

Large-scale geographical effect of isolation on adult sex  
ratios in the rock ptarmigan (*Lagopus muta*)

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## **Abstract**

Recent advances in sex ratio theory show that increased habitat isolation might be causing male-biased adult sex ratios (ASRs) in avian populations. This has raised concerns, since skewed ASRs are frequently seen in small and threatened populations. In this study we determined the ASRs of 20 isolated populations of the red-listed rock ptarmigan in southern Norway and Sweden, using non-invasive genetic sampling. The studied populations inhabit a fragmented mountain system that lay scattered around a large contiguous alpine landmass, which gives the montane landscape a mainland-island structure. The aim was to see if we could observe a pattern of male-skewed ASRs on the most isolated islands in this landscape. We calculated several estimates of isolation, including the islands distance from the mainland, stepping stone distances, and two isolation indexes that incorporate the isolation from other neighbouring islands. We found that an islands distance from the mainland alone correlated positively with male-biased ASRs, and that this correlation increased when stepping stone distances and the proximity to the other neighbouring islands were included when measuring isolation. We found no significant effect of area size of the focal island on skewed ASRs.

This is the first study that highlights the influence of isolation on ASRs on a large geographical scale, as previous studies have been based on local populations or on small geographical scales. Lastly, our findings might contribute to understanding the decline of rock ptarmigan populations observed in Europe over the last decades.

## **Introduction**

Geographic isolation has long been considered a driving force in the evolution and dynamics of species and populations (Fisher 1930; Wright 1943; Mayr 1963; Lomolino et al. 2010), and has played a pivotal role in fundamental ecological frameworks such as the island biogeography theory (MacArthur and Wilson 1967) and metapopulation theory (Levins 1969; Hanski 1991; 1994). Even though isolation events have occurred naturally over the course of time, it has now become a topic of great conservation concern due to the continuing loss and division of natural habitat as a result of human activity (Bierregaard et al. 1992; Kruess and Tschamtker 1994; Prugh et al. 2008; Gonzalez 2013). These habitual changes can create or enlarge geographical barriers that might restrict movement patterns, subsequently affecting the genetics and dynamics of populations. Such negative effects have been observed in numerous animal taxa (Fahrig 2003; Reed 2004; Bellard et al. 2012). Even highly mobile species such as birds can be negatively affected by these changes. Examples include reduced nesting success (Stephens et al. 2003), lower genetic diversity (Caizergues et al. 2003; Bruggeman et al. 2010), population declines (Athrey et al. 2012; Quesnelle et al. 2013; Taylor and Stutchbury 2016), and higher rates of local extinction (Boulinier et al. 2001).

In wild bird populations the adult sex ratio (ASR) might be another demographic parameter which is influenced by isolation. Defined by Mayr (1939) as the sex ratio between all non-juvenile individuals in a population, ASRs have received attention in later years as an important parameter when assessing population development. In birds, a higher proportion of males is commonly seen, probably due to higher female mortality (Donald 2007; Székely et al. 2014). However concerns have been raised about a potential link between male-biased ASRs and avian population declines. Dale (2001) was among the first to thoroughly describe this link, and was later supported by Donald

(2007) who observed a relationship between male-biased sex ratios and small and threatened populations. In particular, the tendency of female birds to be the natal dispersing sex (Greenwood 1980) has been argued to contribute to this skewed sex ratio, seeing that small and isolated populations might be unable to balance the emigration of females with immigration from other populations. This can result in a high proportion of unpaired males, which likely acts as limiting factor of population growth (Dale 2001; Steifetten and Dale 2006). However, much of our knowledge about skewed avian ASRs is derived from studies conducted on a single, or a few or small populations, often looking at changes in sex ratios over time (e.g. McIlhenny 1940; Lens et al. 1998; Donald et al. 2005; Steifetten and Dale 2006; Lehikonen et al. 2008; Veran and Beissinger 2009; Nadal et al. 2016). Thus, we know little of the variation across several isolated habitats within the same landscape.

Knowledge about the effects of isolation on a landscape level might be of high importance in the future, since large scale habitat alterations in the alpine landscape are observed over the last century due to an upward shift of the tree line (Harsch et al. 2009; Kullmann and Öberg 2009). This might pose a threat to many alpine species, since they are often restricted to naturally fragmented patches, such as mountain massifs and mountaintops. An increase in tree line altitude will potentially reduce and fragment these habitats (Chamberlain et al. 2012), gradually increasing their isolation and the amount of surrounding matrix of unsuitable habitat. Among those species vulnerable to tree line shifts, is the rock ptarmigan (*Lagopus muta*), a grouse species endemic to the above-tree line tundra. Together with other alpine birds the rock ptarmigan has declined from the northern European mountains in recent years (Lehikonen et al. 2014). In this study we use non-invasive genetic sampling of faecal pellets and feathers to assess the ASR of the naturally fragmented rock ptarmigan populations in southern Norway and

Sweden. The aim is to see if there is large scale relationship between ASR and geographic isolation. The ASR is expected to be skewed towards males, and this pattern is expected to increase proportionally with increasing isolation.

## **Methods**

### **Study species and study area**

The rock ptarmigan is a medium-sized bird distributed throughout the northern hemisphere, inhabiting montane areas above the tree-line. It is considered non-migratory, but has been observed to perform seasonal migrations between wintering and breeding grounds (Watson and Moss 2008), and dispersal distances up to more than 1000 km have been reported (Salomonsen 1950). The male defends a breeding territory in spring and mates with a single female. Globally it is listed as Least Concern on the IUCN Red List for Threatened Species, but with a decreasing population trend (BirdLife International 2016). In Norway the rock ptarmigan has a naturally fragmented distribution usually consisting of large contiguous mountain areas surrounded by smaller, isolated mountains of varying size. This is particularly evident for the southern region of the Fennoscandian mountain range, where the landscape is configured into a distinct mainland-island structure. Here, a proportionately much larger mountain area could be viewed as the mainland, while mountain fragments, especially to the east and into Sweden, could be viewed as islands. For thematic and logistical purposes we delineated the study area between latitudes 63°50' and 58°25' N (Figure 1).

### **Study design and data collection**

To recreate a mainland-island scenario in our study area, we first defined potential rock ptarmigan habitat as all areas above the tree-line ecotone. To achieve this, a tree-line

layer was constructed with GIS software ArcMap 10.3, based on an interpolation of 357 point observations of tree-line altitudes in Norway and Sweden (Kullman 1979; Moen 1998). This resulted in more than 5900 small and large mountain fragments (range: 0.006-14927.4 km<sup>2</sup>, median 0.051 km<sup>2</sup>), making it difficult to distinguish a mainland-island structure within this multitude of varying sized fragments. To overcome this problem we first removed all fragments smaller than 0.5 km<sup>2</sup>, as we considered these areas to be of minor biogeographical importance. We then applied a buffer to connect mountain fragments which were closer to each other than 2.5 km, in order to treat clusters of nearby mountain tops as one single mountain unit. One could argue that this would create artificial contiguous islands, but distances up to 2.5 km are well within the range of daily dispersal capabilities of rock ptarmigan and other grouse species (Gruys 1993; Martin et al. 2000; Hörnell-Willebrandt et al. 2014; Fuglei et al. 2017), and is thus unlikely to underestimate the effect of isolation. The consolidation of mountain fragments resulted in a large and contiguous mountain area of 42939.8 km<sup>2</sup> (hereafter referred to as the “mainland”) and a total of 315 mountain units (hereafter referred to as “islands”) of varying size (range: 0.5-2492.3 km<sup>2</sup>, median 4.1 km<sup>2</sup>) and degree of isolation (range Euclidean distance to the mainland: 2.5-267.2 km, median: 28.1 km) within the study area (Figure 1). It should be noted that all measures of size and distance are based on the area size above the tree-line and the edge-to-edge distances between these areas. As the buffer was merely a tool to reduce the landscape noise, all measures of size and distance including the buffer zone were not investigated further.

To determine the ASRs on a selected number of islands we used genetic samples from faecal pellets and feathers, collected from islands of varying size and degree of isolation to the mainland. Collection of most data was performed over two years (2015

and 2016) during February – April. Additionally, samples collected from an island in 2013 (Darrud and Storøy 2014) were also included in the dataset. Faecal pellets constituted the bulk of genetic samples (79%) used in analyses, and were sampled at altitudes ranging from 700-1550 m a.s.l. which were either accessed by skis or a snow mobile. An island was normally sampled within one or two days. The collection of samples were conducted at least 100 meters above the tree-line, to minimize the probability of collecting faecal pellets from the willow grouse (*L. lagopus*), which tend to overlap with the rock ptarmigan at its lower altitudinal distribution. All samples were randomly collected, usually spotted as snow-roosts from a distance, and were immediately stored into separate plastic containers. To reduce the risk of collecting several samples from the same individual, the minimum distance between sampled pellets was 500 meters, with the exception of when two or more birds were observed together, or when a cluster of snow-roosts was discovered (i.e. a roosting flock). Feathers were predominantly collected from birds shot by hunters during autumn from 2012-2016, but were also collected on the ground. All sites from which feathers were collected were situated on the mainland.

### **DNA analyses**

DNA from faecal pellets was extracted with Qiaamp® DNA Stool Mini Kit (Qiagen, Cat. No. 51504). We followed the standard protocol supplied by the stool handbook, but in order to increase the amount of extracted DNA the quantity of buffer ASL was changed from 1.4 ml to 1.6 ml. For the DNA extraction from feather we used DNeasy® Blood & Tissue kit (Qiagen, Cat. No. 69506), also following the standard protocol, but adding 5µL of DTT 1M to the ATL buffer during the first step.

For species identification the samples were amplified with species-specific mitochondrial DNA primers Lagsp3F, Lag3R and Mut3R, and PCR conditions from Nyström et al. (2006). The results were read via gel electrophoresis, where rock ptarmigan samples have an amplicon size of 212 bp, while willow grouse have an amplicon size of 154 bp. For sex determination the sex linked CHD genes (see Griffiths et al. 1998) were amplified in a PCR, using forward primer P8mod and reverse primer PU (Pérez et al. 2011). The forward primers were labelled with FAM (blue) fluorescent dye. For the PCR, 12 µl of reactions were prepared: 2 µl DNA template, 3 µl RNase free H<sub>2</sub>O, 6 µl Qiagen master mix, 0.5 µl of 10 µM fluorescent forward primer P8mod, and 0.5 µl of 10 µM reverse primer PU. The PCR run conditions were modified from Pérez et al. (2011) with an annealing temperature of 53 °C instead of 50 °C. To validate the PCRs, a negative (RNase free H<sub>2</sub>O) and a positive control sample (previously analysed *L. muta* DNA) was added to each run. If any positive signal was found in the negative control, the results were removed from the analysis and the run was repeated entirely. Finished PCR products were analysed on an ABI 3130xl genetic analyser, and allele scoring was done with Genemapper software V5.0 from Applied Biosystems. Finally, an individual identity analysis was performed with 14 microsatellites (Costanzi et al. 2018) using Cervus V3.0.7 software (minimum number of matching loci: 7, fuzzy matching allowed: 1), to identify and remove individuals that had been sampled twice or more.

### **Isolation metrics**

We considered the following variables to express isolation for an island, either by itself or in combination: 1) proximity to the mainland, 2) area size of the island, and 3) the proximity to, and the size of other neighbouring islands. Proximity to the mainland was



measured as the Euclidian edge-to-edge distance between the mainland and the focal island, and additionally as the shortest distance between the mainland and the focal island via in-between islands (referred to as stepping stone distance). To calculate the latter, we used a cost distance tool (see Weigelt and Kreft 2013), where we assigned a cost of one unit per km over matrix habitat, and a cost of zero over non-matrix habitat. The second isolation variable, the size of the focal island, was expressed by its area in km<sup>2</sup>.

To also account for isolation from other alpine areas that are not defined as the mainland (i.e. neighbours), we computed two types of isolation indexes. The first index, referred to as the landscape isolation index, is a variant of a proximity index (McGarigal et al. 2002). The index is calculated by dividing the area of a neighbouring island ( $A$ ) by its distance ( $D$ ) to the focal island. This is repeated for several neighbours, and the scores are summed to produce the index. A general formula can be written:

$$\text{Landscape isolation index} = \sum \frac{\log A^a}{\log D^b}$$

where the exponents  $a$  and  $b$  can be varied iteratively until the best model fit is achieved as a means of weighting the variables to avoid the assumption that area and distance play equal roles in determining isolation. To normalize the input variables all data were log transformed.

The second index, referred to as the likelihood index, was developed to incorporate an ecological component into the above model, as isolation, conceived by any given species, is just as much a result of ecological constraints and possibilities, than landscape structure alone. Here, each neighbour to the focal island was appointed two likelihood scores set between 0 and 1: an area score  $A_n$  modelled as a linear function of the neighbour's area, and a distance score  $D_n$  modelled as a logistic function of its distance from the focal island. Low scores indicated neighbours that were small in size

or far away from the focal island, whereas high scores indicated neighbours that were large in size or close to the focal island. For each neighbour the two scores were multiplied, and these resulting index scores were then summed up to obtain a likelihood index for the focal island:

$$Likelihood\ index = \sum A_n * D_n$$

The area score  $A_n$  was based on the assumption that larger neighbouring areas contain more individuals which could potentially immigrate to the focal island. Therefore we assumed that the likelihood of individuals leaving a neighbouring island was linearly proportional to its size. However, we expected very large neighbours to always function as source populations with individuals dispersing at relatively high rates, and therefore all neighbours above a certain area threshold were automatically given a score of 1. This also prevents disproportionately large neighbours (i.e. the mainland) from distorting the index. Although no data exist on what constitutes a source population in the rock ptarmigan, we set the area threshold to 100 km<sup>2</sup>, an area size that potentially could hold up to 600 breeding pairs when based on reported territory sizes of territorial males (see Zohmann and Wöss 2008). Neighbours smaller than the threshold size were given an index score by dividing their area  $A$  with the area threshold:

$$A_n = \begin{cases} \frac{A}{100}, & \text{if } A < 100 \\ 1, & \text{if } A \geq 100 \end{cases}$$

The distance score  $D_n$  was calculated on the assumption that individuals dispersing away from neighbouring islands would have a high likelihood of arriving the focal island when nearby, but a low likelihood when the neighbouring island was located far away. We assumed that the likelihood of arrival followed a logistic distribution:

$$D_n = \frac{1}{1 + \exp\left(\frac{D - a}{b}\right)}$$

We fitted the logistic curve inside a distance-interval set between 0 and 100 km, where location parameter  $a$  and scale parameter  $b$  were modified so that  $D = 0$  received an index score of 0.999, and  $D = 100$  received an index score of 0.001. The curve's breaking points were adjusted to lie around 10 and 90 km. The rationale for the fitting of the curve was that we expected individuals dispersing over matrix habitat to have a reasonably high probability of arriving the focal island up to approximately 10 km, but zero probability at distances more than 100 km. A distance of 10 km is probably well within the perceptual range of the rock ptarmigan making any island easy to locate, whereas islands located more than 100 km away could be compared to “finding a needle in a haystack”.

Moreover, to limit the number of neighbours to include in the index, we implemented a multi-scale analysis, varying the number of nearest neighbours from one up to 10, and selecting the number which explained ASR best. The mainland was included as a potential neighbour in both the isolation indexes.

### **Statistical analysis**

ASRs for each sampled island were expressed as the number of males divided by the total number of samples (Wilson and Hardy 2002), and deviations from an equal sex ratio of 0.5 were assessed with standard Pearson's chi-square tests (Donald 2007) with a significance threshold of 0.05. We used generalized linear models (GLM) to test for any correlation between ASR and the independent variables. In the GLMs we used a binomial distribution, or a quasibinomial distribution if there was evidence of overdispersion, which was assessed by GOF-statistics. The isolation metrics were

analysed against ASRs in single-predictor models, as well as in multi-predictor models. Multicollinearity was assessed using Pearson correlation scores and the variance inflation factor (VIF), where a VIF-scores of 3 were considered the upper limit (Zuur et al. 2010). As estimates for model fit, we calculated McFadden's pseudo- $R^2$ . To avoid overfitting models, we did not include interaction terms, or more than two additive predictors in the multipredictor models. This was done to uphold a recommended ratio of 10:1 between the number of observations ( $n=20$ ) and the number of variables (Babyak 2004).

## **Results**

In total 1220 samples were collected. Based on the genetic analysis, we removed 507 samples from the dataset. These samples were either unreadable (127), identified as willow grouse (312), or detected by the individual identity analysis as duplicates (68). On the islands the sex analysis detected 377 males and 199 females. Here, ASRs were mostly male-biased (see Table 1) with 11 of the 20 islands having significantly skewed ASRs towards males (Pearson Chi Sq.,  $p < 0.05$ ). In the samples collected from the mainland, we identified 66 male and 71 female individuals. Although the mainland has a joint sex ratio of 0.48, the sex ratios varied strongly between their sample locations (Table 1).

To see if there was a correlation between sample dates and ASR, we entered Julian sample dates as an independent variable, and found no significant correlation in either 2015 ( $p = 0.14$ ), 2016 ( $p = 0.66$ ), nor both years counted together ( $p = 0.13$ ). The faecal samples from 2013 were left out of this analysis, as sample dates were not available for this period.

We found evidence of overdispersion in the GLMs, where ASR was a response variable, and thus we applied a quasibinomial distribution to these models. A higher proportion of males were generally seen in the island populations where isolation was measured as high, as all isolation metrics correlated significantly with ASRs (Figure 2). As single predictors, both estimates of proximity to the mainland (Euclidean distance and stepping stone distance) showed significant correlations ( $p < 0.01$ , pseudo  $R^2 = 0.38$  and  $0.48$  respectively). The ASRs also correlated significantly with the landscape isolation index ( $p < 0.001$ , pseudo  $R^2 = 0.52$ ), as well as the likelihood index ( $p < 0.01$ , pseudo  $R^2 = 0.31$ ). The area size of the focal island did not produce any significant correlations, neither as a single predictor, nor as an additive predictor term together with other isolation metrics. For a total overview of model performance, see Table 2.

In the landscape isolation index, the best results were achieved when iterators  $a$  and  $b$  were set to 1 and 0.2, respectively. For both the landscape isolation index and likelihood index, we discovered that correlations with ASRs increased if we excluded islands smaller than  $3 \text{ km}^2$  as neighbours. Most likely, neighbours of such small size potentially hold very few breeding pairs, and rather serves as “biological noise” in the isolation models. Furthermore, we found that the best results were achieved when increasing this threshold to  $4 \text{ km}^2$ . When varying the number of neighbours for the landscape isolation index and likelihood index, the best results were achieved when including the four nearest neighbours (Figure 3).

## **Discussion**

Avian population studies have traditionally focused on basic demographic parameters such as birth and death rates and the emigration and immigration of individuals, but recent studies suggest that ASRs can be of major importance in the population

development of small and isolated populations (Steifetten and Dale 2006; Brooke et al. 2012; Morrison et al. 2016; but see Donald 2007 for review). Skewed sex ratios can emerge from different sets of reasons. For example, some birds are shown to adjust their offspring sex ratio when the environment favours one of the sexes (Rutkowska and Badyaev 2008; Bowers et al. 2015). Moreover, higher mortality in females has been suggested to result in male-biased ASRs (Donald 2007; Székely et al. 2014). However, for small and isolated populations, another likely explanation could be female natal-dispersal (Dale 2001). In small and geographically restricted areas, for example a small island, females are less likely to disperse within the island. Instead, they move across landscape barriers onto other islands or larger contiguous areas, and thus become lost from the population. Higher degrees of isolation then prevent these, or other females from returning to their natal island, and keeps the immigration rates low. Reasons for male-biased rock ptarmigan populations have not been investigated, but female grouse have been observed to be more dispersive than males, both during natal dispersal and seasonal migration (Weeden 1964; Montadert and Léonard 2011; Hörnell-Willebrandt et al. 2014).

### **Isolation metrics**

To measure isolation a wide range of models and methods exist, and choosing a suitable isolation metric is not a straightforward process. Despite being well reviewed (Bender et al. 2003; Kalmar and Currie 2006; Diver 2008; Prugh 2009; Weigelt and Kreft 2013), it is hard to find a consensus on one single method of assessing isolation. Ultimately, determining and creating measures of isolation should depend on species- and landscape-specific attributes (Lomolino 1982; Bender et al. 2003; Matter et al. 2005; Wilson and Bissonette 2009; Kupfer 2012). Little is known on how the rock ptarmigan

perceives isolation and moves in a large fragmented landscape, but it is undoubtedly capable to travel long stretches of contiguous suitable habitat (Salomonsen 1950; Fuglei et al. 2017), as well as long-range dispersal over unsuitable habitat (Gudmunsson 1972). Studies on movement in fragmented landscapes are sparse, but an 18 km wide valley between two mountains was suggested to pose a barrier to gene flow between populations in the Pyrenees (Bech et al. 2009), and a 28 km wide water strait was shown to prevent an unoccupied island from being colonized by nearby populations over a 25-year period (Kaler et al. 2010).

#### *Proximity to the mainland*

Mainland-island structures, such as our study area, are well described in the literature. Most prominently in the island biogeography theory (MacArthur and Wilson 1967), but also as one of the four main types of metapopulations (Hanski and Gilpin 1991; Harrison 1991). In these frameworks, the distance to mainland is often the favoured measure of isolation (e.g. Boback 2003; Lawes et al. 2006; Harvey and MacDougall 2014; Blackburn et al. 2016). This metric is also a sensible isolation measure in respect to skewed ASRs, as the mainland might be a dominant source population in the landscape, and thus harbours a large pool of possible female emigrants, which may uphold an even sex ratio on surrounding islands. However, many of these females are less likely to end up on remote islands, because 1) long-distance dispersal can be costly to the individual, and 2) encountering a remote island requires the individual to travel along a very specific and narrow path. This was also the case in our study, where distance from the mainland correlated positively with male-biased ASRs. Yet, the distance from mainland can be criticized for being somewhat simple. First, it assumes that dispersing individuals ignores other islands along its path by traveling in a straight

line from the mainland to the focal island, and second, it does not consider the impact of neighbouring islands of the focal island to provide female immigrants.

To measure stepping stone distances have been suggested to improve upon the simplicity of the Euclidean distance from the mainland (MacArthur and Wilson 1967; Whittaker and Fernández-Palacios 2007; Weigelt and Kreft 2013), and could be considered a relevant measure of isolation in our study. We found that stepping stone distances performed better than the Euclidean distance as a predictor of male-biased ASRs on the islands. Most likely, moving via stepping stones makes remote islands more accessible and less costly for females that disperse from the mainland. Therefore, some remote islands can have high female immigration, even though the straight line distance from the mainland is large. Although the rock ptarmigan is considered a strong flyer, stepping stone distances are argued to be highly important for species that have difficulties of dispersing long stretches of hostile habitat (Saura et al. 2014).

#### *Isolation indexes*

The landscape isolation metric and the likelihood index serve as alternatives to the mainland-based metrics, as they also incorporate any isolation-reducing effect of neighbouring islands. Metrics similar to the landscape isolation index (i.e. proximity indexes) are used by conservationists to determine isolation for patches in fragmented landscapes (e.g. Massimino et al. 2010; Storck-Tonon and Peres 2017). However, it should be noted that most of these landscapes often are without a large mainland. In our study, the landscape isolation index showed the strongest correlation with male-biased ASR, which indicate that the proximity to the mainland alone does not account for all the factors that lead to skewed ASRs. Thus, an isolated island's ASR may in part be maintained by its closeness to other nearby islands, and not just the mainland. This



makes sense, as large nearby neighbours potentially hold large populations, and thus has the potential to provide the focal island with a higher immigration of female birds. However, in area-based isolation metrics such as the landscape isolation index, the mainland gives a very high isolation-reducing effect as a neighbour due to its size, and might overshadow the effect of other neighbours in the landscape.

This effect of the mainland as a neighbour is greatly reduced in the likelihood index. Here, the effect of other nearby islands on ASR become more evident. The likelihood index shares some similarities with the isolation parameter  $S_i$  from Hanski's metapopulation models (Hanski 1994), but we used a logistic function to reflect dispersal capacities instead of an exponential. A logistic function is suggested when dealing with active dispersers (MacArthur and Wilson 1967; Lomolino et al. 2010). Despite these adjustments to the likelihood index, it did not predict male-skewed ASRs on the isolated islands as strongly as the landscape isolation index. An explanation for this might be the toned-down effect of the mainland in the likelihood index, compared to the landscape index. This further highlights the importance the mainland might have in the landscape as a source pool of females.

#### *Focal area size*

The size of the focal island is traditionally not treated as a measure of isolation, and was considered an immigration-independent variable in the equilibrium theory of island biogeography (MacArthur and Wilson 1967; Brown and Kodric-Brown 1977). However, the size of an island might be important when explaining male-biased ASRs, as 1) smaller islands prove a smaller geographical target, and are less likely to be discovered by a female migrant (referred to as the "target effect" by Whitehead and Jones 1968), and 2) on larger islands females are more likely to perform intra-island

dispersal instead of dispersing onto neighbouring islands. However, in the rock ptarmigan we did not find any significant relationship between focal island size and male-skewed ASRs, neither as a single predictor, nor as an additive term with other isolation metrics. One possible explanation is that the effect of focal area size may be overshadowed by other factors, such as isolation from the mainland, or distance to other neighbouring islands. Small areas with low degree of isolation will despite of their size have high rates of female immigration. In this case, any potential adverse effect of area size on ASRs could be too weak to give a measurable effect in our models. Indeed, nine of the 20 islands in our study are relatively close to the mainland (< 20 km). This is also coherent to area-diversity patterns mentioned in the island biogeography theory, where the area effect is predicted to be stronger on more remote islands (MacArthur and Wilson 1967).

### **Implications for conservation**

During the last decade the rock ptarmigan has experienced a steady decline in Norway (Kålås et al. 2014), and was categorized as Near Threatened in the Norwegian Red List for 2015. Similar declines have also been reported in the European alps (Petronace et al. 2012; Furrer et al. 2016) and in Japan (Nakamura 2007). Several explanations have been proposed to explain declines of rock ptarmigan, including changes in population cycles (Watson et al. 1998; Nielsen 1999), fluctuations in weather (Watson et al. 2000; Novoa et al. 2008) and human disturbances (Bevanger and Brøseth 2001; Watson and Moss 2004; Bech et al. 2012). Furthermore, rock ptarmigan is a popular game bird in Norway and Sweden, and might be vulnerable to overhunting. However, isolation leading to male-skewed ASRs might be an alternative explanation to the decline, as it might reduce the recruitment of new individuals into the populations, and hence limits

population growth. Although several studies have documented genetic impoverishment in isolated grouse populations (see Gregory et al. 2012), reports on sex ratios are few. Some studies suggest a relationship between skewed ASRs and habitat fragmentation in the Chinese grouse (*Bonasa sewerzowi*) and the capercaillie (*Tetrao urogallus*) (Helle et al. 1999; Sun et al. 2003; Morán-Luis et al. 2014).

The observation of a male-biased sex ratio in the most isolated rock ptarmigan populations might be a forewarning that further decline is likely to happen as global warming proceeds and furthers the upward shift of treelines (Harsch et al. 2009; Kullmann and Öberg 2009). We predict that the rock ptarmigan is likely to face reduced habitat area and increased isolation in the future, due to these climate changes, and that this could lead to severe population declines, and even local extinction on the smallest and most isolated islands.

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## Figure legends

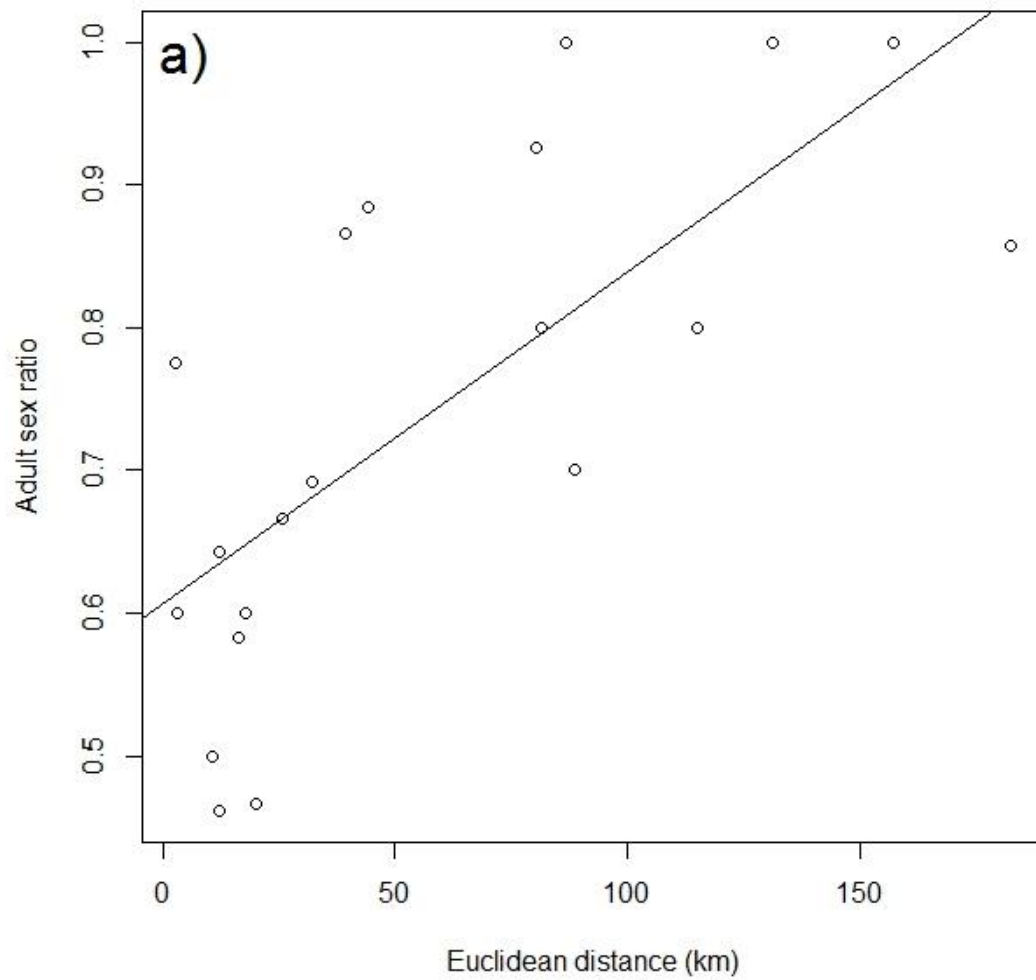
**Figure 1.** A map of the study area, showing in black the isolated mountains selected for sampling, in dark grey the mainland mountain, and in light grey the unsampled isolated mountains.

**Figure 2.** Correlation between different isolation metrics and adult sex ratios: a) the Euclidean distance between the mainland and the focal mountain, b) the shortest distance between the mainland and the mountain via in-between mountains as stepping stones, c) the landscape isolation index and d) the likelihood index. Each data point represents a sampled mountain.

**Figure 3.** Barplots from the multi-scale analysis applied to the two isolation indexes. The figure shows test results from 10 different GLMs with a quasibinomial distribution, where the numbers of neighbours included in each index were varied from one to 10 neighbours.

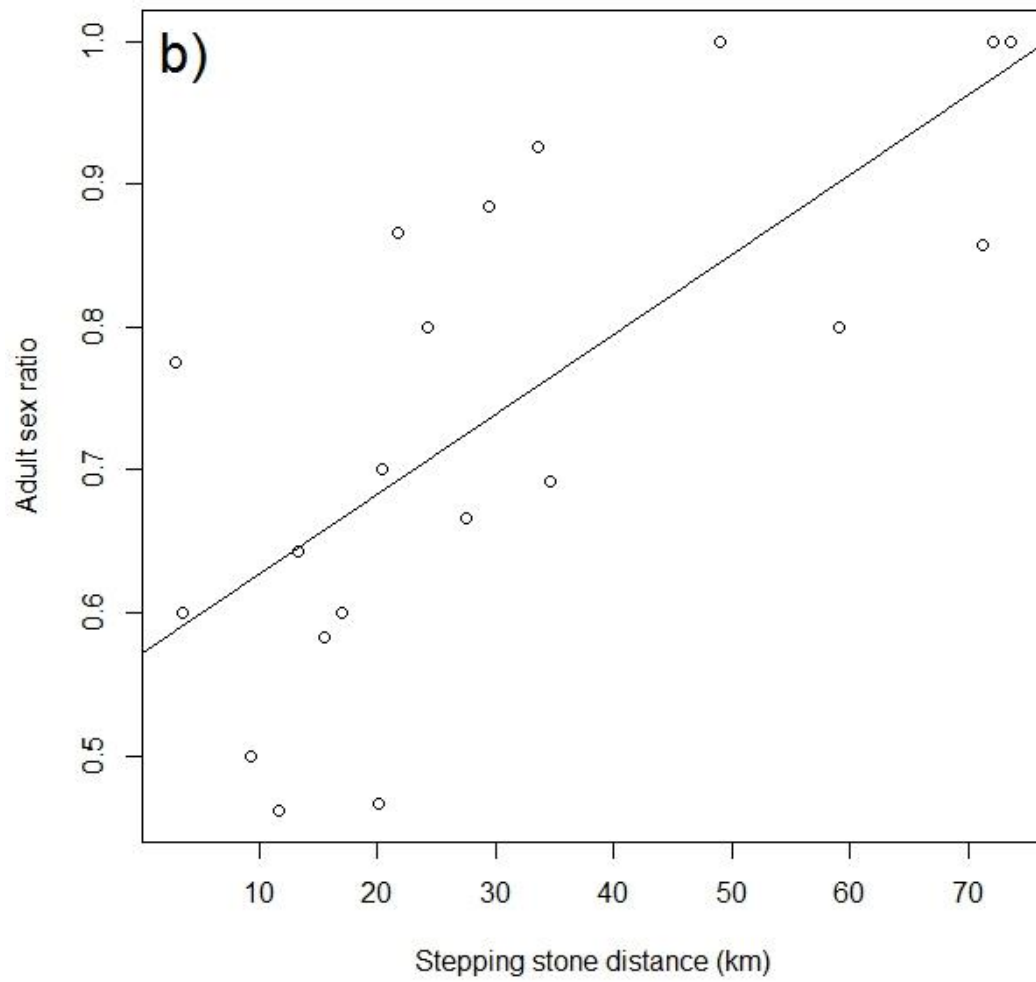


**Figure 1**

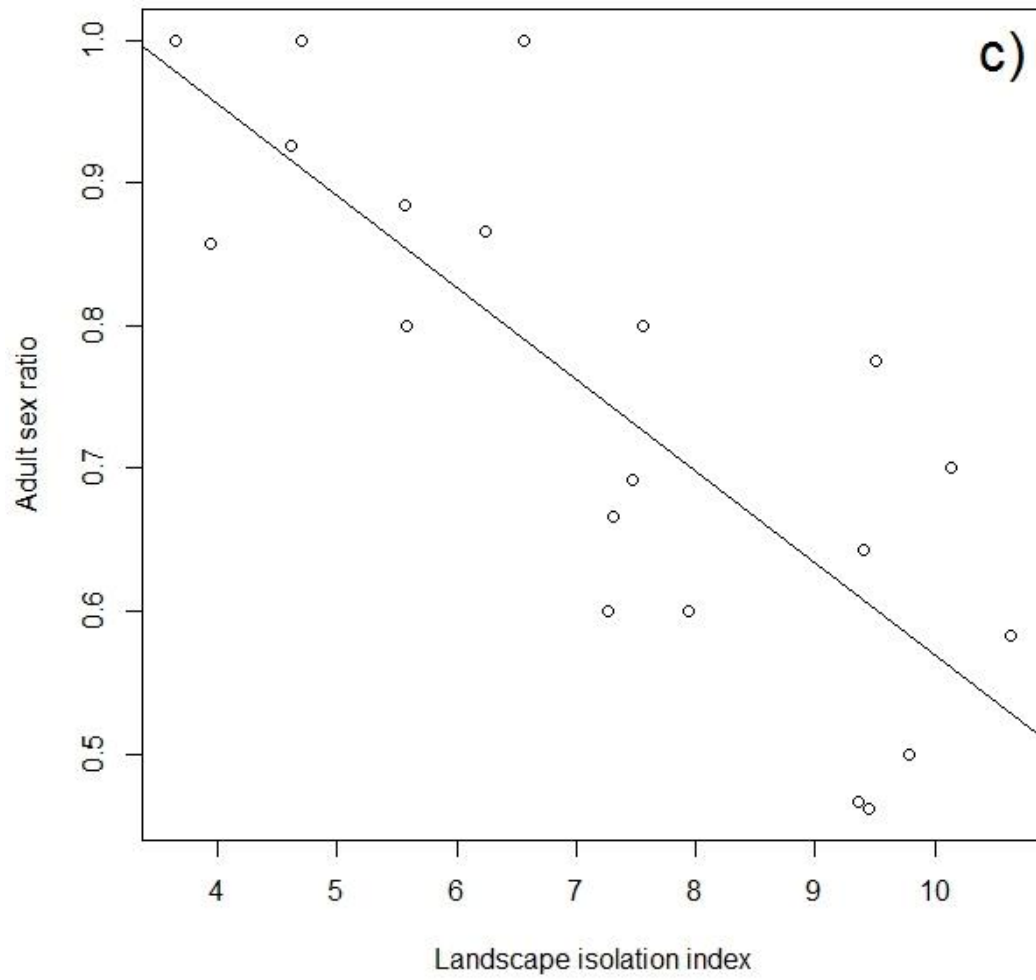


**Figure 2 a**

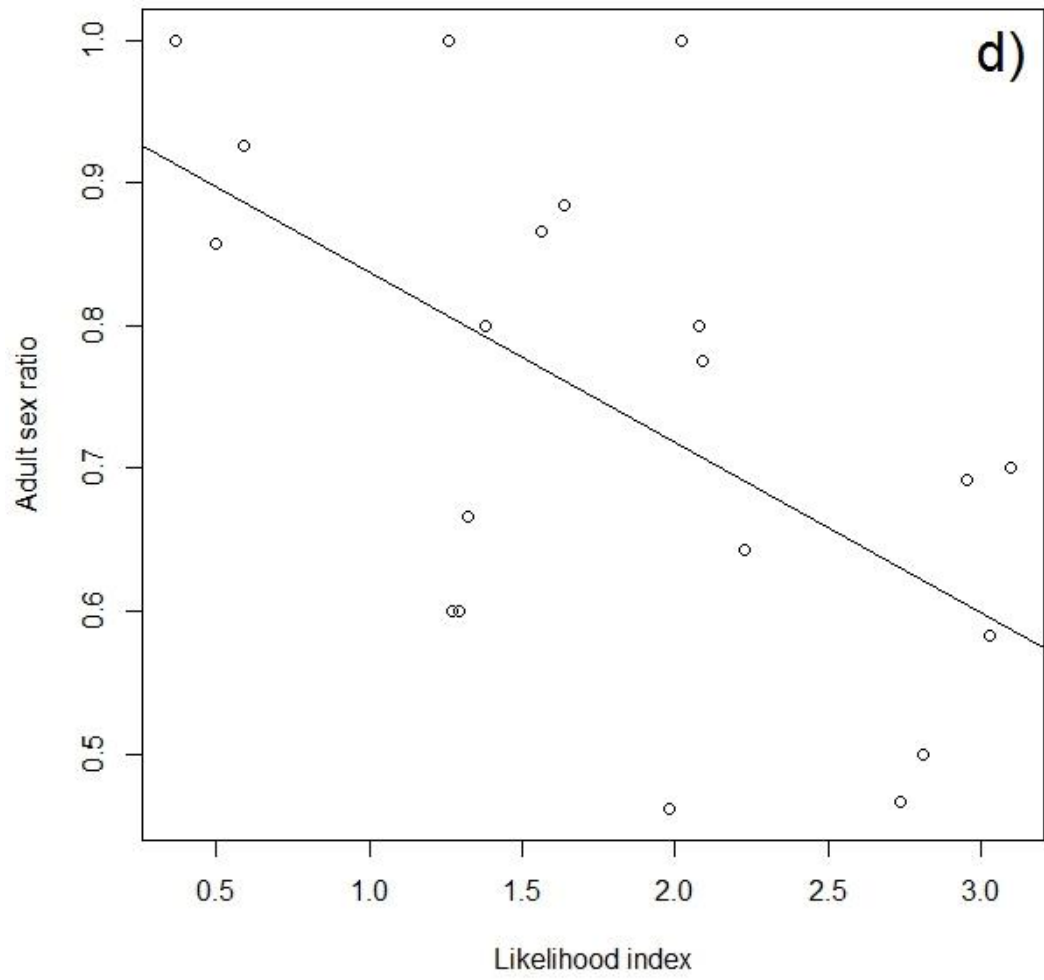




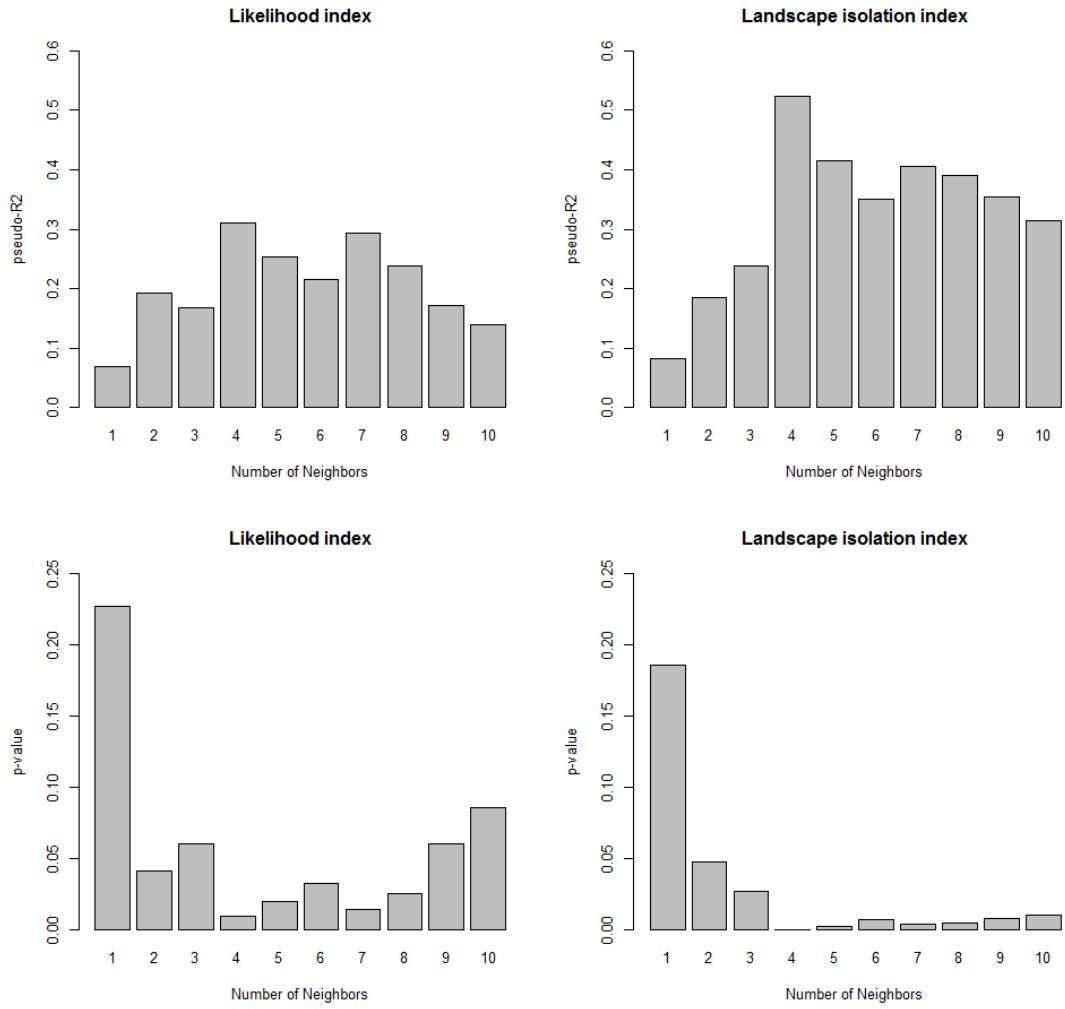
**Figure 2 b**



**Figure 2 c**



**Figure 2 d**



**Figure 3**

**Table 1.** Characteristics of the sampled islands. Adult sex ratios are expressed as the proportion of males in the total number of collected samples (n). Mainland sites are indicated by an asterix.

Location name	Coordinates		n	Adult sex ratios	Isolation (km)	Area (km <sup>2</sup> )
Stavsfjell	59° 38' 37"	8° 5' 40"	16	0.6	2.7	23.56
Norefjell	60° 22' 6"	9° 5' 39"	45	0.69	2.93	294.02
Roan	59° 17' 50"	8° 17' 45"	13	0.46	5.39	158.32
Blefjell	59° 50' 13"	9° 12' 35"	26	0.5	8.86	70.93
Ilfjellet	62° 56' 32"	9° 55' 21"	14	0.64	12.24	38.26
Lifjell	59° 31' 43"	8° 44' 35"	25	0.6	14.57	76.01
Tronfjellet	62° 9' 41"	10° 41' 2"	36	0.58	16.04	24.7
Slagsfjell	61° 22' 50"	10° 4' 53"	15	0.47	19.59	16.27
Nystølfjellet	60° 44' 7"	9° 17' 46"	9	0.67	25.93	31.05
Storhøgda	61° 36' 16"	11° 30' 27"	13	0.69	31.44	20.73
Sørbøfjellet	60° 27' 29"	9° 43' 54"	30	0.87	39.19	83.69
Synnfjell	61° 4' 26"	9° 50' 27"	26	0.88	44.35	28.9
Femundsmarka	62° 8' 26"	12° 13' 29"	27	0.93	77.79	221.89
Sylan	63° 3' 36"	12° 11' 46"	80	0.7	77.89	2492.96
Fulufjellet	61° 31' 7"	12° 40' 57"	21	1	86.82	293.77
Skenørsfjellet	62° 34' 51"	12° 12' 5"	25	0.8	94.81	289.56
Nipfjället	61° 58' 28"	12° 49' 16"	15	0.8	111.88	9.8
Vedungfjällen	62° 2' 3"	13° 14' 53"	23	1	127.4	117
Sånfjället	62° 16' 54"	13° 31' 21"	22	1	155.05	13.52
Skorvdalsfjället	62° 25' 50"	13° 58' 13"	21	0.86	182.13	15.17
Bykleheiane*	59° 26' 42"	7° 5' 18"	32	0.25	-	-
Hallingsskarvet*	60° 50' 2"	7° 47' 26"	16	0.25	-	-
Dalegubben*	62° 13' 49"	6° 22' 20"	7	1	-	-
Reinheimen*	62° 8' 20"	8° 29' 43"	20	0.75	-	-
Dovrefjell*	62° 9' 41"	9° 0' 17"	13	0.62	-	-
Aursjø*	62° 26' 30"	8° 22' 52"	5	0.6	-	-
Ringebu fjella*	61° 33' 44"	10° 21' 10"	3	1	-	-
Vikbekkfjellet*	60° 7' 19"	8° 17' 41"	23	0.52	-	-

**Table 2.** Model results from GLMs, testing the adult sex ratios as the dependant variable against measures of isolation. All tests were run with a quasibinomial distribution. Neighbours over 4 km<sup>2</sup> in area size were used to calculate the likelihood index and landscape isolation index.

	$\beta$	SE	p-value	pseudo R <sup>2</sup>
<b>Single-predictor terms</b>				
Distance to mainland	0.0000128	0.0000039	0.00390	0.38
Stepping stone distance	0.0000384	0.0000107	0.00210	0.48
Area	-0.0000941	0.0002196	0.67331	0.01
Likelihood index	-0.6150000	0.2116000	0.00940	0.31
Landscape isolation index	-0.3271600	0.0741900	0.00034	0.52
<b>Multi-predictor terms</b>				
Distance to mainland	0.0000171	0.0000051	0.00349	0.49
Area	-0.0004163	0.0002123	0.06647	
Stepping stone distance	0.0000384	0.0000110	0.00288	0.48
Area	-0.0000092	0.0001744	0.95869	
Likelihood index	-0.8038367	0.2476659	0.00476	0.38
Area	0.0003018	0.0002122	0.17302	
Landscape isolation index	-0.3904918	0.0772444	0.00010	0.60
Area	0.0003023	0.0001580	0.07270	