Do Major Roads Reduce Gene Flow in Urban Bird Populations?

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Abstract

Background: Although the negative effects of roads on the genetics of animal populations have been extensively reported, the question of whether roads reduce gene flow in volant, urban bird populations has so far not been addressed. In this study, we assess whether highways decreased gene flow and genetic variation in a small passerine bird, the tree sparrow (Passer montanus).

Methodology: We assessed genetic differences among tree sparrows (Passer montanus) sampled at 19 sites within Beijing Municipality, China, using 7 DNA microsatellites as genetic markers.

Results: AMOVA showed that genetic variation between sites, between urban and rural populations, and between opposite sides of the same highway, were very weak. Mantel tests on all samples, and on urban samples only, indicated that the age and number of highways, and the number of ordinary roads, were uncorrelated with genetic differences (FST) among tree sparrows from different urban sites. Birds sampled at urban sites had similar levels of genetic diversity to those at rural sites. There was, however, evidence of some weak genetic structure between urban sites. Firstly, there were significant genetic differences (FST) between birds from opposite sides of the same highway, but no significant FST values between those from sites that were not separated by highways. Secondly, birds from eleven urban sites had loci that significantly deviated from the Hardy–Weinberg equilibrium but no such deviation was found in birds from rural sites.

Conclusion: We cannot, therefore, conclusively reject the hypothesis that highways have no effect on the gene flow of tree sparrow populations. Furthermore, since the significance of these results may increase with time, we suggested that research on the influence of highways on gene flow in urban bird populations needs to be conducted over several decades.

Introduction

The ecological effects of roads have long been identified [1]. The most commonly reported impacts of roads on animal populations include habitat loss, edge effects, genetic isolation, road mortality and increased human access [1–5]. In recent decades, there has been growing interest in how roads act to reduce gene flow between animal populations [6–8]. Reduced dispersal can lead to the loss of genetic diversity through genetic drift [9], which in turn is likely to increase population extinction rates through inbreeding [10,11]. Negative genetic effects of roads on various species, ranging from crickets and ground beetles to amphibians and mammals, have been reported [12–15].

Previous research on the influence of roads on gene flow in animal populations has focused on flightless species [8], to date, no study has addressed the question of whether roads also affect gene flow in volant taxa such as passerine birds. Generally, wider roads with greater volumes of high-speed traffic have a greater effect on animal populations than smaller, less travelled roads [16,17]. Several studies have confirmed that highways play a role in the decline of bird populations in species such as sparrows and blackbirds [18–22]. There is also evidence that proximity to highways decreases the probability of occurrence of both forest and urban birds [23,24]. In light of these facts, there is growing concern that highways could reduce dispersal and gene flow in urban bird populations. In this study, we assess whether highways decreased gene flow and genetic variation in a small passerine bird, the tree sparrow (Passer montanus).

More specifically, we test the following predictions:

1. Genetic differences between tree sparrows at different sites are positively correlated with the number, or age, of multilane highways between sampling sites.
2. Genetic differences between samples from opposite sides of the same highway are greater than those between samples from the same side of a highway.
3. Urban tree sparrows display greater inter-site genetic differentiation and lower genetic diversity than rural tree sparrows.

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Methods

Study species

The tree sparrow is a relatively sedentary species that occurs in a variety of habitats throughout China [25,26]. Tree sparrows have relatively small home ranges with an estimated radius of 100 to 300 m [26,27]. In a previous study, we found that virtually no tree sparrows live alongside major roads in urban Beijing [24]. For these reasons, we chose tree sparrows to examine the effect of urban highways on the population genetics of a volant, but relatively sedentary, bird species.

Sample collection

We collected blood samples from tree sparrows at 14 sites in urban Beijing which has a dense road network with six concentric ring highways and ten intercity highways (Figure 1). We also collected samples from five rural sites outside Beijing. All samples were collected during May 2010; at this time of year juveniles have not yet fledged which reduced the possibility of catching close

Table 1. Microsatellite DNA loci used in the present study.

| Locus   | Reference         | Expected PCR product length (bp) | PCR annealing temperature (°C) |
|---------|-------------------|----------------------------------|-------------------------------|
| HiUS-A  | Primmer et al. 1995 | 141–180                          | 50 °C                         |
| Pdou3   | Griffith et al., 1999 | 109–153                          | 54 °C                         |
| Pdou5   | Griffith et al., 1999 | 210–268                          | 65 °C                         |
| Pdo10   | Griffith et al., 2007 | 113–147                          | 60 °C                         |
| Ase18   | Richardson et al. 2000 | 185–249                          | 54 °C                         |
| WBSW11  | McRae & Amos 1999 | 177–283                          | 55 °C                         |
| Fhu2    | Primmer et al. 1996b | 128–160                          | 58 °C                         |

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Table 2. Genetic variability at seven microsatellite loci in tree sparrows from 19 different sites in urban and rural Beijing, China.

| locus name, repeat motif | HrU5-A | Pdo3 | Fhu2 | Pdo10 | Ase18 | Pdo5 | WBSW11 | All |
|--------------------------|--------|------|------|-------|-------|------|--------|-----|
| **urban sites**          |        |      |      |       |       |      |        |     |
| A                        |        |      |      |       |       |      |        |     |
| n = 28                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.714  | 0.786| 0.607| 0.893 | 0.929 | 0.857| 0.836  | 0.803|
| H⁸                       | 0.809  | 0.869| 0.701| 0.860 | 0.948 | 0.929| 0.905  | 0.860|
| B                        |        |      |      |       |       |      |        |     |
| n = 28                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.733  | 0.733| 0.867| 0.600 | 1.000 | 0.933| 0.700* | 0.795|
| H⁸                       | 0.839  | 0.805| 0.690| 0.775 | 0.963 | 0.917| 0.929  | 0.845|
| C                        |        |      |      |       |       |      |        |     |
| n = 30                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.903  | 0.902| 0.767| 0.601 | 0.933 | 0.905| 0.907  | 0.845|
| H⁸                       | 0.844  | 0.825| 0.708| 0.785 | 0.955 | 0.904| 0.926  | 0.849|
| D                        |        |      |      |       |       |      |        |     |
| n = 24                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.708  | 0.875| 0.708| 0.625 | 0.958 | 0.917| 0.833  | 0.803|
| H⁸                       | 0.843  | 0.853| 0.711| 0.786 | 0.947 | 0.920| 0.948  | 0.858|
| E                        |        |      |      |       |       |      |        |     |
| n = 26                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.885  | 0.846| 0.846| 0.731 | 0.923 | 0.769*| 0.877  | 0.839|
| H⁸                       | 0.841  | 0.859| 0.698| 0.785 | 0.935 | 0.945| 0.937  | 0.850|
| F                        |        |      |      |       |       |      |        |     |
| n = 29                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.655* | 0.897| 0.655| 0.724 | 0.964 | 0.966| 0.759* | 0.802|
| H⁸                       | 0.851  | 0.839| 0.709| 0.843 | 0.950 | 0.936| 0.932  | 0.866|
| G                        |        |      |      |       |       |      |        |     |
| n = 25                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.867  | 0.800| 0.733| 0.600 | 0.933 | 0.933| 0.767  | 0.804|
| H⁸                       | 0.837  | 0.841| 0.747| 0.715 | 0.899 | 0.917| 0.890  | 0.835|
| J                        |        |      |      |       |       |      |        |     |
| n = 37                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.892  | 0.730| 0.622| 0.676 | 0.919 | 0.838| 0.814  | 0.784|
| H⁸                       | 0.855  | 0.863| 0.732| 0.801 | 0.946 | 0.918| 0.923  | 0.861|
| L                        |        |      |      |       |       |      |        |     |
| n = 29                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.724  | 0.793| 0.621| 0.862 | 0.857 | 0.929| 0.790  | 0.796|
| H⁸                       | 0.845  | 0.793| 0.730| 0.858 | 0.936 | 0.913| 0.884  | 0.851|
| M                        |        |      |      |       |       |      |        |     |
| n = 39                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.795  | 0.897| 0.769| 0.897 | 0.923 | 0.872| 0.790* | 0.849|
| H⁸                       | 0.841  | 0.848| 0.737| 0.867 | 0.941 | 0.900| 0.956  | 0.866|
| P                        |        |      |      |       |       |      |        |     |
| n = 28                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.857  | 0.571*| 0.821| 0.786 | 0.857 | 0.821| 0.736  | 0.778|
| H⁸                       | 0.832  | 0.849| 0.714| 0.823 | 0.946 | 0.904| 0.897  | 0.852|
| T                        |        |      |      |       |       |      |        |     |
| n = 26                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.692* | 0.846| 0.808| 0.885 | 0.923 | 0.885| 0.692* | 0.818|
| H⁸                       | 0.829  | 0.864| 0.768| 0.878 | 0.941 | 0.942| 0.864  | 0.869|
| Y                        |        |      |      |       |       |      |        |     |
| n = 35                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.800  | 1.000| 0.733| 0.600*| 0.867 | 0.933| 0.867  | 0.828|
| H⁸                       | 0.761  | 0.816| 0.800| 0.857 | 0.952 | 0.938| 0.943  | 0.866|
| Z                        |        |      |      |       |       |      |        |     |
| n = 33                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.758  | 0.758| 0.606| 0.697*| 0.879 | 0.939| 0.818  | 0.779|
| H⁸                       | 0.825  | 0.848| 0.724| 0.826 | 0.942 | 0.938| 0.930  | 0.861|
| **Rural sites**          |        |      |      |       |       |      |        |     |
| H                        |        |      |      |       |       |      |        |     |
| n = 35                   |        |      |      |       |       |      |        |     |
| H⁰                       | 0.942  | 0.809| 0.714| 0.771 | 0.885 | 0.857| 0.809  | 0.826|
| H⁸                       | 0.854  | 0.861| 0.738| 0.866 | 0.954 | 0.925| 0.948  | 0.878|
| K                        |        |      |      |       |       |      |        |     |
| n = 35                   |        |      |      |       |       |      |        |     |
| H⁰                       | 4.708  | 5.046| 3.586| 4.354 | 6.656 | 6.416| 6.123  | 5.270|

Gene Flow in Urban Bird Populations
Data Analysis

Population differentiation. Pairwise multilocus $F_{ST}$ values were used to estimate genetic differentiation between populations, $F_{ST}$ values were calculated in Arlequin 3.0. Three hierarchical analyses of molecular variance (AMOVA; [32]) using different grouping methods were conducted in Arlequin 3.0. Method A treated all the samples obtained from each site as a single group, Method B treated samples collected on the same side of the same highway as a group and Method C divided all samples into urban and rural groups. Three hierarchical levels were analyzed for all three Methods: 1) variance among groups, 2) variance among individuals within group, 3) variance within individuals. The significances of all $F$ statistics values were tested via 16000 permutations.

Mantel test

The correlation between $F_{ST}$, geographical distance, number of highways, highway age and number of roads between sample sites were tested using a Mantel test carried out in Fstat. Because urban highways have much more traffic than those in rural areas, highways would be expected to be a greater barrier to gene flow in urban than rural tree sparrow habitat. To determine if this was the case we performed two Mantel tests: one on all samples, and a second on urban samples only. By means of a permutation procedure, the significance of the partial regressions between the pairwise $F_{ST}$ values and the following four matrices were tested: geographical distance as measured on a map, number of highways, total age of highways in years and number of roads.

Genetic diversity within populations

The observed and expected heterozygosities were estimated for each locus from each sample site. Significant departures from the Hardy–Weinberg equilibrium were detected applying tests which were carried out with 1000000 steps in the Markov chain and 5000 dememorization steps [33]. A sequential Bonferroni correction for multiple tests [34] was applied to the data from each sample site. The linkage disequilibria between all pairs of loci were tested with a Likelihood-ratio test [35]. All the above statistical analyses were conducted in Arlequin 3.0. Allelic richness was calculated using HP-rare [36] which uses rarefaction to correct for sampling error.

Results

Genetic diversity

All seven microsatellite loci were polymorphic in all samples, with total allelic richness ranging between 4.972 and 5.586 (Table 2). Total observed heterozygosity varied from 0.778 to

| locus name, repeat motif | HrUS-A | Pdop3 | Fhu2 | Pdo10 | Ase18 | Pdop5 | WB5W11 | All |
|-------------------------|--------|-------|------|-------|-------|-------|--------|-----|
| n=29                    | H0     | 0.678 | 0.862| 0.758 | 0.724 | 0.983 | 0.896  | 0.851| 0.822|
|                         | Hf     | 0.794 | 0.854| 0.722 | 0.767 | 0.944 | 0.935  | 0.904| 0.845|
| n=54                    | H0     | 0.870 | 0.824| 0.716 | 0.701 | 0.888 | 0.981  | 0.832| 0.830|
|                         | Hf     | 0.871 | 0.851| 0.734 | 0.751 | 0.937 | 0.924  | 0.936| 0.857|
| W                      | A      | 5.116 | 5.082| 3.693 | 4.876 | 6.607 | 6.282  | 6.573| 5.461|
| n=35                   | H0     | 0.812 | 0.765| 0.763 | 0.706 | 0.835 | 0.860  | 0.809| 0.792|
|                         | Hf     | 0.861 | 0.883| 0.798 | 0.825 | 0.904 | 0.942  | 0.927| 0.877|
| O                      | A      | 5.057 | 5.079| 3.549 | 4.527 | 6.624 | 6.216  | 6.483| 5.362|
| n=32                   | H0     | 0.798 | 0.871| 0.758 | 0.764 | 0.905 | 0.853  | 0.801| 0.821|
|                         | Hf     | 0.894 | 0.854| 0.722 | 0.758 | 0.931 | 0.916  | 0.904| 0.854|

*Heterozygosity values significantly different from those expected under the Hardy–Weinberg equilibrium (P<0.05).
0.849, and the total expected heterozygosity varied from 0.835 to 0.877 (Table 2). There was no significant difference in genetic diversity between urban and rural tree sparrows (Mann-Whitney U test, A: \( P = 0.459 \), Ho: \( P = 0.622 \), He: \( P = 0.559 \)). However, eleven of the 14 urban sites had heterozygosity values significantly different from those expected under Hardy–Weinberg equilibrium \( (P<0.05) \) (Table 2). No such departure from the Hardy–Weinberg equilibrium was found in samples from the rural sites (Table 2).

### Population differentiation

In 171 pairwise tests for genetic differentiation between sample sites, 17 (9.9%) were significant at the \( P<0.05 \) level, 15 (8.7%)

| Grouping method | Source of variation | d.f. | Sum of squares | Variance component | Percentage of variation | F statistics | \( P \) |
|-----------------|---------------------|------|----------------|---------------------|-------------------------|-------------|-----|
| A               | among groups        | 18   | 88.642         | 0.016               | 0.46%                  | 0.005(\( F_{GT} \)) | 0.013 |
|                 | within individuals  | 583  | 2339.103       | 0.591               | 17.21%                 | 0.173(\( F_{IG} \)) | 0.016 |
|                 |                     | 602  | 1703.219       | 2.829               | 82.33%                 | 0.177(\( F_{IT} \)) | 0.017 |
| B               | among groups        | 1    | 6427           | 0.007               | 0.15%                  | 0.002(\( F_{GT} \)) | 0.031 |
|                 | within individuals  | 600  | 2422.217       | 0.604               | 17.51%                 | 0.175(\( F_{IG} \)) | 0.015 |
|                 |                     | 602  | 1703.219       | 2.829               | 82.34%                 | 0.177(\( F_{IT} \)) | 0.018 |
| C               | among groups        | 9    | 47.591         | 0.005               | 0.14%                  | 0.001(\( F_{GT} \)) | 0.012 |
|                 | within individuals  | 592  | 2386.812       | 0.612               | 17.78%                 | 0.178(\( F_{IG} \)) | 0.011 |
|                 |                     | 602  | 1703.219       | 2.829               | 82.08%                 | 0.179(\( F_{IT} \)) | 0.016 |

*\( P<0.05; \) **\( P<0.01; \) ***\( P<0.001; \)

n.s.: no significant difference; The \( F_{st} \) and significance of paired sites with no highway between them are shown in bold.
were significant at the P<0.01 level and 3 (1.8%) were significant at the P<0.001 level (Table 3). Therefore, a total of 20% of paired sites had significant genetic differences. All significant genetic differences occurred between birds from sites on opposite sides of the same highway. There were no significant genetic differences between birds from sites with no highway between them. AMOVA revealed that most (99%) of the variance was explained by within-the-same highway. There were no significant genetic differences occurred between birds from sites on opposite sides of the same highway. This suggests that highways may be responsible for a weak genetic structure in urban tree sparrow populations. Furthermore, although we did not find any significant difference in the genetic diversity of urban and rural tree sparrow populations, we did find significant deviation from the Hardy–Weinberg equilibrium in some urban tree sparrow loci but no such deviation in birds from rural sites. This suggests that urban tree sparrows may be more inbred than those at rural sites. Meanwhile, the Mantel test returned lower P values and higher determination values when applied to urban samples than when applied to all samples, which suggests that highways have a greater effect on gene flow between urban than rural sites. These results suggest that highways, and especially urban highways, have a weak, but detectable, effect on gene flow in tree sparrow populations in Beijing.

There are several possible explanations for these results. The first is that tree sparrows can fly over roads, including highways. The second is population density. Gauffre et al. [37] demonstrated that genetic barrier effects are difficult to detect in species with large effective population sizes. The tree sparrow’s high population density in parks, university campuses and suburban areas of Beijing [38] could, therefore, have reduced the likelihood of detecting genetic differences between sample sites. The third is temporal scale. The effects of genetic isolation typically develop over long periods of time [39,40], but the oldest highway in this study was constructed in 1992 and had been in use for just 20 years. Because the tree sparrow is a relatively long-lived species [41], relatively few generations would have passed since these roads were built. We think it likely that Beijing’s highways are too recent to have produced marked genetic differentiation in resident bird populations. In addition, highways with heavy traffic might have a bigger influence on the dispersal of birds than those with light traffic. High traffic volume is a relatively recent phenomenon in China where only 30 years ago private vehicles were rare compared to European or American cities of similar size. Therefore, Beijing’s road network would be expected to have had less impact on gene flow in urban bird populations than the much older road systems in North America and Europe. All the above reasons could explain why we only detected relatively weak genetic effects.

Therefore, we cannot conclusively reject the hypothesis that highways do not restrict gene flow in urban tree sparrow populations. A time series analysis should be done to test whether the effects of highways on the genetic structure of Beijing’s tree sparrow population will increase with time. Future research on the influence of highways on gene flow in bird populations will need to T

Table 5. Partial regression coefficients (bYj) between FST values and geographical distance, age of highways, number of roads and number of highways respectively, P values for bYj, and determination coefficients.

|                        | samples   | bYj     | P       | Determination (%) |
|------------------------|-----------|---------|---------|-------------------|
| geographical distance  | all samples | 8×10^-6 | 0.458   | 0.000             |
|                        | urban samples | 8×10^-5 | 0.352   | 0.35              |
| age of highways        | all samples | 3.5×10^-5 | 0.601   | 0.001             |
|                        | urban samples | 5×10^-6 | 0.492   | 0.002             |
| number of roads        | all samples | 1.8×10^-5 | 0.551   | 0.000             |
|                        | urban samples | 1×10^-6 | 0.491   | 0.000             |
| number of highways     | all samples | 1.8×10^-4 | 0.302   | 0.007             |
|                        | urban samples | 8.7×10^-4 | 0.097   | 4.98              |
| % of variance explained by the model | all samples | 0.008 |         |                   |
|                        | urban samples | 5.132 |         |                   |
be conducted over several decades to obtain more conclusive results.

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