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Intrinsic factors drive spatial genetic variation in a highly vagile species, the wedge-tailed eagle *Aquila audax*, in Tasmania

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Knowledge of dispersal in a species, both its quantity and the factors influencing it, are crucial for our understanding of ecology and evolution, and for species conservation. Here we quantified and formally assessed the potential contribution of extrinsic factors on individual dispersal in the threatened Tasmanian population of wedge-tailed eagle *Aquila audax*. As successful breeding by these individuals appears strongly related to habitat, we tested the effect of landscape around sampling sites on genetic diversity and spatial genetic variation, as these are influenced by patterns of dispersal. Similarly, we also tested whether habitat intervening sampling sites could explain spatial genetic variation. Twenty microsatellites were scored, but only a small proportion of spatial genetic variation (4.6%) could be explained by extrinsic factors, namely habitat suitability and elevation between sites. However, significant clinal genetic variation was evident across Tasmania, which we explain by intrinsic factors, likely high natal philopatry and occasional long-distance dispersal. This study demonstrates that spatial genetic variation can be detected in highly vagile species at spatial scales that are small relative to putative dispersal ability, although here there was no substantial relationship with landscape factors tested.

Dispersal (defined herein as the movement of individuals from the natal site to another site where breeding occurs, or movement between different sites of breeding) is of fundamental importance to the dynamics of populations. Dispersal may result in reduced species extinction risk by facilitating the colonisation of new habitat patches, and arresting declines in populations through a rescue effect (Brown and Kodric-Brown 1977, Pulliam 1988, Hanski 1998). Likewise, dispersal may prevent inbreeding depression and the loss of genetic diversity (Frankham et al. 2002). Environmental changes – such as habitat fragmentation or climate change – that restrict dispersal by increasing movement costs can thus place significant pressure on populations. Therefore, knowledge of dispersal and population connectivity can be informative regarding population extinction probability (Thomas 2000), can inform conservation practices such as habitat restoration to reinvigorate population connectivity (Huxel and Hastings 1999), and may enable predictions regarding connectivity to be extended to taxa and localities beyond the immediate study system (Cushman and Landguth 2012).

Dispersal capability of species can be influenced by both intrinsic and extrinsic factors. Among intrinsic factors, features such as morphology (e.g. flight musculature), life history, and behaviour (e.g. territoriality) are commonly inferred to influence connectivity in avian taxa. For example, it has been shown that bird species that are sexually dichromatic typically exhibit lower connectivity and greater rates of speciation owing to the fitness benefits conferred to males that are more philopatric and have greater knowledge of resources in their vicinity (Barraclough et al. 1995). Similarly, cooperative breeding among apostlebirds *Struthidea cinerea* results in natal philopatry among both males and females and therefore large, stable groups of closely related individuals with minimal movement of breeding groups between seasons (Woxvold et al. 2006). While extrinsic factors such as landscape can also influence connectivity, fewer studies (e.g. 'landscape genetics'; Manel et al. 2003) assess their influence on connectivity in birds relative to other terrestrial vertebrates, which may reflect perceptions that birds are too vagile to be greatly influenced by such factors, or difficulties conducting studies at appropriate spatial scales for such vagile taxa.

There are two primary means by which landscape can influence dispersal. The first relates to the effect of landscape factors on the directionality and ease of dispersal, where constraints can be imposed by barriers such as rivers, mountain ranges, or anthropogenic habitat fragmentation (Angelone et al. 2011, Hagerty et al. 2011, Díaz-Muñoz 2012, Frantz et al. 2012). Although birds are often less physically

constrained in their movements than other taxa owing to their flight ability, their movement costs are unlikely to be equal across all landscapes. Flight is energetically expensive, and flying over mountains or against prevailing winds will be more physiologically costly than alternatives (Tucker 1971). Furthermore, costs of movement may be expressed on other axes, such as mortality risk from predation while traversing different landscapes (Yoder et al. 2004, Bonte et al. 2012).

The second means by which landscape can influence dispersal is through habitat selection (Alda et al. 2013), whereby an individual may preferentially move through habitat suitable for feeding or breeding, although conversely it may undertake less dispersal in preferred habitats as its needs are readily satisfied (Spear et al. 2010). For instance, the northern bobwhite *Colinus virginianus* may be less likely to leave suitable habitat patches than unsuitable habitat, thus experiencing greater gene flow over longer distances in areas of poor habitat (Berkman et al. 2013). Habitat preferences may vary across populations through processes such as natal habitat preference induction or local adaptation, which may influence the rate and symmetry of gene flow across the landscape and accentuate spatial genetic structure (Benard and McCauley 2008, Dionne et al. 2008). Natal habitat preference induction occurs where a dispersing individual is attracted to environmental cues that were imprinted upon that individual from its natal habitat (Davis and Stamps 2004). Natal habitat preference induction is thought to occur frequently among birds, but field studies remain relatively rare (Chalfoun and Schmidt 2012).

The two mechanisms by which landscape can influence dispersal thus necessitate different study approaches. The study of habitat selection effects requires an emphasis on the landscape surrounding the sites where individuals were sampled, while those pertaining to movement costs may be better explained by landscape heterogeneity between sample sites.

The wedge-tailed eagle *Aquila audax* is a large, highly vagile raptor. It is long lived, and in Tasmania commences reproduction at approx. 4 yr of age, with evidence of monogamy and mate fidelity (Bell and Mooney 1999). The species is also territorial, with territories occupied year-round and containing several nests which may be used alternately across years (Bell and Mooney 1999). In Tasmania it is continuously distributed across a range of habitats, but appears highly dependent on large emergent trees in sheltered areas of mature native forest for breeding (Brown and Mooney 1997). High quality breeding habitat is restricted in distribution across Tasmania (Fig. 1) (Forest Practices Authority 2013). The Tasmanian *A. audax* population has also been listed as endangered due to a low number of breeding pairs, loss and disturbance of breeding habitat, and high rates of unnatural mortality, with an estimated population size of 1000–1500 individuals across 426 breeding territories (Threatened Species Section 2006). As one of Tasmania's only remaining apex predators, it may also be important in maintaining ecosystem structure and function (Estes et al. 2011). The Tasmanian population is genetically depauperate, containing a subset of variation observed from the mainland Australian population, with only a single mitochondrial haplotype observed (Burridge et al. 2013). Consequently, considerable effort is being devoted to its management, particularly the preservation of its breeding habitat (Forest Practices Authority 2013). This management will benefit from landscape genetic research as an additional and independent means to assess factors influencing the dispersal and distribution of individuals in this vulnerable population.

There is no published study of dispersal in Tasmanian *A. audax*. The species is assumed to be capable of dispersal over very long distances, with one recorded terrestrial movement of 800 km in Western Australia (Ridpath and Brooker 1986), and genetically inferred infrequent dispersal across Bass Strait (up to approx. 240 km) over historic (pre-European) timescales (Burridge et al. 2013). If dispersal over these distances occurs regularly in Tasmania, which has a total area of 68 401 km^2, it would be expected that the Tasmanian *A. audax* population is genetically panmictic. However, occasional observations of high vagility in one environment may not necessarily apply to others, nor translate into actual dispersal (changes in the location of reproduction).

It is plausible that the highly specific breeding habitat requirements of Tasmanian *A. audax*, with the relatively constrained distribution of such habitats in Tasmania, might result in some restriction of dispersal due to a strong habitat selection effect. Therefore, it may be hypothesised that proximity to high quality habitat restricts dispersal in *A. audax*, rather than facilitating it as in most other studies of landscape influences on dispersal. The possibility of encountering occupied breeding territories reduces the probability of dispersers stopping at suitable nest sites; however, availability of vacant suitable breeding habitat is unlikely to be highly limited in this population. No trend towards nest establishment in areas of lower habitat suitability has been identified (Forest Practices Authority 2013), and replacement times for adults lost from territories are long – sometimes more than 6 months (Bell and Mooney 1999). Therefore, it is likely that areas of high habitat suitability are frequently vacant and could thus be significantly more probable destinations for dispersers than areas of low habitat suitability. Tasmania is also topographically variable, with a mountainous central region (highest elevation is 1616 m) producing a significant east-west climate gradient and heterogeneity in the distribution of vegetation used by *A. audax*, in addition to that resulting from anthropogenic activities. It is possible that some restriction of *A. audax* dispersal may result from these variables.

Here we employ a genetic approach to investigate the potential influence of landscape on dispersal in Tasmanian *A. audax*. We use polymorphic microsatellite markers to test for spatial patterns of genetic variation in the Tasmanian population and any relationships with heterogeneous landscape features, including output from an *A. audax* breeding habitat suitability model, topographic elevation, and vegetation. We test associations of genetic diversity and genetic structure with landscape factors, addressing both local environmental conditions (habitat at collection site) and matrix quality (habitat intervening collection sites), which are rarely considered together (Pflüger and Balkenhol 2014). We also test for any spatial patterns such as clines or clusters, independent of landscape.

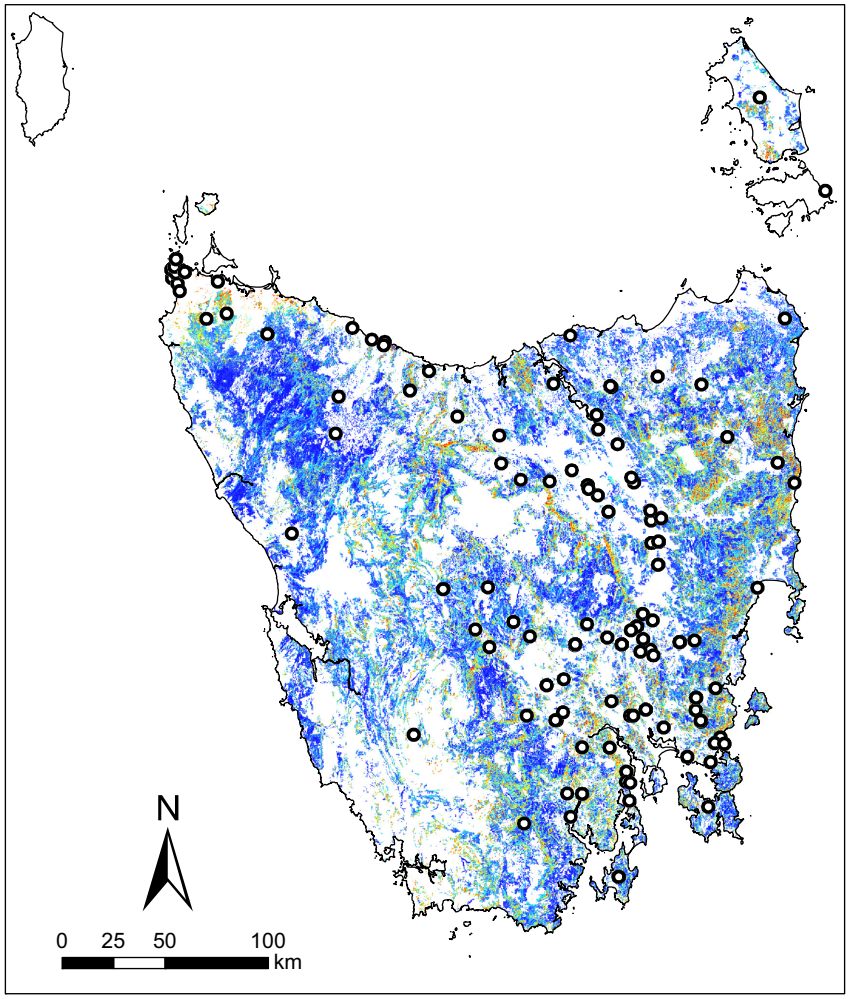

Figure 1. Combined map demonstrating high heterogeneity of *Aquila audax* breeding habitat suitability across Tasmania, modelled by the Forest Practices Authority (2013). Map colours reflect the predicted likelihood of the habitat being suitable for breeding, with warmer colours representing higher likelihood, and cooler colours representing lower likelihood. White areas represent a lack of any habitat predicted to be suitable for breeding. Black open circles represent *A. audax* sample locations (n = 154), with concentrations in areas of human activity.

Methods

Study area and sample collection

The study area encompassed the entire main island of Tasmania as well as proximate islands, and included genetic samples for 228 *A. audax* individuals. Most (n = 143) individuals were sampled opportunistically as mortalities deposited with the State Government. These samples were concentrated around areas of frequent human activity, and were supplemented using museum specimens (n = 50) and shed feathers (n = 35) to fill in distribution gaps (Fig. 1). We were unable to sample nestlings or from occupied nests given the documented negative implications of human visitation to breeding sites (Forest Practices Authority 2013, O'Sullivan 2014). Due to incomplete knowledge of specific breeding territories, it was not possible to associate individuals with particular territories based on sampling locations, and we have no data on which individuals were reproductively active. Age was estimated for some individuals upon collection, and among these there were a high proportion of sub-adults (approx. 68%); however, age estimates were absent from more than half of our samples (Supplementary material Appendix 2). Most (n = 182) individuals were collected after 1996, although the oldest museum specimen was collected in 1944. Tissue sampling from the Government collection followed Burridge et al. (2013). Tissue samples from museum specimens were obtained by slicing several thin (approx. 1 mm) layers of skin from the toe pad (Mundy et al. 1997). A sample size of 154 was used for all analyses requiring spatial information following exclusion of individuals for which reliable locality data were lacking.

DNA extraction and genotyping

DNA extraction from feathers and muscle tissue followed Burridge et al. (2013). Approx. ~4 mm^3 toe pad skin samples were first rehydrated in 9% NaCl solution at 4°C for 48 h. DNA was then extracted using the GENTRA Puregene Tissue Kit, following the 'DNA Purification from Fixed Tissue protocol', with some modification. As DNA yield was expected to be low due to the nature of the samples,

the addition of 0.17 µl glycogen solution as a DNA carrier during DNA precipitation was included. Likewise, DNA was concentrated by hydrating in 30 µl instead of 100 µl.

Twenty polymorphic (based on Tasmanian and mainland Australian samples) microsatellite loci were genotyped (see Austin et al. 2014 for primer notes), and scored using Genemapper ver. 3.7 (Applied Biosystems). A 'multiple tube' approach (Navidi et al. 1992, Pompanon et al. 2005) was employed for non-invasive samples to control for the possibilities of allelic dropout (Walsh et al. 1992, Pompanon et al. 2005), contamination, and the occurrence (or relative prominence) of false alleles produced as biochemical artefacts (Shinde et al. 2003). Each PCR was replicated four times for each genotype, increasing the detectability of errors. If genotypes could not be conclusively inferred across a majority of replicates, no score was recorded for that locus.

Genepop ver. 4.5.1 (Rousset 2008) was used to conduct an exact test of Hardy–Weinberg equilibrium using a Markov Chain algorithm, with 10 000 dememorisation steps, 20 batches, and 5000 iterations per batch. The false discovery rate procedure was performed to counteract the inflated risk of type I error when performing multiple tests of the same null hypothesis (Benjamini and Yekutieli 2001). Genotypes among these loci were previously demonstrated to be independent (Austin et al. 2014).

Spatial data analysis

Spatial genetic patterns without consideration of landscape

A spatial principal component analysis (sPCA) was conducted using the R package 'adegenet' (Jombart 2008). sPCA scores summarise spatial autocorrelation that can represent two types of spatial structure: global structure (positive spatial autocorrelation), where there are broad-scale patterns such as genetic differentiation between multiple spatial groups, or a genetic cline, and local structure (negative spatial autocorrelation), where there are genetic differences among neighbours. Monte-Carlo tests were conducted with 10 000 permutations to enable significance testing of local and global spatial structure.

Spatial landscape data

Landscape data were used for comparisons with individual genetic diversity and pairwise individual genetic distance. These consisted of Tasmanian *A. audax* breeding habitat suitability model output (Forest Practices Authority 2013), a digital elevation model, and a series of vegetation categories hypothesised to be important to Tasmanian *A. audax* breeding and foraging. All viewing and manipulation of spatial data were performed using ArcGIS ver. 10.2 (ESRI) unless otherwise specified. The breeding habitat suitability models were based on the locations of all known Tasmanian wedge-tailed eagle nests, and predict areas of suitable breeding habitat (Forest Practices Authority 2013). There were separate models for areas under 850 m elevation, over 700 m elevation, and for the north-west of Tasmania; these models were combined into a single GIS layer prior to analysis, using mean suitability for areas where the models overlapped. The GEODATA 9 second digital elevation model ver. 3 (Geoscience Australia 2008) presents elevation data in metres at a grid cell size of 9 seconds (approximately 250 m). Tasveg 2.0 (DPIPWE 2009) is a digital map of Tasmania's vegetation, from which eight vegetation categories of hypothesised relevance to Tasmanian *A. audax* (Bell and Mooney 1999) were selected: breeding habitat comprising dry eucalypt forest, wet eucalypt forest, rainforest, and non-eucalypt forest; and foraging habitat comprising native grassland, agricultural land, cleared land, and scrub.

Measures of individual genetic variation

Individual heterozygosity was used to measure genetic diversity for site-based landscape genetic analysis, calculated as 1-homozygosity by locus (Aparicio et al. 2006) using the 'GENHET' function (Coulon 2010) in R. A pairwise matrix of the proportion of shared alleles (D_{ps}) among individuals (Forstmeier et al. 2012) was calculated using Microsatellite Analyser (Dierenger and Schlötterer 2003) for use in both site-based and between-site landscape genetic analyses.

Site-based analyses

Landscape variables surrounding sample locations were tested for their influence on variation in individual heterozygosity and pairwise genetic distance (D_{ps}). Landscape variables were quantified within a 3.1 km buffer around each sample location using Geospatial Modelling Environment (GME) ver. 0.7.2.1 RC2 (<www.spatialecology.com>), reflecting the approximate home range (20–30 km^2) of eagles in regions containing good quality habitat, comprising a majority of the population (Bell and Mooney 1999). Within each buffer, total habitat suitability (calculated as sum of habitat suitability map grid cell values), mean elevation, and total area of each vegetation category were quantified. Additionally, easting and northing were included as separate factors as a means to quantify spatial genetic variation.

To determine the relative importance of each site-based variable as a predictor of individual heterozygosity, a Random Forests regression analysis (Breiman 2001) was conducted using the 'randomForest' package (Liaw and Wiener 2002) in R. Both node purity and percentage increase in mean squared error were employed as measures of relative importance of predictor variables. Simple linear regressions were conducted to quantify the relationships between individual heterozygosity and its most important predictors. To determine the best site-based predictors of pairwise genetic distance, a distance-based redundancy analysis (dbRDA) (Legendre and Anderson 1999) was conducted using the DISTLM routine in PERMANOVA+ for PRIMER (PRIMER-E). All landscape variables were modelled against the matrix of pairwise D_{ps} across 10 000 permutations using the 'step-wise' selection procedure and the AIC_c selection criterion (Hurvich and Tsai 1989). Sequential tests were conducted to provide AIC_c scores for the best predictors, and the proportion of total variation explained by these predictors was quantified and displayed on a dbRDA plot.

Between-site analyses

A series of pairwise distance matrices were produced using Circuitscape ver. 4.0 (McRae et al. 2008) that describe the relative resistance to gene flow between sites based on the hypothesised effects of three different landscape features: 1) breeding habitat suitability, 2) elevation, and

3) an isolation-by-distance (IBD) null model. Resistance surfaces based on vegetation categories were relatively homogeneous and were thus highly correlated with the null model (IBD), and were excluded from analysis. An input grid cell size of 1 km was chosen for resistance surfaces, as this is small enough to maintain a sufficient level of detail without attributing undue influence to fine-scale variation in landscape, and is relatively efficient computationally.

The original breeding habitat suitability model scores for each cell (0–1) were retained for the construction of resistance surfaces. However, because 'no data' cells (i.e. cells containing zero suitable habitat according to the habitat suitability model) are interpreted by Circuitscape as having infinite resistance, we adjusted 'no data' cells over land and inland water bodies to 0.0001, such that areas of no or minimal habitat suitability had low rather than infinite resistance, and areas of high habitat suitability had high resistance. This resistance surface reflects a hypothesised scenario where dispersing individuals would tend to stop at suitable habitat, provided it is not already occupied by a resident breeding pair, and pass unsuitable habitat. The inverse scenario, where higher habitat suitability is more conducive to dispersal, was also assessed. Elevation values were categorised and represented as integers, such that the lowest elevation cells had values of 1, and cells at higher elevations had values through to 10; elevation categories were divided equally across the full range of elevation in Tasmania. This resistance surface reflects a hypothesised scenario where areas of high elevation provide greater resistance to *A. audax* dispersal. All cells representing marine waters were assigned 'no data' values such that gene flow could only be modelled as occurring over land (or inland water bodies). Because of this, four individuals collected from proximate islands were excluded from this analysis (final $n = 150$).

Circuitscape calculated resistance between each pair of sample sites, and was run separately for each input resistance surface, producing a matrix of pairwise resistance for each resistance hypothesis. Circuitscape was also run inversely on the habitat suitability model input, interpreting the values as conductance instead of resistance. A total of four gene-flow-resistance (or 'landscape distance') matrices were constructed for analysis. Principal coordinate analysis (PCoA) was conducted on each resistance matrix (Borcard and Legendre 2002), using the 'ecodist' package (Goslee and Urban 2007) in R, and the first principal coordinate was used as a low-dimensional representation of resistances between collection sites. Each landscape resistance predictor was then tested against the matrix of D_{ps} using dbRDA as above.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.610pf> (Kozakiewicz et al. 2017).

Results

15 loci were polymorphic and allele frequencies did not deviate from Hardy–Weinberg equilibrium following false discovery rate correction (Supplementary material Appendix 1 Table A1). This suggests that genotyping error rates were low, and that factors that could bias the estimation of gene flow were absent.

Spatial patterns of genetic variation

Significant positive spatial autocorrelation was detected (Monte-Carlo test with 10 000 permutations, $p = 0.0001$), with comparatively large positive eigenvalues of the first two principal components from sPCA (Supplementary material Appendix 1 Fig. A1). Negative spatial autocorrelation was non-significant ($p = 0.5337$). Consequently, lagged scores of the first two principal components were plotted over the sampled area (Fig. 2). The first principal component shows clear evidence of a genetic cline from north-west to south-east, indicated as a graduation from large positive scores to large negative scores (Fig. 2A). The second principal component, which explains less of the spatial structure overall (Supplementary material Appendix 1 Fig. A2), suggests that individuals from north-west and northern central Tasmania are somewhat genetically distinct from those elsewhere (Fig. 2B).

Site-based analyses

Random Forests regression analysis ranked easting and then northing as the most important predictors of individual heterozygosity (Supplementary material Appendix 1 Fig. A3). Additionally, easting and northing remained the two highest ranking predictors when the Random Forests analysis was repeated, while the ranks of all the other variables were not consistent between repeat analyses. Simple linear regressions showed that individual heterozygosity increases from west to east ($r^2 = 0.068$; $p = 0.001$) (Supplementary material Appendix 1 Fig. A4), and decreases from south to north ($r^2 = 0.031$; $p = 0.030$) (Supplementary material Appendix 1 Fig. A5). There was relatively little differentiation of importance between the landscape variables, and these were also ranked inconsistently between the mean squared error and node purity measures. This indicates that spatial variation in individual heterozygosity is unrelated to the landscape variables measured within the 3.1 km buffers at each collection site. Similarly, only easting was detected as a predictor of pairwise genetic distance among individuals from dbRDA, explaining 3.5% of the total variation in genetic distance (Supplementary material Appendix 1 Fig. A6).

Between-site analyses

dbRDA of principal coordinates derived from matrices of resistance to dispersal indicated that elevation and habitat suitability as conductance were the best predictors of pairwise individual genetic distance (Supplementary material Appendix 1 Fig. A7). Habitat suitability as conductance (i.e. where suitable habitat encourages rather than restricts dispersal) was the stronger of the two predictors, explaining 2.9% of the total variation. Euclidean distance was not a significant predictor of genetic distance between individuals.

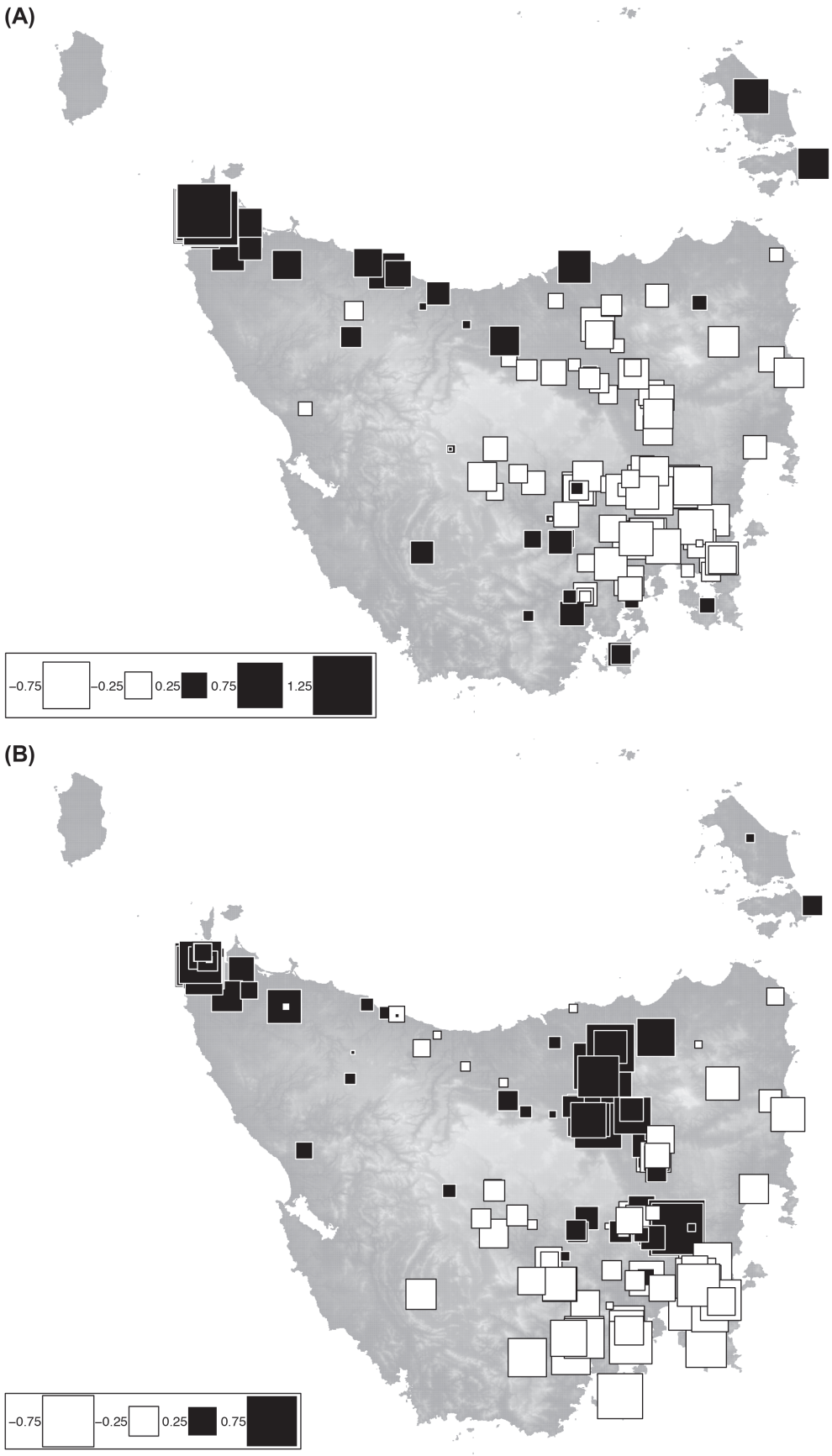

Figure 2. First (A) and second (B) spatial principal component lagged scores for each Tasmanian *A. audax* individual, plotted according to sample location. Cell size and colour are scaled relative to lagged scores, which represent spatial genetic variation across the population. A genetic cline from north-west to south-east is evident from both spatial principal components.

Discussion

We identified significant spatial genetic variation in Tasmanian *Aquila audax*, which appeared to be clinal with respect to geography, from north-west to south-east Tasmania. This was indicated primarily by spatial principal component analysis, but also by the significance of easting and northing control variables during site-based landscape genetic analysis. However, there were no relationships between either individual heterozygosity or pairwise genetic distance and landscape variables surrounding sampling locations. The latter result was not expected given that birds frequently display strong preferences for specific breeding habitats (Cody 1985, Jones 2001, Davis and Stamps 2004), and in heterogeneous landscapes this can produce patterns of spatial genetic variation (Kawecki and Ebert 2004). Tasmanian *A. audax* displays a strong requirement for emergent old-growth eucalypts in native forests on slopes sheltered from the prevailing weather (Brown and Mooney 1997), and this habitat is heterogeneous in distribution across Tasmania (Fig. 1). A small component of total genetic variation could be explained by extrinsic factors intervening sample sites, such as habitat suitability for eagle breeding and elevation (4.6%). Therefore, Tasmanian *Aquila audax* movement appears spatially constrained, in a manner weakly influenced by the extrinsic factors tested.

A population is unlikely to exhibit spatial structuring of genetic variation where individuals regularly disperse large distances relative to the population range, unless external factors are acting on that population to influence the directionality of dispersal (Beveridge and Simmons 2006, White et al. 2009). Despite *A. audax* having a continuous distribution across Tasmania and exhibiting high vagility elsewhere, a genetic cline was detected. The strength of this cline was low (as indicated by regressions against easting and northing), but the existence of any significant pattern is indicative of some limitation to dispersal. Therefore, long-distance dispersal events, such as those implied by Ridpath and Brooker (1986) and Burridge et al. (2013), are relatively uncommon within Tasmania, or are directionally biased.

Geographic clines in genetic variation can result from adaptation to environmental gradients, but these are more often manifest on large geographical scales, such as those involving latitudinal variation in climate (Moran et al. 1989, James et al. 1997). Adaptation along a climatic gradient therefore seems unlikely to be a driver of genetic variation in Tasmanian *A. audax*. Clines can also occur following genetic admixture, where individuals from two or more genetically differentiated populations begin interbreeding (Freedman et al. 2010, Burridge et al. 2015). This effect usually occurs following the removal of a barrier that had previously separated populations, yet for Tasmanian *A. audax* there are no obvious factors that could have completely precluded dispersal between two necessarily geographically proximal populations. Multiple waves of dispersal to Tasmania from mainland Australia could produce a genetic cline radiating from the area in Tasmania where mainland individuals most recently arrived (Vandewoestijne and Van Dyck 2010). As the Tasmanian population is genetically less diverse than the mainland population (Burridge et al. 2013), an area receiving migrants from the mainland would be of higher genetic diversity than the remainder of the Tasmanian population. However, north-west Tasmania, from which the observed genetic cline radiates and represents a plausible arrival destination for mainland individuals, has lower genetic diversity (individual heterozygosity) than the remainder of Tasmania. Additionally, Burridge et al. (2013) found no evidence of further gene flow between the Tasmanian and mainland populations after an initial, relatively recent colonisation of Tasmania.

Genetic cline through intrinsic factors – behaviour, territoriality

Rather than reflecting extrinsic or historical factors, the genetic cline in Tasmanian *A. audax* may be best explained by intrinsic factors, particularly behaviour. Direct observations in similar species indicate a propensity for natal philopatry (Newton 1979, Steenhof et al. 1984, Whitfield et al. 2009). Direct observations and genetic analyses have identified occasional long distance natal dispersal in raptors that otherwise exhibit strong philopatry, from which spatial patterns of genetic variation – such as isolation-by-distance and genetic clines – have been observed (Martínez-Cruz et al. 2004, Alcaide et al. 2009, Mira et al. 2013, Nemesházi et al. 2016). This suggests that the genetic patterns in Tasmanian *A. audax* also reflect a degree of philopatric behaviour and occasional dispersal. There is no evidence for difference in nesting habits and potential imprinting that could contribute to spatial genetic structuring within Tasmanian *A. audax*. Future research has recently been approved to quantify natal dispersal in this species using telemetry.

Methodological considerations

Storfer et al. (2007) highlighted the importance of sampling design in landscape genetics, which should reflect the questions to be addressed. The necessarily opportunistic nature of the sampling for this study has the potential to introduce bias, as some individuals may not have been sampled in locations that are representative of the landscape in their territories, or near the centres of their territories. Similarly, juveniles may range much more widely than adults (Bell and Mooney 1999), exacerbating this potential problem. As breeding habitat was a primary focus of the landscape genetic analyses, and the site-based approach was dependent on inferences regarding breeding territory size, a sampling approach that focused on nest sites would have been ideal. Similarly, inferences of geographic structure could be biased by the sampling of non-resident individuals (Ogden et al. 2015), although currently there is little knowledge of juvenile dispersal in this species. However, the potential for nest abandonment by Tasmanian *A. audax* resulting from experimenter visitation during the breeding season (i.e. when individuals are present at nests) appears very real (Forest Practices Authority 2013, O'Sullivan 2014), precluding such an approach. We re-ran analyses on subsets of individuals to test their sensitivity to the inclusion of subadults and found no substantial change in our results (Supplementary material Appendix 3).

Conclusions and future directions

This study represents a rare example of landscape genetics conducted on a highly vagile avian taxon. Although we did not identify any landscape features that were dominant explanations for genetic variation among Tasmanian *A. audax*, this is not to say that studies of other avian taxa, volant or otherwise, will yield similar results. Molecular data indicate that the Tasmanian *A. audax* population represents a recent founding by a small number of mainland Australian individuals. Against this background, spatial genetic variation ascribable to landscape may be yet to manifest (Landguth et al. 2010, Epps and Keyghobadi 2015). Relationships between genetic variation and landscape variables have been detected in other avian taxa (Lindsay et al. 2008, Unfried et al. 2012), and many more studies have revealed spatial patterns in highly vagile birds that have not been formally tested under a landscape genetics framework (Martínez-Cruz et al. 2007, Alcaide et al. 2009, Barlow et al. 2011, Mira et al. 2013). Additional landscape genetic studies on birds are critically needed to help formulate general ecological theory in landscape genetics. In the broadest sense, this study adds to the wider literature by suggesting that landscape-scale genetic structuring can exist in highly vagile birds, although this structure may be better explained by intrinsic rather than extrinsic factors in this case.

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Supplementary material (Appendix JAV-01326 at <www.avianbiology.org/appendix/jav-01326>). Appendix 1–3.