Reduced gene flow in a vulnerable species reflects two centuries of habitat loss and fragmentation

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Abstract. Understanding the effects of landscape modification on gene flow of fauna is central to informing conservation strategies that promote functional landscape connectivity and population persistence. We explored the effects of large-scale habitat loss and fragmentation on spatial and temporal patterns of gene flow in a threatened Australian woodland bird: the Grey-crowned Babbler Pomatoxostomus temporalis. Using microsatellite data, we (1) investigated historical (i.e., pre-fragmentation) and contemporary (i.e., post-fragmentation) levels of gene flow among subpopulations and/or regions, (2) identified first-generation migrants and likely dispersal events, (3) tested for signatures of genetic bottlenecks, (4) estimated contemporary and historical effective population sizes, and (5) explored the relative influences of drift and migration in shaping contemporary population structure. Results indicated that the functional connectivity of landscapes used by the Grey-crowned Babbler is severely compromised in the study area. The proportion of individuals that were recent immigrants among all subpopulations were low. Habitat fragmentation has led to a clear division between subpopulations in the east and west, and the patterns of gene flow exchange between these two regions have changed over time. The effective population size estimates for these two regions are now well below that required for long-term population viability ($N_e < 100$). Demographic history models indicate that genetic drift was a greater influence on subpopulations than gene flow, and most subpopulations show signatures of bottlenecks. Translocations to promote gene flow and boost genetic diversity in the short term and targeted habitat restoration to improve landscape functional connectivity in the long term represent promising conservation management strategies that will likely have benefits for many other woodland bird species.

Key words: ancestry; bottleneck; functional connectivity; region; subpopulation.
In populations with small effective population sizes ($N_e <100$; $N_e$, heuristically, is the number of individuals that contribute to the next generation), a population is expected to experience inbreeding depression and loss of critical functional genes (e.g., immunity) through genetic drift over a period of five, or fewer, generations (Frankham 1995, Frankham et al. 2014). Reduced fitness as a result of inbreeding can have negative implications for a species’ reproductive rate, population size, and likelihood of long-term population persistence (Keller 1998). Although population sizes when $N_e > 100$ should limit loss of fitness over five generations to ≤10%, it is widely accepted that much larger population sizes (e.g., $N_e > 1000$) are required to maintain a population’s ability to adapt to environmental change (Jamieson and Allendorf 2012, Frankham et al. 2014).

Dispersal of individuals promotes gene flow among habitat patches and is crucial for recolonizing suitable vacant habitat, maintaining genetic diversity, and mitigating extinction risk (Bowler and Benton 2005). The degree to which landscapes facilitate the movement of populations, individuals, and ultimately genes (Taylor et al. 1993) is influenced by landscape connectivity. Landscape connectivity has two components: Structural connectivity refers to the physical elements and configuration of the landscape, while functional connectivity refers to an animal’s ability to move through the landscape (Tischendorf and Fahrig 2000). While it follows that structural connectivity influences functional connectivity, functional connectivity is a more direct measure of the capacity of a population to persist in modified landscapes (Uezu et al. 2005, FitzGibbon et al. 2007). Thus, an understanding of functional connectivity in fragmented landscapes can be central to the successful implementation of conservation management actions for threatened taxa (Fahrig 2007, Sunnucks 2011).

Increasing rates of gene flow among vulnerable and declining populations (e.g., via genetic rescue or genetic restoration) can counteract genetic drift, reduce inbreeding depression, and boost genetic diversity (Frankham 2015, Hoffmann et al. 2015, Whiteley et al. 2015). There is a growing body of evidence that demonstrates the positive outcomes of gene flow, including genetic rescue, for small, inbred populations (Frankham 2015, Whiteley et al. 2015). Maintaining large metapopulations and promoting functional connectivity between small and isolated population subunits (i.e., maintaining metapopulation processes) is therefore predicted to promote species persistence under increasing human-induced pressures from landscape modification, extreme events, and climate change uncertainties (Nimmo et al. 2016).

The Grey-crowned Babbler *Pomatostomus temporalis* is a cooperatively breeding woodland bird found on mainland Australia (i.e., excluding the island State of Tasmania) and in southern New Guinea, and which has been adversely affected by human-induced reductions in landscape connectivity (Adam and Robinson 1996, Blackmore et al. 2011, Stevens et al. 2016). Cooperatively breeding birds typically have much smaller effective breeding populations than pair breeding species when compared to total population size (Frankham 1995). As such, cooperative breeders present ideal models with which to investigate the effects of reduced gene flow on populations before they become critically endangered.

We investigated the effects of landscape-scale habitat loss and fragmentation on spatial and temporal patterns of gene flow in a threatened woodland bird. We analyzed microsatellite data of the Grey-crowned Babbler to (1) investigate the levels of historical (i.e., pre-fragmentation) and contemporary (i.e., post-fragmentation) gene flow among subpopulations and/or regions, (2) identify first-generation migrants and likely dispersal events, (3) screen for signatures of genetic bottlenecks, (4) estimate contemporary and historical effective population sizes, and (5) explore the relative influences of drift and migration in shaping contemporary population structure. In doing so, our primary goal is to provide recommendations for management actions that would promote functional connectivity and population persistence in this and other woodland-dependent bird species.

**Materials and Methods**

**Study species**

The Grey-crowned Babbler was historically common across much of eastern Australia (Department of Environment and Heritage 2013), but has undergone a major range contraction and population declines of over 90% across the southern...
extent of its distribution as a consequence of habitat loss and fragmentation (Robinson 1993, 2006, Environment Conservation Council 2001, Environment Australia 2011). In the mid-1800s, extensive clearing of native vegetation for anthropogenic purposes such as agriculture and mining began in earnest. This clearing continued, such that ~14% of native habitat now remains in this region (Environment Conservation Council 2001). In southern parts of its range, the Grey-crowned Babbler is now restricted to roadside or riparian vegetation, small adjacent remnant woodland patches within farmland (<0.5 ha), and habitat edges of the few remaining larger conservation reserves (>5 ha; Robinson 2006).

Because of their complex social structures and mating systems, cooperatively breeding bird species can be particularly vulnerable to habitat loss and fragmentation (Blackmore et al. 2011, Harrison et al. 2013). Grey-crowned Babblers typically live in groups of up to 12 individuals (avg. ~5 individuals; Stevens et al. 2015) and occupy territories of between 2 and 53 ha in size (Higgins and Peter 2003, Blackmore and Heinsohn 2008). Groups usually consist of a dominant breeding pair and past offspring that delay dispersal from natal territories for up to three years to help raise young (Blackmore et al. 2011). High levels of genetic relatedness across local neighborhoods suggest most dispersal occurs over relatively short distances (<2 km; Koenig et al. 1992, Blackmore et al. 2011, Stevens et al. 2016).

**Sampling**

The study region encompassed an area of ~22,250 km² in north-central and northeast Victoria, Australia (Fig. 1). Potential sites were identified based on long-term survey records (Robinson 1993, Tzaros 1995, 2001, Davidson and Robinson 2009; N. Lacey, unpublished data; D. Robinson, unpublished data). Call playback confirmed the presence and size of a Grey-crowned Babbler family group at each potential site. Territory occupancy was verified from nesting activity, and site locations were recorded using a Geographic Positioning System. An on-ground search using call playback was conducted in areas of habitat within a 2 km radius of each study territory to determine distances to adjacent groups. An average Euclidean distance of 979.8 m separated sampled groups and their closest neighboring Grey-crowned Babbler group, measured between group centroids (usually a nest). Structural connectivity distances between sampled groups were estimated using the distance-measuring tool available in Google Earth satellite data 2015 to calculate the cumulative straight-line distance total between pairs of groups through visibly connected habitat areas of tree cover. Sampling was undertaken at 39 sites selected from three geographic regions: west (n = 15, Kerang; Boort), southeast (n = 12, Violet Town; Lurg), and northeast (n = 12, Peechelba; Rutherglen; Chiltern; Fig. 1). The sampling period incorporated two annual breeding seasons between June 2010 and April 2012. Birds were lured into mist nets using call playback. Each individual was banded with a metal leg band provided by the Australian Bird and Bat Banding Scheme and a unique combination of three colored plastic leg bands for identification in the field. Individuals were measured and a blood sample (~70 μL) collected from the brachial vein using a VITREX capillary tube. Blood was transferred to a Whatman FTA Card and stored at room temperature in paper envelopes. We sampled 135 Grey-crowned Babbler individuals from 39 discrete family groups.

**Molecular sexing, genotyping, and genetic marker behavior**

All Grey-crowned Babbler individuals were screened by polymerase chain reaction (PCR) and sexed using a standard molecular protocol (Griffiths et al. 1998). DNA isolates were subsequently genotyped for 13 Grey-crowned Babbler microsatellite loci by the Australian Genomic Research Facility on an AB3730 capillary sequencer and analyzed using GeneMapper 3.7 (Applied Biosystems, Foster City, California, USA). Extraction protocol, primer sequences, PCR conditions and protocols, and appropriate genetic marker behavior checks are described in Stevens et al. (2016).

Since many population genetic analyses assume independence of individuals, in cooperatively breeding systems inclusion of close relatives has the potential to introduce some bias and contribute to patterns of population genetic structure. However, the potential influence of including close relatives should be greater on analyses conducted at the site level (<0.5 km; Stevens et al. 2016) than at the subpopulation or regional level (which pools multiple sites). Previously we found...
that removing closely related individuals did not substantially alter inferences (e.g., patterns of diversity, genetic structure, relatedness; Stevens et al. 2016), so we chose to retain all individuals to retain maximum power.

Analyses were based on six subpopulations: (1) Kerang north; (2) Kerang south and Boort; (3) Violet Town south; (4) Lurg, Violet Town north, and Peechelba; (5) Rutherglen; and (6) Chiltern (Fig. 1). Subpopulations were defined according to geography and genetic substructure previously described in Stevens et al. (2016). Given Kerang north and Rutherglen (previously identified as sharing membership to the same genetic cluster) are separated by a very large geographic distance (>100 km), we chose to treat them as separate subpopulations here in order to be able to measure the extent of gene flow between them (Fig. 1). Similarly, Violet Town south and Kerang south/Boort (which share membership to the same genetic cluster in the TESS analysis; Stevens et al. 2016) are also separated by a very large geographic distance (>100 km), and so were treated as separate subpopulations in gene flow analyses (Fig. 1).

Contemporary gene flow and migration among subpopulations

Contemporary (previous 2–3 generations) levels of gene flow between all subpopulation pairs...
(n = 36 possible pairwise comparisons) were assessed using BayesAss v 3.1.1 (Wilson and Rannala 2003). As the average reproductive lifespan of the Grey-crowned Babbler is five years and they exhibit overlapping generations (Counsilman and King 1977), we presumed contemporary gene flow levels to represent the 10–15 yr prior to sampling and therefore reflect genetic processes following extensive habitat fragmentation in the study area which have occurred within the past 200 yr (Bradshaw 2012).

BayesAss uses a Bayesian method with Markov chain Monte Carlo (MCMC) simulations to provide estimates of the mean and 95% confidence intervals (CI). BayesAss assumes both linkage equilibrium and that migration and genetic drift do not change subpopulation allele frequencies over the previous 2–3 generations, and relaxes assumptions of Hardy–Weinberg equilibrium (HWE) within populations (Wilson and Rannala 2003). Research has shown that BayesAss analyses may result in incorrect estimations of migration rates which arise from bimodality of the inference that models produce, as well as the effects of weak population structure (Meirmans 2014). Stevens et al. (2016), however, reported strong genetic structure across our study area. To increase the statistical power and inference reliability of BayesAss output, we followed Meirmans (2014) further suggestions and ran over 30 repeats and did not average results, instead reporting the most biological meaningful and repeated results. These methods assist parameter optimization and ensure convergence. Furthermore, in instances where model assumptions may be violated, such as cooperatively breeding species, accurate estimates can still be obtained if migration rates are low (Faubet et al. 2007). Markov chain Monte Carlo mixing parameter values for migration rates (gene flow), allele frequencies, and inbreeding coefficients were adjusted to 0.50, 0.95, and 0.50, respectively, to achieve recommended acceptance rates (Wilson and Rannala 2003). We performed $3 \times 10^6$ MCMC iterations with $10^6$ iterations to discard as burn-in. Each run was initialized with different starting-seed values to achieve consistency of mean parameter estimates between runs (Wilson and Rannala 2003).

Initial identification of putative first-generation immigrants and their inferred origins also used BayesAss. To validate BayesAss ancestry assignments, we conducted a second method implemented in Geneclass2 (Piry et al. 2004) using a Bayesian approach (Rannala and Mountain 1997) with a Monte Carlo resampling algorithm (Paetkau et al. 2004). We tested 10,000 simulated individuals with a type I error threshold of 0.05 and used a likelihood ratio $L_{\text{home}}/L_{\text{max}}$. This ratio is computed from the likelihood of the population from which the individual was sampled ($L_{\text{home}}$) over the highest likelihood value among all population samples ($L_{\text{max}}$), including the population of the individual (Piry et al. 2004). The likelihood ratio of $L_{\text{home}}/L_{\text{max}}$ has more statistical power to identify non-resident individuals among populations than using only $L_{\text{home}}$ (Piry et al. 2004). Both assignment methods assume all possible source populations have been sampled. Although some disparate Grey-crowned Babbler groups exist between our populations (Robinson, unpublished data), ancestry analyses allowed us to identify general pathways of dispersal and to make direct comparisons of ancestry assignments between methods.

Detecting temporal gene flow and gene flow patterns between east and west regions

Common methods for enabling direct comparisons of temporal gene flow levels include comparing BayesAss and Migrate-n estimates. Recent studies have shown that such comparisons may not always reflect biological reality (Faubet et al. 2007, Meirmans 2014, Samarasin et al. 2017). For instance, Samarasin et al. (2017) suggest that in scenarios where there has been a recent decline in migration, Migrate-n will underestimate historical migration rates (i.e., Migrate-n will be biased to recent parts of the $4N_e$ time period). Furthermore, in the same situation, BayesAss will overestimate recent migration rates (Samarasin et al. 2017). Therefore, we undertook a qualitative investigation into long-term and contemporary gene flow (connectivity) occurrence between regions. We also looked for any pattern variation in potential gene flow occurrence over time. We ensured greater robustness in the results by pooling our data which reduced the number of group comparisons to two regions rather than all possible pairs of subpopulations, while increasing the number of individuals within a group (east: $n = 84$; west: $n = 51$; Meirmans et al. 2014; Fig. 1).

We used Migrate-n to estimate mutation-scaled, long-term gene flow rates between the two regions.
To reduce the number of potential parameters relative to the number of loci and improve statistical power (Kuhner 2009), we set parameters to include symmetrical gene flow. We used the Brownian motion model with \( F_{ad} \) calculations of \( \theta \) and \( M \) as starting parameters, and Metropolis-Hastings sampling and uniform prior distributions to estimate \( \theta \) (range, 0–100; delta, 10) and \( M \) (range, 0–500; delta, 50). The Markov chain settings recorded 10⁴ steps from 1 long chain of 10⁵ sampled steps, and a search strategy following a static heating scheme using four temperatures (1.0, 1.5, 3.0, and 1,000.0) to examine the genealogical space more effectively (Beerli 2006, 2009). Runs were replicated twice to ensure posterior probabilities stabilized.

We used the commonly used method for estimating unscaled long-term gene flow rates (Chiucchi and Gibbs 2010, Dutta et al. 2013, Wood et al. 2017) by multiplying the mutation-scaled long-term gene flow rates (\( M \)) generated in Migrate-n with a typical vertebrate microsatellite mutation rate (0.001; Ellegren 2000, Schlötterer 2000). Meanwhile, estimates of contemporary gene flow rates were obtained with BayesAss using the same methods as described above, but for east and west regions. We present means and CIs for Migrate-n and BayesAss in our results.

**Long-term and contemporary effective population sizes of east and west regions**

We derived the long-term effective population sizes from \( \theta \) values produced in Migrate-n for east and west regions. To obtain a measure of contemporary effective population size (\( N_{eD} \)), we estimated the effective number of breeders (\( N_{eD} \); related to inbreeding and reflecting the parental generation) using the single-sample linkage disequilibrium-based method implemented in LDNe (Waples and Do 2008). A recent study suggests that LDNe analysis can be unreliable for sample sizes <30 (Tallmon et al. 2010). To increase the robustness of estimates of effective population size (Tallmon et al. 2010), we ran analyses for our pooled data set of east and west regions. We estimated \( N_{eD} \) using three different rates for the inclusion of rare alleles (\( p_{crit} \): 0.05; 0.02; and 0.01), which allowed for comparisons of consistency across results. We report estimates from the criterion \( \geq 0.05 \) as these provide a reasonable balance between maximum precision and minimal bias with polymorphic loci such as microsatellites (Waples and Do 2008).

**Modeling population history**

The genealogical history of the six subpopulations was investigated to estimate whether drift was more important than immigration in shaping contemporary population structure (Ciofi et al. 1999). Two models of population history, drift vs. immigration–drift equilibrium (gene flow), were assessed in 2-Mod v 0.2 following the methods of Ciofi et al. (1999). Both models are based on population allele frequencies. The drift model computes allele frequencies as a product of pure drift with little evidence of gene flow between populations. The gene flow model works on an equilibrium principle between immigration and genetic drift to evaluate allele frequency within populations. The likelihood of each model’s fit to the data is estimated using MCMC methods which compare estimates between models and provide probabilities of the goodness of fit for each (Ciofi et al. 1999). Simulations of MCMC were run for 10⁵ iterations, discarding the initial 10% of results as burn-in to avoid possible bias from start conditions. The analysis was repeated three times to validate results.

**Signature of bottlenecks within subpopulations**

To investigate whether the six subpopulations had experienced genetic bottlenecks, we ran a two-phase mutation model (TPM) in Bottleneck v 1.2.02 (Cornuet and Luikart 1996). This method investigates whether observed heterozygosity within each subpopulation was higher than would be expected for populations in mutation–drift equilibrium and can be used to detect bottlenecks over the last 2–4\( N_e \) generations (Cornuet and Luikart 1996, Luikart et al. 1998). The proportion of stepwise mutation model in the TPM was set to 70%.

**RESULTS**

**Contemporary gene flow and migration among subpopulations**

Very low levels of contemporary gene flow (i.e., the proportion of individuals within a subpopulation that are immigrants) per generation were recorded between six subpopulations of Grey-crowned Babblers over the previous 2–3 generations using BayesAss (Table 1). Estimates ranged from 0.01 to 0.19, with most rates being ≤0.03 (Table 1). Two population pairs showed strong evidence of gene flow (CIs did not include
Table 1. Estimates of recent (previous 2–3 generations) mean gene flow rates per generation among six Grey-crowned Babbler subpopulations.

| Destination of gene flow | Origin of gene flow | Kn    | KsB   | Vs    | LVP   | Rg    | Ch    |
|--------------------------|---------------------|-------|-------|-------|-------|-------|-------|
| Kn                       | (0.84 to 0.98)      | 0.02  | (−0.02 to 0.05) | 0.02  | 0.02  | 0.02  | 0.02  |
| KsB                      | 0.01                | 0.69  | (0.02 to 0.99) | 0.02  | 0.02  | 0.02  | 0.02  |
| Vs                       | 0.03                | (0.11 to 0.27) | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  |
| LVP                      | (−0.02 to 0.08)     | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| Rg                       | (−0.01 to 0.03)     | (−0.01 to 0.05) | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| Ch                       | (−0.01 to 0.04)     | (−0.01 to 0.06) | (−0.01 to 0.04) | 0.01  | 0.01  | 0.01  | 0.01  |

Notes: Values indicate the mean proportion of individuals within subpopulations in rows (Destination of gene flow) that are immigrants from subpopulations in columns (Origin of gene flow). The 95% confidence intervals of gene flow rates are in parentheses, and immigration CI values that do not cross zero are in bold type. Proportions of non-migrants are on the diagonal. Subpopulations are Kerang north (Kn); Kerang south/Boort (KsB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); and Chiltern (Ch). Values were calculated using BayesAss (Wilson and Rannala 2003).

Initial ancestry assignments from BayesAss identified 10 individuals as likely first-generation immigrants. Eight out of the 10 individuals were adult birds (≥2nd-year bird), and this cohort was male-biased ($n = 7/1$ sex ratio). The two remaining birds, one male and one female, were first-year birds.

The Geneclass2 ancestry assignment method identified nine possible first-generation immigrants ($P < 0.05$). Seven of the nine birds identified were adults, and two were immature. Three out of the nine birds were also identified as likely migrants with BayesAss (one adult; two immature). Geneclass2 results also supported a male bias among adult immigrants ($n = 6/1$ sex ratio; Table 2).

Detection of temporal gene flow and pattern variation between east and west regions

We found evidence of symmetrical long-term gene flow between the east and west regions (Fig. 1, Table 3). Contemporary gene flow occurrence was evident in the direction of the east region to the west, but no evidence for contemporary gene flow occurring from the west to the east region (CIs included zero; Fig. 1, Table 3).

Long-term and contemporary effective population sizes

Mutation-scaled, long-term effective population size estimates ($\theta$) were higher in the east (5.89) than in the west (4.29; Table 3). Contemporary LD-based effective population size estimates ($N_{eD}$) were also higher in the east than in the west region and were smaller than their respective sample size ($n$; east: $n = 83$, $N_{eD} = 19.7$; west $n = 51$, $N_{eD} = 17.0$; Table 3).

Demographic history of subpopulations

The pure drift model was identified as the most plausible model given the genetic history of subpopulations (probability, drift = 0.70; gene flow = 0.30). This result suggests that levels of gene flow among these subpopulations are not sufficient to counteract genetic drift.

Bottleneck signatures within subpopulations

Under the TPM, four of the six populations showed evidence of genetic bottlenecks: Kerang south/Boort; Violet Town south; Lurg/Violet Town north/Peechelba; and Rutherglen (Table 4).

Discussion

Our study demonstrates that the contemporary functional connectivity of landscapes used by the Grey-crowned Babbler in the southern parts of its range is likely compromised relative to historical levels. The change in gene flow pattern over time shows that contemporary migration of individuals from the west to the east region has decreased to a level that provides no
evidence of its occurrence. Demographic history models indicated that genetic drift was a greater influence on the species than gene flow across the study region, and most subpopulations show signatures of bottlenecks. Effective population size estimates of less than 100 for the regions are now well below what is required for long-term population viability (Frankham 1995).

Gene flow decline despite evidence of long-distance dispersal

Although evidence was found for continuing contemporary gene flow in an east-to-west direction, the few long-distance dispersal events observed from the west to the east did not support evidence for continuing occurrences in this latter direction. In fact, overall contemporary gene flow levels remain very low or non-existent between the east and west regions (<2 effective migrants per generation). We suggest that the evidence of gene flow from the west to the east found in contemporary immigration rates between subpopulations Kerang south/Boort to Violet Town south (Table 1) may be a remnant of historical connectivity. Additionally, the highest number of samples for any of the genetic clusters

| Route | n | Long-term m | Contemporary m | Recipient θ | Recipient NeD |
|-------|---|-------------|---------------|-------------|--------------|
| East to West | 51 | 0.015 (0.01–0.02) | 0.066 (0.03 to 0.10) | 4.29 (2.07–6.47) | 17.0 (13.90–20.90) |
| West to East | 84 | 0.015 (0.01–0.02) | 0.018 (–0.02 to 0.04) | 5.89 (3.53–8.20) | 19.70 (16.50–23.50) |

Notes: Shown are the sample size (n); long-term (Migrate-n; Beerli 2006) and contemporary (BayesAss; Wilson and Rannala 2003) levels of gene flow per generation (m); and mutation-scaled, long-term effective population size (θ; Migrate-n) and contemporary effective number of breeders (NeD; related to inbreeding and reflecting the parental generation; LDNe; Waples and Do 2008) for the recipient population. Values shown in parentheses are 95% CIs, and values for m that do not cross zero are in bold type.

Table 2. First-generation immigrants identified among six genetic subpopulations of the Grey-crowned Babbler in southern parts of its range

| Individual | Sample location | Origin of ancestry | Probability of ancestry | Log(L) of ancestry | Sex | Age class | Approximate Euclidean distance (km) |
|------------|-----------------|--------------------|-------------------------|------------------|-----|-----------|----------------------------------|
| VB085†     | Vs              | KsB                | 0.979                   | 16.804           | Male | Adult    | 170                              |
| VB086†     | Vs              | KsB                | 0.956                   | 19.156           | Male | Adult    | 170                              |
| VA135†     | Vs              | KsB                | 0.950                   | 21.958           | Male | Adult    | 220                              |
| VB087†     | Vs              | KsB                | 0.946                   | 20.761           | Male | Adult    | 215                              |
| VB088†     | Vs              | KsB                | 0.934                   | 20.036           | Female| Adult | 12                               |
| VA092†     | Vs              | KsB                | 0.910                   | 18.517           | Male | Immature | 15                               |
| VA134†     | Vs              | KsB                | 0.892                   | 17.066           | Male | Immature | 20                               |
| CH006†‡    | Ch              | Rg                 | 0.872                   | 21.507           | Male | Adult    | 37                               |
| VA091†‡    | Vs              | LVP                | 0.794                   | 16.804           | Male | Immature | 220                              |
| RH035†‡    | Rg              | KsB                | 0.845                   | 19.156           | Male | Adult    | 215                              |
| CK042‡     | LVP             | Ch                 | 0.794                   | 17.066           | Female| Adult | 15                               |
| RE131‡     | Kn              | Rg                 | 0.872                   | 21.507           | Male | Adult    | 37                               |
| RR040‡     | LVP             | Rg                 | 0.845                   | 19.156           | Female| Adult | 12                               |
| CH004‡     | Rg              | Ch                 | 0.872                   | 21.507           | Male | Adult    | 220                              |
| VT068‡     | Vs              | LVP                | 0.845                   | 17.066           | Male | Adult    | 15                               |
| RI008‡     | Ch              | Rg                 | 0.872                   | 17.066           | Male | Adult    | 37                               |

Notes: Values indicate the probability (BayesAss; Wilson and Rannala 2003) and/or the log likelihood (log(L); Geneclass2; Piry et al. 2004) of an individual being a first-generation immigrant. Euclidean distances are approximations and measured from the individual's sampling location to the closest sampled family group associated with the putative subpopulation of origin. Subpopulations are Kerang north (Kn); Kerang south/Boort (KsB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); and Chiltern (Ch). All log(L) and probability values were below the significance threshold (P < 0.05). Results are shown in descending order based on probability, then log(L), values.

† Individual identified using BayesAss.
‡ Individual identified using Geneclass2.
Table 4. Models of bottleneck signatures for Grey-crowned Babblers sampled from six sub-populations.

| Subpopulation | n     | k   | TPM |
|---------------|-------|-----|-----|
| Kn            | 25.85 | 5.46| 0.66|
| KsB           | 76.00 | 7.23| <0.01|
| Vs            | 20.00 | 4.69| <0.01|
| LVP           | 73.54 | 7.08| <0.01|
| Rg            | 41.85 | 6.77| 0.05|
| Ch            | 32.00 | 5.77| 0.25|

Notes: Subpopulations are Kerang north (Kn); Kerang south/Boort (KsB); Violet Town south (Vs); Lurg/Violet Town north/Peechelba (LVP); Rutherglen (Rg); and Chiltern (Ch). Values are mean number of individuals sampled per locus (n); mean observed number of alleles (k); and significant values (P < 0.05) for the two-phase mutation model (TPM). Computations were calculated in Bottleneck v 1.2.02 (Cornuet and Luikart 1996).

found in our study area was evident in the Kerang south/Boort subpopulation (n = 38; Stevens et al. 2016). These data could potentially skew our results, indicating that the birds in Violet Town south (n = 4) from the same genetic cluster are from the western Kerang south/Boort subpopulation. Some evidence of the same genetic cluster was also recorded in the Chiltern subpopulation (n = 2), and being in the east region and geographically closer, the Violet Town south birds may have originated from Chiltern. In these northeastern areas, greater levels of structural connectivity, that is, more available tree cover, are provided by dispersed tree cover (Stevens et al. 2016), and roadside and riparian corridors (Fig. 1; K. Stevens, personal observation).

The variation in contemporary patterns of gene flow between the east and west, and the (potential) change in gene flow patterns over time, may also be a consequence of higher levels of available habitat in the east region. Grey-crowned Babblers in this region may exhibit increased fitness and greater mobility and be capable of flying further or more often. Higher population levels in the east also require more available habitat, and fitter birds in this region could utilize the higher levels of functional habitat connectivity to move west. By contrast, birds in the west region may not be as mobile due to a lack of habitat and lower levels of functional connectivity across their region, potentially producing negative effects on their fitness and movement between habitat patches. Less mobile species often rely on corridors as conduits for dispersal, and these types of habitat linkages can be crucial to animal movement through fragmented landscapes, particularly in agricultural systems (van der Ree and Bennett 2001, Gillies and St. Clair 2008, Vergara et al. 2013). Ongoing gene flow may be better facilitated by the presence of both corridors and dispersed (stepping-stone) habitat connectivity in fragmented systems.

If our estimates of contemporary gene flow levels between east and west regions are overestimated as a result of recent declines in migration rates as studies suggest (Samarasin et al. 2017), this could mean gene flow between east and west regions is potentially occurring at even lower levels than our estimates show (Fig. 1, Table 3). Under such a scenario, there is an even more pressing need to instigate targeted conservation management efforts for these birds in our study area. Similar studies on metapopulations that are reliant on relatively stable sources of habitat have shown that habitat loss and fragmentation are associated with decreased wildlife immigration and survival (Catlin et al. 2016). Populations that experience high levels of habitat disturbance can become demographically and genetically isolated as a result of reduced dispersal and gene flow. Although our study showed evidence for some long-distance (~220 km) emigration from the west to the east region, potentially facilitated by extant riparian habitat connectivity between major rivers in the area (e.g., Murray and Goulburn rivers; Fig. 1), the overall rate of observed gene flow may be insufficient to mitigate the detrimental effects of small population sizes on the long-term genetic viability of these subpopulations (Weeks et al. 2011, Segelbacher et al. 2014).

Signatures of genetic bottlenecks and small effective population sizes

Signatures of genetic bottlenecks likely reflect declines in population size and/or reduced gene flow (Cornuet and Luikart 1996, Broquet et al. 2010). Detectable signatures of bottlenecks generally become apparent when high levels of population decline have occurred or numbers of breeding individuals are reduced to unsustainable levels (i.e., $N_e < 100$ individuals; Peery et al. 2012). Strong evidence of longer-term signatures of bottlenecks in most subpopulations supports...
the small $N_e$ estimates and evidence of drift. Our results are consistent with those of other studies on species experiencing major population declines resulting from recent isolation and/or population collapse as a consequence of habitat loss and fragmentation (Bender et al. 1998, Fahrig 2001, Radford et al. 2005).

Small $N_e$ and severe reductions in $N_e$ can lead to a loss of fitness through inbreeding depression and reduced evolutionary potential (Frankham et al. 2014). For species of conservation concern, identifying populations which have small $N_e$ and that show evidence of recent bottlenecks is crucial for effective conservation decisions (McCusker et al. 2014). Long-term and contemporary estimates of effective population sizes were higher for the east region than for the west, but were well below the level predicted to limit loss of fitness to $\leq 10\%$ over five generations (Frankham et al. 2014). The census population in the southern extent of the species’ range is estimated at $\leq 2000$ individuals (Davidson and Robinson 2009). Samples used in this study were collected within the same census population, and hence, our results may reflect a concerning trend across the entire population, and which is below the number required for the future genetic viability of these populations (i.e., $N_e > 1000$; Frankham et al. 2014).

Influences of drift rather than migration shaping contemporary population structure

Despite evidence for dispersal over large geographic distances, the higher probability of genetic drift influencing Grey-crowned Babbler population structure in the study area will likely outweigh the level of migration required for mutation–drift equilibrium (Luikart et al. 1998). This finding is consistent with earlier studies indicating that habitat fragmentation implications include disrupted dispersal of the Grey-crowned Babbler (Environment Australia 2011). Other studies investigating the effects of habitat modification on species’ population genetic structure and functional connectivity report similar detrimental effects (Dutta et al. 2013, Harrisson et al. 2013, McCusker et al. 2014). Declines in genetic exchange between small populations are likely to be associated with increased levels of inbreeding and elevated risk of local extinction as subpopulations lose genetic diversity (Sunnucks 2011).

Analyses indicated that Kerang south/Boort was no longer receiving gene flow from other subpopulations, which suggests a decrease in genetic exchange from this subpopulation. Long-term census records have shown population decline and extirpation of Grey-crowned Babbler groups from habitat patches in these areas particularly (Tzaros 1995, 2001, Stevens et al. 2015). The lack of immigration from Kerang south/Boort, population decline, and local extinctions is a concerning trend. This concern is further compounded given the drift model estimated there was a 70% probability that drift had occurred. Such evidence strongly indicates Kerang south/Boort is exposed to an increasing threat of inbreeding and drift, and its long-term viability is questionable without intervention (Volpe et al. 2014, Weeks et al. 2015). As such, we identify the Kerang south/Boort population as a management priority within our study area.

Conclusion and Recommendations

An understanding of the role of landscape connectivity among spatially structured and declining populations is required to inform effective conservation measures that promote genetic variation and population demographic viability (Amos et al. 2014). Differences in gene flow patterns over time that were observed here suggest that these regions are now, or are becoming, isolated, and are consistent with a loss of functional connectivity resulting from large-scale habitat loss and fragmentation since the mid-1800s in this area (Fig. 1, Table 3). Given a lack of functional landscape connectivity is a likely driver in this threatening process, there is potential to reverse this decline in gene flow. Across the Lurg area for instance, long-term ($>22$ yr) and large-scale ($>1500$ ha) habitat restoration has led to a substantial increase in woodland bird species diversity and richness, including the Grey-crowned Babbler (Thomas 2009, Veskit et al. 2015). Ongoing research into the long-term effects of habitat restoration for the Grey-crowned Babbler in these areas demonstrates an increase in population size (2001–2008, mean = 59; 2009–2015, mean = 106) with the average group size increasing by 0.8 birds (Thomas 2009, Veskit et al. 2015; Lacey, unpublished data, Moylan, unpublished data). Although substantial areas of revegetated habitat support population
increases in woodland fauna within the Lurg area (Vesk et al. 2015), this is a localized phenomenon within our study region. There remain large gaps in structural connectivity and a lack of habitat availability between subpopulations elsewhere, which may explain the low levels of contemporary gene flow between them. With similar habitat restoration effort within targeted areas, woodland species could experience an increase in gene flow levels.

Our study suggests that loss of functional connectivity of landscapes has had negative consequences for the future genetic viability of the Grey-crowned Babbler in the southern part of its range. The current status of the species in the study area is symptomatic of faunal declines in fragmented systems (Radford et al. 2005). In our focal area, there are a suite of other woodland birds that are likely threatened by the same or similar processes (Amos et al. 2012). The Grey-crowned Babbler is an exemplar in this context as its cooperatively breeding behavior makes it especially susceptible to the influences of habitat fragmentation owing to a substantially reduced \( N_e \) (relative to total population size; Sunnucks 2011). Under these circumstances, actions to promote/enhance gene flow for the fragmentation-sensitive Grey-crowned Babbler are likely to also have benefits for other threatened species, including species with less sensitive breeding strategies such as pair breeders.

Efforts that promote species genetic viability, such as conservation translocations and habitat connectivity enhancement, require information about functional connectivity and genetic variability of populations (Weeks et al. 2011). The data we have presented are highly relevant for targeting revegetation programs between subpopulations that have become disconnected, but could also be used to inform carefully managed translocation programs (Weeks et al. 2011, Volpe et al. 2014). Translocations for genetic rescue/restoration purposes are increasingly being considered as a potentially powerful management strategy for boosting fitness and genetic diversity of small, isolated populations (Hoffmann et al. 2015, Weeks et al. 2015, Whiteley et al. 2015). Arguments warning against translocations often suggest that mixing genes between previously genetically isolated populations will lead to outbreeding depression (Storfer 1999). However, evidence of historical genetic connectivity across the study region indicates that efforts to increase functional connectivity would be highly unlikely to result in negative fitness consequences for the Grey-crowned Babbler (Frankham et al. 2011, Frankham 2015). Intervention programs, such as human-assisted translocations, could potentially be implemented across the southern parts of the Grey-crowned Babbler’s range as an interim measure until habitat revegetation can provide functional landscape connectivity in these areas (Clarke et al. 2002). Such management interventions may be necessary to avoid localized extinctions as have been observed in other highly fragmented parts of the species range (e.g., south-coastal Victoria, southeast South Australia; Barrett 2003, Department of Environment and Heritage 2013, Department of Land, Water, Environment and Planning 2017). Increasing structural landscape connectivity to facilitate gene flow for Grey-crowned Babblers is also likely to provide long-term benefits for other woodland bird species that are affected by loss of habitat in the same areas (Clarke and Oldland 2007).

Subpopulations in this fragmented landscape present a model for species that persist at the extremes of their range. But perhaps more importantly, here they also present a transferable model with broad applicability for many declining bird species. This study has detailed how genetic approaches can be used to drive intervention-orientated conservation programs that aim to facilitate long-term gene flow in a contemporary landscape.

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LITERATURE CITED

Adam, P., and D. Robinson. 1996. Negative effects of fuel-reduction burning on the habitat of the Grey-crowned Babbler Pomatostomus temporalis. Victorian Naturalist 113:4–9.

Amos, J. N., et al. 2012. Predicting landscape-genetic consequences of habitat loss, fragmentation and mobility for multiple species of woodland birds. PLoS ONE 7:e30888.

Amos, J. N., et al. 2014. Species- and sex-specific connectivity effects of habitat fragmentation in a suite of woodland birds. Ecology 95:1556–1568.

Banks, S. C., et al. 2005. The effects of habitat fragmentation on the social kin structure and mating system of the Agile Antechinus, Antechinus agilis. Molecular Ecology 14:1789–1801.

Barrett, G. 2003. The new Atlas of Australian birds. Royal Australasian Ornithologists Union, Melbourne, Victoria, Australia.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22:341–345.

Beerli, P. 2009. How to use Migrate-n or why are Markov chain Monte Carlo programs difficult to use? Pages 42–79 in G. Bertorelle, M. W. Bruford, H. C. Hauffe, A. Rizzoli, and C. Vernesi, editors. Population genetics for animal conservation. No. 17 of Pages 42.

Bender, D. J., T. A. Contreras, and L. Fahrig. 1998. Habitat loss and population decline: a meta-analysis of the patch size effect. Ecology 79:517–533.

Blackmore, C., and R. Heinsohn. 2008. Variable mating strategies and incest avoidance in cooperatively breeding Grey-crowned Babblers. Animal Behaviour 75:63–70.

Blackmore, C. J., R. Peakall, and R. Heinsohn. 2011. The absence of sex-biased dispersal in the cooperatively breeding Grey-crowned Babbler. Journal of Animal Ecology 80:69–78.

Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80:205–225.

Bradshaw, C. J. A. 2012. Little left to lose: deforestation and forest degradation in Australia since European colonization. Journal of Plant Ecology 5:109–120.

Broquet, T., et al. 2010. Genetic bottlenecks driven by population disconnection. Conservation Biology 24:1596–1605.

Catlin, D. H., et al. 2016. Metapopulation viability of an endangered shorebird depends on dispersal and human-created habitats: Piping Plovers (Charadrius melodus) and prairie rivers. Movement Ecology 4:6.

Chiucchi, J. E., and H. L. Gibbs. 2010. Similarity of contemporary and historical gene flow among highly fragmented populations of an endangered rat-tlesnake. Movement Ecology 19:5345–5358.

Ciofi, C., M. A. Beaumont, I. R. Swingland, and M. W. Bruford. 1999. Genetic divergence and units for conservation in the Komodo Dragon Varanus komodoensis. Proceedings of the Royal Society B 266: 2268–2274.

Clarke, R. H., R. L. Boulton, and M. F. Clarke. 2002. Translocation of the socially complex Black-eared Miner Manorina melanocephala: implications for habitat restoration. Wildlife Research 34:253–261.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Cousilnami, J. J., and B. King, 1977. Ageing and sexing the Grey-crowned Babbler (Pomatostomus temporalis). Bird Behavior 1:23–41.

Davidson, I., and D. Robinson. 2009. Conservation plan for the Grey-crowned Babbler population in the Boort-Loddon district. Department of Natural Resources and Environment, Melbourne, Victoria, Australia.

Department of Environment and Heritage. 2013. Grey-crowned Babbler (eastern subspecies): vulnerable species listing. New South Wales Government, Australia. http://www.environment.nsw.gov.au/determinations/GreycrownedBabplerVulSpListing.htm

Department of Land, Water and Environment Planning. 2017. Flora and Fauna Guarantee Act 1988; threatened species list July 2017. Victorian Government. https://www.environment.vic.gov.au/conserving-threatened-species/flora-and-fauna-guarantee-act-1988

Dutta, T., et al. 2013. Gene flow and demographic history of Leopards (Panthera pardus) in the central Indian highlands. Evolutionary Applications 6:949–959.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends in Genetics 16:551–558.

Environment Australia. 2011. Action plan for Australian birds 2010. Commonwealth Government of Australia, Canberra, ACT, Australia.

Environment Conservation Council. 2001. Box-Ironbark Forests and Woodlands Investigation: Final Report. Environmental Conservation Council, Victoria, Australia.

Fahrig, L. 2001. How much habitat is enough? Biological Conservation 100:65–74.
Fahrig, L. 2007. Non-optimal animal movement in human-altered landscapes. Functional Ecology 21: 1003–1015.
Faubet, P., R. Waples, and O. Gaggiotti. 2007. Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. Molecular Ecology 16:1149–1166.
FitzGibbon, S. I., D. A. Putland, and A. W. Goldizen. 2007. The importance of functional connectivity in the conservation of a ground-dwelling mammal in an urban Australian landscape. Landscape Ecology 22:1513–1525.
Frankham, R. 1995. Effective population-size/adult-population size ratios in wildlife: a review. Genetic Research 66:95–107.
Frankham, R. 2015. Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24:2610–2618.
Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170:56–63.
Frankham, R., et al. 2011. Predicting the probability of outbreeding depression. Conservational Biology 25:465–475.
Fuhlendorf, S. D., A. J. W. Woodward, D. M. Leslie, and J. S. Shackford. 2002. Multi-scale effects of habitat loss and fragmentation on Lesser Prairie-chicken populations of the US Southern Great Plains. Landscape Ecology 17:617–628.
Gillies, C. S., and C. C. St. Clair. 2008. Riparian corridors enhance movement of a forest specialist bird in fragmented tropical forest. Proceedings of the National Academy of Sciences of the United States of America 105:19774–19779.
Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A simple DNA test to sex most birds. Molecular Ecology 7:1071–1075.
Hanski, I. 1998. Metapopulation dynamics. Nature 396:41–49.
Hanski, I., T. Pakkala, M. Kuussaari, and G. C. Lei. 1995. Metapopulation persistence of an endangered butterfly in a fragmented landscape. Oikos 72:21–28.
Harrisson, K. A., et al. 2013. Disrupted fine-scale population processes in fragmented landscapes despite large-scale genetic connectivity for a widespread and common cooperative breeder: the Superb Fairy-wren (Malurus cyaneus). Journal of Animal Ecology 82:322–333.
Higgins, P. J., and J. M. Peter, editors. 2003. Handbook of Australian, New Zealand and Antarctic Birds volume 6: Pardalotes to Shrike-Thrushes. Oxford University Press, Melbourne, Victoria, Australia.
Hoffmann, A., et al. 2015. A framework for incorporating evolutionary genomics into biodiversity conservation and management. Climate Change Responses 2:1.
Jamieson, I. G., and F. W. Allendorf. 2012. How does the 50/500 rule apply to MVPs? Trends in Ecological Evolution 27:578–584.
Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of Song Sparrows (Melospiza melodia). Evolution 52:240–250.
Koenig, W. D., F. A. Pitelka, W. J. Carmen, R. L. Mumme, and M. T. Stanback. 1992. The evolution of delayed dispersal in cooperative breeders. Quarterly Review of Biology 67:111–150.
Kuhner, M. K. 2009. Coalescent genealogy samplers: windows into population history. Trends in Ecological Evolution 24:86–93.
Luikart, G., F. Allendorf, J. Cornuet, and W. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238–247.
McCuSker, M. R., N. E. Mandrak, B. Egeh, and N. R. Lovejoy. 2014. Population structure and conservation genetic assessment of the endangered Pugnose Shiner, Notropis anogenus. Conservation Genetics 15:343–353.
Meirmans, P. G. 2014. Nonconvergence in Bayesian estimation of migration rates. Molecular Ecological Resources 14:726–733.
Nimmo, D. G., A. Haslen, J. Q. Radford, M. Hall, and A. F. Bennett. 2016. Riparian tree cover enhances the resistance and stability of woodland bird communities during an extreme climatic event. Journal of Applied Ecology 53:449–458.
O’Grady, J. J., et al. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133:42–51.
Ortego, J., M. F. Aguirre, V. Noguerales, and P. J. Cordero. 2015. Consequences of extensive habitat fragmentation in landscape-level patterns of genetic diversity and structure in the Mediterranean Esparto Grasshopper. Evolutionary Applications 8:621–632.
Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13:55–65.
Pavlacky, D. C., et al. 2012. Anthropogenic landscape change promotes asymmetric dispersal and limits regional patch occupancy in a spatially structured bird population. Journal of Animal Ecology 81: 940–952.
Peery, M. Z., et al. 2012. Reliability of genetic bottleneck tests for detecting recent population declines. Molecular Ecology 21:3403–3418.

Piry, S., et al. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536–539.

Radford, J. Q., A. F. Bennett, and G. L. Cheers. 2005. Landscape-level thresholds of habitat cover for woodland-dependent birds. Biological Conservation 124:317–337.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America 94:9197–9201.

Robinson, D. 1993. Habitat requirements of a threatened grassy woodland bird, the Grey-crowned Babbler. National Estates Grant Program and Royal Australasian Ornithologists Union, Melbourne, Victoria, Australia.

Robinson, D. 2006. Is revegetation in the Sheep Pen Creek area, Victoria, improving Grey-crowned Babber habitat? Ecological Management and Restoration 7:93–104.

Saccheri, I., et al. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494.

Samarasin, P., B. J. Shuter, S. I. Wright, and F. H. Rodd. 2017. The problem of estimating recent genetic connectivity in a changing world. Conservation Biology 31:126–135.

Schlötterer, C. 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365–371.

Segelbacher, G., et al. 2014. Analyses of historical and current populations of Black Grouse in central Europe reveal strong effects of genetic drift and loss of genetic diversity. Conservation Genetics 15:1183–1195.

Stevens, K. P., K. A. Harrisson, R. H. Clarke, R. Cooke, and F. E. Hogan. 2016. Genetic structure and sex-biased dispersal of a declining cooperative-breeder, the Grey-crowned Babbler, Pomatostomus temporalis, at the southern edge of its range. Emu 116:323–332.

Stevens, K. P., G. J. Holland, R. H. Clarke, R. Cooke, and A. F. Bennett. 2015. What determines habitat quality for a declining woodland bird in a fragmented environment, the Grey-crowned Babbler Pomatostomus temporalis, in south-eastern Australia? PLoS ONE 10:e0130738.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation 87:173–180.

Sunnucks, P. 2011. Towards modelling persistence of woodland birds: the role of genetics. Emu 111:19–39.

Sullmon, D. A., et al. 2010. When are genetic methods useful for estimating contemporary abundance and detecting population trends? Molecular Ecology Resources 10:684–692.

Taylor, P. D., L. Fahrig, K. Henein, and G. Merriam. 1993. Connectivity is a vital element of landscape structure. Oikos 68:571–573.

Thomas, R. 2009. Regent Honeyeater habitat restoration project Lurg hills, Victoria. Ecological Management Restoration 10:84–97.

Tischendorf, L., and L. Fahrig. 2000. On the usage and measurement of landscape connectivity. Oikos 90:7–19.

Tzaros, C. 1995. Population monitoring of the Grey-crowned Babbler (Pomatostomus temporalis) in central Victoria. Department of Conservation and Natural Resources, Bendigo, Victoria, Australia.

Tzaros, C. 2001. Field surveys and population monitoring of the Grey-crowned Babbler Pomatostomus temporalis in the Loddon and Murray Valley regions, north-west Victoria. Department of Natural Resources and Environment, Melbourne, Victoria, Australia.

Uezu, A., J. P. Metzger, and J. M. E. Vielliard. 2005. Effects of structural and functional connectivity and patch size on the abundance of seven Atlantic forest bird species. Biological Conservation 123:507–519.

van der Ree, R., and A. F. Bennett. 2001. Woodland remnants along roadsides: A reflection of pre-European structure in temperate woodlands? Ecological Management Restoration 2:224–226.

Vergara, P. M., C. G. Perez-Hernandez, I. J. Hahn, and J. E. Jimenez. 2013. Matrix composition and corridor function for Austral Thrushes in a fragmented temperate forest. Landscape Ecology 28:121–133.

Vesk, P. A., et al. 2015. Demographic effects of habitat restoration for the Grey-crowned Babbler Pomatostomus temporalis, in Victoria, Australia. PLoS ONE 10:e0130153.

Villard, M.-A., M. K. Trzcinski, and G. Merriam. 1999. Fragmentation effects on forest birds: relative influence of woodland cover and configuration on landscape occupancy. Conservation Biology 13:774–783.

Volpe, N. L., A. S. Hadley, W. D. Robinson, and M. G. Betts. 2014. Functional connectivity experiments reflect routine movement behavior of a tropical hummingbird species. Ecological Applications 24:2122–2131.

Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.
Weeks, A. R., et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Application 4: 709–725.

Weeks, A. R., et al. 2015. Conserving and enhancing genetic diversity in translocation programmes. Pages 127–140 in D. Armstrong, A. M. Hayward, D. Moro, and P. Seddon, editors. Advances in reintroduction biology of Australian and New Zealand Fauna. CSIRO Publishing, Melbourne, Victoria, Australia.

Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to the rescue. Trends in Ecological Evolution 30:42–49.

Wilson, G. A., and B. Rannala. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191.

Wood, D. A., et al. 2017. A century of landscape disturbance and urbanization of the San Francisco Bay region affects the present-day genetic diversity of the California Ridgway’s Rail (*Rallus obsoletus obsoletus*). Conservation Genetics 18:131–146.