Range shifts or extinction? Ancient DNA and distribution modelling reveal past and future responses to climate warming in cold-adapted birds

Running head: Avian palaeogenetics of climate change

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ABSTRACT

1   Global warming is predicted to cause substantial habitat rearrangements, with the most severe effects
2   expected to occur in high-latitude biomes. However, one major uncertainty is whether species will be
3   able to shift their ranges to keep pace with climate-driven environmental changes. Many recent studies
4   on mammals have shown that past range contractions have been associated with local extinctions rather
5   than survival by habitat tracking. Here, we have used an interdisciplinary approach that combines
6   ancient DNA techniques, coalescent simulations and species distribution modelling, to investigate how
7   two common cold-adapted bird species, willow and rock ptarmigan (*Lagopus lagopus* and *Lagopus*
8   *muta*), respond to long-term climate warming. Contrary to previous findings in mammals, we
9   demonstrate a genetic continuity in Europe over the last 20 millennia. Results from back-casted species
10   distribution models suggest that this continuity may have been facilitated by uninterrupted habitat
11   availability and potentially also the greater dispersal ability of birds. However, our predictions show that
12   in the near future, some isolated regions will have little suitable habitat left, implying a future decrease
13   in local populations at a scale unprecedented since the last glacial maximum.

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INTRODUCTION

How organisms will respond to future global warming is a topic of considerable interest in conservation management, and much effort is spent to explore different scenarios about the future state of species distributions. Among the major uncertainties are if, and at what speed, species will be able to shift their ranges in order to keep pace with changes in available habitat (e.g. Kerr *et al.* 2015). Thomas *et al.* (2004) predicted that 9-32 % of all species will go extinct due to climate induced habitat loss by 2050, assuming that they are able to colonise new areas as soon as these become climatically suitable. However, if this assumption is not met, the proportion of predicted species extinctions increases to 34-58 % (Thomas *et al.* 2004). Successful range shifts could be highly dependent on the geographic connectivity between suitable habitat patches (Engler *et al.* 2009; Hodgson *et al.* 2012), and it has been suggested that only the populations already inhabiting refugial areas are the ones that survive range contractions (Bennett *et al.* 1991). These refugial populations may also obstruct the subsequent arrival of individuals from more remote areas through intra-specific competition (Hewitt 1996; Hewitt 1999). It has previously been difficult to evaluate whether range contractions actually do correspond to population contractions (i.e. habitat tracking), since modern phylogeographic analyses only give information about the surviving lineages. Lately though, ancient DNA techniques have made it possible to analyse temporal changes in genetic variation of cold-adapted taxa, from the last Ice Age up until today. Interestingly, these results have often shown that local extinctions and population turnovers, rather than survival by habitat tracking, were the dominant processes during past climate warming (e.g. Dalén *et al.* 2007; Lagerholm *et al.* 2014; Palkopoulou *et al.* 2016). This raises concerns from a conservation perspective, since such local extinctions following habitat loss could lead to ensuing losses of unique genetic variation. This is especially the case for the future viability of cold-adapted species, since the effects of on-going climate warming are expected to be most severe in northern biomes (Pithan & Mauritsen 2014; Serreze & Barry 2011) and their ranges are already in a contracted state since the end of the last glaciation (Stewart *et al.* 2010). In order to make reliable predictions of future distributions and ensure effective conservation planning, it is therefore important to increase the knowledge about species' habitat tracking abilities (Kerr *et al.* 2015; Urban 2015).

  Two cold-adapted bird species that co-occurred with the iconic Ice Age mammal fauna are the willow ptarmigan (*Lagopus lagopus*) and rock ptarmigan (*Lagopus muta*). Since ptarmigan are important prey

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60   in the arctic ecosystem (e.g. Hansen *et al.* 2013; Nyström *et al.* 2005), and also have slightly different

61   environmental preferences, they together provide a good case-study to evaluate how cold-adapted

62   species are affected by climate-induced habitat shifts. Further, an important feature that distinguishes

63   them from previously investigated taxa is their flight capability which, although they have a sedentary

64   lifestyle, potentially could make dispersal to new areas less dependent on habitat connectivity (Pedersen

65   *et al.* 2004).

66   Today, the European ranges of ptarmigan are restricted to high altitude and/or high latitude regions,

67   with rock ptarmigan occupying the more alpine and sparsely vegetated areas (Fig. 1), but during the last

68   glacial period both species were widely distributed across the tundra-steppes of midlatitude Europe (e.g.

69   Tyrberg 1998). Morphological analyses of fossil remains of the two sister species have shown that both

70   were morphologically distinct and probably had greater body weights than their modern counterparts

71   (Bochenski 1985; Potapova 1986; Stewart 1999). However, it has not yet been established whether these

72   populations represented specialised lineages that went extinct during end-Pleistocene climate warming,

73   approximately 10 thousand years ago, or if the glacial populations in fact are the direct ancestors of

74   modern European *L. lagopus* and *L. muta* (Höglund *et al.* 2013).

75   In this study, we have used an interdisciplinary approach consisting of ancient DNA analyses,

76   Bayesian inference of coalescent simulations and back-casted species distribution modelling to analyse

77   temporal changes in genetic variation and available habitat over the last 20 millennia. We evaluate two

78   contrasting historic scenarios: 1) The glacial fossil remains from midlatitude Europe represent lineages

79   that went extinct during the rapid warming of the early Holocene, and modern European ptarmigan

80   populations have thus been established by postglacial immigrants from the Siberian population; or 2)

81   The widespread glacial populations in Europe managed to track their shifting habitats into alpine and/or

82   northern regions. In addition to investigating past responses, we also use species distribution modelling

83   to forecast how global warming will affect ptarmigan distributions in the future, and discuss how

84   knowledge of the past can influence conservation planning.

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90   **MATERIALS AND METHODS**

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92   **Genetic analyses**

93   *Samples and DNA Extraction*

94   We collected *Lagopus* spp. bones from 73 Late Pleistocene individuals, ranging in age between ~ 11 to

95   27 thousand calendar years before present (cal kBP), from 10 paleontological sites across Europe (Table

96   S1). DNA was extracted from bone powder following Palkopoulou *et al.* (2013). In order to create a

97   reference dataset of the modern Holarctic genetic variation within both species, sequences from various

98   locations (Table S2 and S3) were downloaded from GenBank (see the Supporting Information). This

99   modern dataset was further complemented with modern samples from 34 willow and 13 rock ptarmigan

100   samples (Table S2 and S3), from which we extracted DNA following either the Palkopoulou *et al.*

101   (2013) protocol or the Qiagen DNeasy tissue kit according to Segelbacher *et al*. (2002).

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103   *DNA Amplification and Sequencing*

104   A total of 240 bp of the mitochondrial control region was amplified in three partially overlapping

105   fragments, using genus-specific primers developed for the study (Table S4). Some of the modern

106   samples were analysed at Uppsala University, Sweden, and polymerase chain reactions (PCRs) were

107   then made using the primers and protocol developed by Baba *et al*. (2001). Additional details regarding

108   DNA extraction, PCR amplification and sequencing are presented in the Supporting Information.

109   The pre-PCR work on the Late Pleistocene fossil remains were made in the ancient DNA laboratory at

110   the Swedish Museum of Natural History, which is physically isolated from the facilities for modern

111   material and post-PCR. All working surfaces and equipment were regularly sterilised with UV light,

112   bleach or hydrochloric acid, and all extraction and amplification reactions were made with blank

113   controls in order to monitor possible contamination. Further, at least two independent amplifications

114   were done in order to allow identification of erroneous bases caused by misincorporation during the

115   PCR process.

116   The obtained sequences were aligned and edited using the software SeqMan in the package Lasergene

117   v8.1.5 (DNASTAR) or the Muscle alignment algorithm in the package CodonCode Aligner 2.0.6

118   (CodonCode, USA). BioEdit v1.7.3 (Hall 1999) was then used to align our data with the downloaded

119   sequences from GenBank and build complete Holarctic datasets for willow and rock ptarmigan,

120 respectively. These datasets were subdivided into the following geographic regions: Europe, consisting

121 of Western Europe (west of the White Sea) and Western Russia (between the White Sea and the Urals);

122 Siberia (Russia east of the Urals) together with North America; and Iceland, Svalbard and Greenland.

123 The wide geographic areas of the two latter groups were due to a corresponding labelling of many of the

124 downloaded GenBank sequences.

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126 *Phylogenetic and Demographic analyses*

127 For each species, a temporal statistical parsimony network was created with the R-script TempNet v1.4

128 (Prost & Anderson 2011) in order to display the haplotypes found in Late Pleistocene Europe, as well as

129 in modern populations world-wide. To further investigate the amount of population genetic structure in

130 the two species and evaluate the similarity between ancient European and modern Eurasian samples, we

131 used Arlequin v3.5.1.2 (Excoffier & Lischer 2010) to compute analyses of molecular variance

132 (AMOVA). Based on distance matrices of pairwise differences and 10,000 permutations, we analysed

133 the following geographic and temporal hierarchical groupings: [modern and ancient Europe vs. modern

134 Siberia]; [modern Europe and Siberia vs. ancient Europe]; [modern Europe vs. modern Siberia and

135 ancient Europe].

136 Bayesian Skyline plots were constructed in BEAST v1.8.0 (Drummond *et al.* 2005; Drummond *et al.*

137 2012) to analyse population size changes over time in Western Europe (i.e. only Europe west of the

138 White Sea) for both species. We used the HKY+G nucleotide substitution model, as suggested by the

139 hLRT criterion in MrModeltest v2.3 (Nylander 2004), five groups, and a strict molecular clock with

140 mutation rates estimated from the data. Each analysis was run for 70 million generations, with

141 parameters logged every 1,000 generation, and the results were checked in TRACER v1.5 (Rambaut &

142 Drummond 2007) to ensure convergence and effective sampling of all parameters.

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144 *Approximate Bayesian Computation*

145 A model choice analysis by approximate Bayesian computation (ABC) coupled with coalescent

146 simulations was carried out for both species, in order to evaluate our two proposed hypotheses regarding

147 the most likely ancestral population to modern European birds. The simulated system was the same for

148 both species, and was based on the Eurasian dataset (see Table S2 and S3) with three statistical groups.

149 Modern Europe was set to coalesce with one of the two alternative ancestral populations between 12 and

30 cal kBP, which in turn subsequently coalesced with the third remaining population between 30 and 120 cal kBP (Fig. 2).

After determining the most likely historic scenario for the origin of the modern European populations, we carried out another model choice analysis by ABC in order to investigate in more detail the demographic history of Western European (Europe west of the White Sea: Table S2 and S3) ptarmigan during the rapid climate warming at the Pleistocene-Holocene transition. Four different demographic scenarios were evaluated: a constant population size through time, a population increase or decrease, and a bottleneck (Fig. S1). From the chosen scenario we then estimated the parameters of interest, most notably effective population sizes at different time periods.

We performed the approximate Bayesian computation, including coalescent simulations and model choice analysis (scenario comparison), in the program BaySICS (Sandoval-Castellanos *et al.* 2014). The scenario comparison was made using Bayes factors, obtained for every pair of scenarios, using three different methods: a direct approach, an adjusted method (in which model likelihoods were weighted by an Epanechnikov kernel), and a logistic regression (Fagundes *et al.* 2007).

The analyses also included 50 replicates with increasing acceptance thresholds to assess the consistency. Pilot simulations and analyses were carried out in order to choose a proper set of summary statistics and to define optimal prior distributions (see Table S5). One million simulations were run in the pilot and the final simulations, respectively, and 5,000 of the accepted simulations were retained for further analysis. For both species, effective population sizes were first simulated from exponential priors (with λ=250,000) in order to sample homogeneously over a logarithmic scale to track a wide range of values, before employing uniform priors in the final simulations. The mutation rate had a uniform prior of 2.5 to 25 % substitutions per million years (Myr^{-1}), covering the previously published rate for the two species of 6.5 % Myr^{-1} (Drovetski 2003) as well as the possibility of elevated rates in ancient DNA data sets (e.g. Ho *et al.* 2011). Additionally, in order to study the robustness of the results to parameter uncertainties, we performed replicates of the entire analysis considering very large mutation rates (up to 90 % Myr^{-1}) as well as a genetic differentiation within modern Europe that could have begun at the onset of the Holocene climate warming. Finally, we cross-validated our results by analysing pseudo-observed datasets (PODs) in order to estimate the probability of making incorrect model choices.

Species Distribution Modelling

Distribution and climatic data

The modern Eurasian ranges of willow and rock ptarmigan (Fig. 1) were compiled from the IUCN database (BirdLife BirdLife_International 2012a; BirdLife BirdLife_International 2012b), with a subsequent correction of the distribution in Russia according to Potapov and Potapov (2011). Paleontological sites with *Lagopus* spp. remains were georeferenced from Tyrberg (1998; 2008) and classified in 1,000 year intervals, to be used as past presence data (Fig. 1).

The climatic variables used in the models were mean temperature and the sum of precipitation, estimated for the summer (June, July, August and September) and winter (December, January, February and March) as well as for the whole year (Fig. S2). These were selected due to their well-known ability to explain plant species distributions, i.e. the main limiting resources for ptarmigan (Espíndola *et al.* 2012; Maiorano *et al.* 2013). Past climate data sets were created from a global ocean–atmosphere climate model from the Hadley Centre (HadCM3) with a temporal resolution of 1,000 years, ranging from the present and back to 20 cal kBP, and corrected for anomalies such as in Maiorano *et al.* (2013). We added a mask on the environmental variable corresponding to the Late Pleistocene ice sheets, with glacial data downloaded from NOAA's National Climatic Data Center (http://www.ncdc.noaa.gov/), as well as digitized from Darnault *et al.* (2012). Data on current climate (averaged from 1950 to 2000) and future climate projections (averaged from 2070 to 2100) were obtained from the Climatic Research Unit (Mitchell *et al.* 2004). For future climatic projections, we used data from the HadCM3 climate model in order to be consistent with past data. Two different IPCC (Intergovernmental Panel on Climate Change) future climatic scenarios were considered: the B1 scenario and the A2 scenario (IPCC 2001). Past, present and future environmental data were mapped over the European and Eurasian extent on a 0.5x0.5' resolution grid.
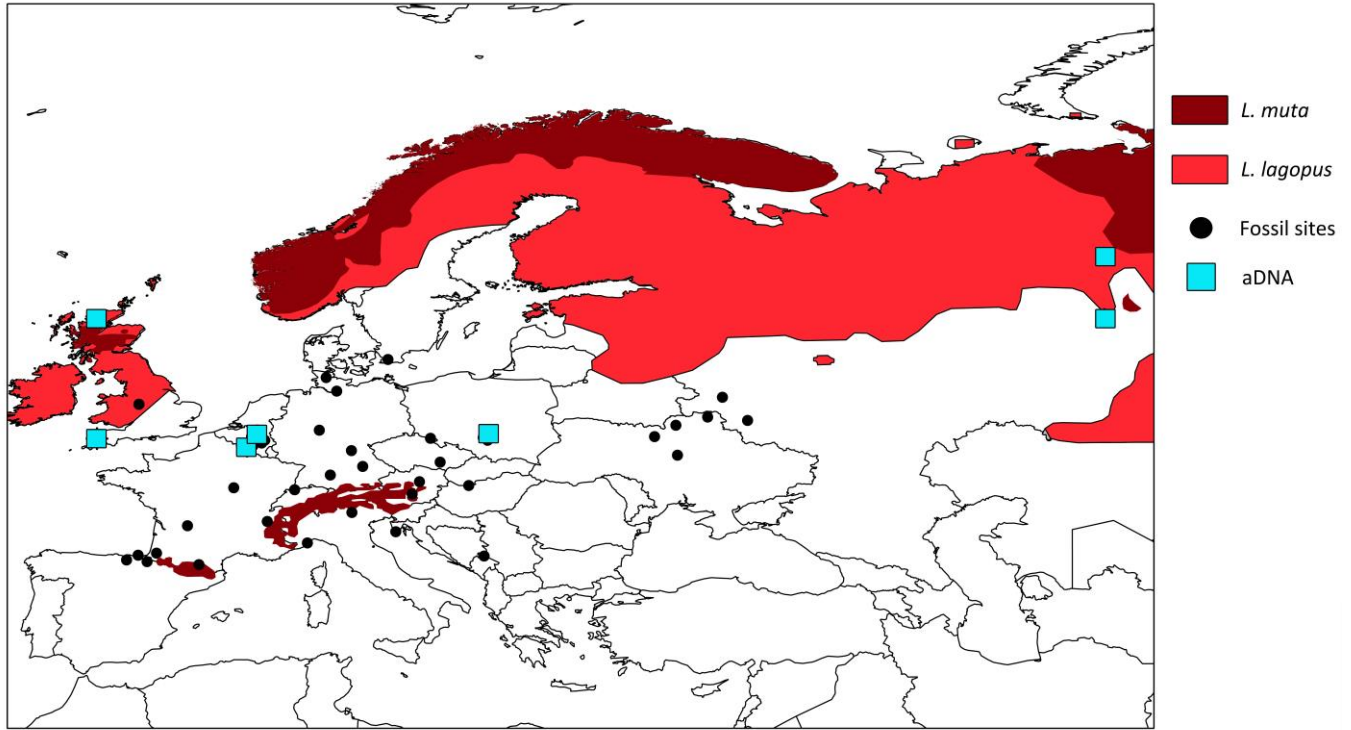
Models

The models were calibrated on current species presence/absence data and climatic conditions, using the R package BIOMOD (R Core Team 2014; Thuiller *et al.* 2009). Training data (species presence/absence and environmental covariates) were sampled randomly within species' modern distribution ranges. In a preliminary analysis we compared the predictive performances of different sampling strategies that differed regarding *i*) the size of the sample and *ii*) the spatial extent of the sample (European versus

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210 Eurasian extent). Since the current climatic conditions in Siberia are considered to resemble those in
211 Europe during the Last Glacial Maximum, training the models on the whole Eurasian continent
212 (including Siberia) might increase the predictive power of ptarmigan occupancy for European
213 distributions during the Late Pleistocene period. Further, as an addition to the modern presence/absence
214 data, we also investigated if we could improve the model predictive performance by adding climate data
215 from the georeferenced fossil sites to the training data sets.

216 Different modelling techniques were fitted to the training data sets (see Supporting Information) and
217 their predictions were evaluated on present and past data sets. For present data, the Area Under the
218 Curve (AUC) metric was computed on cross-validated test data sets and on a sample of 1,000 test data
219 points randomly sampled within the European region. Model predictions of the past were evaluated
220 using fossil records as a test data set, and by computing sensitivity.
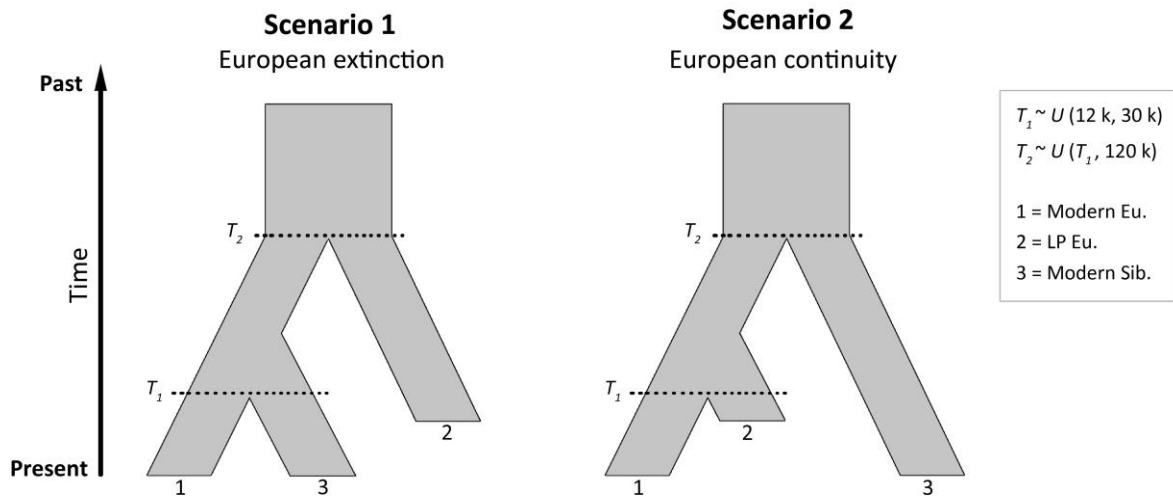
221 The Ensemble method in BIOMOD, that includes the uncertainty in predictions arising from the
222 choice of model algorithm, was finally selected to predict ptarmigan species probabilities starting from
223 the present and then for every 1,000 year back to 20 cal kBP. Predictions were also made of the future
224 ranges of ptarmigan for the years 2070-2100. For each time period, landscape metrics were calculated
225 for patches of favourable habitats at the 0.5x0.5' resolution. We estimated the percentage of favourable
226 pixels in the landscape and the degree of fragmentation, given by the number and the average area of
227 suitable habitat patches within the region, using the R library *SDMtools* (McGarigal *et al.* 2002;
228 VanDerWal *et al.* 2012). A pixel was classified as favourable when the probability of presence was
229 above 0.5.

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Figure 1 Present European distribution of willow (*L. lagopus*) and rock (*L. muta*) ptarmigan, paleontological sites with *Lagopus* fossil remains (filled circles) used in the species distribution model training (obtained from Tyrberg 1998; Tyrberg 2008), and the sites from where successful ancient DNA sequences were retrieved (blue squares).

Figure 2 Models and associated priors used in the coalescent simulations of the origin of European ptarmigan populations. Scenario 1 corresponds to an extinction of the Late Pleistocene (LP) European population, followed by a post-glacial colonisation from Siberia. Scenario 2 corresponds to a genetic continuity in Europe over the Pleistocene-Holocene transition, meaning that ptarmigans were able to follow the geographic shifts in suitable habitat that was associated with post-glacial climate warming. The same models are used for both willow and rock ptarmigan. See Tables S2 and S3 for details on the grouping of samples.

RESULTS

Phylogenetic and Demographic analyses

We successfully retrieved ancient DNA sequences from 42 Late Pleistocene *Lagopus* spp. bones, originating from 7 European palaeontological sites (Table S1 to S3). Of these, 27 were found to be willow ptarmigan (from 6 sites) and 15 rock ptarmigan (from 5 sites). The modern data set included 36 willow and 64 rock ptarmigan sequences sampled from their complete Holarctic distributions (Table S2 and S3).

In both species, two major haplogroups were found in the modern dataset; a western (Europe) and an eastern (Russia/Siberia and North America) (Fig. 3). Furthermore, an intermediate group was found for rock ptarmigan, consisting of birds from Iceland, Svalbard and Greenland. The two major haplogroups

were also found in the Late Pleistocene dataset, although that only included European samples, and in both ptarmigan species two haplotypes were shared between the modern and the glacial populations (Fig. 3). In the modern European populations, we observed a phylogeographic structure in both willow and rock ptarmigan (Fig. S3, Tables S2 and S3). However, this was not the case for the glacial European population of the respective species, where haplotypes were instead distributed more or less randomly among geographic regions (Fig. S3, Tables S2 and S3).

The analyses of molecular variance showed that, for both species, the structure that maximised the genetic differentiation among groups (*i.e.* had the highest F_{CT} value) was with modern and ancient European samples together in one group, and modern Siberian samples in a separate (willow ptarmigan $F_{CT} = 0.24$, $p = 0.04$; rock ptarmigan $F_{CT} = 0.57$, $p = 0.03$). The other hierarchical groupings analysed produced F_{CT} values below 0.03 (willow ptarmigan) and 0.22 (rock ptarmigan), and were non-significant.

The Skyline plots constructed in BEAST did not reveal any large temporal changes in effective population size within Western Europe, for either species. However, a comparison between the two species' Skyline plots suggests that willow ptarmigan has always been the more abundant species throughout the investigated time period (Fig. S4).

Approximate Bayesian Computation

The scenario comparison yielded a strong support for the second historic scenario, in which modern European populations descend from the sampled Late Pleistocene European populations, for rock ptarmigan (Bayes factor, BF, = 5.6 - 7,506), but not for willow ptarmigan (BF = 0.5 - 0.9) (Table S6). The 50 simulation replicates with different acceptance thresholds also produced similar results for rock ptarmigan, all supporting the same scenario, while for willow ptarmigan the best supported scenario shifted among replicates (i.e. with the thresholds used).

A similar result was obtained from the model choice for demographic scenarios (Table S7), where the rock ptarmigan analyses gave a modest but consistent support (except with the logistic regression) for a population growth in Western Europe following the onset of Holocene climate warming (BF = 2.0 - 3.3). For willow ptarmigan, none of the analysed scenarios gained a higher support than the alternatives. Replicates including population structure during the Holocene and high mutation rates also confirmed the described pattern (data not shown). The estimation of female effective population size in Western

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290 Europe prior to the Pleistocene-Holocene transition suggested that willow ptarmigan was more common
291 than rock ptarmigan (Fig. S5).

292 We found a very good fit of the chosen scenarios to the actual data, where the observed values of the
293 summary statistics frequently fell in the 95% highest density intervals (HDI) of their respective
294 predictive distributions (the distributions of the summary statistics after rejection; Figs. S6 to S9).

295 The pseudo-observed datasets (PODs) analyses showed that the statistical power for choosing the right
296 scenario was between 0.65 and 0.85, and increased to 0.88-0.94 when the observed Bayes factor was
297 used as a reference (i.e. only the PODs with values equal to or larger than the observed one were taken
298 into account). The first estimate reflects the probability of choosing the correct scenario without
299 considering the obtained results, whereas the latter could be interpreted as the probability that our
300 scenario choice is correct, given the observed support.

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302 **Species Distribution Modelling**

303 *Model calibration and evaluation*

304 Not surprisingly, models performed better in predicting past species distributions when they were
305 trained on fossil records, regardless of sample size (Fig. S10). Also, the predictive performance of the
306 past for models trained on fossil records varied with sample size: performance decreased when
307 increasing sample size. Models trained on the smallest sample size (n = 500) were therefore the best
308 whatever the geographical extent of the training data set. In contrast, performance to predict the present
309 was lower for models trained on fossil records, but increased with increasing sample size. However,
310 since models trained on fossils and on small sample size still performed well to predict in present time,
311 we chose this strategy to predict ptarmigan distributions for the whole time period. Finally, models
312 trained on the whole Eurasian distribution did not outperform models trained on Europe, except for the
313 past predictions of rock ptarmigan (*L. muta*). In the final analyses for both species, we therefore used
314 models trained on the European distribution, including fossil sites, with a sample size of 500. Details on
315 the contribution of each ecological variable to the model, as well as species' response curves along main
316 gradients are provided in the Supporting Information (Figs. S11 and S12).

317 *Past ptarmigan distributions*

318 For each time period, the proportion of suitable habitat in Europe was four times higher for willow
319 ptarmigan than for rock ptarmigan (41 % and 10 % in mean over the last 20 thousand years,

320   respectively, see Figs. 3 and 4). However, for willow ptarmigan, the projections showed that the
321   proportion of suitable habitat in the landscape is more restricted today (34 % suitable pixels) than at any
322   time before. The period with most suitable habitat for this species was between 5 and 13 cal kBP
323   (although a decrease in availability was observed at 10 cal kBP), and reached the maximum predicted
324   extent at 13 cal kBP with 45 % suitable pixels. For rock ptarmigan, the proportion of suitable habitat
325   was higher today and at 1 cal kBP (11 % and 11.5 %, respectively) than during the period between 2 to
326   17 cal kBP, with a minimum availability at 14 cal kBP (6 % suitable pixels). Before that, the habitat
327   availability was higher than today, with a maximum predicted extent occurring at 20 cal kBP with 19 %
328   suitable pixels.

329   Regarding the continuity of suitable habitat patches, the predicted range of both ptarmigan species
330   were found to be less fragmented today than during the last 20 thousand years, although similar values
331   as today were estimated for the period 18 to 20 cal kBP (Figs. 3 and 4). The maximum predicted
332   fragmentation occurred at 12 cal kBP and 13 cal kBP for willow and rock ptarmigan, respectively,
333   corresponding approximately to the warm Bølling-Allerød interstadial. During the whole time period,
334   rock ptarmigan had a more fragmented available habitat than willow ptarmigan, with a higher number of
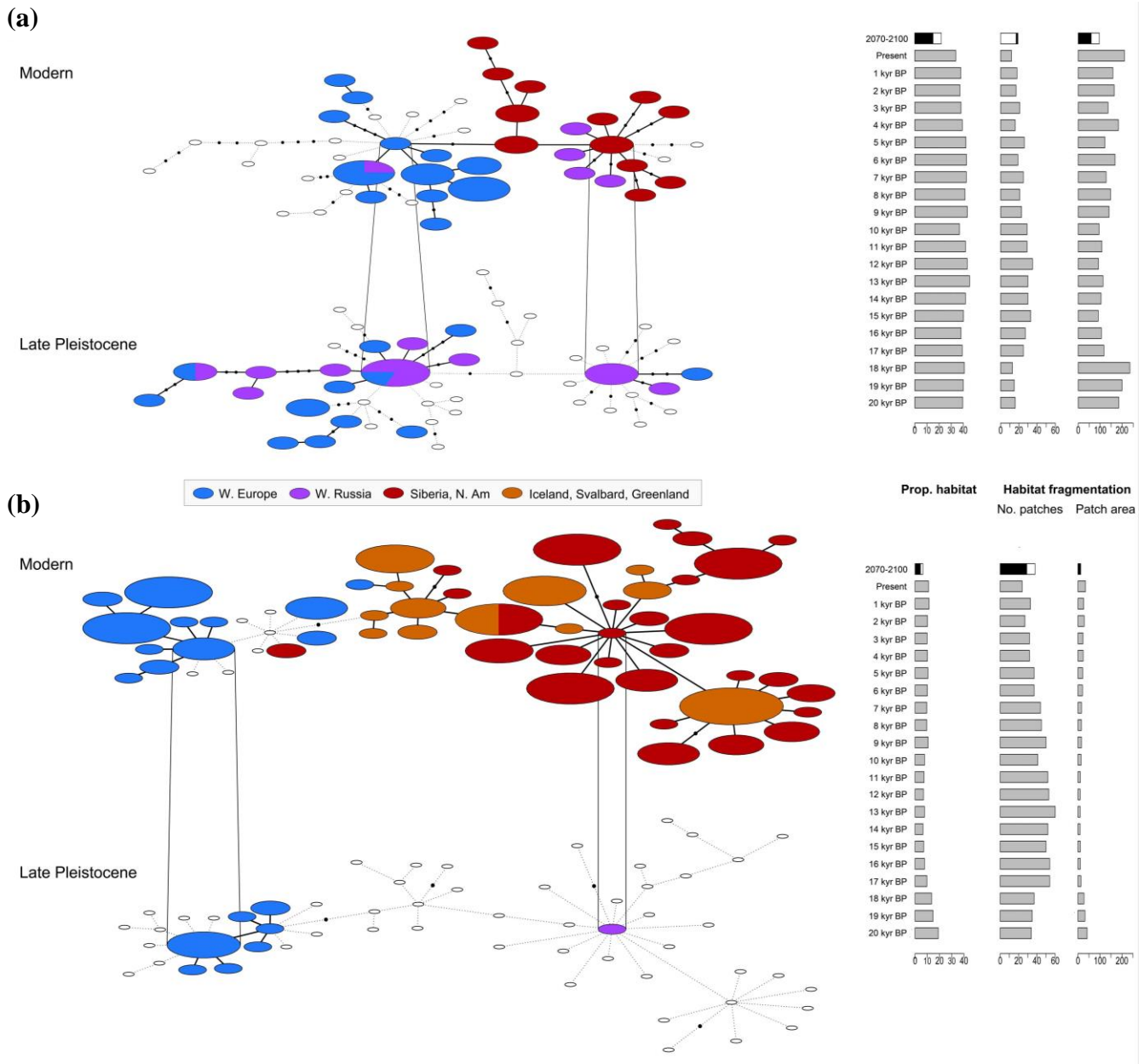335   suitable patches but a smaller mean patch area.

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337   *Future ptarmigan distributions*
338   For the time period 2070 to 2100, our models (based on climate emission scenario B1 and A2) projected
339   that the proportion of available habitat for both species will reach the lowest levels observed during the
340   last 20 thousand years (Figs. 3 and 4). For willow ptarmigan, the proportion of suitable pixels was
341   predicted to be reduced by at least 1/3 from today, whereas nearly half of the habitat currently available
342   for rock ptarmigan was predicted to disappear by 2070-2100. Also, future habitats for both species were
343   predicted to be more fragmented than today (Figs. 3 and 4), with the number of available patches
344   becoming as many as estimated for 1 cal kBP (*i.e.* more than today) and the mean patch area being as
345   small as the minimum observed during the Late Pleistocene (which occurred approximately between 10
346   to 17 cal kBP).

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Figure 3 Temporal network of all haplotypes found in modern and Late Pleistocene willow (a) and rock (b) ptarmigan. Vertical lines connect the haplotypes that are shared between both time periods, while empty circles indicate a missing haplotype in one time period that is present in the other. Haplotypes separated by one mutation are connected with a line, while black dots show additional mutations. The number of individuals sharing a haplotype is reflected by its size, with the largest reflecting ≥10 individuals. See Fig. S3 and Tables S2 and S3 for haplotype information. Barplots to the right show temporal changes in the proportion of available habitat and the degree of habitat fragmentation in Europe, illustrated as number of patches and median patch area, as estimated

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358 from the SDM for each species. The future projections, averaged for 2070 to 2100, are shown for both climate

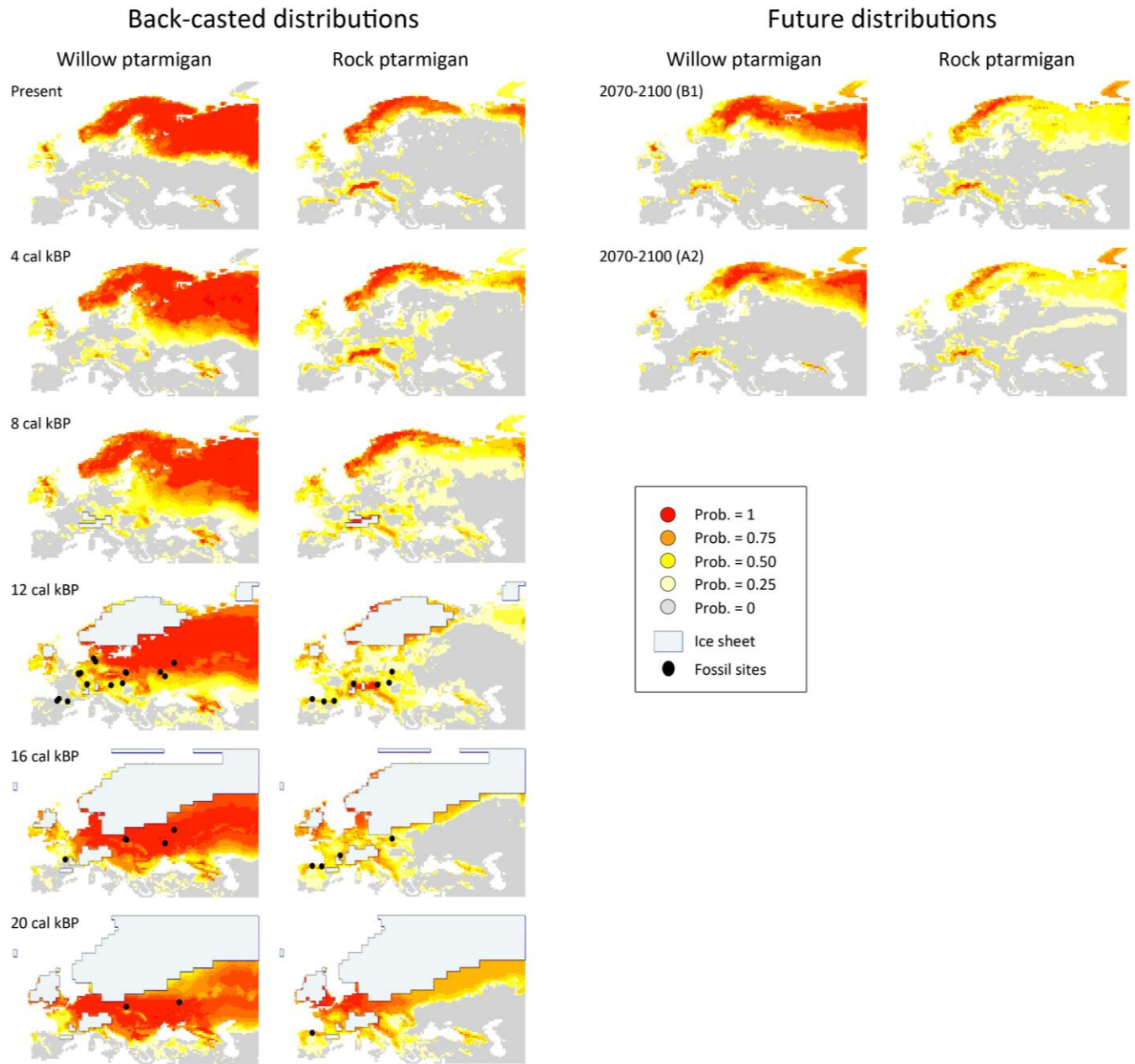359 scenario B1 (white bar) and A2 (black bar).

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Figure 4 Projected occurrence probabilities (from 0 to 1) for willow ptarmigan (left panel) and rock ptarmigan (right panel), at present and back to 20 thousand calendar years before present (cal kBP). Fossil site locations of different ages that have been used in the training and testing data sets are illustrated with filled circles. Also shown to the right are predictions of future distributions, averaged for the period 2070 to 2100, based on climate scenario B1 and A2.

DISCUSSION

The modern Holarctic mitochondrial genetic variation in the two ptarmigan species was divided into two major haplogroups; a western and an eastern, and these were also found in the Late Pleistocene European data sets. If populations managed to track their habitat across the Pleistocene-Holocene transition, we would expect that the modal haplotypes in the modern European populations would also be found in the glacial populations, due to genetic founder effects known to occur during postglacial colonisations of new areas (e.g. Hewitt 1999). Interestingly, this is what we observed for both willow (*Lagopus lagopus*) and rock (*Lagopus muta*) ptarmigan, where the modal modern European haplotypes were identical to the most common haplotypes in the two glacial datasets. A genetic continuity in Europe from the Late Pleistocene up until today was also the best scenario in the analyses of molecular variance (AMOVA), where the most probable genetic structure was when glacial and modern European samples were grouped together, while modern Siberian samples made up a separate group. Furthermore, the Bayesian coalescent simulations also gave support to the scenario of long-term genetic continuity in European rock ptarmigan. However, no clear support for either of the different analysed scenarios could be retrieved from the Bayesian coalescent simulations of the willow ptarmigan dataset.

In congruence with the genetic results, the back-casted species distribution models revealed that although environmental conditions in Europe have varied substantially over the last 20 millennia, there have always existed suitable ptarmigan habitats somewhere, which is an important prerequisite for population survival into the present. Based on the two sister species' modern habitat preferences, we could assume that the climate warming at the end of the last Ice Age would have resulted in somewhat different population responses. However, our genetic results show that both seem to have been able to track their shifting habitats to high latitude and altitude regions in Europe, keeping pace with the rapidly changing environment. This continuity is in contrast to the dynamics observed in earlier studies on mammals (e.g. Dalén *et al.* 2007; Lagerholm *et al.* 2014; Palkopoulou *et al.* 2016) and might be explained by the ptarmigans' flight capability, which although they are not strong flyers would have made them less dependent on the connectivity between suitable habitat patches. On the other hand, the previously reported body size reduction of both species (Bochenski 1985; Potapova 1986; Stewart 1999) could suggest that their survival was not just facilitated by successful dispersal, but might also have been coupled with adaptation, or adjustment by phenotypic plasticity (Gienapp *et al.* 2008). For example,

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404 ptarmigan might have changed their diet to match the habitat alterations during post-glacial climate
405 warming (Stewart 1999; Stewart 2007).

406 Among the analysed Late Pleistocene *Lagopus* spp. fossil remains, the majority turned out to be from
407 willow ptarmigan (*L. lagopus*). The species was also found to have had approximately 4 times more
408 available habitat than glacial rock ptarmigan, as well as a higher genetic variation (Fig. 3 and Table S5)
409 and larger effective population size (Figs. S4 and S5). Our results therefore suggest that willow
410 ptarmigan was the dominant *Lagopus* species in the tundra-steppes of midlatitude Europe during the
411 cold Late Pleistocene, which is also in line with the demographic inferences made from modern
412 genomes (Kozma *et al.* 2016). Possibly, rock ptarmigan has a more restricted climatic niche than its
413 sister species, and by being dependent on rocky, high alpine terrain it thus had access to fewer suitable
414 areas during the Late Pleistocene, when the major European mountain ranges were glaciated. Consistent
415 with this, the ABC results also indicated that Western European rock ptarmigans experienced a
416 population increase after the transition to the warmer Holocene period (Table S7). The niche separation
417 of the two species into lower and high alpine habitats can also be seen today, where willow ptarmigan is
418 absent from the high mountains of the Alps and the Pyrenees although the distribution models suggest
419 the climate in these areas to be favourable (Figs. 1 and 4). The rock ptarmigan's dependency on rocky
420 terrain would also imply that the species had a more fragmented range than willow ptarmigan during the
421 Late Pleistocene. The back-casted species distributions supports this idea, showing that willow
422 ptarmigan had more continuous patches of available habitat than rock ptarmigan (Figs. 3 and 4).

423 Although rock ptarmigan appear to have had a more fragmented distribution in Ice Age Europe than
424 willow ptarmigan, the general lack of phylogeographic structure within the glacial data sets of both
425 species indicates that there were no major barriers to dispersal between different parts of the range. The
426 observed differentiation between modern European populations of willow and rock ptarmigan (Fig. S3)
427 is therefore probably a result of their recent range contractions and isolation in different Holocene
428 interglacial refugia (Hewitt 1996). Consequently, the modern subspecies red grouse (*L. lagopus scotica*),
429 an endemic to the British Isles previously proposed to have diverged from other willow ptarmigan
430 before the Last Glacial Maximum (Huntley *et al.* 2013), thus appears to have evolved during the last 11
431 thousand years.

432 Our findings of successful range shifts during past climate changes are encouraging for the future
433 viability of both ptarmigan species, which like many other cold-adapted species are threatened by

434   predicted habitat decreases due to global warming (e.g. Sala *et al.* 2000; Smith *et al.* 2013; Stewart *et al.*
435   2010). However, an ability to respond to climate changes in the past does not necessarily mean that
436   similar habitat tracking will occur in the future. In many regions, the distributions of these species are
437   already at the limits of possible areas to colonise, and so they are restricted to Arctic regions or southern
438   alpine "sky islands" (Hampe & Jump 2011). Our forecasts of ptarmigan distributions in the years 2070-
439   2100 show that global warming will further reduce the amount of available habitat, and also
440   substantially increase the degree of fragmentation. In parts of the current European distribution, there
441   might only be very small, if any, areas left that fulfil the environmental requirements of willow or rock
442   ptarmigan. Populations in these areas, such as in the isolated British Isles, might therefore need to adapt
443   to a warmer environment in order to survive. Interestingly, the lack of moulting to a white winter
444   plumage observed in the red grouse (*L. lagopus scotica*) could possibly be such an ongoing adaptation.
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