of our study area (black square). (b) Ecological niche model, representing the resistance surface While generally levels of genetic differentiation and population structure are low, our ENM revealed clearly delimited habitat areas. On an individual level, we found models based on IBD to be marginally more explaining than models based on IBR, leading to the conclusion that the landscape's resistance arguably did not exert a meaningful effect. On a subpopulation level, LCP lengths were the most explanatory models for spatial genetic variation. While this could be taken as an indicator for the presence of barriers, we assume the observed patterns to result from short-distance dispersal of Black Grouse, as short distance dispersal could lead to global IBD patterns with effects of IBR on local scales only (Blair et al. 2012). Taken together, our results indicate the spatial genetic variation to be driven by IBD on the individual level and by IBR on the subpopulation level. Models however were not distinctly different, indicating either a cumulative effect or the presence of additional, not yet included factors. Finally, contrary to our expectations, effective resistances as circuit theory-based approach were outperformed by LCP lengths as cost-distance approach. This was unexpected, as circuit theory assumes no prior knowledge of individuals when moving through the landscape and therefore seems better suited as a model for Black Grouse dispersal. This result might be explained by the landscape restricting dispersal through topographical criteria (high mountain ridges and steep valleys), only allowing for dispersal route, which was best described by the LCPs.

3.3. Paper III: Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system

In **paper I** and **paper II**, we investigated drivers of genetic variation on a spatial scale, which we could attribute to landscape characteristics. The resistance of a landscape affects the individual's movements and thus migration rates between subpopulations. Migration rates are presumably drivers of genetic differentiation between subpopulations, hence they play an important role in the assessment of a metapopulation's viability. Ultimately, increasing genetic differentiation will lead to further isolation of subpopulations as gene flow is not sufficient to preserve genetic diversity and therefore adaptive potential. Smaller, isolated and less divers subpopulations will experience an increased extinction risk (Frankham et al. 2010). Understanding a metapopulation's genetic differentiation and its drivers and trends therefore is a priority target for conservation and can only be achieved by temporal analyses.

The Black Forest Capercaillie population, as outlined in chapter 1.6., has experienced a drastic decline over the past century. Whether this decline in numerical population size was accompanied by an increase in genetic differentiation, and if so, what conservation actions might be a worthwhile endeavor to halt or decrease genetic differentiation, were important questions raised by conservationists. The metapopulation system itself consists of four varyingly good connected subpopulations. Given the distances to neighboring Capercaillie occurrences, this metapopulation system can be considered as an island population, making it a perfect focal system to study genetic differentiation as a response to migration rates.

We acquired data from a previous sampling (the historic dataset, from 1999 to 2004, 213 samples) and a recent sampling (the recent dataset, from 2013 to 2017, 1278 samples). Both datasets originate from genotyping of non-invasive samples (feces and feathers), using eleven microsatellite loci. In a first step, we compared population structure between the two time periods. While we did not detect genetic differentiation or population structure in the historic dataset, evidence was present in the recent dataset, revealing an increase in genetic differentiation over these approximately 15 years.

In a second step, we designed forward-in-time individual-based stage-based simulations to simulate genetic differentiation of future generations using several scenarios of migration rates. Forward-intime simulations were seeded with the recent dataset and run for 35 years to the year 2050. At the end of each simulation, we extracted measurements of genetic differentiation between subpopulations (averaged, as these simulations are stochastic and therefore 1000 iterations were run for each scenario). The simulations were individual-based, meaning that the genotype and fate of each individual (the initial founder population and all offspring) was tracked. The simulations were build using a stage-based transition model at its core. Therein, three Capercaillie life stages were defined as: 1) juvenile, 2) adult females and 3) adult males. Transition rates (individual probabilities to move from one stage to the next or survive within one stage for a year) were informed by estimates from a nearby comparable population (Grimm and Storch 2000). We evaluated our simulation set up by running simulations seeded with the historic dataset forward in time, applying recent migration rates, and compared results to the recent dataset. Our set up proved to be appropriate to generate plausible results. We used a power analyses to determine that 1000 iterations were enough to find differences in genetic differentiation between scenarios. We built five scenarios per simulation (Table 1). **Table 1** Scenarios used in the simulations. The specific migration rates (m) used within the simulation aredisplayed per pairing of subpopulations (N North, C Central, E East, S South), e.g. migration rate from North toSouth as m_Ns

scenario	verbal description		
simulation HR (historic to recent)			
scenario HR_1: no migration	This scenario implemented no migration between the four subpopulations, assuming isolation. $m_{all} = 0$.		
scenario HR_2: evidence-based estimates of migration	This scenario was built on estimates of migration rates that are derived from analyses of recent migration and genetic population structure. Estimates were adjusted for sample size. $m_{CN} = 0.1$, $m_{CE} = 0.05$, $m_{CS} = 0.01$, $m_{EC} = 0.15$, $m_{ES} = 0.2$, $m_{SE} = 0.15$, all other $m = 0$		
Scenario HR_3: ideal migration	This scenario implemented identical migration between all subpopulations, in both directions, with the migration rate resembling the maximum migration rate used for scenario HR_2. $m_{all} = 0.15$.		
simulation RF (recent to future)			
scenario RF_1: no migration	Migration rates identical to simulation 1 scenario HR_1.		
scenario RF_2: evidence-based estimates of migration	Migration rates identical to simulation 1 scenario HR_2.		
scenario RF_3: ideal migration	Migration rates identical to simulation 1 scenario HR_3.		
scenario RF_4: isolation of East	This scenario implemented isolation of the subpopulation East, hence exploring the differentiation between the remaining three subpopulations in case of extinction of subpopulation East. Migration rates identical to scenario RF_2, except all migration rates from and to subpopulation East were set to 0.		
scenario RF_5: re-establishment of connectivity to North	This scenario implemented the re-establishment of connectivity from and to subpopulation North, via increased migration rates. Specifically, $m_{_{NC}}$ was introduced as well as a uniform migration between the subpopulations North and East. $m_{_{NC}} = 0.1$, $m_{_{NE}} = 0.05$, $m_{_{CN}} = 0.1$, $m_{_{CE}} = 0.05$, $m_{_{CS}} = 0.01$, $m_{_{EN}} = 0.05$, $m_{_{EC}} = 0.15$, $m_{_{ES}} = 0.2$, $m_{_{SE}} = 0.15$, all other $m = 0$		

Scenario RF_4 and scenario RF_5 explored effects of two realistic yet hypothetical migration scenarios on genetic differentiation. Given the low population numbers in subpopulation East, stochastic effects could lead to its extinction, which we simulated in scenario RF_4. Our results showed reduced migrations rates also between the other subpopulations, leading to two conclusions: first, the subpopulation East seems to act as an important core area for gene flow and second, events affecting one subpopulation could very well have consequences for the rest of the metapopulation system.

Contrary to scenario RF_4, scenario RF_5 explored effects of a potential conservation action, the reestablishment of gene flow to subpopulation North. As subpopulation North was already becoming differentiated in the past decades, results of our simulation showed that the reestablishment of gene flow would be a conservation action capable of delaying further genetic differentiation.

We found no populations structure in the historic dataset, only a slight differentiation of subpopulation North was indicated. When analyzing the recent dataset however, we found clear signals for a pronounced population structure with subpopulation North being differentiated. These results lead to the conclusion that over these approx. 15 years, genetic differentiation increased to recent levels. Conservation geneticists should therefore treat first signs of genetic differentiation, as were found in the historic dataset, as early warning signals and focus on the functional connectivity between the affected subpopulations.

Our results emphasize the preservation of connectivity within metapopulation systems in order to preserve gene flow and prevent genetic differentiation. We showed that within such systems, events affecting one subpopulation can have far reaching consequences for the whole metapopulation system. We further proved the effectiveness and usefulness of our simulation set up for conservation genetics and promote further use of simulations in the assessment of conservation actions.

4. Synthesis and prospect

This thesis is the combined work of several conservation genetic studies, each with different objectives, aims and methods. The two study systems face numerous challenges common for metapopulation systems of ground based species with mediocre dispersal capabilities. All studies were driven by conservation practitioners' needs and designed so that management questions could be answered and conservation strategies could be concluded. Accordingly, results of this thesis have already been adopted in conservation management and policy. As such, Styrian land-use planning concepts for example were directly informed by results of **paper I** and **paper II** (Amt der Steiermärkischen Landesregierung Abteilung 17 Landes- und Regionalentwicklung 2019). Besides the practical implications, important scientific insights could have been achieved as the two focal systems are representative for many metapopulation systems of species with limited dispersal capabilities. By assessing the drivers of spatial genetic variation (**paper II**) and simulating the effects of migration on genetic differentiation (**paper III**), this thesis significantly advances our understanding of the genetic makeup of metapopulation systems.

4.1. Implications for conservation of grouse

Metapopulations of grouse are oftentimes threatened und hence of immediate conservation concern (Storch 2007b). As the afore mentioned changes in mountainous areas are proceeding or even picking up in pace, alpine grouse metapopulations are in the need of well-informed integral management concepts.

Our ENM for the Styrian Black Grouse showed habitats to be confined to suitable subalpine areas in higher altitudes. While this was expected, the spatial modelling approach combined with cost distance and effective resistance approaches revealed the overall connectivity within the metapopulation system. Together with analyses of population structure and gene flow, we found that especially subpopulation OSW is of high concern, as 1) it is geographically separated by a major valley (the Mur-Mürz-Furche), 2) no immigration into that subpopulation was detectable and 3) it is already the most differentiated subpopulation. Although the general genetic variability of Styrian Black Grouse is rather promising (comparable with continuous populations in Scandinavia, Höglund et al. 2007; Corrales and Höglund 2012), our results should be viewed as first warning signs for increasing differentiation of OSW (as shown in **paper III**). As a response to the ongoing changes in mountainous areas, Black Grouse are generally expected to move towards higher elevations (Canonne et al. 2021; Schai-Braun et al.

2021). Such response however is limited by the available habitat in higher altitudes. Especially regions with lower mountain ridges will therefore experience a decline in overall habitat area, leading further to reduced population numbers and increased fragmentation. As shown in **paper II**, conservation strategies targeting this problem should make use of landscape modelling techniques, as the genetic viability of Black Grouse was partially explained by landscape effects.

The same conclusions hold true for Black Forest Capercaillie. This metapopulation has now dramatically declined for years (Coppes et al. 2019). Populations numbers are low, potentially comparable with the situation of the Cantabrian Capercaillie (Tetrao urogallus cantabricus), which was recently assessed as 'Critically Endangered' by the Spanish administration (Jiménez et al. 2022). Preservation and increase of population size are obvious prerequisites for successful conservation, and annual survival and recruitment have been identified in several studies as main drivers for Capercaillie population dynamics (Jiménez et al. 2022 and references therein) and therefore present themselves as priority targets in conservation strategies. Yet, our simulations showed that even in the case of stable population numbers, genetic differentiation between subpopulation can increase, jeopardizing all conservation efforts and hence the metapopulation's long-term survival (paper III). We therefore argue for focusing on subpopulation connectivity, for the Black Forest Capercaillie as well as for all other comparable metapopulation systems. Enhancing gene flow by conservation actions targeting corridors and stepping stones between subpopulations can reduce or even stop the process of ongoing genetic differentiation. For the Black Forest specifically, connectivity to subpopulation North should be focused as this subpopulation showed the highest differentiation. Additionally, subpopulation North is partially resident in the Black Forest National Park, benefiting from enhanced conservation. So having the connectivity improved, subpopulation North could potentially emerge as a source population, significantly contributing to the metapopulation's viability.

In our human-dominated landscape, preservation of habitats is key. Habitats are however already fragmented and populations consequently small. As our simulations showed (**paper III**), already minor amounts of gene flow might be able to counteract genetic differentiation and subsequent effects of reproductive isolation. In order to preserve connectivity, information about habitat areas and unsuitable land in between is necessary. Conservation strategies should therefore address the following targets: 1) Further habitat loss and increase of distance between habitats must prevented. 2) Corridors between subpopulations must be defined, oftentimes containing stepping stone habitats (suitable areas that are too small to harbor self-sustaining populations but provide food resources and shelter for dispersing individuals). And 3), barriers to gene flow (direct or indirect) must be identified and removed. Ultimately, as large-scale recreation of suitable habitats for a wide range of species is

rather unlikely in our highly used landscape, the key for long-term conservation lies within the preservation and recreation of connectivity between subpopulations.

4.2. Parameterizing resistance surfaces

Parameterizing resistance surfaces is a crucial step in any landscape genetic analysis (Spear et al. 2016). Resistance surfaces are, however, used in several ways in landscape genetic analyses. A first distinction can be made by the use of resistance surfaces within landscape genetic studies: resistance surfaces are either used to 1) compare several resistance surfaces to find the best supported one and then infer conclusions based on that resistance surface or 2) used within the analytical pipeline to infer conclusions about something else. While the first approach is often used when testing for the effect of single variables on spatial genetic patterns, the second approach is found more often in studies addressing broader questions within conservation genetics. It is therefore obvious that there is no single optimal approach to parameterize resistance surfaces. Additionally to the study design, the species' biology and movement ecology as well as other various factors influence the decision about an approach to parameterize a resistance surface (Spear et al. 2010).

Once popular approaches, expert-based models (Epps et al. 2007; Shirk et al. 2010) were replaced by correlative models (Wang et al. 2008; Milanesi et al. 2017b). While ENMs still succeed in predicting occurrence of species (Lee-Yaw et al. 2021) – which is what there were developed for –, their adequacy in predicting resistances to movement is however more and more discussed (Keller et al. 2013). The underlying question comes down the drivers of a species' movement. Species might select their habitat based on different criteria than what drives their movement through the landscape. Birds are understandably difficult to tackle in landscape genetic studies and consequently under-represented (Kozakiewicz et al. 2018). The Black Grouse however is a ground based species and individual movement is believed to be highly driven by landscape characteristics, namely protection against predation and supply of food resources. Accordingly, land cover was proven to be the most important variable for movement in the closely related Capercaillie (Kämmerle et al. 2021). Although creating and testing a resistance surface for each of variable separately could have been possible and was common in the past decades, recent studies argue to create composite resistance surfaces (Peterman and Pope 2020). Thus, we calculated an exhaustive ENM, including all variables potentially relevant for Black Grouse movement. ENMs were also already successfully applied in Capercaillie to parameterize resistance surfaces (Milanesi et al. 2017a).

Resistance surfaces will remain key tools within landscape genetic studies. Advancements in computation as well as data generation (remote sensing, individual tracking data, etc.) however will allow for new parameterization approaches to emerge. Independently from the approach chosen, resistance surfaces must be translated into pairwise data to represent measures of connectivity between individuals and sites (Spear et al. 2016). Researchers can choose between several concepts, with the two most prominent ones probably being cost-distance approaches (like LCPs) and circuit theory approaches. While the choice of approach can be based on good assumptions, our results in **paper II** showed that unnoted factors might lead to wrong conclusions. Thus, decisions for approaches must be based on careful considerations and researchers might generally want to apply more than one approach.

4.3. Simulations enable analyses on temporal scales

In the current state of conservation genetics, good evidence on a population's genetic makeup can be achieved thanks to vast improvements on analytical methods and the combination of population genetics with landscape characteristics. Conservation strategies are often informed by results describing the status quo of populations. Hereby, spatial scale is acknowledged within most assessments. The temporal scale however has mostly been ignored so far. Repeated sampling over time allows at least for past to present comparisons and conclusions about trends and their potential causes, exceeding one-time analyses (Schwartz et al. 2007). Yet within conservation genetics, studies aim to conclude on conservation implications in the form of management actions (Holderegger et al. 2019). It seems obvious that conservation genetic studies therefore want to test for the potential longterm effects of such actions, something that can be achieved by using simulations. Simulations were traditionally applied in genetics as tools to model and understand molecular biological processes (Hoban et al. 2012). Hereby, simulations were often built to generate pseudo data under certain constrains and compare that pseudo data with real data to infer about underlying principles and processes. Besides, simulations were also used frequently in the evaluation of analytical methods, as authors were able to explore the effects of parameters or test statistical methods in their power to detect built in signals in the pseudo data (Hoban et al. 2012). More recently though, the advancement of methods now allows for seeding simulations with field data. Forward-in-time simulations can then be applied, projecting individuals into the future by using individual-, population- or species-specific demographic parameters, resulting in the demographic and genetic makeup of future generations (Hoban et al. 2012; Hoban 2014). By designing the simulations and parameters, researchers are able to basically simulate any given scenario.
In **paper III**, we were able to employ simulations to test the effect of migration rates between subpopulations on genetic differentiation of future generations. Our results showed that decreased migration rates between subpopulations result in increasing genetic differentiation between those subpopulations, as would be expected under genetic drift. However, we also found genetic differentiation between the other subpopulations being affected. This result clearly indicates that changes in migration between two subpopulations can have subsequent effects on the whole metapopulation. Our study hereby provides important insights into the functionality of metapopulation systems and advances our understanding of such.

While this study was focused on genetic differentiation, also other genetic variables of a species' response can be simulated. E.g., in a recent study on Grey Wolf (*Canis lupus*), we simulated genetic diversity of an European subpopulation applying different scenarios of population dynamic parameters seeded with recently sampled genotypes (Institute of Wildlife Biology and Game Management IWJ 2022), using the simulation engine Vortex (Lacy and Pollak 2021). Besides their high potential, simulations are still not widely used in conservation genetics (Andrew et al. 2013), especially within studies focused on conservation strategies. Reasons are probably twofold:

First, in order to build a simulation, precise knowledge of the species demographic and population dynamic parameters are necessary. In order to simulate a realistic genetic response of individuals, simulations need to incorporate fully fledged population models. Therefore, age-based or stage-based models are often used, requiring demographic parameters for all ages/life stages and subpopulations (e.g. survival rates, reproduction rates, mating systems, etc.). As such data is sparse and mostly not available for species of conservation concern, models cannot be implemented easily. The recent advancement of using integrated population modeling (IPMs) however offers promising new opportunities. IPMs are now increasingly used in grouse (Rotelli et al. 2021; Jiménez et al. 2022). Their Bayesian nature allows for estimation of demographic parameters while accounting for stochasticity. As simulations of genetic parameters also want to be stochastic (to adequately model processes like genetic drift), such a Bayesian framework appears to be perfectly suited to be merged with genetics. Combining Bayesian IPMs with genetic simulation engines might therefore be a promising endeavor advancing conservation genetics as a whole.

Second, the limited accessibility and necessary skills to build simulations probably present major challenges (Hoban 2014). Although known for more than a decade, most simulation software still remains reserved to users with a relatively strong background in bioinformatics and basic scripting to build the simulations but also loop over several scenarios and hundreds of iterations given their stochastic nature (Parobek et al. 2017). With increasing quantity of data, simulations will however

become more and more important in conservation genetics. Testing for the effects of conservation actions presents itself as a promising tool within conservation management. Package-based solutions (for programming languages, e.g., Rmetasim, Strand 2002) offer great flexibility, but need good documentation for users to understand all parameters and functions. Contrary, stand-alone software (e.g. Vortex, Lacy and Pollak 2021) is easier to use but oftentimes limited in its applicability, given parameters and functions are predefined and user input is restricted. Developers of simulation software must therefore prioritize especially usability and documentation, to improve both their understandability as well as their applicability and prevent simulation software from becoming a 'black box'. As an example, the R package skeleSim (Parobek et al. 2017) is on the right track. In the near future, especially the prediction of effective population size (N_E) within simulations presents itself as a priority target given the importance of N_E as a parameter within conservation genetics (Wang et al. 2016; Hoban et al. 2021). Currently, prediction of N_E is only marginally implemented (Vortex for example is using a temporal N_E estimation which is based on simulated heterozygosity even when field data is supplied) and most package-based simulation engines still need to add methods of estimation of N_E. In the long term, genetic simulations will also probably be combined with more advanced approaches for modelling population dynamics (e.g. IPMs) and landscape effects, advocating further for complete documentation and usability (Epperson et al. 2010; Hoban 2014). In order for simulations to be used up to their full potential, some development still needs to be done - but once simulation software is more widely applicable, simulations will be an integral part of design and management of conservation strategies.

4.4. Conservation genetics in genomic times

With the advancement of methodologies, conservation genetics (henceforth including traditional genetic methods and genomic approaches) as a scientific field has moved into the genomic era. While underlying concepts and principles stay the same, new methods are developed at a fast pace. These offer a wide range of new opportunities, tackling old and new research questions (reviewed by Holderegger et al. 2019; Hohenlohe et al. 2021 and references therein). Comparisons of traditional and high-throughput (sometimes called next-generation) sequencing (HTS) methods tend to demonstrate a finer resolution of HTS results (Hunter et al. 2018), yet challenges remain and traditional methods (such as the analysis of tandem repeats, most prominent of which are microsatellites) still offer unmatched possibilities thanks to their unique characteristics. In a recent comparison between microsatellites and single nucleotide polymorphisms (SNPs), Zimmerman et al. (2020) found a generally comparable performance, with SNPs outcompeting microsatellites in

identifying groups within a North-American grouse species. However, SNPs were generated from blood samples – a source of DNA which is oftentimes not available for species of conservation concern due to sampling restrictions or the species' elusiveness. While recent advances look promising for future use of HTS on non-invasive samples (Carroll et al. 2018; Hohenlohe et al. 2021), currently their application is impeded by the overall higher costs and their complexity both in terms of laboratory work and analyses (Holderegger et al. 2019). In a current study applying double digest restriction-site associated DNA (ddRAD) sequencing on Capercaillie and Black Grouse samples, both invasively and non-invasively sampled, we aim at comparing results generated through HTS with results from traditional methods and assess the feasibility of HTS through ddRAD sequencing for conservation practice. While still ongoing, preliminary results emphasize the barrier to entry as library preparation is theoretically and practically more challenging than well-known, established PCR protocols and due to its technical requirements associated with higher overall costs. Correspondingly, analyses to generate reliable data require a profound understanding not only in molecular biology but also in bioinformatics, given most libraries to map or build loci de novo assume a strong background in various coding languages and easy-to-use stand-alone software is yet to be developed.

While HTS approaches will replace some traditional methods in many ways in the long term, traditional methods will see ongoing usage especially in the near future. Justification is manifold, as these methods are well established and come with a common consensus on analysis and interpretation, are easily applicable as infrastructure (facilities) exists and offer services at continuously reduced costs, are well-understood by an increasing body of practitioners and ultimately enable specific research questions due to their characteristics (e.g. the highly polymorphic nature of microsatellites) that won't be achievable otherwise. Although HTS methods are frequently discussed to potentially replace traditional methods, it is quite possible that there will rather be a synthesis. HTS and other new methods could be used to analyze traditional markers (i.e. microsatellites amplified by using a genotyping-by-sequencing pipeline, Curto et al. 2019) or both could be used in a complementary fashion in comprehensive research projects.

4.5. Informing practice: bridging an increasing gap

While conservation genetics has been rapidly evolving in the past century, its results, concepts and methods have not been adequately transferred into conservation practice and policy (Santamaría and Méndez 2012). Many fields remain in which the rapid progress of science paired with the complexity of the subject has led to ignorance or – even worse – misunderstanding of important genetic concepts, illustrated by examples like the correct estimation and interpretation of effective population size or

application of HTS approaches (Garner et al. 2020; Hohenlohe et al. 2021). Given the complexity of the more recent HTS methods both in terms of laboratory work and analyses (Holderegger et al. 2019), the gap between conservation science and practice is likely to increase with the advancement of genomic approaches on a short-term basis. Yet, sufficient transfer of knowledge is a prerequisite for scientific studies to contribute to society and challenges like the biodiversity crisis.

Attempts to bridge this gap are currently discussed within the conservation genetics community (e.g. in Hoban et al. 2020). Hohenlohe et al. (2021) present four recommendations, summarized in brief as: 1) the development of professional relations between conservation geneticists and practitioners, 2) the guidance of conservation genetics by management questions, 3) improved training for aspiring students and long-term practitioners and 4) the development of streamlined methods to reduce the barrier of entry.

Following these recommendations, important advances can be achieved by adapting conservation genetic study design. However, two rather systemic problems will continue to contribute to the gap. On the one hand, academic researchers are currently barely incentivized to engage in conservation practice (Shafer et al. 2015) as funding as well as employment opportunity are oriented towards scientific performance rather than real-world impact, while on the other hand conservation practitioners are oftentimes not trained geneticists and do not command over time and resources to educate themselves (Holderegger et al. 2019; Garner et al. 2020). Consequently, the translation of conservation genetic science into practice and policy is of high relevance and should be naturally considered as a critical step within conservation genetic research. Whereas traditional science oftentimes ends with scientific publications – maybe including a chapter on conservation recommendations – conservation geneticists now need to acknowledge the transfer of results as a natural milestone within conservation research.

4.6. Final conclusions

In the light of the current biodiversity crisis, conservation genetics as an interdisciplinary field is needed more than ever. Results and insights from mission-driven conservation genetic studies are key to the achievement of sustainable development and especially SDG 15 'Life on Land'. By focusing on umbrella species, conservation actions can be valuable additions to preserve genetic diversity within species and biodiversity as a whole. As such, all parts of this dissertation were directly informed by management needs of conservation practitioners (as is integral for mission-driven sustainability research, International Science Council 2021). Newly developed approaches (e.g. the simulation engine or the analyses of isolation by resistance) were combined with well-established methods to lower the barrier and enable understanding of results and implementation of management strategies based on those results.

However, with the technological advancement and increasing complexity of methods, the gap between science and practice is prone to become larger. It should therefore be a key task for any conservation geneticist to focus on transferring conservation genetic insights and results into conservation practice and policy. Knowledge transfer should be viewed as an integral milestone of conservation genetic studies, and research must not end with a paper published. Accordingly, study design, results and interpretations were transferred into conservation practice and policy, making use of science-to-professional talks, magazines, workshops or trainings. While technological advancement is crucial and certainly promising, the solution we are looking for to bridge the gap is not made of results or textbooks, but the cooperation between people working within conservation (science as well as practice and policy) themselves.

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6. Publications

Paper I

Sittenthaler M, Kunz F, Szymusik A, Grünschachner-Berger V, Krumböck S, Stauffer C, Nopp-Mayr U (2018). **Fine-scale genetic structure in an eastern Alpine black grouse** *Tetrao tetrix* **metapopulation**. Journal of Avian Biology 49:e01681. <u>https://doi.org/10.1111/jav.01681</u>

Paper II

Kunz F, Klinga P, Sittenthaler M, Schebeck M, Stauffer C, Grünschachner-Berger V, Hackländer K, Nopp-Mayr U (2022). Assessment of drivers of spatial genetic variation of a ground-dwelling bird species and its implications for conservation. Ecology and Evolution 12: e8460. https://doi.org/10.1002/ece3.8460

Paper III

Kunz F, Kohnen A, Nopp-Mayr U, Coppes J (2021). **Past, present, future: tracking and simulating** genetic differentiation over time in a closed metapopulation system. Conservation Genetics 22:355– 368. <u>https://doi.org/10.1007/s10592-021-01342-5</u>

6.1. Paper I

Fine-scale genetic structure in an eastern Alpine black grouse Tetrao tetrix metapopulation

Sittenthaler M, Kunz F, Szymusik A, Grünschachner-Berger V, Krumböck S, Stauffer C, Nopp-Mayr U (2018). Journal of Avian Biology 49:e01681. <u>https://doi.org/10.1111/jav.01681</u>

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Article

Fine-scale genetic structure in an eastern Alpine black grouse *Tetrao tetrix* metapopulation

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Understanding genetic consequences of habitat fragmentation is crucial for the management and conservation of wildlife populations, especially in case of species sensitive to environmental changes and landscape alteration. In central Europe, the Alps are the core area of black grouse Tetrao tetrix distribution. There, black grouse dispersal is limited by high altitude mountain ridges and recent black grouse habitats are known to show some degree of natural fragmentation. Additionally, substantial anthropogenic fragmentation has occurred within the past ninety years. Facing losses of peripheral subpopulations and ongoing range contractions, we explored genetic variability and the fine-scale genetic structure of the Alpine black grouse metapopulation at the easternmost fringe of the species' Alpine range. Two hundred and fifty tissue samples and non-invasive faecal and feather samples of eleven a priori defined subpopulations were used for genetic analysis based on nine microsatellite loci. Overall, eastern Alpine black grouse show similar amounts of genetic variation ($H_0 = 0.65$, $H_F = 0.66$) to those found in more continuous populations like in Scandinavia. Despite of naturally and anthropogenically fragmented landscapes, genetic structuring was weak (global $F_{ST} < 0.05$), suggesting that the actual intensity of habitat fragmentation does not completely hamper dispersal, but probably restricts it to some extent. The most peripheral subpopulations at the edge of the species range show signs of genetic differentiation. The present study gives new insights into the population genetic structure of black grouse in the eastern Alps and provides a more fine-scale view of genetic structure than previously available. Our findings will contribute to monitor the current and future status of the population under human pressures and to support supra-regional land use planning as well as decision making processes in responsibilities of public administration.

Keywords: microsatellites, population structure, conservation genetics, habitat fragmentation, gene flow, Austria



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Introduction

Conservation and management of landscapes, wildlife populations and species can be improved by studying the genetic diversity, structure, and the extent of fragmentation of wildlife populations (Frankham 2003). Fragmentation and loss of wildlife habitats may reduce gene flow, leading to lower genetic diversity, higher inbreeding rates and genetic drift (Höglund et al. 2007, Bech et al. 2009). Gene flow among populations is mostly determined by the dispersal capability of a species, by landscape connectivity and environmental conditions. Lack of gene flow may lead to reproductive isolation, and may reduce the potential of populations to adapt to environmental changes. In the worst scenario this may lead to extinction on the long term (Westemeier et al. 1998, for a review see Keller and Waller 2002, Frankham 2005, Keyghobadi 2007, Frankham et al. 2010). Populations composed of disconnected subunits are more prone to extinction than larger, continuously distributed populations. Connectivity among fragmented habitat patches is important for population persistence, especially in a metapopulation system, where small peripheral subpopulations are considered to be more susceptible to isolation from larger central subpopulations (Frankham et al. 2010, Bush et al. 2011). Understanding the genetic consequences of habitat fragmentation and habitat loss is particularly important in case of species sensitive to environmental and landscape changes and having limited dispersal capacities.

The black grouse Tetrao tetrix is a galliform species with a distribution range from Great Britain to Siberia (BirdLife International 2016). It is listed in Annex I and II of the EU Birds Directive (Directive 2009/147/EC). In central Europe, core areas of black grouse distribution are in the Alps (Glutz von Blotzheim et al. 1973, Klaus et al. 1990), where the species mainly inhabits the treeline ecotone (Sachser et al. 2017). According to Storch (2007a), black grouse might be taken as an indicator species of this ecotone. The elevational zone of the tree line (Körner 2003) frequently provides a mixture of open habitat patches and patches covered by woody plants. The resulting edge effects reduce locomotive efforts of black grouse to satisfy its' feeding, resting, and cover demands (Hannon and Martin 2006, Patthey et al. 2012, Schweiger et al. 2012, Sachser et al. 2017). Dwarf shrubs (particularly Ericaceae) are important food sources for black grouse, either being directly consumed by adults or offering arthropod biomass for juveniles (Wegge and Kastdalen 2008, Signorell et al. 2010, Patthey et al. 2012). Apart from nutritional aspects, dwarf shrubs provide shelter from aerial (Aquila chrysaetos, Accipiter gentilis) and terrestrial predators (Vulpes vulpes), particularly for hens during the time of chicken rearing. Woody plants that overtop the snow layer in winter act as food sources and shelter as well. However, a dense closure of the tree canopy is avoided by the species (Rotelli 2004, Patthey et al. 2012, Schweiger et al. 2012, Immitzer et al. 2014, Sachser et al. 2017). Open habitat patches on convex parts of the terrain are preferred lekking sites, where males display on arenas that

are visited by females. The species shows a sex-biased dispersal with philopatry of males and natal dispersal of (young) females (Caizergues and Ellison 2002, Corrales and Höglund 2012). On average, female dispersal occurs over distances of 8 km (Willebrand 1988, Caizergues and Ellison 2002, Warren and Baines 2002, Marjakangas and Kiviniemi 2005). Compared to other alpine galliform species with overlapping niches like capercaillie *Tetrao urogallus* or rock ptarmigan *Lagopus muta helvetica*, black grouse shows an intermediate dispersal capability: average juvenile dispersal distances of 5 km have been reported for capercaillie females (Storch 1993, Beshkarev et al. 1995) and 18 km for rock ptarmigan females (Novoa et al. 2005, Bech et al. 2009).

Black grouse dispersal seems to be limited by high altitude mountain ridges (Caizergues and Ellison 2002) as the birds preferrably move along similar elevations. Consequently, recent black grouse habitats are assumed to show some degree of natural fragmentation and isolation in the Alps. Together with this natural fragmentation, changes in land use drive the amount and distribution of available habitats for the species. Abandonment of Alpine pastures (Groier 2010) and subsequent successional processes together with impacts of climate change on plant community distribution (Theurillat and Guisan 2001, Gehrig-Fasel et al. 2007) result in a loss of open habitats and in altitudinal shifts of the tree line ecotone. Schaumberger et al. (2006) and Zurell et al. (2012) modelled potential effects of climate change with its complex interplay of demographic processes and habitat availability and they predicted distinct range contractions of black grouse in the future. Within the modelled scenarios, Schaumberger et al. (2006) calculated a loss of 98% of well-suited black grouse habitats due to climate change in an Alpine Austrian study area. Furthermore, suitable habitats within the Alps become increasingly separated by human settlements, agricultural areas, expanding skiing areas, wind power facilities and other human activities (Ingold 2005, Arlettaz et al. 2007, Immitzer et al. 2014, Coppes et al. 2017).

Considering this ongoing habitat fragmentation and the limited dispersal capabilities of this species, the Alpine black grouse population is hypothesized to comprise several interconnected subpopulations. These subpopulations are partially separated by more than 10 km and thus form a metapopulation system (Storch and Segelbacher 2000, Höglund et al. 2007). Within this metapopulation, black grouse are decreasing in several Alpine countries (e.g. France), but are assumed to be stable in numbers at a national scale in Austria (Storch 2007b, BirdLife International 2016). However, considerable population declines and extinction of local occurrences took place at smaller scales, particularly at the Austrian eastern Alpine edge of black grouse distribution (Wöss and Zeiler 2003, Nopp-Mayr and Grünschachner-Berger 2011). Facing these losses and ongoing range contractions of the species, reduced effective dispersal as well as pronounced genetic structuring could be expected, which could derive either from isolation by distance (IBD; Wright 1943) or from barriers to gene flow.

On a Europe-wide scale, Höglund et al. (2007) already compared genetic diversity of continuous, contiguous, and isolated black grouse populations. Small and isolated lowland populations (Larsson et al. 2008) significantly differed from contiguous and continuous populations in terms of standard genetic diversity measures. Caizergues et al. (2003) provided evidence for fragmentation in contiguous populations in the Alps, as shown for south-western Alpine black grouse occurrences. Similar results have been found for Alpine capercaillie: genetic differentiation increased along a gradient of connectivity from continuous populations to metapopulation systems to isolated populations (Segelbacher and Storch 2002, Segelbacher et al. 2003). Whereas ecological niche models (Sachser et al. 2017) and larger scale habitat distribution models (Grünschachner-Berger 2013) have been developed for black grouse populations in the northern and eastern Alps, little is known about population genetic structure there.

Determining genetic diversity and genetic population structure is essential for managing declining peripheral populations in fragmented landscapes. To gain further insight into the fine-scale genetic structure of the Alpine black grouse metapopulation system, we conducted a genetic analysis of Austrian black grouse samples using polymorphic microsatellite markers. Our study encompasses the easternmost edge of the species' Alpine range, an area that has suffered from substantial anthropogenic fragmentation in the past ninety years (e.g. abandonment of Alpine pastures, expansion of human settlements and infrastructures, increased recreational use; Wöss and Zeiler 2003, Nopp-Mayr and Grünschachner-Berger 2011). The aims of our study were 1) to evaluate the genetic diversity of black grouse along the eastern edge of the Alpine population and to compare the genetic variability of different mountain ranges, 2) to describe the extent of fragmentation by addressing the amount of population differentiation among and within mountain ranges and 3) to explore local population structuring within the Alpine metapopulation focusing on a relatively small scale in the eastern Alps.

Based on our results, we build up a reference data set for future monitoring of changes in genetic diversity and population structure of black grouse within the Alpine population. Finally, we discuss implications for the conservation and the future situation of black grouse in the Alps.

Material and methods

Study population and sampling localities

We conducted our study in Austria, where black grouse mainly occurs in the Alps at the treeline belt and on adjacent alpine meadows at an altitude of approximately 1000–2500 m a.s.l. We subdivided the eastern Alpine black grouse occurrence into eleven subpopulations (mountain ranges) (Fig. 1 and Table 1) and assigned our sampling locations accordingly. The classification of subpopulations was based on the following key factors: 1) topographic separation of black grouse habitats (like separation by large valleys or high mountain ridges), which may function as potential barriers (Caizergues and Ellison 2002); 2) average black grouse dispersal distances of juvenile females of ca 8 km addressing female biased dispersal in grouse species (Willebrand 1988, Caizergues and Ellison 2002); and 3) output of an expert-based species distribution model (Grünschachner-Berger 2013).

Sample collection in the field and sample storage

For genetic analyses, we collected a total of 250 samples invasively and non-invasively. We received muscle tissue samples from 106 male black grouse shot during the hunting season of the years 2013, 2015 and 2016. Additionally, we collected 132 fresh (≤ 5 d old) faeces (intestinal droppings) and 12 feathers in the field during winter and lekking season in 2015 and 2016. Collection of faecal samples took place within 5 d after snowfall. Muscle tissue was stored in ethanol (> 96%), feathers were stored in paper envelopes at room temperature, and faeces were kept frozen at -20° C until DNA extraction.

DNA extraction and microsatellite genotyping

DNA of muscle tissue and feather samples was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich). With feathers, the tip of the calamus and the superior umbiculus (Horváth et al. 2005) were cut into small pieces and homogenised in a ball mill before digestion. For feather samples digestion was extended to 24 h. Faecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with slight modifications: a fragment of each dropping (about the size of 1.0–1.5 cm) was first incubated overnight at 55°C in 4 ml ASL buffer before adding the InhibitEX tablet. Secondly, we increased the final incubation time to 5 min before elution of the DNA extract with 2 × 70 µl AE buffer.

Samples were genotyped at nine di- and tetra-nucleotide microsatellite loci (Supplementary material Appendix 1 Table A1): TUT1, TUT2, TUT3 and TUD6 (Segelbacher et al. 2000); BG6, BG15, BG18, BG19 (Piertney and Höglund 2001). The microsatellites were amplified in three multiplex reactions (reaction volume 10 µl), containing 1 µl template DNA, 5 µl SuperHot Mastermix (Genaxxon, Germany), 1.6 pmol of each primer and 0.1 µg µl⁻¹ Bovine Serum Albumine (BSA, Sigma-Aldrich, USA). Amplifications were done using an initial denaturation for 15 min at 95°C followed by 38 cycles of 30 s at 95°C, 1 min at annealing temperature, 1 min at 72°C, and a final extension step for 30 min at 72°C. For microsatellites TUD6, BG6 and BG19, annealing temperature was at 57°C, for BG15, BG16 and BG18 at 53°C, and for TUT1, TUT2 and TUT3 at 60°C.

For sex identification of non-invasively collected samples, we amplified a fragment of the chromo-helicase-DNA-binding (CHD) gene with the primer pair 1237L and 1272H (Kahn et al. 1998). The PCR was set up in 10 µl volumes containing 1 µl DNA, 0.05 u µl⁻¹ peqGOLD Taq-DNA-Polymerase (peQLab, Germany), 3 pmol of each primer,

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Figure 1. Sample locations represented by black dots and a priori delineated *Tetrao tetrix* subpopulations (black outlined polygons). Grey shaded areas represent black grouse habitat distribution within the Austrian federal state Styria (Grünschachner-Berger 2013). Abbreviation of subpopulations: LIN=Liezen North, HSW=Hochschwab West, HSS=Hochschwab South, OSW=East Styria/Wechsel, KOR=Koralm, GLS=Gleinalm/Stubalm, ZIO=Zirbitz East, ZIW=Zirbitz West, TUR=Turrach, TAU=Tauern, AUS=Aussee; insets show the European distribution of black grouse (BirdLife International and Handbook of the Birds of the World 2016) and the study area location in Europe (black rectangle) and Austria.

Table 1. Summary of genetic diversity indices of *Tetrao tetrix* samples (n = 195) genotyped at nine microsatellite loci for putative subpopulations and for inferred genetic clusters and admixed subpopulations: number of unique genotypes (*N*), number of males/females (M/F), mean number of alleles per locus (N_A), allelic richness (A_B), private allelic richness (A_e), observed and expected heterozygosity (H_{CY} H_E), inbreeding coefficient (F_{IS}). Significant deviations from Hardy–Weinberg proportions are marked in bold (considering Benjamini–Yekutieli FDR for multiple testing). P_e : population estimates (Grünschachner-Berger unpubl.).

Subpopulation/cluster	ID	P_{e}	Ν	M/F	N_A	A_{R}	A_P	H_{o}	$H_{\scriptscriptstyle E}$	F_{IS}
Aussee	AUS	1.200	7	7/0	4.8	3.8	0.37	0.65	0.66	0.02
Liezen North	LIN	450	5	4/1	3.9	3.5	0.14	0.69	0.63	-0.09
Hochschwab South	HSS	925	13	12/1	5.2	3.7	0.14	0.76	0.71	-0.07
Hochschwab West	HSW	925	13	9/4	5.2	3.8	0.09	0.68	0.70	0.02
Tauern	TAU	6.850	56	56/0	7.5	3.8	0.14	0.66	0.69	0.04
East Styria/Wechsel	OSW	400	41	25/16	6.1	3.5	0.16	0.60	0.64	0.07
Turrach	TUR	850	4	4/0	3.7	3.7	0.15	0.75	0.69	-0.08
Zirbitz West	ZIW	225	11	2/9	4.4	3.3	0.05	0.51	0.58	0.12
Zirbitz East	ZIO	225	7	4/3	4.7	3.8	0.08	0.59	0.68	0.12
Gleinalm/Stubalm	GLS	700	23	18/5	5.8	3.6	0.05	0.62	0.68	0.09
Koralm	KOR	150	15	10/5	4.7	3.2	0.05	0.61	0.62	0.03
Cluster 1 (green)	AUS/LIN/HSS/TAU/TUR	_	85	83/2	8.4	5.4	0.47	0.68	0.70	0.03
Cluster 2 (yellow)	OSW	_	41	25/16	6.3	4.8	0.30	0.61	0.65	0.06
Cluster 3 (red)	ZIO/GLS/KOR	-	45	32/13	7.1	5.3	0.31	0.60	0.68	0.12
Admixed 1	ZIW	-	11	2/9	4.7	4.6	0.11	0.57	0.61	0.05
Admixed 2	HSW	_	13	9/4	5.0	4.7	0.06	0.63	0.66	0.04

0.8 mM dNTP mix, 1 μ l 10 × reaction buffer S (peQLab, Germany), 0.1 μ g μ l⁻¹ BSA. Conditions for the PCR amplification were an initial denaturation for 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 45 s at 49°C, 1 min at 72°C and a final extension step for 10 min at 72°C.

Negative controls were included throughout the extraction procedure and PCR amplifications to check for possible contaminations. Furthermore, pre- and post-PCR pipetting was carried out in different laboratory rooms.

Fragment length analysis was performed on an ABI PRISM[®] 3130 automated DNA sequencer (Applied Biosystems, Foster City, USA) by a commercial provider. Alleles were analysed and scored manually using Peak Scanner 2.0 software (Applied Biosystems, Foster City, USA).

For feather and faecal samples a consensus genotype was determined following the multiple-tube approach by Navidi et al. (1992) and Taberlet et al. (1996). A heterozygote genotype was accepted when each of both alleles was recorded at least twice and a homozygote genotype after at least three independent replications of a single allele. Loci, which could not be scored after five PCR replicates were coded as missing values. Samples that did not amplify at more than six out of nine loci were excluded from further analysis.

Data analysis

Although we collected faeces and feathers over a wide area, we could not a priori exclude multiple sampling of one individual. Hence, we checked for matching pairs of genotypes and excluded duplicates from the data set. We calculated the probability of identity (P_{ID}) and the probability of identity for siblings ($P_{ID:ibc}$), which is a more conservative upper bound for the probability that two individuals share the same genotype (Taberlet and Luikart 1999, Waits et al. 2001), using the software GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

Prior to calculating diversity measures and analysing population structure, the data set was checked for errors, and tests for model assumptions (no linkage disequilibrium, Hardy–Weinberg proportions) were conducted. Using Micro-Checker 2.2.2 (Van Oosterhout et al. 2004) we checked our microsatellite data set for presence of stuttering, large allelic dropout and presence of null alleles. Deviations from Hardy–Weinberg proportions and linkage disequilibrium (*LD*) for each pair of loci were tested with Genepop 4.2 (Raymond and Rousset 1995, Rousset 2008) using the default Markov chain parameters (1000 step dememorisation, 100 batches, 1000 iterations per batch) followed by a correction method (false discovery rate FDR) (Benjamini and Yekutieli 2001, Narum 2006) for multiple testing calculated in R 3.3.2 statistic software (R Core Team).

Genetic variation, differentiation and population structure analysis

Genetic variation was quantified using standard summary statistics computed with the program GenoDive 2.0b23

(Meirmans and Van Tienderen 2004). Allele frequencies, mean number of alleles per locus (N_A) , observed (H_O) and expected (H_E) heterozygosity were calculated, as well as the inbreeding coefficient (F_{IS}) to examine the degree of inbreeding within the assumed subpopulations. Allelic richness (A_R) , i.e. an index of the number of alleles corrected for sample size using rarefaction, and the number of private alleles (A_P) corrected for sample size, were estimated using HP-RARE 1.1 (Kalinowski 2005).

We investigated population divergence conducting analyses both with and without predefined subpopulation assumptions. We conducted hierarchical analyses of molecular variance (Amova) in Arlequin 3.5.2.2 (Excoffier et al. 2005) to calculate the proportion of genetic variation within and between subpopulations. Genetic differentiation of a priori defined subpopulations was calculated by pairwise F_{ST} values and associated p values with the program GenoDive 2.0b23 (Meirmans and Van Tienderen 2004) running 10 000 permutations. Again, p values were adjusted for multiple testing using Benjamini–Yekutieli FDR.

We used Structure 2.3.4 (Pritchard et al. 2000) to perform Bayesian clustering analyses to ascertain the number of genetic clusters (K). In a first step the admixture ancestry model with correlated allele frequencies was used, without any prior population information. Secondly, we used sampling locations as prior information applying the 'locprior' model (Hubisz et al. 2009) implemented in Structure. This model supports clustering of data sets with a population structure that is too weak to be found applying standard models (Hubisz et al. 2009). The initial burn-in period consisted of 200 000 iterations and the number of Markov chain Monte Carlo (MCMC) repetitions was 600 000. The Kvalue was set from 1 to 8 and 30 different runs were executed for each K. Considering unbalanced sampling, Structure analyses were run adopting the alternative ancestry prior suggested by Wang (2017). We applied Structure Harvester Web ver. 0.6.94 (Earl and vonHoldt 2012) to determine the most likely number of clusters by calculation of delta K proposed by (Evanno et al. 2005) and as estimated by mean log likelihood LnP(K). Structure results were post processed (summation and comparison of multiple runs across K values) and plotted graphically with Clumpak (Kopelman et al. 2015). Individuals with mixed ancestry, which could not clearly be assigned to one of the clusters, were re-run with individuals from each of the probable clusters to confirm their assignment and grouped accordingly for further analyses. The output of clustering analysis for each predefined sampling area/subpopulation was visualized by calculating the average assignment values per cluster (Fig. 2).

To infer genetic clusters incorporating both allele frequencies and a spatial statistical model, a Geneland 4.0.6 (Guillot et al. 2005a, b) analysis implemented in R statistic software (R Core Team) was performed. We ran the uncorrelated and correlated frequency model and allowed the number of clusters to vary between 1 and 8 in 20 independent runs, each with 10⁴ iterations and a thinning of 100. An uncertainty of sampling coordinates of 1 km was assumed.

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Figure 2. Bayesian clustering analysis implemented in Structure for K=2 and K=3 of 195 individual *Tetrao tetrix* genotypes with an admixture model using the 'locprior' information. (a) Bar plot: each colour represents one cluster (*K*), vertical bars show proportional membership of individuals (grouped by putative subpopulations) to the corresponding cluster. Data shown are aggregated data from 30 independent runs. (b) Delta *K* values (black) and mean log likelihood scores meanLnP(K) (grey) for cluster 1–8 to find the most likely number of clusters. (c) Map of study area showing the average proportional cluster membership of each putative *Tetrao tetrix* subpopulation (black outlined polygons) as revealed by Structure analysis for K=3.

Black grouse frequently show home-ranges of 200 up to 500 ha (depending on habitat quality, Klaus et al. 1990). One square kilometre is therefore a plausible order of magnitude to account for a potential spatio-temporal bias of sampling. The choice of K was based on the histogram of estimated clusters along the chain and the highest average posterior probability for each run.

Population genetic structure was further analysed applying a multivariate method, namely a discriminant analysis of principal components (DAPC) (Jombart et al. 2010). This approach does not require assumptions regarding Hardy– Weinberg equilibrium, linkage disequilibrium, or underlying population models (Jombart et al. 2010). DAPC first conducts a principal component analysis on the data, followed by a linear discriminant analysis on the retained principal components generating new synthetic variables: so called discriminant functions. Discriminant functions combine the most between group variation while within group variation is minimized (Jombart et al. 2010). The analysis was conducted with the package 'adegenet' 2.0.1 (Jombart 2008, Jombart and Ahmed 2011) using R statistic software (R Core Team). As prior information about group membership is necessary for conducting a DAPC, predefined subpopulations were used as groups.

Analogous to a priori defined subpopulations, we quantified genetic diversity, departures from Hardy–Weinberg proportions and genetic differentiation for each cluster inferred by clustering analyses (Table 1 and 2).

Isolation by distance, spatial autocorrelation

In case of presence of isolation by distance (IBD), an overestimation of genetic structure inferred by Bayesian clustering methods can occur (Schwartz and McKelvey 2009). To test if the genetic relationships among black grouse in our study are likely governed by IBD we tested for correlation between pairwise geographic and genetic distance among individuals by performing a Mantel test (Mantel 1967) with the program GenAlEx 6.5 (Peakall and Smouse 2006, 2012) using 9999 permutations. We used this program to estimate the spatial

Table 2. Pairwise F_{ST} comparisons (above the diagonal) and their corresponding p values (below the diagonal) for *Tetrao tetrix* clusters and admixed subpopulations inferred by cluster analysis. Significant values are set in bold (considering Benjamini–Yekutieli FDR for multiple testing).

Cluster	Cluster 1 (green)	Cluster 2 (yellow)	Cluster 3 (red)	Admixed 1 (ZIW)	Admixed 2 (HSW)
Cluster 1 (green)	•	0.016	0.009	0.011	0.042
Cluster 2 (yellow)	< 0.001	•	0.007	0.030	0.058
Cluster 2 (red)	0.001	0.028	•	0.016	0.030
Admixed 1 (ZIW)	0.041	0.002	0.030	•	0.016
Admixed 2 (HSW)	< 0.001	< 0.001	0.004	0.126	•

extent of genetic structure and dispersal by conducting a spatial autocorrelation analysis for 15 distance classes. We chose variable distance classes, because sample size was not evenly distributed across distances. We used 9999 random permutations to test the null hypothesis of random distribution of genotypes, and used 10 000 bootstrap iterations to estimate 95% confidence intervals around the autocorrelation coefficient r.

Results

Multilocus genotyping

For a total of 241 samples, a microsatellite profile was generated, resulting in an average genotyping success rate of 94.40%, with feather samples having the lowest rate (83.33%). With a probability of identity (P_{ID}) of 4.4×10^{-9} (nine loci) and 1.5×10^{-6} (six loci), and the probability of identity for siblings (P_{IDiibs}) of 5.6×10^{-4} (nine loci) and 5.9×10^{-3} (six loci), our set of microsatellites had enough power to distinguish between closely related black grouse individuals. Based on this, a total of 46 recaptures of 27 individuals could be identified. All recaptures matched at all nine loci and took place in the same respective sample area as the initial capture (data not shown). Finally, 195 unique genotypes (44 females, 151 males), which at least differed at two heterozygote loci, were considered for further population genetic analysis.

Population genetic analysis and genetic diversity

All nine microsatellite loci were polymorphic with allele numbers ranging from 3 to 15 per locus (mean = 8.67). After correction for multiple testing, within subpopulations only three, within genetic clusters only one of the 396 pairwise loci Fisher exact probability tests of deviation from genotypic equilibrium were significant. Overall, only one of 36 pairwise comparisons was significant. As loci involved differed across subpopulations, not indicating physical linkage between loci, these loci were retained for further analyses. Neither a large allele dropout nor stuttering were detected in the data set, but Micro-Checker detected the possible presence of null alleles at one locus (TUT1) in seven localities. The omittance of TUT1 did not change the conclusions of the results of further analyses, we therefore included the locus in our data set. For the same microsatellite locus deviations from Hardy-Weinberg proportions were detected in two putative subpopulations and three clusters after correcting for multiple testing and global tests indicated significant deviation from Hardy–Weinberg proportions probably due to the presence of null alleles at TUT1 (Supplementary material Appendix 1 Table A1). Relatively low F_{IS} values indicate random mating. The subpopulations and genetic clusters in the east (OSW) and some in the south (ZIW, ZIO, GLS, KOR) showed the highest F_{IS} values (Table 1). Mean observed heterozygosity (H_0) and expected heterozygosity (H_E) across all samples was 0.65 and 0.66, respectively. When separated into geographic subpopulations and genetic clusters, values of allelic richness and heterozygosity were relatively similar between the groups (Table 1). Number of private alleles per subpopulation and cluster was highest in the north-west.

Population structure and genetic differentiation

Running Structure without the 'locprior' information yielded no clear genetic distinction among any of the sampling sites (subpopulations) with all individuals being partially, but wellbalanced assigned to all clusters (data not shown). Using the 'locprior' model, we determined that the most likely number of genetic cluster was K=2 (delta K=6.04), but delta K was also increased for K=3 (delta K=5.95). Mean log likelihood values LnP(K) indicated K=2 and K=3 as the most likely number of clusters, thus we show both variants (Fig. 2b). Applying the alternative ancestry prior (Wang 2017) did not change the optimal number of K or cluster assignment.

On a larger scale, the genetic clusters are in accordance with the putative subpopulations: in case of 3 clusters, the first cluster (green) consisted of a group comprised of the northwestern subpopulations AUS, LIN, HSS, TAU and TUR; the second cluster (yellow) consisted of the single easternmost subpopulation (OSW); and the third cluster (red) consisted of the southernmost subpopulations KOR, GLS and ZIO. The remaining individuals of ZIW and HSW displayed admixed ancestry and could not clearly be assigned to one of the 2 or 3 clusters (Fig. 2a, c). Hierarchical STRUCTURE analysis did not resolve cluster assignments of these individuals sufficiently (data not shown), but they more likely group with the first cluster (green), rather than with individuals of OSW (yellow cluster). We treated them as extra (admixed) groups when calculating diversity measures and genetic differentiation of the genetic clusters (Table 1 and 2).

Running the uncorrelated frequency model in Geneland, we did not find any genetic distinction (K=1). Applying

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the correlated model, Geneland identified 6 clusters and the second most likely number of clusters was 5 (Fig. 3; Supplementary material Appendix 1 Fig. A1 and A2 and Table A3), which in principle align with the clustering found by Structure analysis: again, samples from sites AUS, LIN, TAU and TUR cluster together; in the northern area two additional clusters were built comprised of HSS, HSW and one TAU sample, although based on only small differences in membership probabilities (Supplementary material Appendix 1 Fig. A2). The easternmost area, OSW, again formed a delimited cluster and the orange cluster in the south of the study area was comprised of samples from KOR, GLS and some of ZIO, corresponding to the red cluster inferred by Structure. Individuals of ZIW and some individuals of ZIO subpopulation formed one additional cluster in the south. Overall, Geneland supported Structure results, presenting admixed subpopulations of Structure as additional clusters.

Scatterplots of the first principal components of the DAPC (Fig. 4) revealed highly overlapping ellipses, which corresponded to the putative subpopulations. This indicated weak genetic structure with the Tauern subpopulation (TAU) located in the centre. Again, the highest differentiation based on the first principal component was visible for the easternmost subpopulation (OSW) and the southernmost subpopulations (KOR, GLS, ZIW and ZIO), supporting results of the other clustering methods. The proportion of variance conserved by PCA principal components is 93.4%.

Analysis of molecular variance between subpopulations and genetic clusters showed highest genetic variation within individuals (93.6%), and a small but significant amount of genetic variation between subpopulations and clusters with 3.1 and 1.7%, respectively (Supplementary material Appendix 1 Table A2).

The degree of genetic differentiation between subpopulations and genetic clusters inferred by Structure and supported by Geneland was generally low, with F_{ST} values ranging from 0.00 to 0.12 and from 0.01 to 0.06, respectively. Global F_{ST} values were below 0.05 (Supplementary material Appendix 1 Table A2), meaning that only a small proportion of the genetic variation was due to differentiation among subpopulations and clusters. From 55 pairwise comparisons, 17 were significant after correction for multiple testing, and only 20 comparisons had a higher F_{ST} value than 0.05, indicating at least some degree of differentiation (Table 3) between subpopulations. For the genetic clusters, six out of ten pairwise comparisons had significant F_{ST} values.

The highest differentiation existed between birds of the northern localities, the southwestern localities, and black grouse individuals of the southeast (OSW).

Spatial genetic structure

A Mantel test showed a weak, but statistically significant IBD with a correlation coefficient for the geographic and genetic data of 0.049 ($R^2 = 0.002$, p = 0.005). Spatial autocorrelation among all individuals was significant (heterogeneity test p < 0.01). Over shorter distances (0 to 45 km) fine-scale spatial autocorrelation analysis showed a pattern of decreasing relatedness with increasing geographic distance. Within 5-, 15-, 25- and 45-km distance classes there was a significant correlation (p < 0.004; Fig. 5). At 51 km *r* reached a value of zero, indicating the approximate range of positive spatial genetic structuring (i.e. IBD) in our studied black grouse population. Until distance class 65 km, the relationship between *r* and distance was not significantly negative. In general, *r* values were quite small, indicating weak correlation between genetic and geographic distances.

Discussion

Overall, eastern Alpine black grouse show similar amounts of genetic variation and a similar degree of inbreeding to



Figure 3. Bayesian clustering analysis implemented in Geneland for K=6 of 195 individual *Tetrao tetrix* genotypes. Geneland map of cluster membership and map of study area including putative subpopulations and individual samples represented by coloured dots according to cluster assignment based on membership probabilities revealed by Geneland analysis.



Figure 4. Scatterplot of a discriminant analysis of principal components using a priori defined black grouse subpopulations as prior clusters. Labels indicate subpopulation names, colours represent genetic clusters inferred by Structure (green=cluster 1, yellow=cluster 2, red=cluster 3, grey=no clear assignment). Insets show the eigenvalues of the discriminant functions (DA) and the principal component analysis (PCA). X- and y-axes represent the first two principal components.

those found in black grouse in continuous populations in Scandinavia (Höglund et al. 2007, Corrales and Höglund 2012), in contiguous populations in northern Scotland (Höglund et al. 2011) and in other Alpine populations (Caizergues et al. 2003, Höglund et al. 2007, Larsson et al. 2008). Compared to isolated populations in western or central Europe (Larsson et al. 2008, Svobodova et al. 2011, Segelbacher et al. 2014), black grouse in the eastern Alps exhibit higher genetic diversity, which is comparable to Scandinavian populations. This high genetic variation may be explained by an admixture and maintenance of different lineages from different glacial refugia (Provan and Bennett 2008, Corrales et al. 2014) and by a historical connection of the north-eastern Alpine black grouse to the main range of the species (Caizergues et al. 2003, Corrales et al. 2014).

Within our study area, a slightly lower degree of genetic diversity and a higher inbreeding coefficient was found for the southern- and eastern-most subpopulations, representing the margin of the species' Alpine distribution range. As highlighted by Wöss and Zeiler (2003) and Nopp-Mayr and

Table 3. Pairwise F_{ST} comparisons (above the diagonal) and their corresponding p values (below the diagonal) for 11 putative *Tetrao tetrix* subpopulations. Significant values are set in bold (considering Benjamini–Yekutieli FDR for multiple testing).

Subpopulation ID ^a	AUS	LIN	HSS	HSW	TAU	OSW	TUR	ZIW	ZIO	GLS	KOR
AUS	•	0.017	0.016	0.022	0.000	0.039	0.015	0.063	0.010	0.007	0.032
LIN	0.166	•	0.026	0.037	0.005	0.026	0.063	0.052	0.044	0.035	0.083
HSS	0.153	0.073	•	0.015	0.012	0.040	0.064	0.094	0.050	0.012	0.056
HSW	0.075	0.029	0.077	•	0.020	0.041	0.041	0.066	0.021	0.010	0.058
TAU	0.444	0.321	0.051	0.003	•	0.030	0.023	0.050	0.020	0.011	0.032
OSW	0.008	0.090	< 0.001	< 0.001	< 0.001	•	0.102	0.060	0.035	0.028	0.073
TUR	0.220	0.027	0.004	0.038	0.080	< 0.001	•	0.123	0.058	0.056	0.087
ZIW	0.005	0.016	< 0.001	<0.001	< 0.001	< 0.001	0.004	•	0.002	0.067	0.099
ZIO	0.272	0.022	0.003	0.071	0.041	0.014	0.025	0.406	•	-0.002	0.018
GLS	0.264	0.027	0.076	0.120	0.013	< 0.001	0.008	< 0.001	0.526	•	0.010
KOR	0.038	0.005	< 0.001	< 0.001	< 0.001	< 0.001	0.006	< 0.001	0.143	0.105	•

^a Subpopulation ID corresponds to the putative subpopulation's geographical location as shown in Fig. 1.



Figure 5. Spatial autocorrelation coefficient (r) for 15 distance classes (in km) for 195 black grouse samples and 9 microsatellite loci. Solid line = r with error bars, dashed lines = 95% confidence interval for the null hypothesis of no existing spatial genetic structure.

Grünschachner-Berger (2011), distinct population declines and extinction of local occurrences have already taken place at this southeastern border of black grouse distribution. Pairwise F_{ST} values (global $F_{ST} < 0.05$) indicated only a slight to moderate genetic differentiation between the subpopulations. This was in line with the Amova yielding a very high proportion of variation within individuals (94%), compared to the variation found between subpopulations. In respect to high polymorphism and the use of multilocus genotypes, a F_{ST} value of 0.05 can already be interpreted as threshold for important genetic distinction (Balloux and Lugon-Moulin 2002, Bird et al. 2011). This threshold was exceeded by several pairs of subpopulations. Both Bayesian clustering methods and the multivariate approach with a DAPC further supported these results, suggesting an existing but weak structure. Moreover, some individuals (subpopulation HSW and ZIW) appeared admixed, which may be interpreted as gene flow occurrence. As shown in the DAPC plot, the Tauern subpopulation (TAU) lies in the center of all subpopulations, probably acting as an important connecting link in this metapopulation network.

Even though to a small extent, all analyses gave evidence that subpopulations on the edge of the species range (OSW, KOR, GLS, ZIO, ZIW) show first signs of genetic differentiation, most likely due to habitat fragmentation, followed by reduced effective dispersal and gene flow. Although Mantel test and spatial autocorrelation revealed a significant geographic structure of black grouse in our study area, only a very small portion of the differentiation could be explained by geographic distance. The genetic differentiation is thus likely the result of naturally caused fragmentation and/or fragmentation caused by human activities. Although we cannot quantify the contribution of human-induced vs natural fragmentation to the observed differentiation, there are some areas, where one factor seems to dominate. For example, it is highly probable that the adjacent areas ZIO and ZIW are mainly naturally separated by a high mountain ridge. The height of the ridge would not urgently mean a barrier in the central parts of the Austrian Alps, but obviously represents a natural barrier on the given location. In fact, the mountain ridge 'Zirbitz' is the highest elevation at the given pre-alpine surrounding. For other subpopulations, both natural and human-induced fragmentation seems to be relevant, e.g. for OSW, HSS, and GLS. These subpopulations a separated by large distances, by large river valleys with a dense network of human settlements and other human infrastructures and by distinct losses of available habitats (abandonment of alpine pastures, emergence of ski-lifts etc.).

The genetic composition and the geographic distribution of subpopulations suggest, that although Alpine black grouse live in naturally and anthropogenically fragmented landscapes, the actual degree of fragmentation does not completely hamper dispersal, but probably restricts it to some extent. According to Wright (1964), one effective disperser per generation is theoretically necessary to prevent higher degrees of population differentiation. As stated by Caizergues and Ellison (2002), black grouse move along suitable habitat patches, exceeding maximum distances of 20 km in some cases, and they cross valleys by traversing low mountain passes. Therefore, the unexpected low degree of differentiation in Alpine black grouse may be attributed to a stepping stone model of dispersal as described by Kimura and Weiss (1964). This was also suggested by Corrales and Höglund (2012) for a continuously distributed black grouse population in northern Sweden and for Alpine capercaillie (Segelbacher and Storch 2002). For the Cantabrian capercaillie, Fameli et al. (2017) did not find distinct genetic structuring despite of anthropogenic fragmentation of the area as well, suggesting maintenance of connectivity. The spread of genes obviously exceeds the assumed dispersal capabilities of black grouse, spanning from 5 to 29 km for females, which is the farther dispersing sex in black grouse, and reaching up to 8.5 km for males (Caizergues and Ellison 2002, Warren and Baines 2002). In the Czech Republic, Svobodova et al. (2011) found relatively high population differentiation (with F_{ST} values up to 0.23) between neighboring mountain ranges, indicating little dispersal between them. This could be a future scenario for black grouse in the Alps, if stepping stones

will not be conserved and further established. As shown for a Central European capercaillie population (Segelbacher et al. 2003), formerly connected populations are now genetically isolated and reduced in population size and consequently exhibit lower levels of genetic diversity. This could lead to an irreversible extinction vortex (Gilpin and Soulé 1986).

The exchange between neighboring areas through a dense network of stepping stones across the landscape should allow for effective dispersal even in presence of substantial fragmentation. It should be maintained and further promoted, predominantly, where genetic differentiation is already measurable. In our case, this is particularly important for the most peripheral occurrences of black grouse in the south and east of our study area. For future perspectives, location of dispersal corridors, dispersal time and tolerance towards different kinds of human fragmentation and disturbance need to be identified more explicitly (Immitzer et al. 2014, Coppes et al. 2017). Projects, which potentially further contribute to the isolation of remaining black grouse occurrences should be planned and evaluated carefully. This could be done at first by considering the output of our study in supra-regional land use planning and in creating preclusion maps for building projects. These maps should be available for project solicitors applying for the approval of planned projects. In a second step, such land plans should be used for decision making processes in the responsibilities of public administrations. Although planned projects have currently to be subjected to environmental impact assessments and their approval by public administrations depends on their virtual impact, genetic consequences have largely not been considered as referring data were lacking.

Interpreting current genetic patterns, the time lag between genetic effects and processes of habitat fragmentation should be considered. Fragmentation and isolation of subpopulations might have occurred too recently to be detectable using molecular genetics. In this case, the degree of genetic structure and the high genetic diversity presented in our study might not capture actual consequences of fragmentation. According to Frankham et al. (2010), genetic effects might be detectable only after a few generations when using neutral genetic markers. As habitat fragmentation is an ongoing process, it may be difficult to relate delayed genetic effects to different measures that have caused habitat fragmentation. Analysis of historic samples, e.g. museum specimens from the same area would provide information on earlier genetic diversity and structure and they would allow for an evaluation of recently gained data, as it was done for black grouse in the Netherlands (Larsson et al. 2008), northern Germany and Denmark (Segelbacher et al. 2014), and Ukraine (Pavlovska and Höglund 2015). Recovery of genetic variation is a remarkably slow process. Loss of genetic diversity has a longlasting effect, which is hardly reversible. We thus strongly recommend the maintenance of an intact stepping stone network and the set-up of a monitoring scheme to observe ongoing fragmentation processes. Moreover, latest trends of population isolation and its effects on genetic composition should be assessed regularly (Höglund 2009).

The present study gave new insights into the population genetic structure of black grouse in the eastern Austrian Alps and it provides a more fine-scale view of genetic structure than previously available. Nevertheless, a larger study encompassing the entire range of the species in the Alps is necessary to broaden our knowledge on this species and its conservation.

Our results should be interpreted as first warning signals and they should be used as a baseline for population genetic monitoring for black grouse in the eastern Alps and other Alpine populations. This would help to monitor the current and future status of the black grouse population under human pressure, to support supra-regional land use planning as well as decision making processes in the responsibility of public administration.

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Supplementary material (Appendix JAV-01681 at <www. avianbiology.org/appendix/jav-01681>). Appendix 1.

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Supplementary material

Supplementary material - Appendix 1

Table A1 Genetic diversity statistics of black grouse samples (N=195) genotyped at 9 microsatellite loci: number of alleles per locus (N_A), allele ranges (in basepairs), observed and expected heterozygosity (H_O , H_E), inbreeding coefficient (F_{IS}), deviations of Hardy-Weinberg proportions (*HW*) are marked with an asterisk (p values adjusted for multiple testing using Benjamini-Yekutieli FDR).

Locus	N _A	Allele ranges	Ho	H _E	F _{IS}	HW
TUT1	9	205-237	0.55	0.81	0.32	*
TUT2	3	132-144	0.25	0.24	-0.02	ns
TUT3	7	154-178	0.73	0.72	-0.01	ns
BG15	12	164-208	0.78	0.72	-0.08	ns
BG16	7	148-172	0.69	0.70	0.02	ns
BG18	10	143-183	0.86	0.82	-0.05	ns
TUD6	7	183-197	0.72	0.69	-0.04	ns
BG19	8	158-190	0.48	0.50	0.04	ns
BG6	15	188-288	0.76	0.75	-0.01	ns
overall	8.67		0.65	0.66	0.02	*

Table A2 Analysis of molecular variance (*AMOVA*) for black grouse samples. Two structures were analysed: (1) clusters inferred by cluster analyses, (2) putative subpopulations.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	p value	Fixation index
(1)						
Between clusters	4	26.56	0.051	1.68	<0.001	$F_{ST} = 0.017$
Between individuals within clusters	190	594.54	0.143	4.70	<0.001	$F_{IS} = 0.048$
Within individuals	195	554.50	2.843	93.62	<0.001	$F_{IT} = 0.064$
Total	389	1,175.59	3.037			
(2)						
Between subpopulations	10	61.60	0.095	3.14	<0.001	$F_{ST} = 0.031$
Between individuals within subpopulations	184	559.49	0.098	3.24	0.013	$F_{IS} = 0.033$
Within individuals	195	554.50	2.843	93.61	<0.001	$F_{IT} = 0.064$
Total	389	1,175.59	3.037			



Figure A1 Graphical output of GENELAND analysis to choose the best number of clusters of 195 black grouse samples.

Pun	Number of	Average log posterior
NUII	populations	probability
00	0	4005 750
20	6	-1935.756
13	6	-1953.885
16	6	-1963.969
4	6	-1973.352
6	6	-1993.062
18	6	-2043.045
12	6	-2045.508
8	6	-2059.959
9	6	-2070.438
3	6	-2086.643
7	6	-2103.621
1	6	-2122.914
5	6	-2128.190
14	6	-2183.804
11	5	-2205.300
2	6	-2206.328
17	6	-2213.829
15	5	-2236.006
19	5	-2294.664
10	5	-2323.737

Table A3 GENELAND runs and their average log posterior probability for inference of optimal number of clusters.



Figure A2 Posterior probability of cluster membership for the most likely number of clusters K=6 and K=5 inferred from GENELAND analysis.
6.2. Paper II

Assessment of drivers of spatial genetic variation of a ground-dwelling bird species and its implications for conservation

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>> pages 62:78

RESEARCH ARTICLE

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Assessment of drivers of spatial genetic variation of a grounddwelling bird species and its implications for conservation

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Abstract

In modern wildlife ecology, spatial population genetic methods are becoming increasingly applied. Especially for animal species in fragmented landscapes, preservation of gene flow becomes a high priority target in order to restore genetic diversity and prevent local extinction. Within Central Europe, the Alps represent the core distribution area of the black grouse, Lyrurus tetrix. At its easternmost Alpine range, events of subpopulation extinction have already been documented in the past decades. Molecular data combined with spatial analyses can help to assess landscape effects on genetic variation and therefore can be informative for conservation management. Here, we addressed whether the genetic pattern of the easternmost Alpine black grouse metapopulation system is driven by isolation by distance or isolation by resistance. Correlative ecological niche modeling was used to assess geographic distances and landscape resistances. We then applied regression-based approaches combined with population genetic analyses based on microsatellite data to disentangle effects of isolation by distance and isolation by resistance among individuals and subpopulations. Although population genetic analyses revealed overall low levels of genetic differentiation, the ecological niche modeling showed subpopulations to be clearly delimited by habitat structures. Spatial genetic variation could be attributed to effects of isolation by distance among individuals and isolation by resistance among subpopulations, yet unknown effects might factor in. The easternmost subpopulation was the most differentiated, and at the same time, immigration was not detected; hence, its long-term survival might be threatened. Our study provides valuable insights into the spatial genetic variation of this small-scale metapopulation system of Alpine black grouse.

KEYWORDS

conservation genetics, ecological niche modeling, isolation by distance, isolation by resistance, *Lyrurus tetrix*, maximum likelihood population effects (MLPE) models

Florian Kunz and Peter Klinga: Equally contributing first authors.

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1 | INTRODUCTION

Spatial population genetic methods are increasingly used in modern wildlife ecology and conservation. Particularly for species in fragmented landscapes, maintaining gene flow is of high relevance to preserve genetic diversity and minimize extinction risks of populations and species (Frankham et al., 2010). Various frameworks and concepts can be applied to identify the spatial distribution of genetic data (Sexton et al., 2014; Wagner & Fortin, 2013; Wang & Bradburd, 2014) and are the basis to understand the structure of populations and infer management strategies. Isolation by distance (IBD) describes the positive relationship between genetic differentiation and geographic distance (usually driven by a species' dispersal; Wright, 1943), a pattern commonly observed in panmictic populations (Sexton et al., 2014). However, the spatial genetic structure of wildlife species can be affected by several co-occurring factors and processes beyond Euclidean distances (Balkenhol et al., 2016). Therefore, the concept of isolation by resistance (IBR) is of particular interest in wildlife conservation genetics (McRae, 2006). IBR describes the relationship between genetic differentiation and landscape resistance and can be affected by various factors hindering the chance of migration and dispersal through the environment (Wagner & Fortin, 2013; Wang & Bradburd, 2014). Apart from intrinsic, species-specific drivers such as dispersal strategies (Corrales & Höglund, 2012; Lampert et al., 2003) or dispersal capabilities (Bech et al., 2009), extrinsic factors like landscape topography, vegetational cover, and anthropogenic factors might shape the extent of gene flow and spatial genetic variation (Cushman, 2006). In order to maintain gene flow, preservation and reestablishment of connectivity are primary targets in wildlife conservation (Kettunen et al., 2007). It is thereby essential for conservation management to understand the drivers of spatial genetic variation, especially for connectivity assessments and conservation strategies for grounddwelling, elusive species.

Forest grouse (Galliformes, Tetraoninae) are such species. Many populations of these birds are of high conservation concern due to declining trends and increasing habitat fragmentation (Storch, 2007). Well-documented dispersal capabilities combined with general site fidelity of adult individuals result in genetic structure on a fine spatial scale (Klinga et al., 2015; Rutkowski et al., 2017; Sittenthaler et al., 2018), making grouse important model systems to study drivers of spatial genetic variation. The black grouse (Lyrurus tetrix) was specifically targeted by several genetic studies as it is of high conservation concern (Corrales et al., 2014; Höglund, 2009; Rutkowski et al., 2018). Having a distribution range from Great Britain to Siberia, it shows a worldwide decreasing population trend (BirdLife International, 2016), and especially European populations declined dramatically or became extinct in the past decades (Höglund et al., 2007; Larsson et al., 2008; Rutkowski et al., 2018; Segelbacher et al., 2014; Watson & Moss, 2008). Most of the remaining populations are either isolated or exist within a metapopulation context (Caizergues et al., 2003; Höglund et al., 2007). It is consequently listed in Annex I and II of the EU Birds Directive (Directive 2009/147/EC), and special

conservation measurements must be taken to ensure its long-term survival. Core areas of the black grouse Central European distribution are located in the Alps (BirdLife International, 2016; Klaus et al., 1990), where the species shows a strong affinity to the tree-line ecotone (Sachser et al., 2017). This ecosystem is mainly characterized by a patchy mixture of open, grassy vegetation and woody plants with varying but typically low canopy closure. Alpine black grouse usually avoid patches with a dense tree canopy closure (Immitzer et al., 2014; Patthey et al., 2012; Sachser et al., 2017; Schweiger et al., 2012), and open, elevated habitat patches are preferred sites for lekking. Dispersal of black grouse is typically sex-biased with natal dispersal of females and philopatry of males (Caizergues & Ellison, 2002; Corrales & Höglund, 2012). Female dispersal usually occurs over distances of up to 8 km (Caizergues & Ellison, 2002; Marjakangas & Kiviniemi, 2005; Warren & Baines, 2002; Willebrand, 1988). Although in rare events, black grouse traverse longer distances in flight (potentially enabling gene flow over impermeable landscapes), it is in general a sedentary bird species, responding sensitively to the spatial structure of habitats. Being mainly ground-dwelling (Klaus et al., 1990), black grouse therefore serves as an indicator species for its ecosystem (Storch, 2007), and habitat factors are assumed to be key factors for movement behavior and dispersal.

Black grouse habitats within the Alps are naturally separated by high mountain ridges and low valleys (Caizergues & Ellison, 2002). Over the last decades, abandonment of alpine pastures (Groier, 2010) and impacts of climate change affected the plant community distribution (Gehrig-Fasel et al., 2007: Theurillat & Guisan, 2001). which resulted in a distinct loss of open habitats and in altitudinal shifts of the tree-line ecotone (Tasser et al., 2007), significantly reducing the available habitat for black grouse. Furthermore, habitats became increasingly fragmented by human settlements, agricultural areas, expanding skiing areas, wind power facilities, and other human activities (Arlettaz et al., 2007; Coppes et al., 2017; Immitzer et al., 2014; Ingold, 2005). These effects become particularly important at the marginal areas of the species' distribution. For the easternmost black grouse occurrences of the Alpine distribution, situated in the Austrian province of Styria (Figure 1), genetic differentiation into distinct clusters has already been observed (Sittenthaler et al., 2018), and multiple extinction events of marginal subpopulations have been documented in the past decades (Wöss & Zeiler, 2003). It remained unclear whether the spatial genetic variation was driven by the mere geographic distance or the resistance of the landscape. Yet, such knowledge is of major importance to infer targeted conservation actions, in order to preserve threatened populations and to adjust ongoing landscape planning processes.

Here, we aimed to study the population genetic structure and habitat suitability of a Central European black grouse metapopulation system to infer drivers of spatial genetic variation and to understand their effects on the conservation status of the species. We modeled connectivity using least-cost-path (LCP) lengths and effective resistances and parameterized regression-based landscape genetic analyses among individuals and subpopulations. Our study helps to understand drivers of the genetic structure of Alpine black FIGURE 1 Results of population genetic analyses, ecological niche modeling, and landscape genetic approaches on 195 Styrian black grouse individuals. (a) Digital elevation model of the study area Styria, with all 195 individuals, classified in 10 subpopulations (black outline, 5-km buffer around presence points, identified by Sittenthaler et al., 2018) and four clusters (green-, yellow-, orange-, and gray-colored areas of suitable habitat, as identified in this study). Least-cost-paths by Linkage Mapper 1.1 were classified into five quantiles of effective resistances calculated by Circuitscape 4.0. The inset shows the area of the Alps (dark gray) provided by the European Environment Agency and the location of our study area (black square). (b) Ecological niche model by MaxEnt 3.4.1, representing the resistance surface

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(b) Ecological niche model / resistance model



grouse populations at the edge of their range. This is the basis to infer conservation strategies and can help to prevent the loss of this characteristic bird species of Alpine ecosystems.

2 | METHODS

2.1 | Study site and collection of samples

Samples of black grouse were obtained from the entire Austrian province of Styria (Figure 1), representing the easternmost occurrence of the species' Alpine distribution range (BirdLife International, 2016). The study area shows a high portion of mountain areas, ranging from 200 to almost 3000 m.a.s.l., a high cover of conifer forests (>55%), a prominent portion of alpine meadows (7%), and gradients between alpine and pannonic climate (Land Steiermark, 2019). Black grouse occurrences are structured in subpopulations based on topographical criteria and average dispersal distances (Sittenthaler et al., 2018). Several subpopulations at the edges of the distribution range have already gone extinct (Wöss & Zeiler, 2003), and the remaining 10 subpopulations form a metapopulation system (Table 1; Sittenthaler et al., 2018). We used genetic data of black grouse individuals from a previous population genetic survey from all subpopulations (Sittenthaler et al., 2018). Samples were obtained from feces, feathers, and muscle tissue (n = 250) and stored in ethanol (for muscle tissue) and frozen (for feces and feathers). DNA extraction and polymerase chain reaction (PCR) amplification were performed as described in Sittenthaler et al. (2018). Each individual

TABLE 1 Characterization of the subpopulations within the metapopulation system of black grouse in Styria

Subpopulation	Abbreviation	P_	N	cluster assignment	Н.	Н_	F.,
		. F			0	F	- 15
Aussee	AUS	1200	7	Inneralpine	0.65	0.66	0.02
Liezen North	LIN	450	5	Inneralpine	0.69	0.63	-0.09
Hoschschwab South	HSS	925	13	Inneralpine	0.76	0.71	-0.07
Hochschwab West	HSW	925	13	Inneralpine	0.68	0.70	0.02
Tauern	TAU	6.850	56	Inneralpine	0.66	0.69	0.04
East Styria/Wechsel	OSW	400	41	Eastern	0.60	0.64	0.07
Turrach	TUR	850	4	Inneralpine	0.75	0.69	-0.08
Zirbitzkogel	ZIK	500	18	Zirbitzkogel	0.54	0.62	0.12
Gleinalm/Stubalm	GLS	700	23	Southern	0.62	0.68	0.09
Koralm	KOR	150	15	Southern	0.61	0.62	0.03

Note: Overall F_{IS} : -0.04; Overall F_{IT} : 0.04; Overall F_{ST} : 0.08.

Cluster assignment based on Sittenthaler et al. (2018), results from *memgene* and indices of fixation and differentiation. Population size estimates are rough expert-based estimates to characterize the subpopulations.

Abbreviations: F_{IS} , inbreeding coefficient; H_{E} , expected heterozygosity; H_{O} , observed heterozygosity; N, number of individual genotypes; P_{E} , population size estimate (Sittenthaler et al., 2018).

	From			
То	Inneralpine	Eastern	Southern	Zirbitzkogel
Inneralpine	0.700 (±0.040)	0.046 (±0.100)	0.251 (±0.112)	0.006 (±0.012)
Eastern	0.021 (±0.040)	0.812 (±0.248)	0.154 (±0.258)	0.013 (±0.025)
Southern	0.030 (±0.044)	0.019 (±0.037)	0.941 (±0.057)	0.010 (±0.020)
Zirbitzkogel	0.018 (±0.034)	0.056 (±0.133)	0.239 (±0.122)	0.687 (±0.040)

TABLE 2 Migration rates as estimated by BayesAss 3.0.4 with 95% credible intervals among the genetic clusters of black grouse as in Table 1

Note: Significant values based on the credible intervals are in bold emphasis.

was genotyped at nine microsatellite loci using a multiple tubes approach for noninvasive samples (Navidi et al., 1992; Taberlet et al., 1996). A total of 195 individuals were fully genotyped. A consensus genotype was accepted when at least two (for heterozygote loci) or three (for homozygote loci) independent replications of a single allele were recorded.

2.2 | Population genetic analysis

Summary statistics were calculated per subpopulation using the R package *hierfstat* 0.5-7 (Goudet, 2005). In addition to F_{ST} values (Weir & Cockerham, 1984), we calculated the pairwise fixation indices G_{ST} (Nei & Chesser, 1983) and G'_{ST} (Hedrick, 2005) and the differentiation index D_{Jost} (Jost, 2008), using the R package *diveRsity* 1.9.9 (Keenan et al., 2013). As G''_{ST} (Meirmans & Hedrick, 2011) is not implemented within *diveRsity*, we used the R package *mmod* 1.3.3 (Winter, 2012) and calculated bias-corrected confidence intervals following the method implemented in *diveRsity*. For all indices, confidence intervals were based on 10,000 bootstrap iterations. Although correlated (Pearson's correlation coefficients ranging from 0.8 to 0.9), these indices quantify complementary aspects of population structure and should therefore be considered separately for subsequent analyses

(Jost et al., 2018; Meirmans & Hedrick, 2011). To assess clustering within the genetic dataset, a principle component analysis (PCA) was calculated using the R package adegenet 2.0.1 (Jombart, 2008; Jombart & Ahmed, 2011) in addition to the discriminant analysis of principle components (DAPC) and Structure analyses by Sittenthaler et al. (2018). Given the previously reported low amount of genetic differentiation among subpopulations (Sittenthaler et al., 2018), we further used the R package memgene 1.0.1 (Galpern et al., 2014) to explore spatial genetic patterns in detail. memgene was specifically designed to detect and visualize weak or cryptic structure within a genetic pattern by using Moran's eigenvector maps (MEMs; Galpern et al., 2014), thus being a suitable approach to detect genetic structure in our study system. We used the function mgQuick to assess population structure, with the response variable being the proportions of shared alleles D_{PS} (calculated with memgene) among individuals. Subpopulations in our study area were assigned to clusters based on the combined interpretation of Structure and DAPC results by Sittenthaler et al. (2018), our memgene analysis and significant indices of genetic fixation and differentiation.

Furthermore, we estimated recent migration rates to analyze potential asymmetric migration using BayesAss 3.0.4 (Wilson & Rannala, 2003). Migration rates were calculated between clusters based on the analyses of population genetic structure (Table 2). We

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conducted 10 independent repeats of $50 * 10^6$ iterations (including $5 * 10^6$ iterations burn-in) with a sampling frequency of 2000, each initiated with a different random seed for each dataset. In order to keep the acceptance rates for proposed changes between 40% and 60%, delta values were adjusted to $\Delta m = 0.1$, $\Delta a = 0.3$, and $\Delta f = 0.7$. Convergence of chains was confirmed using Tracer 1.7.1 (Rambaut et al., 2018) and by checking for concordance between repeats. We used the Bayesian deviance as calculated by Meirmans (2014) in R 3.6.0 (R Core Team, 2019) to search for the best fitting model (the one with the lowest Bayesian deviance was selected) (Faubet et al., 2007). Credible intervals (Cls 95%) of migration rates were calculated as standard deviation multiplied by 1.96 as described in the program's manual. Migration rates that included zero within their 95% Cl were considered not significant.

2.3 | Ecological niche modeling and resistance surface

In order to parameterize a model representing the resistance of the landscape to movement and dispersal for black grouse, we used a correlative ecological niche model (ENM). The process of parameterization of resistance models is broadly discussed (Mateo-Sánchez et al., 2015; Milanesi, Holderegger, Caniglia, et al., 2017; Roffler et al., 2016; Wang et al., 2008), and several approaches have been suggested. Black grouse are mainly ground-dwelling and react sensitively to habitat structures. Movement and dispersal are most probably directly linked to habitat factors, as suitable habitats provide food resources and protection (against predators and adverse weather conditions). Therefore, we assume that the resistance of a landscape to movement and dispersal is best reflected by the distribution of suitable habitat areas (Milanesi, Holderegger, Caniglia, et al., 2017). Furthermore, ENMs have already been used successfully to parameterize resistance surfaces for the closely related Western capercaillie (*Tetrao urogallus*) (Milanesi et al., 2017). Accordingly, we selected 15 topographic, climatic, and land cover variables that might affect dispersal and movement (Table 3).

The topographic variables (altitude, slope, exposure, and ruggedness, Sappington et al., 2007) were calculated based on the digital elevation model. The climatic variables were taken from the official geodata catalog of climate of the province of Styria (GIS-Steiermark, 2018). The land cover dataset was based on an extensive land cover classification (Wrbka et al., 2002). It comprises 42 landscape types, which were grouped into the eight categories relevant for black grouse (Table 4): summits and glaciers; subalpine grasslands (including pastures and meadows); continuous forests; lowland forest patches; submountainous grasslands; lowland grasslands and pastures; lowland arable land; and human settlements and industrial areas. We included two variables representing the distance to the land cover type positively (subalpine grasslands) or negatively

TABLE 3 Environmental input data used for the ecological niche modeling of black grouse in Styria with MaxEnt 3.4.1 (Phillips et al., 2006; Phillips & Dudík, 2008)

Environmental variable	Final model contribution (%)	Source
Distance to subalpine grasslands	55.7	Derived from the land use classification
Altitude	37.8	Derived from a digital elevation model (DEM) by LiDAR data (Land Kärnten, 2015)
Land use classification	4.3	Classified into eight categories based on Wrbka et al. (2002)
Distance to human settlements and industrial areas	1.6	Derived from the land use classification
Ruggedness, vector ruggedness measure (VRM)	0.7	Derived from the DEM following Sappington et al. (2007), neighborhood size: 11
Aspect	-	Derived from the DEM
Slope	_	Derived from the DEM
Buffered single tree individuals above 1200 m.a.s.l.	-	Derived from LiDAR data (GIS-Steiermark, 2018), includes vegetation between 6 and 15 m height outside of areas classified as forest
Distance to single tree individuals	_	Derived from the single tree individuals
Climatic variables (duration of vegetation period, precipitation per season, days of frost, and days of snow cover)	-	Klimaatlas Steiermark/climate data (GIS-Steiermark, 2018)
Tree composition	_	Waldatlas Steiermark/forest data (GIS-Steiermark, 2018)
Tree height	-	Waldatlas Steiermark/forest data (GIS-Steiermark, 2018)

Note: Final model contribution gives the relative contribution of the variable to the final model. Most important variable based on jackknife tests was altitude.

TABLE 4	Summary of land use classification by Wrbka et al.
(2002) into	eight categories relevant for black grouse in Styria used
in the prese	nt study

Land use category used in the present study	Land cover (%)	Identifier of Wrbka et al. (2002)
Summits and glaciers	3.2	101
Subalpine grasslands and pastures	7	102, 103
Continuous forests	22.8	201
Lowland forest patches	35.3	202, 203, 204, 205
Submountainous grasslands and pastures	8.6	301, 302, 303
Lowland grasslands and pastures	11.3	304, 305, 307, 312, 313
Lowland arable land	9.9	401, 402, 404, 405, 406, 407, 411, 604
Human settlements and industrial areas	1.9	701, 702, 703, 704, 705, 706

Note: Land cover displays the proportion of study area covered by the respective category.

(human settlements and industrial areas) affecting black grouse distribution. Hence, we accounted for potential push or pull effects of these areas. As the inclusion of local habitat structures is crucial for ENMs to parameterize resistance surfaces (Milanesi, Holderegger, Bollmann, et al., 2017), we included variables representing tree height, tree composition, and the existence of single tree individuals. Tree height and composition directly link to black grouse habitat preferences and were based on the official geodata catalog of forestry of the province of Styria (GIS-Steiermark, 2018). Single trees in open subalpine areas might also positively affect habitat suitability by offering resting sites and food resources. We therefore generated a dataset representing single trees in subalpine areas by extracting vegetation between 6 and 15 m height outside of the land cover categories "continuous forest" and "lowland forest patches" from light detection and ranging (LiDAR) data (GIS-Steiermark, 2018). Additionally, we calculated distance to single trees to assess potential pull effects. Based on Pearson's correlation coefficient, we excluded highly correlated variables (coefficients \geq |0.7|). For all data, we used a resolution of 100 m grain size. Preparation steps and further spatial analyses were done in ArcGIS 10.5 (ESRI, 2016). The study area was buffered 20 km around the political boundary of Styria, allowing the analyses to explore areas of biological relevance beyond administrative borders.

The ENM was calculated using maximum entropy modeling implemented in MaxEnt 3.4.1 (Phillips et al., 2006; Phillips & Dudík, 2008). The underlying principle of maximum entropy uses machine learning concepts to minimize the difference between two probability density functions of environmental variables, one based on our presence locations and the other one based on the entire study area (background locations) (Elith et al., 2011). We calibrated models with varying sets of environmental variables and regularization parameters and combinations of features (Merow et al., 2013; Phillips et al., 2017). We followed a stepwise top-down procedure of model selection, evaluating model fit and adequacy by their average area under the receiver operating characteristics curve (AUC) value through cross-validation and together with regional experts as recommended by Morales et al. (2017). The final model parameters were set to 20 replications of 5,000 iterations, and the regularization parameter was set to 1.5. To account for a potential sampling bias, we included background manipulation via a Gaussian kernel density of sampling locations calculated with SDMtoolbox 2.2 (Brown, 2014; Brown et al., 2017) as bias file. The final ENM was inverted into a resistance surface using SDMtoolbox 2.2. Additionally, we created an alternative resistance surface based on an inverted ENM of altitude only (altitude_inv), as altitude was the most explanatory variable beside land cover classification in the ENM.

2.4 | Measures of IBR

We applied two distinctly different approaches to extract distances and resistance values of the resistance surface that might explain IBR: (1) LCP lengths were extracted according to the cost distance approach (Adriaensen et al., 2003), and (2) effective resistances were calculated according to the circuit theory approach (McRae et al., 2008). Whereas the cost distance approach assumes an individual's full a priori knowledge of the landscape when calculating the LCP, circuit theory assumes random movement and therefore yields higher connectivity where higher redundancy in travel routes exist (McClure et al., 2016). LCPs and effective resistances between subpopulations (areas defined as suitable habitat within a conservative 5 km buffer around individual presence points; Figure 1) were generated using the geographical information system routine within LinkageMapper 1.1 (McRae & Kavanagh, 2011) and PinchPoint Mapper (McRae, 2012) (making use of Circuitscape 4.0; McRae et al., 2013).

2.5 | Identifying spatial genetic pattern

At the individual level, we used the function mgLandscape within *memgene* to address whether IBD or IBR might explain the spatial genetic pattern. This function computes LCPs from provided resistance surfaces to extract MEM eigenvectors and subsequently performs a regression framework. We used D_{PS} as response variable and the following landscape distances as predictors (Table 5): Euclidean distances resembling IBD (Euc. dist.), our resistance surface based on the ENM resembling IBR (res. surface), and the resistance surface based on altitude alone (altitude_inv). By including altitude as a predictor, we assessed whether IBR effects are driven by the complex ENM (including topography, climate, and land cover) or by altitude alone (irrespective of anthropogenic influence).

At the subpopulation level, we contrasted IBD versus IBR using the regression framework within the function mgLandscape_list

TABLE 5 Comparison of the proportion of spatial genetic variation (R_{adj}^2) among black grouse individuals in Styria explained by Moran's eigenvector maps derived from different models

Model	[abc]	P[abc]	[a]	P[a]	[c]	P[c]	[b]	[d]
Euc. dist.	0.080	0.001	0.052	0.001	0.005	0.060	0.023	0.920
res. surface	0.074	0.001	0.047	0.001	0.003	0.126	0.024	0.926
altitude_inv	0.055	0.001	0.028	0.001	-0.001	0.631	0.029	0.945

Note: The table describes the proportion of variation in pairwise genetic distances that can be attributed to the different spatial predictors [abc] and to the particular pattern in the landscape resistance surface [a], the coordinates of the individuals in the landscape resistance surface [c], or to confounded pattern of the landscape resistance surface and coordinates [b]. Additionally, residuals not explained by spatial predictors are reported [d]. P[abc], P[a], and P[c] represent the *p* values of each calculated proportion. Tested models are Euclidean distances (Euc. dist.), pairwise least-cost-path (LCP) lengths between individuals across the resistance surface based on the ENM (res. surface), and pairwise LCPs between individuals across a resistance surface based on altitude only (altitude_inv).

by Polato et al. (2017). Although following the same approach as mgLandscape within memgene, this adapted function allowed us to test the aforementioned indices of pairwise genetic fixation and differentiation as response variables against pairwise geographic distances as predictors. The pairwise Euclidean distances (IBD), LCP lengths (IBR), and effective resistances (IBR) were used as predictors. Additionally, we calculated maximum likelihood population effects (MLPE) models (Clarke et al., 2002) implemented in the R package ResistanceGA (Peterman, 2018). MLPE models account for nonindependence of pairwise distance data due to population effects and have been identified as best-suited regression-based approaches for model selection (Shirk et al., 2017, 2018). We used the same response and explanatory variables as for the mgLandscape_ list approach. Due to strong correlations between the explanatory variables and the small sample size within each model, each variable was tested separately resulting in 20 models (five response variables and three explanatory variables plus a null model assuming that the response variable is constant for the explanatory variable). We then applied deltas and weights of the Akaike Information Criterion corrected for small sample sizes (AICc; Anderson & Burnham, 2002) and R^2 to compare the candidate models and select the best model (Row et al., 2017).

3 | RESULTS

3.1 | Spatial genetic structure

Overall, a low amount of genetic differentiation among subpopulations was detected. Although the PCA could not resolve a clear cluster assignment (Figure 2), the spatial genetic structure detected by the mgQuick approach of *memgene* (Figure 3) indicated the presence of clusters. The first *memgene* variable explaining the highest amount of spatial genetic variation found the subpopulation OSW to be distinct. The second variable suggested a cluster of the northern subpopulations, and subpopulation ZIK seemed to be connected to the southeastern ones. The third variable showed mixed results for the northern subpopulations, and ZIK seemed to be distinct from the southeastern subpopulations. Significant indices of genetic fixation and differentiation (Table 6 and Table A1) provided further evidence for genetically discrete clusters; both OSW and ZIK were differentiated. Taken together, our data and the Structure and DAPC results by Sittenthaler et al. (2018) imply that the ten subpopulations can be differentiated into four clusters (Table 1). The subpopulations AUS, LIN, HSS, HSW, TAU, and TUR are situated in the Central Alps and together formed the Inneralpine cluster. The southern subpopulations GLS and KOR formed a cluster called Southern. The easternmost subpopulation OSW was the most differentiated and formed its own cluster Eastern. The subpopulation ZIK showed ambiguous results and was therefore assigned its own cluster Zirbitzkogel.

The proportion of genetic variation found by memgene that can be attributed to spatial patterns (R_{adi}^2 , Galpern et al., 2014) was 0.07, indicating weak overall genetic structure. This is in line with the indices of fixation and differentiation showing overall low fixation and differentiation (range of values for F_{ST} : 0.001–0.103; G_{ST} : 0.001-0.053; G'_{ST}: 0.007-0.207, G''_{ST}: 0.008-0.306; D_{Jost}: 0.001-0.124). Results of the first and second memgene variables (explaining 28% and 19% of the spatial genetic variation, respectively) resolved the four genetic clusters, with ambiguous assignments for ZIK. In estimation of migration rates, BayesAss chains converged well, and log-likelihood and Bayesian deviance were comparable between repeats. The estimates indicate unidirectional migration patterns between the clusters (Table 2). Individuals appeared to be migrating from Southern into the Zirbitzkogel and Inneralpine clusters. Migration rates from Southern to Eastern were not significant. Therefore, no immigration into Southern and Eastern was found.

3.2 | Ecological niche modeling and resistance surfaces

All climatic variables were excluded due to their high correlation with altitude prior to the parameterization of the ENM. The final ENM (Figure 1) comprised the following five environmental variables reflecting relevant topographical and land cover criteria for black grouse (model contributions in parentheses): distance to subalpine grasslands (55.7%); altitude (37.8%); land use classification (4.3%); distance to human settlements and industrial areas (1.6%); and terrain ruggedness (0.7%) (Table 3). The model corresponded well to regional expert assessment and showed an averaged test AUC of 0.954, which indicated strong model fit and high predictive performance. The single most important variable in terms of information not covered by other variables was altitude.



FIGURE 2 Principal component analysis with four retained PCs of the 195 Styrian black grouse genotypes. PC1 (x axis; 3.9% explained variance) versus PC2 (y axis; 3.6% explained variance) (top) and PC1 (x axis, 3.9%) versus PC3 (y axis, 3.5%) (bottom). Different colors indicate the assignment of subpopulations to four clusters

FIGURE 3 Spatial genetic structure of the 195 Styrian black grouse samples as found by *memgene* 1.0.1 (Galpern et al., 2014). Circles of similar size and color indicate individuals with similar scores (large black and large white circles describe opposite extremes). The first *memgene* variable explains 28% of the spatial genetic variation and the second and third variable 19% and 15%, respectively. Colored polygons indicate the assignment of subpopulations to the four clusters. Axes in UTM WGS84



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TABLE 6 Pairwise F_{ST} (Weir & Cockerham, 1984) and G''_{ST} (Meirmans & Hedrick, 2011) comparisons among black grouse subpopulations in Styria

Subpopulation ID	AUS	LIN	HSS	HSW	TAU	OSW	TUR	ZIK	GLS	KOR
AUS	_	0.044	0.044	0.073	0.008	0.119	0.040	0.121	0.034	0.091
LIN	0.017	_	0.070	0.116	0.026	0.081	0.156	0.132	0.120	0.221
HSS	0.016	0.026	_	0.047	0.040	0.121	0.198	0.223	0.040	0.162
HSW	0.022	0.037	0.015	-	0.069	0.124	0.136	0.144	0.039	0.171
TAU	0.001	0.005	0.012	0.021	-	0.093	0.079	0.116	0.037	0.100
OSW	0.039	0.026	0.040	0.041	0.030	_	0.306	0.141	0.085	0.203
TUR	0.015	0.063	0.064	0.041	0.023	0.103	_	0.273	0.190	0.242
ZIK	0.040	0.045	0.076	0.048	0.038	0.050	0.094	_	0.123	0.177
GLS	0.007	0.035	0.012	0.010	0.011	0.028	0.056	0.040	_	0.036
KOR	0.032	0.083	0.056	0.058	0.032	0.073	0.087	0.065	0.010	_

Note: F_{ST} values below the diagonal and $G_{ST}^{\prime\prime}$ above. Significant values based on 95% bias corrected confidence intervals in bold.

Response variable	Explanatory variable	ΔAICc	w	R ²
F _{ST} (Weir & Cockerham, 1984)	LCP length	0.00	0.60	0.17/0.66
	Euclidean dist.	0.97	0.37	0.16/0.65
	Effective resist.	6.09	0.03	0.10/0.58
	Null model	8.84	0.01	0.00/0.57
G _{ST} (Nei & Chesser, 1983)	LCP length	0.00	0.61	0.19/0.66
	Euclidean dist.	1.01	0.37	0.16/0.65
	Effective resist.	6.49	0.02	0.10/0.56
	Null model	9.54	0.01	0.00/0.55
G' _{ST} (Hedrick, 2005)	LCP length	0.00	0.59	0.20/0.64
	Euclidean dist.	0.88	0.38	0.19/0.63
	Effective resist.	6.65	0.02	0.10/0.53
	Null model	9.57	0.00	0.00/0.52
G'' _{ST} (Meirmans & Hedrick, 2011)	LCP length	0.00	0.59	0.20/0.64
	Euclidean dist.	0.87	0.38	0.19/0.64
	Effective resist.	6.66	0.02	0.10/0.54
	Null model	9.66	0.00	0.00/0.53
D _{Jost} (Jost, 2008)	LCP length	0.00	0.28	0.07/0.38
	Euclidean dist.	0.17	0.26	0.06/0.37
	Null model	0.39	0.23	0.00/0.34
	Effective resist.	0.44	0.23	0.06/0.33

TABLE 7 Maximum likelihood population-effects models for the black grouse subpopulations in Styria, ranked by weights (w) of the delta of the corrected Akaike Information Criterion for small sample sizes (Δ AICc) and R^2 (marginal/ conditional)

Note: Response variables were fixation and differentiation indices of genetic distances; explanatory variables were a null model, Euclidean distances (Euclidean dist.), least-cost-path (LCP) lengths based on the ecological niche model (ENM) (LCP length), and effective resistances (Effective resist.).

3.3 | Identifying spatial genetic pattern

Within the mgLandscape approach on the individual level, MEM eigenvectors derived from Euclidean distances ([abc], Table 5) between individuals explained a slightly higher proportion of spatial genetic variation ($R_{adi,[abc]}^2 = 0.08$) than MEM eigenvectors derived from the resistance surface based on the ENM and the resistance surface based on altitude alone $(R_{adj,[abc]}^2 = 0.074 \text{ and } 0.055, \text{ respectively})$. The fraction of genetic distance that is explained by the model [a] is notably higher than the fraction explained by coordinates [c], indicating good model fit (Table 5). Although Euclidean distances (testing for the effect of IBD) are therefore

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preferred over the resistance surface based on the ENM (testing for the effect of IBR), the small difference in the proportions of spatial genetic variation explained by the spatial predictors [abc] suggests that IBD and IBR effects cannot be easily distinguished. Among subpopulations, the mgLandscape_list approach was not able to detect significant Moran's eigenvectors, as the spatial signal within the pairwise genetic distance matrices was presumably too weak. All MLPE models, however, showed positive signs of relationships between the predictors and dependent variables. The predictors were significant ($\alpha = 0.05$) for all models except the ones built with D_{Jost} as dependent variable. The models using LCP length as predictor were preferred for all indices of fixation or differentiation (Table 7).

4 | DISCUSSION

We applied several consecutive analyses to disentangle the drivers of spatial genetic variation within an Alpine black grouse metapopulation system at the easternmost edge of the species' distribution. Although low levels of population differentiation and only a slight difference among models testing for IBD or IBR were found, our results provide valuable insights into the spatial genetic pattern of this small-scale metapopulation system with a high conservation concern.

4.1 | Migration rates and population structure

The migration rates estimated by BayesAss indicated emigrating individuals from the two clusters, Eastern and Southern. However, BayesAss estimates should be viewed with caution as the maximum proportion of immigrated individuals within a cluster is assumed to not exceed one third of its size (Faubet et al., 2007). Although the overall genetic differentiation is low within our study system, black grouse are sedentary birds with intermediate juvenile dispersal (Caizergues & Ellison, 2002; Marjakangas & Kiviniemi, 2005; Warren & Baines, 2002), presumably not violating this assumption. Additionally, BayesAss decreases in accuracy when sample sizes are differing among subpopulations (Meirmans, 2014). Although this is the case in our study (as is for the most studies on rare and elusive species), BayesAss estimates correspond well to our other results. Especially the subpopulation OSW (Eastern cluster) appears to be of high concern. Separated by a major valley (the Mur-Mürz-Furche), it is the most differentiated subpopulation within the metapopulation, and no immigration from other subpopulations was found. Losing connection to the metapopulation system, subpopulation OSW might end up in reproductive isolation. Given ongoing range contraction through the loss and degradation of habitat (Gehrig-Fasel et al., 2007; Groier, 2010; Tasser et al., 2007; Theurillat & Guisan, 2001) and increasing disturbance within the remaining habitats (Arlettaz et al., 2007; Coppes et al., 2017; Immitzer et al., 2014; Ingold,

2005), the subpopulations' long-term survival is therefore threatened (Frankham et al., 2010). Our results might be an early warning signal (Kunz et al., 2021), as extinction events of isolated black grouse populations have been observed in various cases over the past decades (Höglund, 2009; Höglund et al., 2007 and references therein).

Individuals from the Southern cluster seem to be migrating into the Zirbitzkogel and Inneralpine cluster. The subpopulations within the Southern cluster are situated at the administrative border, and it is very likely that they are connected to black grouse populations in Carinthia. Especially the subpopulation KOR might therefore act as an important stepping stone. Surprisingly, no migration was found between the Zirbitzkogel and the Inneralpine cluster. Considering the landscape's permeability, we therefore assume individuals emigrating from the Southern cluster to either settle within Zirbitzkogel or continue dispersing into the Inneralpine cluster. Its role as potential stepping stone for black grouse populations in Carinthia still remains unresolved, and more samples are needed, spanning a wide geographic region. As we only found unidirectional migration, unknown effects might be leading individuals to emigrate and, at the same time, prevent immigration.

4.2 | Drivers of black grouse spatial genetic variation

We found clear positive significant relationships of genetic differentiation and geographic distances (LCP length and effective resistances) for all our models. On an individual level, analyses resulted in models based on IBD being marginally more explanatory than models based on IBR. The proportion of shared alleles was better explained by the model including the pairwise Euclidean distances among individuals than by the model including the pairwise LCPs across the resistance surface. Pairwise genetic data are known to be noisy, and therefore, inferences are often challenging (Peterman & Pope, 2020). Among individuals, the landscape's resistance arguably did not exert a meaningful effect. The similar performance of the tested models of IBD and IBR might rather indicate a cumulative effect on gene flow, which seems reasonable for a species with restricted dispersal capabilities. Both models were superior to a model based solely on altitude.

On a subpopulation level, the *memgene* analysis was not able to reproduce the patterns found among individuals, which might derive from the fact that *memgene* is working best when genetic distances are more pronounced among individuals than among subpopulations (P. Galpern, pers. comm.). The MLPE models showed the LCP lengths to be the best explaining predictors. Taken together, our results suggest the spatial genetic pattern in the studied black grouse metapopulation system to be driven by IBD among individuals and by IBR effects among subpopulations. Our results did not show distinct differences among models, as shown by the small delta AICc and the proportion of explained variance. Additional factors not represented WILEY_Ecology and Evolution

within our chosen approach might be affecting genetic differentiation beyond geographic distances. We purposely excluded highly variable short-term environmental factors. Anthropogenic factors and disturbances (e.g., frequencies of hikers and dogs and forestry) might as well exert effects on the spatial genetic variation of black grouse (Arlettaz et al., 2007; Coppes et al., 2017; Immitzer et al., 2014; Ingold, 2005). Studies quantifying these effects for black grouse are still lacking as data of these factors are sparse and mostly not available for larger regions.

Although our results could be taken as indication for the presence of barriers between subpopulations, we assume the observed patterns to be a consequence of unidirectional dispersal and short-distance dispersal of black grouse. Unidirectional dispersal is common for metapopulation systems experiencing source-sink dynamics (Kawecki, 2004). We found patterns of unidirectional dispersal for several pairs, with especially the outermost subpopulations not receiving alleles from the larger, more central subpopulations. Although dispersal in black grouse is female-based (Lebigre et al., 2010), no clear evidence has been found for femalebased dispersal affecting black grouse spatial genetic variation (Corrales & Höglund, 2012). Female-based dispersal rather seems to counteract differentiation effects (Lebigre et al., 2008, 2010). Instead, short-distance dispersal in general is assumed to lead to a global IBD pattern (overall subpopulations), with potential IBR effects being present at local scales only (Blair et al., 2012). We therefore assume our observed pattern of spatial genetic variation to be a result of short-distance dispersal. Detection of effects of recent barriers, however, might be difficult, as for short-distance dispersing species, such effects need several generations to manifest (Landguth et al., 2010). Within our study area, habitat segregation as an ongoing process might be too recent yet to lead to distinct genetic differences. Additionally, a network of remaining patches of suitable habitats between subpopulations serving as stepping stones might have prevented subpopulations from distinct differentiation in the past. In the light of increasing habitat loss and fragmentation, it becomes vital to reassess population structure and connectivity on a regular basis, in order to understand a species' response to landscape features and detect potential barriers for gene flow.

A key component within landscape genetic analyses is the parameterization of the resistance surface. In the past decades, expert-based resistance surfaces were widely applied to extract measures of geographic distances (Epps et al., 2007; Shirk et al., 2010). More recently, correlative ENMs have increasingly been used due to their continuous and objective nature (Milanesi, Holderegger, Caniglia, et al., 2017; Wang et al., 2008). Although ENMs succeed in identifying habitats of species, they were, however, suspected to inaccurately predict landscape elements that are essential during movement or dispersal (Keller et al., 2013). As an alternative, resistance surfaces produced through optimization approaches were suggested (Mateo-Sánchez et al., 2015). There is, however, no single optimal approach applicable for all circumstances. Instead, the parameterization of resistance models depends on various factors, including the study objectives and the species' biology (Spear et al., 2010). As black grouse is mainly ground-dwelling and dispersal is generally low, movement and dispersal behavior are assumed to be driven by habitat structures, especially the availability of food resources and protection (against predators and adverse weather conditions). Accordingly, the resistance of a landscape can be assumed to be reflected by the spatial distribution of suitable areas that offer such resources at finer scales (Milanesi, Holderegger, Caniglia, et al., 2017). We consider black grouse to exhibit back-and-forth movements driven by the landscape's suitability (Baguette & Van Dyck, 2007; Van Dyck & Baguette, 2005). We therefore based our resistance model on a validated correlative ENM by using a vast amount of presence data and potential variables, accounting for spatial autocorrelation and multicollinearity and applying stepwise top-down selection of variables and parameters. This allowed us to model a composite resistance surface prior to extracting distance measures instead of using single environmental variables, as recently recommended (Peterman & Pope, 2020).

Interestingly, effective resistances as circuit theory-based measurements for IBR were outperformed in all analyses by the cost distance-based models (LCP lengths) between subpopulations. Dispersal in black grouse most likely happens at an individual's prereproductive stage (Caizergues & Ellison, 2002; Corrales & Höglund, 2012) and is not traditionally passed on over generations. Therefore, one might expect circuit theory-based approaches to be more suited, as these approaches presume that individuals have no prior knowledge of the landscape apart from their immediate surroundings and incorporate redundancy in pathways between source and destination. Yet, LCP length showed higher explanatory power. We assume this to be due to dispersal between pairs of subpopulations being geographically restricted (by high mountain ridges and valleys densely populated by humans) and therefore often only allowing for one dispersal route, which seemed to be represented by the LCPs.

4.3 | Consequences for conservation

Black grouse were historically widespread in Europe, ranging from Alpine areas to lowland habitats, yet human landscape alteration within the last centuries in Central Europe resulted in the species to retract to the subalpine tree-line ecotones (Sachser et al., 2017). Our ENM clearly shows current habitats to be restricted to those areas. The landscape is highly fragmented, with unsuitable areas to some extend exceeding dispersal distances (approximately 8 km; Caizergues & Ellison, 2002; Marjakangas & Kiviniemi, 2005; Warren & Baines, 2002; Willebrand, 1988). Such areas are predominately major valleys of several kilometers widths, characterized by low altitude and high density of anthropogenic settlements and infrastructure or high mountain ridges. Connectivity of subpopulations seems to follow a metapopulation network (Sittenthaler et al., 2018), with corridors alongside the LCPs.

The easternmost occurrences of black grouse in our study area also represent the easternmost Alpine distribution of the species (BirdLife International, 2016) and losses of connectivity in this region might not be compensated, as shown by past extinction events (Wöss & Zeiler, 2003). Despite large valleys representing barriers to connectivity, other barriers like power lines might impede successful dispersal by causing collision mortality (Baines & Andrew, 2003 and references therein). Thus, two major conservation targets should be particularly addressed for this high priority conservation zone: (1) prevention of a further increase of distances between patches of high habitat suitability paired with establishment of potential stepping stones; this includes all management actions, which aim at an improvement or maintenance of high-quality habitat patches for the target species (e.g., alpine pasturing, no further development for recreational issues, and reduction of human disturbances; Immitzer et al., 2014; Sachser et al., 2017; Schweiger et al., 2012). (2) Removal of any additional barrier effects, for example, deriving from power lines. Our results indicate that habitat management and species conservation actions need to be based on landscape ecological analyses, which have in turn to be translated into landscape planning processes.

5 | CONCLUSION

For the Alpine black grouse metapopulation system, preservation of gene flow appears as a primary conservation target (Caizergues et al., 2003; Höglund, 2009). Extinction events of several occurrences in the past decades (Wöss & Zeiler, 2003) and recent genetic differentiation (Sittenthaler et al., 2018) highlight the need for improved connectivity between subpopulations (Höglund et al., 2007). Within in-situ conservation and landscape planning, Euclidean distances between habitats of subpopulations are often considered and compared with average and maximum dispersal distances of the targeted species (Segelbacher & Storch, 2002; van Strien et al., 2015), thereby accounting for IBD. This approach is uncoupled from any underlying landscape characteristics. We showed that IBR effects between local subpopulations should be considered. Therefore, our ENM provides a valuable addition to landscape planning processes. Overall, Alpine black grouse in the Austrian province of Styria, situated at the eastern border of the species' Alpine distribution, exist within a metapopulation system with currently moderate levels of differentiation. However, the easternmost subpopulation OSW, separated from the Inneralpine occurrences by a major valley, shows first signs of isolation and should be monitored with special attention to prevent its extinction in the upcoming years.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Florian Kunz: Conceptualization (equal); formal analysis (equal); writing – original draft (lead); writing – review and editing (lead). Peter Klinga: Conceptualization (equal); formal analysis (equal). Marcia Sittenthaler: Conceptualization (equal); writing – review and editing (equal). Martin Schebeck: Writing – review and editing (equal). Christian Stauffer: Writing – review and editing (equal). Veronika Grünschachner-Berger: Funding acquisition (lead). Klaus Hackländer: Writing – review and editing (equal). Ursula Nopp-Mayr: Conceptualization (equal); supervision (lead); writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

Input files for ecological modeling: owned by the government of the federal state Styria, Austria, accessible upon request; digital elevation model accessible under https://www.data.gv.at/. Geographic locations of sampling sites: sensitive information, accessible upon request. STR genotypes: provided on Dryad under https://doi. org/10.5061/dryad.3ffbg79k3.

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TABLE A1 (Continued)

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APPENDIX A

TABLE A1 Pairwise indices of genetic fixation anddifferentiation among black grouse subpopulations in Styria,rounded to three digits

	F _{ST}	G _{ST}	G' _{ST}	G″st	D _{Jost}
AUS vs. LIN	0.017	0.008	0.037	0.044	0.002
AUS vs. HSS	0.016	0.007	0.037	0.044	0.000
AUS vs. HSW	0.022	0.012	0.062	0.073	0.010
AUS vs. TAU	0.001	0.001	0.007	0.008	0.000
AUS vs. OSW	0.039	0.021	0.100	0.119	0.028
AUS vs. TUR	0.015	0.006	0.034	0.040	0.000
AUS vs. ZIK	0.040	0.022	0.102	0.121	0.004
AUS vs. GLS	0.007	0.006	0.029	0.034	0.002
AUS vs. KOR	0.032	0.017	0.076	0.091	0.009
LIN vs. HSS	0.026	0.012	0.059	0.070	0.008
LIN vs. HSW	0.037	0.020	0.098	0.116	0.026
LIN vs. TAU	0.005	0.004	0.022	0.026	0.002
LIN vs. OSW	0.026	0.015	0.067	0.081	0.008
LIN vs. TUR	0.063	0.026	0.133	0.156	0.010
LIN vs. ZIK	0.045	0.025	0.110	0.132	0.007
LIN vs. GLS	0.035	0.021	0.102	0.120	0.045
LIN vs. KOR	0.083	0.043	0.188	0.221	0.054
HSS vs. HSW	0.015	0.007	0.040	0.047	0.007
HSS vs. TAU	0.012	0.006	0.034	0.040	0.006
HSS vs. OSW	0.040	0.020	0.103	0.121	0.042
HSS vs. TUR	0.064	0.030	0.174	0.198	0.037
HSS vs. ZIK	0.076	0.039	0.193	0.223	0.046
HSS vs. GLS	0.012	0.006	0.034	0.040	0.006

	F _{ST}	G _{ST}	G' _{st}	G'' _{ST}	D_{Jost}
HSS vs. KOR	0.056	0.028	0.139	0.162	0.027
HSW vs. TAU	0.021	0.011	0.059	0.069	0.028
HSW vs. OSW	0.041	0.021	0.106	0.124	0.047
HSW vs. TUR	0.041	0.021	0.118	0.136	0.033
HSW vs. ZIK	0.048	0.025	0.122	0.144	0.009
HSW vs. GLS	0.010	0.006	0.033	0.039	0.004
HSW vs. KOR	0.058	0.030	0.146	0.171	0.059
TAU vs. OSW	0.030	0.016	0.079	0.093	0.047
TAU vs. TUR	0.023	0.012	0.068	0.079	0.005
TAU vs. ZIK	0.038	0.021	0.098	0.116	0.022
TAU vs. GLS	0.011	0.006	0.032	0.037	0.010
TAU vs. KOR	0.032	0.018	0.084	0.100	0.011
OSW vs. TUR	0.103	0.053	0.270	0.306	0.094
OSW vs. ZIK	0.050	0.027	0.118	0.141	0.055
OSW vs. GLS	0.028	0.015	0.072	0.085	0.036
OSW vs. KOR	0.073	0.039	0.172	0.203	0.080
TUR vs. ZIK	0.094	0.049	0.237	0.273	0.124
TUR vs. GLS	0.056	0.030	0.166	0.190	0.035
TUR vs. KOR	0.087	0.043	0.210	0.242	0.062
ZIK vs. GLS	0.040	0.022	0.104	0.123	0.014
ZIK vs. KOR	0.065	0.035	0.149	0.177	0.034
GLS vs. KOR	0.010	0.006	0.030	0.036	0.002

Note: Significant values via 95% bias-corrected confidence intervals indicated in bold.

6.3. Paper III

Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system

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RESEARCH ARTICLE



Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system

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Abstract

Genetic differentiation plays an essential role in the assessment of metapopulation systems of conservation concern. Migration rates affect the degree of genetic differentiation between subpopulations, with increasing genetic differentiation leading to increasing extinction risk. Analyses of genetic differentiation repeated over time together with projections into the future are therefore important to inform conservation. We investigated genetic differentiation in a closed metapopulation system of an obligate forest grouse, the Western capercaillie *Tetrao urogallus*, by comparing microsatellite population structure between a historic and a recent time period. We found an increase in genetic differentiation over a period of approximately 15 years. Making use of forward simulations accounting for population dynamics and genetics from both time periods, we explored future genetic differentiation by implementing scenarios of differing migration rates. Using migration rates derived from the recent dataset, simulations predicted further increase of genetic differentiation by 2050. We then examined effects of two realistic yet hypothetical migration scenarios on genetic differentiation. While isolation of a subpopulation led to overall increased genetic differentiation, the re-establishment of connectivity between two subpopulations maintained genetic differentiation at recent levels. Our results emphasize the importance of maintaining connectivity between subpopulations in order to prevent further genetic differentiation and loss of genetic variation. The simulation set-up we developed is highly adaptable and will aid researchers and conservationists alike in anticipating consequences of conservation strategies for metapopulation systems.

Keywords Capercaillie · Black Forest · rmetasim · Forward simulation · Conservation · Population structure

Introduction

Many wildlife species of high conservation concern face threats from habitat loss and habitat degradation (Lowe et al. 2005; Brook et al. 2008), subsequently resulting in population fragmentation. As populations become increasingly fragmented, they may enter a metapopulation system

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of geographically distinct subpopulations. Without sufficient migration, effects of genetic drift will result in increasing genetic differentiation between subpopulations, leading to reduced genetic diversity and increased extinction risk of small subpopulations (Frankham et al. 2010). Establishing or enhancing sufficient gene flow within such metapopulation systems, however, can delay or even reverse increasing differentiation (Lowe and Allendorf 2010), and therefore presents itself as a primary conservation strategy (Holderegger et al. 2019).

Decisions on the conservation of threatened metapopulations are ideally informed by predictions based on empirical estimates of migration rates and genetic differentiation between subpopulations (Bennet 2003; Kettunen et al. 2007). Conservation genetic analyses are essential to gain the basic insights needed for such decisions. Repeatedly performing genetic analyses over several time periods recognizes trends, which exceed one-time analyses (Schwartz et al. 2007). In addition to comparisons of past and present genetic data, which facilitate valuable conclusions regarding observed genetic differentiation and its drivers, future projections might be particularly informative to support conservation decisions.

Parameterized with recent field data, such future projections allow for different scenarios to be designed and simulated forward in time. However, designing meaningful scenarios and parameterizing simulations is far from trivial. Populations of conservation concern are often reduced in size, display sex and stage biases, or suffer from reduced gene flow to neighbouring populations (i.e. increasing isolation) (Frankham et al. 2010). Therefore, most assumptions commonly used for simulations, like panmictic mating within infinitely large populations, usually do not hold (Hoban 2014). It is thus crucial to design simulations based on realistic life history parameters and demographics, while at the same time balance model complexity with the risk of underfitting (Hoban 2014). A simulation engine should allow for the specification and variation of these parameters when simulations are seeded with field data of species of conservation concern (Hoban et al. 2012; Hoban 2014). Simulations based on realistic scenarios ultimately enable researchers and conservationists to pinpoint priority areas for conservation actions and evaluate their potential effectiveness. Therefore, simulations of genetic data together with population models represent excellent tools to investigate genetic differentiation between populations and subpopulations, particularly within closed metapopulation systems.

A well suited model system to study and simulate effects of different migration scenarios on genetic differentiation is the Western capercaillie (Tetrao urogallus) in the Black Forest (Germany). Due to its specific range and habitat requirements, the capercaillie is considered as an umbrella species for forests rich in biodiversity (Suter et al. 2002; Pakkala et al. 2003), serving as a flagship species for conservation management (Mollet et al. 2008; Suchant and Braunisch 2008). Worldwide, capercaillie occurs over a large range (Coppes et al. 2015). However, in Western- and Central Europe populations are mainly restricted to mountain ranges and many of them are declining or became extinct in the past (Klaus et al. 1989; Storch 2001; Coppes et al. 2015). Here, we focus on the capercaillie population in the Black Forest, which underwent a severe decline in the past decade (Coppes et al. 2019). This population is well suited for studying genetic differentiation as: (1) it represents a metapopulation system consisting of four subpopulations; (2) the metapopulation system can be considered as a closed system, as distances to the next adjacent populations exceed the species' dispersal capacities; (3) it has been under investigation for over two decades, enabling the assessment of genetic differentiation over time.

We tracked genetic differentiation of capercaillie over time within the Black Forest metapopulation system, using two datasets from different time periods (a historic dataset, sampled from 1999 to 2004; and a recent dataset, sampled from 2013 to 2017). We then ran individual-based forward simulations informed by these field data, implementing different scenarios. Thereby, we aimed to predict genetic differentiation as a function of migration patterns and to model effects of isolation or of the re-establishment of connectivity on future genetic differentiation. We therefore included two from today's perspective realistic yet hypothetical scenarios, one simulating the extinction of one subpopulation, the other one simulating re-establishment of connectivity within the metapopulation. This allowed us to derive conservation actions and to highlight the strength of the simulation engine as a tool to analyse metapopulation systems.

Methods

Study area and focal species

This study was performed in the Black Forest, a lower mountain range (up to 1500 m a.s.l.) in south-western Germany (Fig. 1). The area harbors a population of capercaillie, a large forest dwelling grouse, inhabiting open to semi-open conifer-dominated forests at elevations above 800 m a.s.l. (Klaus et al. 1989; Storch 2001; Graf et al. 2009; Zohmann et al. 2014). Capercaillie numbers and range in the Black Forest have been declining over the past decades. While in 1900 the number of lekking males was estimated to 3800 individuals (Coppes et al. 2019), it declined to around 1300 individuals by mid-century (Roth et al 1990) and only 570 individuals in 1971 based on the first census across the Black Forest (Roth 1974). Since 1983, yearly censuses indicated a further decrease to a historic minimum of 167 lekking males in 2018 (Coppes et al. 2019). Since 1989, the range of capercaillie in the Black Forest decreased from 607 km² in 1993 to 344 km² in 2018 (Coppes et al. 2019). The main cause for this decline is assumed to be habitat deterioration (Kämmerle et al. 2020), but also increasing predation pressure (Kämmerle et al. 2017), increasing human disturbance (Coppes et al. 2017) and climate change (Braunisch et al. 2013) are considered as driving factors. Capercaillie mainly disappeared from small and isolated patches of its range (Kämmerle et al. 2017), while remaining occurrences are increasingly fragmented from each other (Coppes et al. 2019). Currently, the capercaillie population of the Black Forest is split into four, geographically separated subpopulations (i.e. North, Central, East, South, see Fig. 1), which are delineated by topography and landscape characteristics and by median dispersal distances (5-10 km, Storch and Segelbacher 2000). A previous study, including data from 1999 to 2004, indicated effects of barriers for gene flow

Fig. 1 Capercaillie distribution (black) and subpopulations (black dotted line), connected via corridors (grey, from Braunisch et al. 2010) in the Black Forest, south-western Germany. White dots roughly indicate core sample areas of the recent dataset (individual locations not shown)



between the northern and the southern part of the Black Forest area while overall genetic differentiation was found to be weak (Segelbacher et al. 2008). As dispersal between the Black Forest and its nearest neighbouring populations (i.e., Vosges mountains in France, Jura mountains in Switzerland) is highly unlikely due to large distances and unsuitable landscape features (i.e. intensive agricultural land, settlements), the four subpopulations within the Black Forest should be considered as a closed island population (Segelbacher et al. 2003).

Sampling and genotyping of historic and recent data

Datasets from two time periods were included in our analysis: faecal and feather samples collected from 1999 to 2004 (from here on referred to as historic dataset) and from 2013 to 2017 (from here on referred to as recent dataset). For the historic dataset, sampling and laboratory procedures are described in Segelbacher et al. (2008). Molted feathers of 213 individuals were collected non-invasively from 1999 to 2004 and were genotyped at ten microsatellites. In order to correspond to the recent dataset, we excluded one individual that showed missing values at more than three loci, resulting in 212 individuals used in the simulations runs (Table 1).

The recent dataset comprised 1278 faecal samples, which were collected non-invasively from December to April in the years 2013 to 2017. Sampling was conducted up to five days after the last snowfall to ensure high quality of DNA. Samples were genotyped at eleven pre-selected microsatellites (for details see the online appendix). Following the multipletubes approach (Navidi et al. 1992; Taberlet et al. 1996), a consensus genotype was accepted when at least two out of three replicates resulted in the same alleles for heterozygote loci and three out of three replicates for homozygote loci. In case of ambiguous results, additional three replicates were done and samples that still remained ambiguous after these six replicates were discarded afterwards. To check for contamination, negative controls were included in each extraction and PCR batch. Extractions and amplification were performed in separate rooms with regularly sterilized equipment. Samples that failed to amplify at more than three loci were excluded from further analyses.

Genetic diversity, population structure and recent migration rates

To check whether the used microsatellites were appropriate for distinguishing individual genotypes, the probability of identity (P_{ID}) and the probability of identity of siblings (P_{IDsib}) (Waits et al. 2001) were calculated using GenAlEx 6.503 (Peakall and Smouse 2006, 2012). To compare genetic diversity between datasets (sharing four loci), summary statistics were calculated per locus using GenAlEx 6.503 and allelic richness was calculated per locus and subpopulation using PopGenReport 3.0.4 (Adamack and Gruber 2014). Both datasets were checked for deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium using Genepop 4.2 on the web (Raymond and Rousset 1995; Rousset 2008) with default Markov Chain parameters (1000 step dememorisation, 100 batches, 1000 iterations). We corrected for multiple testing (sensu Narum 2006) by using the false discovery rate approaches by Benjamini and Hochberg (1995) and Benjamini and Yekutieli (2001), calculated in R 3.6.0 (R Core Team 2019). Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) was used to test for large allele dropout, presence of null alleles, and stuttering.

Summary statistics of genetic diversity were calculated using GenAlEx 6.503. Allelic richness and private allelic richness were calculated using HPrare 1.1 (Kalinowski 2005).

Genetic population structure of each dataset was inferred using multiple approaches. We performed a Bayesian clustering approach on each dataset, implemented in Structure 2.3.4 (Pritchard et al. 2000), to estimate the number of clusters K and assign individuals accordingly, based on their genotypes. Each analysis was run with 200,000 burn-in and 500,000 MCMC iterations, with 25 iterations for each K from 1 to 7. Information about sampling location (population origin) was implemented using the locprior option (Hubisz et al. 2009). As sample sizes were uneven between subpopulations, an alternative ancestry prior (separate alpha for each subpopulation, and initial alpha of 0.25) was used (Wang 2017). The most likely number of clusters was estimated considering both delta K (Evanno et al. 2005) and mean log likelihood LnP(K) (Wang 2017), calculated by Structure Harvester Web 0.6.94 (Earl and VonHoldt 2012). Post-processing and visualization of runs were done using Clumpak (Kopelman et al. 2015). Population structure was further analysed using the multivariate discriminant analysis of principal components (DAPC, Jombart et al. 2010) implemented in R package adegenet 2.1.1 (Jombart 2008; Jombart and Ahmed 2011) in R 3.6.0. This multivariate

Table 1 Summary statistics of both datasets (HIS = historic dataset, REC = recent dataset) per subpopulation

	N		N _A		A _R		pA _R		H ₀		H _E		F _{IS}	
	HIS	REC	HIS	REC	HIS	REC	HIS	REC	HIS	REC	HIS	REC	HIS	REC
North	60	62	4.6	5.6	4.24	5.19	0.23	0.25	0.513	0.578	0.506	0.626	- 0.026	0.083
Central	33	31	3.9	4.9	3.84	4.85	0.09	0.08	0.537	0.680	0.500	0.662	- 0.050	- 0.030
East	34	41	4.3	5.3	4.18	5.03	0.27	0.01	0.522	0.677	0.516	0.666	- 0.032	- 0.016
South	85	137	4.5	6.4	4.04	5.34	0.21	0.34	0.561	0.673	0.537	0.672	- 0.052	- 0.003
sum/avg	212	271	4.3	5.6	4.08	5.10	0.20	0.17	0.533	0.652	0.515	0.656	- 0.040	0.008

Different sets of microsatellites were used for HIS and REC (HIS: TUT3, TUT4, BG15, BG18, BG4, BG5, BG6, TUT1, TUT2, TUT10; REC: sTUT3, sTUT4, BG15, BG18, sTUD1, sTUD3, sTUD4, sTUD5, sTUD6; online appendix table 1)

N number of individuals, N_A mean number of alleles overall microsatellites, A_R allelic richness and pA_R private allelic richness for a sample size of 26 individuals, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} fixation index. Rounded to two or three digits. Significant F_{IS} values in bold

method first transforms the data using a principal component analysis, followed by a discriminant analysis on the retained components. The resulting discriminant functions visualize the highest between-group variation, while at the same time minimizing within-group variation (Jombart et al. 2010). DAPCs were run including a priori information (population origin) and based on k-means clustering implemented in adegenet. To address pairwise differentiation between populations, we calculated mean pairwise G"_{ST} (Meirmans and Hedrick 2011) as fixation index and mean pairwise Jost's D_{ast} (Jost 2008) as differentiation index with the R package mmod 1.3.3 (Winter 2012). We calculated 95% confidence intervals based on 1000 bootstrapped iterations, and corrected for bias following the method implemented in diveRsity 1.9.9 (Keenan et al. 2013). An AMOVA was calculated using Arlequin 3.5.2.2 with 10,000 permutations.

We used BayesAss 3.0.4 (Wilson and Rannala 2003) to estimate recent migration rates and 95% credible intervals. We conducted ten independent repeats of 50 \times 10⁶ iterations (including 5 \times 10⁶ iterations burn-in) with a sampling frequency of 2000, each initiated with a different random seed for each dataset. In order to keep the acceptance rates for proposed changes between 40 and 60%, delta values were adjusted to $\Delta m = 0.1$, $\Delta a = 0.17$ and $\Delta f = 0.17$. Convergence of chains was confirmed using Tracer 1.7.1 (Rambaut et al. 2018) and by checking for concordance between repeats. We used the Bayesian deviance (Meirmans 2014) to search for the best fitting model (selecting the one with the lowest Bayesian deviance) (Faubet et al. 2007).

Forward simulation of migration scenarios

We performed forward simulations, using individual-based models implemented in the R package rmetasim 3.1.7 (Strand 2002). rmetasim provides a flexible environment that incorporates demographic parameters, migration rates and genetic parameters in the modelling procedure. It offers the possibility to seed simulations using existing genotype data. Stepwise forward simulations can then be run, tracking the fate and genotype of individuals per step, which in our case refers to one year (Strand 2002). After running the simulations, we extracted the genotypes of all individuals in each population and calculated observed and expected heterozygosity using adegenet and pairwise G''_{ST} and Jost's D_{est} using mmod 1.3.3.

We built a stage-based transition model for capercaillie including three stages: juveniles, adult females and adult males. Although some studies indicate slight differences in juvenile survival rates between sexes (Wegge 1980; Klaus et al. 1989; Hörnfeldt et al. 2001), we lumped sexes into a single stage to reduce model complexity. Demographic parameters of capercaillie are multifactorial and differ between years and habitats (Klaus et al. 1989; Grimm and Storch 2000; Kangas and Kurki 2000; Åhlen et al. 2013; Jahren et al. 2016; Augustine et al. 2020). We therefore based the parameterisation on estimates reported from a nearby comparable population (in the Bavarian Alps, Grimm and Storch 2000). Survival rates were set to 0.73 and 0.85 for adult females and males respectively, and 0.36 for juveniles. Reproduction rate was set to 1.5, based on mean clutch size (7), clutch survival (0.65), hatching success (0.95) and chick survival rate (0.342, averaged over both sexes, Grimm and Storch 2000).

Density dependence is assumed to negatively affect survival and reproduction in capercaillie (Kangas and Kurki 2000; Sachot et al. 2006). We thus considered effects of density dependence by reducing female and juvenile survival rates and reproduction rate at carrying capacity. Carrying capacities of subpopulations were estimated by dividing patch sizes by the average size of a female's home ranges (following Sachot et al. 2006), which resulted in 100 to 400 individuals for each subpopulations (North: 400, Central: 100, East: 100 and South; 200). However, the resulting estimates should be seen as rough proxies, as the extent of available habitats can vary as a consequence of changing forest management practices. The simulations were initially seeded using the genotypes, sexes, and subpopulation assignment of the historic and recent datasets. We used all loci represented in the datasets and assumed a general mutation rate of 0.00045 (Whittaker et al. 2003), following the stepwise mutation model.

We ran two simulations, each implementing several scenarios (Table 2). We initialized the first simulation (historic to recent, abbreviated as HR) with the historic dataset and then ran the simulation for 15 years. We implemented three scenarios and compared their results with the genetic differentiation from the recent dataset: The first scenario did not comprise migration between the subpopulations (HR_1: no migration). The second scenario contained migration rates that were derived from empirical analyses of recent migration using BayesAss (HR_2: evidence-based estimates). In the third scenario, we predefined identical migration rates between all subpopulations corresponding to the highest empirically observed migration rate (HR_3: ideal migration). This allowed us to explore a theoretical minimum and maximum differentiation between subpopulations and to evaluate the simulations' set-up (i.e. by comparing scenario HR_2 to the recent dataset). We then initialized the second simulation (recent to future, abbreviated as RF) with the recent dataset, and ran the simulation for 35 years into the future. Thereby, we implemented the same three scenarios as for the HR simulation and two further scenarios derived from recent conservation considerations. This allowed us to contrast potential effects of different migration scenarios on the population differentiation in approximately 2050.

Table 2 Migration scenarios used in the two simulations

scenario	verbal description
simulation HR (historic to recent)	
Scenario HR_1: no migration	This scenario implemented no migration between the four subpopulations, assuming isolation. $m_{all} = 0$
Scenario HR_2: evidence-based estimates of migration	This scenario was built on estimates of migration rates that are derived from analyses of recent migration and genetic population structure. Estimates were adjusted for sample size. $m_{_{CN}}=0.1$, $m_{_{CE}}=0.05$, $m_{_{CS}}=0.01$, $m_{_{EC}}=0.15$, $m_{_{ES}}=0.2$, $m_{_{SE}}=0.15$, all other $m=0$
Scenario HR_3: ideal migration	This scenario implemented identical migration between all subpopulations, in both directions, with the migration rate resembling the maximum migration rate used for scenario HR_2. $m_{all} = 0.15$
simulation RF (recent to future)	
Scenario RF_1: no migration	Migration rates identical to simulation 1 scenario HR_1
Scenario RF_2: evidence-based estimates of migration	Migration rates identical to simulation 1 scenario HR_2
Scenario RF_3: ideal migration	Migration rates identical to simulation 1 scenario HR_3
Scenario RF_4: isolation of East	This scenario implemented isolation of the subpopulation East, hence exploring the differentiation between the remaining three subpopulations in case of extinction of East. Migration rates identical to scenario RF_2, except all migration rates from and to East were set to 0
Scenario RF_5: re-establishment of connectivity to North	This scenario implemented the re-establishment of connectivity from and to sub- population North, via increased migration rates. Specifically, $m_{_{NC}}$ was introduced as well as a uniform migration between North and East. $m_{_{NC}}=0.1$, $m_{_{NE}}=0.05$, $m_{_{CN}}=0.1$, $m_{_{CE}}=0.05$, $m_{_{CS}}=0.01$, $m_{_{EN}}=0.05$, $m_{_{EC}}=0.15$, $m_{_{ES}}=0.2$, $m_{_{SE}}=0.15$, all other $m=0$

The specific migration rates (m) used within the rmetasim simulation are displayed per pairing of subpopulations (N North, C Central, E East, S South), e.g. migration rate from North to South as m_{-NS}

For scenario RF_1, we had to increase the female survival rate slightly to 0.77, so that the simulation would not result in subpopulation numbers too low to be analysed-especially for the recently small subpopulation Central, which was initialized with only 31 individuals (Table 1). We implemented scenario RF 4 to explore effects of isolation/ extinction of the subpopulation East. This subpopulation has experienced the largest decline in the past decades and showed the lowest number of lekking males in 2018 (Coppes et al. 2019). In contrast, we implemented scenario RF_5 to explore effects of an increased migration from and to subpopulation North. According to our analyses of historic and recent population structure, gene flow from and to North markedly decreased in recent times. Therefore, scenario RF_5 explores the potential conservation action of re-establishing gene flow from and to North.

As rmetasim is a stochastic simulation program, we replicated each scenario of both simulations 1000 times (confirmed by an a priori power analyses using the R package pwr 1.2.2, Champely 2018). We analysed each replicate by calculating observed and expected heterozygosity using adegenet 2.1.1, and the pairwise fixation index G''_{ST} as well as the pairwise differentiation index Jost's D_{est} using mmod 1.3.3. We calculated the mean and the 95% confidence intervals (function: qnorm) over all 1000 replicates per scenario (Hoban et al. 2012).

Results

Microsatellite data and observed genetic diversity

After correcting the historic dataset for multiple testing, no deviations from Hardy–Weinberg equilibrium and no evidence for linkage disequilibrium were found within subpopulations. Micro-Checker 2.2.3 indicated the presence of null alleles at locus BG5. Repeating further analyses with omission of this locus did not change the conclusion of results, thus we show results based on data including this locus.

Within the recent dataset, the genotyping success rate was 88%. Locus sTUT2 showed high amounts of missing values (> 30% in all four subpopulations) and was therefore discarded from further analyses. In addition, locus sTUT1 deviated from Hardy–Weinberg equilibrium, Micro-Checker 2.2.3 indicated the presence of null alleles. Therefore, sTUT1 was also discarded from further analyses, reducing the number of microsatellite loci to nine in the recent dataset. We found no evidence for linkage disequilibrium.

The remaining loci in both datasets were powerful enough to detect individuals (historic dataset: $P_{ID} = 5.6 \times 10^{-7}$, $P_{IDsib} = 2.1 \times 10^{-3}$; recent dataset: $P_{ID} = 1.7 \times 10^{-8}$, $P_{IDsib} = 7.1 \times 10^{-4}$). The loci TUT3, TUT4, BG15 and BG18 (shared in both datasets) displayed highly comparable variability, as shown by their allelic richness per subpopulation. In general, the recent dataset was more variable than the historic dataset due to a high allelic richness of locus sTUD5 compared to a lower allelic richness of the loci BG6 and TUT10 (Table 1 in the online appendix).

Genetic diversity was generally higher in the recent dataset compared to the historic dataset (Table 1). However, this result might be affected by the overall higher genetic variability of the microsatellites used in the recent dataset, rather than biological reasons. There was no difference between subpopulations in both datasets. F_{IS} values were not significant for all but one subpopulation (North in the recent dataset), indicating random mating within subpopulations.

Population structure and genetic differentiation from past to present

Results of Structure 2.3.4 without a priori information on sample origin did not show any differentiation of individuals into subpopulations for both datasets (not shown). Using the locprior models for the historic dataset, the most likely number of clusters K ranged from 1 to 3 (with LnP(K) indicating one cluster while ΔK indicated three clusters). Irrespective of the number of clusters, Structure could not distinguish between subpopulations, as all individuals shared comparable proportions of clusters (Fig. 1 on the online appendix).

Within the recent dataset using locprior informed models, the most likely number of clusters K was not clear either. K ranged from 5 to 6 (with LnP(K) indicating five clusters, while Δ K indicated six clusters). Individual assignments however displayed a specific pattern of differentiation between the subpopulations (Fig. 2). While North appeared differentiated as a whole, some individuals of Central appeared to be similar to individuals of East. This indicated migration from East to Central. South displayed an admixed pattern, with individuals appearing similar to individuals of East as well.

The DAPCs showed an increase in differentiation between subpopulations from the historic dataset to the recent dataset, especially for the subpopulations North and Central (Fig. 2). Explained variance retained by the PCA principal components were 96% for the historic dataset and 90% for the recent dataset. The proportion of reassignment, which is the ability of the DAPC to reassign individuals into their original clusters, increased from 61 (historic dataset) to 75% (recent dataset), indicating an increased strength of the genetic signal of differentiation.



Fig. 2 DAPC and Structure results for the historic and recent datasets. DAPCs are shown for the first vs. the second discriminant function (x-axis and y-axis, respectively), Structure results are shown for the most probable number of clusters K inferred by Evanno's ΔK

(Evanno et al. 2005) and LnP(K) (Wang 2017) (K=3 for the historic dataset and K=5 for the recent dataset) with the models including location information. Further Structure plots for K=3-7 for both datasets are shown in the online appendix, Fig. 4



◄Fig. 3 Comparison of pairwise G^{''}_{ST} and bias corrected confidence intervals over datasets and simulation scenarios. a Contrasts the genetic differentiation of the historic dataset, the recent dataset and the scenario RF_2 (recent to future), which is based on recent migration rates. b Shows the scenarios of simulation HR (historic to recent) and contrasts those to the recent dataset. c Shows the scenarios of simulation RF (recent to future)

The comparison of the mean pairwise fixation index G''_{ST} (Meirmans and Hedrick 2011) and differentiation index Jost's D_{est} (Jost 2008) revealed an increase at all pairings from the historic dataset to the recent dataset (Fig. 3a). Both indices were highly positively correlated (hence results for Jost's D_{est} are presented in the online appendix, Fig. 4a).

AMOVAs for the historic dataset and the recent dataset both yielded high amounts of variance within individuals, with only 1.69% of variance (p < 0.001) for the historic dataset and 2.88% (p < 0.001) for the recent dataset due to differences between subpopulations (Table 2 in the online appendix).

Log likelihood was comparable between BayesAss runs, as was Bayesian deviance. Proportions of nonmigrants per subpopulation did not approach either 66 or 100%, altogether indicating a good fit of the Bayes-Ass model (Meirmans 2014). Significant recent migration (Table 3) was highest between East and South (both directions, $m_{BayesAss} = 0.11/0.13$) and from East to Central ($m_{BayesAss} = 0.13$). While uni-directional significant migration rates were found from South and Central to North and from Central to South, they manifested themselves only in low rates (ranging from $m_{BayesAss} = 0.07$ to 0.04).

Simulations of genetic differentiation

The scenarios for both simulations resulted in stable population numbers, and therefore proved to be useful for subsequent analyses. The three scenarios used for simulation HR resulted in different pairwise indices of genetic fixation and differentiation, although partially overlapping confidence intervals indicate variability (Fig. 3b). Scenario HR_1 (no migration) and scenario HR_3 (ideal migration) resulted in highest and lowest pairwise indices, respectively. Indices resulting from scenario HR_2 (evidence-based estimates) were comparable to the indices calculated from the recent dataset. Therefore, our simulation parameters (including the evidence-based estimates for migration rates) proved to be appropriate to generate plausible results of forward simulated population structure.

Scenario RF_1 (no migration) and scenario RF_3 (ideal migration) resulted in the lowest and highest indices of genetic fixation and differentiation (Fig. 3c). Compared to recent pairwise indices of fixation and differentiation (Fig. 3b), indices approximately doubled over the simulated 35 years. Scenario RF_2 (evidence-based estimates)

predicted increasing genetic fixation and differentiation when current migration rates are maintained. Scenario RF_4 (isolation of East) predicted higher pairwise indices of differentiation compared to scenario RF_2. The simulation yielded population numbers for East ranging from less than 10 individuals to 0. This was in line with the scenario assumption of isolation and subsequent extinction of subpopulation East. However, pairwise indices of fixation and differentiation based on such low numbers are not informative and are thus not displayed in detail. Scenario RF_5 (reestablishment of connectivity to North) resulted in overall reduced population differentiation, with some pairwise indices of fixation and differentiation being comparable to recent levels.

Observed and expected heterozygosity per subpopulation indicated an increasing trend with increasing migration rates (Table 3 in the online appendix). Within simulation HR, scenarios HR_1 and HR_2 resulted in slightly reduced heterozygosity whereas heterozygosity of scenario HR_3 remained unchanged, compared to the heterozygosity of the historic dataset. Within simulation RF, scenarios RF 1 and RF 2 again resulted in slightly reduced heterozygosity whereas heterozygosity of scenario RF_3 remained unchanged, compared to the heterozygosity of the recent dataset. Within scenario RF 4, heterozygosity slightly decreased in the remaining subpopulations, while within scenario RF_5, heterozygosity in the subpopulation North increased, compared to the heterozygosity of scenario RF_2. However, observed differences in heterozygosity were generally small.

Discussion

Comparing two datasets, sampled in two different time periods, we were able to trace changes in genetic differentiation between subpopulations within a closed metapopulation system. Combining this with individual-based forward simulations allowed us to explore future genetic differentiation as a consequence of different migration scenarios. Our simulations have shown that genetic differentiation within a metapopulation system is highly dependent on gene flow. With about 15 years between the two sampling periods (roughly 2000 to 2015), we found already increased genetic differentiation between the subpopulations. Simulations for another 35 years (to 2050) revealed a further increase in genetic differentiation if migration patterns stay the same.

Tracking genetic differentiation from past to present

Our results revealed an increase in population structure and genetic differentiation in Black Forest capercaillie within Table 3Migration ratesestimated by BayesAss 3.0.4based on the recent datasetwith 95% credible intervals inparentheses, rounded to threedigits

То	From			
	North	Central	East	South
North	0.844 (±0.070)	0.060 (±0.052)	$0.024 (\pm 0.035)$	0.072 (±0.066)
Central	0.018 (±0.032)	0.825 (±0.076)	0.128 (±0.079)	$0.030(\pm 0.053)$
East	0.015 (±0.028)	$0.060(\pm 0.064)$	0.812 (±0.086)	0.112 (±0.083)
South	$0.011 (\pm 0.020)$	0.035 (±0.034)	0.133 (±0.049)	0.821 (±0.052)

Significant values based on credible intervals in bold. Given as fraction of individuals within a subpopulation (per row) that migrated from a source subpopulation (in columns)

a short period of approximately 15 years (Fig. 2). For the historic dataset, we found no signs of population structure or increased differentiation between specific subpopulations, which is in line with previous studies (Segelbacher et al. 2008). While the slight differentiation of the subpopulation North corresponded to our expectations, high rates of historical migration could have been counterbalancing differentiation effects (Segelbacher et al. 2008), ultimately preserving a nearly panmictic structure (Lowe and Allendorf, 2010). In the recent dataset, however, subpopulation North appeared differentiated from the three other subpopulations. Subpopulation North is geographically separated from the other subpopulations by a large valley with low mountains and poor habitat suitability for capercaillie (Braunisch and Suchant 2007; Coppes et al. 2019). Additionally, increasing habitat deterioration (Kämmerle et al. 2020) and human disturbance (Coppes et al. 2017) might act as driving factors of the observed increase in subpopulation differentiation. The subpopulations South and Central appeared still wellconnected with high bi-directional migrations rates, while migration from East to Central was one-directional. These results indicate that the differentiation between North and the other subpopulations has increased due to limited migration between the subpopulations. Conservationists should thus treat these first signs of differentiation (Segelbacher et al. 2008) as early warning signs of declining functional connectivity and plan management strategies to increase migration rates or to reduce factors hindering migration of the target species.

Our HR simulation showed that the increased differentiation can be attributed at least partially to the ongoing segregation of subpopulations and subsequent loss of gene flow. Comparing scenarios of evidence-based migration rates with simulated optimal conditions (i.e. HR_2 vs. HR_3, Fig. 3b) highlights the effect of migration rates on genetic differentiation. The long-term negative population trend of capercaillie in the Black forest, paired with ongoing habitat contractions (Coppes et al. 2019), resulted in small and fragmented subpopulations. However, within a metapopulation system, migration between subpopulations is essential to compensate for small population sizes and increased extinction risks (Frankham et al. 2010). While single large populations can still harbour low levels of genetic diversity when being isolated (Rutkowski et al. 2017), well-connected metapopulation systems can preserve higher levels of genetic variability even when subpopulation sizes are low (Alstad 2001; Allendorf et al. 2012). Accordingly, we found a slight trend of increasing levels of heterozygosity with increasing migration rates. Yet, the scenario with optimal migration rates (HR_3) could only maintain heterozygosity on historic levels, while the scenario using evidence-based migration rates (HR_2) resulted in decreased heterozygosity compared to historic levels.

When assessing population structure and genetic differentiation, potential time lags have to be considered. The manifestation of signals in genetic patterns is highly dependent on a species' dispersal capability (Landguth et al. 2010). Given median dispersal distances of capercaillie of about 5 to 10 km (Storch and Segelbacher, 2000), the detected increase in differentiation might not be related to specific fragmentation events in the past 15 years, but rather represents cumulative effects within a much longer time period.

Simulating genetic differentiation from present to future

Forecasting genetic differentiation using evidence-based migration rates, we observed a further increase by approximately 2050 (Fig. 3a). However, it is likely that our forecasts based on these rates might even underestimate genetic differentiation. The migration rates were appropriate in simulating the historic dataset to recent times, yet the population decline has accelerated over the entire study period (Coppes et al. 2019), thereby reducing gene flow to a larger extent. Additionally, our simulations featured stable population numbers (cf. Grimm and Storch 2000), further raising serious conservation concerns. Keeping population numbers stable, which is per se a challenging conservation target, evidently does neither prevent further genetic differentiation sufficiently nor preserve the metapopulation's genetic variability.

By implementing different migration rates between the subpopulations, we explored two realistic scenarios. Scenario RF_4 addressed effects of a potential extinction of one

subpopulation (East) on the genetic differentiation between the remaining subpopulations. Considering the low population size of the subpopulation East (Coppes et al. 2019), stochastic effects could have a high impact on the subpopulation, and potentially lead to its extinction. Although initiated with recent migration rates for the remaining pairs of subpopulations, the simulation resulted in levels of genetic differentiation that are comparable to the scenario with no migration (RF_1). The subpopulation East therefore seems to act as an important core area for gene flow, connecting at least the southern subpopulations. The genetic differentiation between the subpopulations Central and North increased as well in this scenario (compared to RF_2), although the subpopulation East is not directly connecting these two subpopulations. However, with the simulated extinction of the subpopulation East, subpopulation Central became relatively isolated as well, probably driving the observed increase in genetic differentiation. This scenario clearly indicates that changes in one subpopulation might have far-reaching effects on all other subpopulations within a metapopulation system.

Addressing a potential conservation action, we simulated effects of an improved connectivity from and to the subpopulation North on the entire metapopulation system within scenario RF_5. Based on existing data of habitat suitability (Braunisch and Suchant 2007) and functional connectivity for capercaillie in the Black Forest (Braunisch et al. 2010) as well as on our own analyses, the re-establishment of corridors to the subpopulation North and particularly corridors between North and Central appeared to be important for preventing further isolation. Our simulations showed a general positive effect of this management action in terms of overall decreased genetic differentiation compared to RF 2. While all pairwise comparisons of subpopulations including the subpopulation North were particularly affected, also the genetic differentiation between the remaining subpopulations in the southern regions of the Black forest was reduced. Additionally, heterozygosity was higher especially for the subpopulations North and Central, compared to scenario RF_4. This again supports the idea, that differentiation between all pairs of subpopulations in an entire metapopulation system might be affected by changes in one single subpopulation.

Increasing migration rates between subpopulations may only lead to maintaining rather than reducing genetic differentiation in the future. Landguth et al. (2010) found a strong nonlinear relationship between losing a historic barrier's signal in the genetic pattern and the species' dispersal capabilities. In highly mobile species, the signal will be lost within several generations. By contrast, species with limited dispersal capabilities were found to accomplish the same within tens or hundreds of generations. This might also be true for capercaillie with comparatively low median dispersal distances of 5 to 10 km (Storch and Segelbacher 2000). Accordingly, dispersal of two male individuals from the subpopulation North to the subpopulation East and of one female individual from the subpopulation East to the subpopulation Central has been found, thereby representing rare cases of migration between subpopulations (with n = 3 out of 1278 samples).

Conclusions for conservation

In the light of the drastic decline in population size and habitat area of capercaillie in the Black Forest (Coppes et al. 2019), conservation strategies must be planned and implemented urgently. While we found genetic differentiation to be driven by migration rates between subpopulations, preservation and increase of population size are important prerequisites to enable migration. Yet our simulations clearly showed that genetic differentiation can increase severely even in the case of stable population numbers. We therefore emphasize the need for conservation strategies that aim at re-establishing functional connectivity between subpopulations. Related actions might include improving habitat suitability within corridors or creating stepping stones. Our simulations have shown that increased migration rates between subpopulations can counteract genetic differentiation even in metapopulation systems with realistic demographic parameters and low population numbers. Re-establishment of functional connectivity could help maintaining genetic differentiation at its current level. Further, we strongly recommend the continuation of periodic genetic monitoring which will allow for adjusting and improving the predictions of future genetic differentiation along with predicting consequences of conservation actions.

Individual-based simulations, as used in the present study, can be valuable tools for both scientists and practitioners (Epperson et al. 2010; Hoban 2014; Holderegger et al. 2019). The flexibility of simulations allows for a variety of scientific questions to be examined and for conservation actions to be designed and tested (Hoban et al. 2012). We showed how simulations can support analyses of genetic differentiation in metapopulations systems and how realistic scenarios can provide valuable insights for species conservation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All authors approved this version of the manuscript for publication.

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1 **Online Appendix** to

2

Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system

5

6 Kunz, F., Kohnen, A., Nopp-Mayr, U., Coppes, J.

7

8 Laboratory procedure of recent dataset

9 DNA from feathers was extracted using the QIAmp DNA MicroKit (Quiagen, Hilden
10 Germany) following the manufacturer's protocol. DNA from faeces was extracted using the
11 QIAmp DNA Stool Mini Kit (Quiagen, Hilden, Germany) following the adaptions of
12 Sittenthaler et al. (2018).

Samples were genotyped using the eleven microsatellite loci sTUT1, sTUT2, sTUT3, sTUT4, 13 sTUD1, sTUD3, sTUD4, sTUD5, sTUD6, BG15 and BG18 based on Jacob, Debrunner, 14 Gugerli, Schmid, & Bollmann (2010) and the sexing locus CHD-1 (Kahn et al. 1998). 15 Amplification was performed in a 10 µl reaction volume containing 5 µl SuperHotStart 16 Mastermix (Genaxxon BioScience, Ulm, Germany), 0.8 – 1.7 µM of each primer and 1 µl of 17 template DNA. Polymerase chain reaction (PCR) was done using the following conditions: 18 19 initial denaturation for 15 min at 95 °C, 38 cycles of denaturation for 30 s at 95 °C/annealing for 1 min at 56 °C/extension for 1 min at 72 °C, followed by a final extension for 30 min at 20 72°C. PCR products were run on an ABI 3130 DNA Analyser (Applied Biosystems, Darmstadt, 21 Germany) and analysed using GeneMapper 4.0 (Applied Biosystems, Darmstadt, Germany). 22

23





Fig. 1 Comparison of Structure results (K = 3 to 7) including location information (using
locprior models) for the historic dataset and the recent dataset



28

Fig. 2 Comparison of pairwise Jost's D_{est} and bias corrected confidence intervals over time periods and scenarios. A) contrasts the genetic differentiation of the historic dataset, the recent dataset and the scenario RF_2 (recent to future), which is based on recent migration rates. B)
shows the scenarios of simulation HR (historic to recent) and contrasts those to the recent
dataset. C) shows the scenarios of simulation RF (recent to future)

34

Table 1 Allelic richness per locus-subpopulation combination for each dataset, calculated with
the R package PopGenReport 3.0.4. (Adamack and Gruber 2014). N_A (number of alleles per
locus overall subpopulations), shared loci are highlighted in grey. Rounded to three digits. Note
that the two loci sTUT1 and sTUT2 were excluded from the recent dataset before analysis due
to high proportion of missing values (sTUT2) and the presence of null alleles (sTUT1).

40

historic dataset						recent dataset							
	NA	North	Central	East	South	mean		NA	North	Central	East	South	mean
TUT3	4	3.97	3.65	3.65	3.63	3.73	sTuT3	4	3.92	2.97	3.53	3.97	3.60
TUT4	5	2.42	2.66	4.26	2.30	2.91	sTuT4	4	2.00	2.00	2.00	2.67	2.17
BG15	3	3.00	3.00	3.00	3.00	3.00	BG15	3	3.00	3.00	3.00	3.00	3.00
BG18	7	6.26	5.94	5.56	5.66	5.85	BG18	7	6.68	5.95	5.82	5.76	6.05
BG4	7	5.82	5.00	5.50	5.70	5.51	sTuD1	6	4.77	4.00	3.92	4.50	4.30
BG5	8	6.73	4.92	5.56	5.55	5.69	sTuD3	6	5.84	4.67	5.52	5.10	5.28
BG6	2	1.95	1.00	1.63	1.89	1.62	sTuD4	10	5.50	5.88	5.78	7.24	6.10
TUT1	8	5.70	4.85	4.99	5.72	5.32	sTuD5	11	9.21	8.75	9.46	9.33	9.17
TUT2	6	4.00	4.65	3.87	4.27	4.20	sTUD6	9	5.20	5.67	5.06	6.13	5.49
TUT10	2	1.88	2.00	1.86	2.00	1.94							

- 41
- 42

Table 2 AMOVA results for the historic and the recent dataset, with results from recent dataset

in square brackets. Rounded to three digits. Significant values due to 10,000 permutations arein bold.

46

Source of Fixation Sum of Variance Percentage p value variation variation index squares components Among 20.33 0.045 1.69 < 0.001 $F_{ST} = 0.017$ subpopulations [39.98] [0.089] [<0.001] [0.029] [2.88] Among individuals 491.98 $F_{IS} = -0.025$ -0.065 0 0.94 within [788.23] [0.056] [1.83] [0.019] [0.07] subpopulations $F_{IT} = -0.007$ Within 529.5 2.688 98.31 0.77 individuals [0.047] [770.5] [2.935] [95.28] [<0.001] 1041.82 2.669 Total [1598.71] [3.08]

48 Table 3 Observed and expected heterozygosity in the simulations HR (historic to recent) and 49 RF (recent to future), compared with the historic and recent datasets, rounded to three digits. 50 Note that the two simulations cannot be compared directly, as their initiating datasets (historic 51 and recent) differ in the used microsatellite loci. Also results for East in scenario RF_4 are not 52 shown, as this scenario simulated isolation and extinction of East.

53

		North	Central	East	South			
simulation HR (historic to recent)								
hists.	Ho	0.513	0.537	0.522	0.561			
nistoric	H _E	0.506	0.500	0.516	0.537			
	Ho	0.497	0.492	0.500	0.531			
HK_1	H _E	0.483	0.448	0.472	0.518			
	Ho	0.499	0.509	0.519	0.530			
HK_Z	HE	0.488	0.491	0.510	0.523			
	Ho	0.516	0.521	0.522	0.530			
HK_3	H _E	0.511	0.513	0.515	0.523			

simulation RF (recent to future)

	Ho	0.578	0.680	0.677	0.673
recent	H _E	0.626	0.662	0.666	0.672
RF_1	Ho	0.602	0.574	0.594	0.640
	HE	0.594	0.529	0.559	0.624
RF_2	Ho	0.617	0.642	0.652	0.655
	H _E	0.609	0.627	0.641	0.646
DF 2	Ho	0.656	0.661	0.660	0.661
KF_S	H _E	0.651	0.655	0.654	0.656
	Ho	0.606	0.567	-	0.637
KF_4	HE	0.596	0.500	-	0.614
DE E	Ho	0.632	0.651	0.656	0.656
NF_3	HE	0.626	0.644	0.651	0.650

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- Alpine black grouse *Tetrao tetrix* metapopulation. J Avian Biol 49:e01681.
- 68 https://doi.org/10.1111/jav.01681

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Appendix A: SCI publication list

- Griesberger P, Kunz F, Obermair L, Hackländer K (submitted) **Spatial distribution of hunting affects the impact of roe deer (***Capreolus capreolus***) on forest vegetation in selected regions of Upper Austria.** Diversity.
- Mattsson B, Mateo-Tomás P, Aebischer A, Rösner S, Kunz F, ... Viñuela J (2022) Enhancing monitoring and transboundary collaboration for conserving migratory species under global change: the priority case of the red kite. J. Environ. Manage. 317: 115345
- Kunz F, Klinga P, Sittenthaler M, Schebeck M, Stauffer C, Grünschachner-Berger V, Hackländer K,
 Nopp-Mayr U (2022) Assessment of drivers of spatial genetic variation of a ground-dwelling bird species and its implications for conservation. Ecol. Evol. 12: e8460
- Kunz F, Kohnen A, Nopp-Mayr U, Coppes J (2021) Past, present, future: tracking and simulating genetic differentiation over time in a closed metapopulation system. Cons. Gen. 22: 355–368
- Nopp-Mayr U, Kunz F*, Suppan F, Schöll E, Coppes J (2021) Novel application and validation of a method to assess visual impacts of rotating wind turbine blades within woodland areas. PFG 89: 1-14 *Equally contributing first author
- Kunz F, Gamauf A, Zachos FE, Haring E (2019) Mitochondrial phylogenetics of the Goshawk Accipiter [gentilis] superspecies. J. Zool. Syst. Evol. Res 57: 942-958
- Sittenthaler M, Kunz F, Szymusik A, Grünschachner-Berger V, Krumböck S, Stauffer C, Nopp-Mayr U (2018) **Fine-scale genetic structure in an Eastern Alpine black grouse (***Tetrao tetrix***) metapopulation**. J. Avian. Biol. 49: jav-01681

Academic awards

- 2019 Best Poster Award Fourth Annual Meeting in Conservation Genetics
- 2019 Granser United Global Academy Award for research within sustainable hunting
- 2019 Young Opinion Research Award by the CIC (Int. Council for Game and Wildlife Conservation)
- 2018 Best Poster Award NOBIS Austria (Network of Biological Systematics)

Appendix B: selected transfer of knowledge

- Kunz F (2022) Warum Vernetzung des Lebensraums wichtig ist. Der Anblick 07/2022, 12-14, ISSN 0003-2824
- Pfeifer M, Huber T, Grünschachner-Berger V, Kunz F (2022) Die Auerhühner: Wie steht's und was ist noch geplant? Vorarlberger Jagd Jan/Feb 2022, 10-12
- Kunz F (2022) Eine Einführung in die "Henderlgenetik". Der Anblick 04/2022, 14-17, ISSN 0003-2824
- Kunz F (2022) Jagd und Wildtiergenetik ein Widerspruch? Der Anblick 02/2022, 6-9, ISSN 0003-2824
- Kunz F, Schneider L, Griesberger P (2022) **Den Wildtieren auf der Spur.** Teaching at schools, science to public, repeatedly in Vienna and its environs
- Kunz F (2022) Wildtiergenetik Genetik im Natur und Artenschutz. Lecture to professionals in an advanced training program, Vienna
- Nöbauer S, Knufinke JF, Griesberger P, Kunz F (2022) Warum trägt ein Hirsch ein Halsband? Information stand and talk at the Long Night of Science, Science to Public event
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