Genetic Composition of Communal Roosts of the Eurasian Magpie (*Pica pica*) Inferred from Non-Invasive Samples

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Genetic Composition of Communal Roosts of the Eurasian Magpie (\textit{Pica pica}) Inferred from Non-invasive Samples

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Many animal species form communal roosts in which they aggregate and sleep together. Several benefits of communal roost have been suggested, but due to lack of data on relatedness among group members, it is unknown whether these benefits can be amplified by the formation of kin-based communal roosts. We investigate the genetic composition of two winter roosts of Eurasian Magpies (\textit{Pica pica}), using microsatellite markers on non-invasive samples. Using permutation tests by reshuffling the alleles presented in the roosts, we determined that individuals in the communal roosts of magpies were not more related than expected by chance, suggesting that kinship may not be a driving force for the formation of communal roosts in magpies. However, the pairwise relatedness and estimated relationship based on a maximum likelihood approach revealed that the roosts involve both kin and non-kin. Relatedness coefficients varied widely within a roost, indicating that family subgroups form a small proportion of the total number of birds in a roost. Our results suggest that ecological benefits of communal roost in animals are sufficient for the evolution of communal roosts without any involvement of kinship.

**Key words:** communal roosting, genetic relatedness, microsatellite, non-invasive sampling, \textit{Pica pica}

**INTRODUCTION**

Communal roosting is the phenomenon in which large numbers of individuals aggregate and sleep together, and is commonly found in mammals (e.g. Lewis, 1995; Anderson, 1998) and birds (reviewed in Beauchamp, 1999). However, the function and evolution of communal roosting are not clearly understood, particularly in birds, although several hypotheses on its benefits have been suggested, such as thermoregulation (e.g. McGowan et al., 2006), predation avoidance by dilution effect (Gadgil, 1972; Bertram, 1978; see Eiserer, 1984) or by vigilance effect (Lendrem, 1983, 1984; Dominguez, 2003), enhanced food availability by information sharing (Ward and Zahavi, 1973; Rabenold, 1983; Bosakowski, 1984; Marzluff et al., 1996), and monitoring the mating status of potential mates (Møller, 1985; Blanco and Tella, 1999).

However, being in a group may also incur costs, such as increased competition for food (Janson and van Schaik, 1988; Isbell, 1991), disease transmission (Hoogland, 1979; Brown and Brown, 1986), and predation risk for the individuals at the periphery of the group (Balmford and Turyaho, 1992; Lingle, 2001). The balance between the costs and benefits of group roosting is expected to be affected by the degree of relatedness between roosting individuals (Parker et al., 1995). For example, if a roost is composed of kin, any ecological benefits gained by communal roosting may be amplified by inclusive fitness that originates from genetic relatedness among the members. Conversely, if the function of a roost is to provide an arena in which to choose or to switch mates (Møller, 1985), low genetic relatedness in a roost would minimize inbreeding (Burland et al., 2001). In previous studies of two bird species, genetic analyses helped enhance the understanding of the adaptive mechanisms responsible for communal roosting; a study on the black vulture concluded that sharing information at communal roosts is shaped by kin selection (Rabenold, 1983, 1987), while similar information-sharing in roosts of the common raven was explained without invoking kin selection (Parker et al., 1994).

Despite the importance, direct measurement of genetic relatedness among roost members has been rare (Rabenold, 1983, 1987; Parker et al., 1994, 1995), perhaps because genetic sampling requires capturing individuals, which may dramatically disturb the composition and structure of the roosting group (Mallet et al., 1987). Non-invasive genetic sampling (reviewed in Waits et al., 2005) has provided methods for assessing the genetic composition of roosting groups without any disturbance. To date, there has been one paper in which non-invasive sampling was used to assess roosting behavior, without genetic analysis on the

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relationships among roosting members (Rudnick et al., 2008).

In the present study, we investigated the genetic relationship between individuals in wintering roosts of the Eurasian magpie (Pica pica). Our study represents the first report to apply a non-invasive method to investigate the genetic composition of communal roosts. We discuss the contribution of our findings to understanding of roosting behavior of animals, based on the hypotheses proposed in earlier studies.

**MATERIALS AND METHODS**

**Location of roosts and faecal sampling**

Faecal samples were collected from two winter roosts in Seoul, Korea; Hye-Hwa (HH) and Sun-Reung (SR) in December 2006 and January 2007, respectively. At both sites, magpies roost in trees along a sidewalk. We sampled at one side of the roost trees, and the number of individuals at roost branches on our sample sites was approximately 20–30 for HH and 10–20 for SR. We placed a layer of aluminum foil under the roost branches. As soon as individuals defecated, the portion of aluminum foil containing the fresh feces was cut out and put into a sterile 15 mL plastic tube. The fecal collection was carefully conducted to minimize contamination of samples and maximize DNA preservation; all steps were performed wearing sterile gloves and samples were frozen within one hour of collection and stored in –20°C until DNA extraction. A total of 18 and 11 samples were collected from HH and SR, respectively. Video recording of roosting birds indicated that the defecation interval of each individual was more than two hours (our unpublished data), so we sampled approximately for one hour at each site to minimize the possibility of sampling of the same individual multiple times.

**DNA extraction and PCR**

Genomic DNA was extracted from faecal samples with QIAamp Stool Kit (QIAGEN) with modifications suggested by Regnaut et al. (2006). Polymerase chain reaction (PCR) was performed for five microsatellite loci: Ppi1, Ppi2, Ppi3, Ppi4 and Ase18 (Table 1). PCR was performed with 3 μl DNA extract, 1.1 μl of 2 mM dNTPs, 1.1 μl of 10× buffer, 0.3 μl of 50 mM MgCl₂, 0.5 μl of 10× BSA, 0.1 μl of each 2 mM primer, 0.5 μl of IRDye® infrared dye (LI-COR, Lincoln, NE) and 0.4 μl Hot Start Taq Polymerase (Cosmo Genetech, Seoul, Korea) and adjusted to a total volume of 11 μl with sterilized, filtered water. Thermal protocol for PCR was as follows; initial denaturation for 3 min at 90°C; 10 cycles of 30 sec at 90°C, 45 sec at 53°C decreasing by 1.0°C each cycle and 60 sec at 72°C; 40 cycles of 30 sec at 94°C, 45 sec at 45°C, 60 sec at 72°C; and final extension for 7 min at 72°C. We repeated PCR more than five times per sample per locus to reduce the risk of null alleles or scoring errors.

**Scoring and statistical analyses**

PCR products were visualized with DNA Analyser Model 4300 and scored with Saga® Software (LI-COR). Eight samples in HH and one sample in SR were not used in further analyses due to poor quality of extracted DNA. Ten individuals from each roost group (HH and SR) were used in the analyses. The software Cervus 3.0 was used to test whether five loci was sufficient to obtain accurate relatedness values in each group. Based on the assumption that closely related individuals are more likely to produce homozygous offspring (Mathieu et al., 1990), the null hypothesis of unrelatedness, as expected in a random sample from a panmictic population, is rejected when the observed relatedness value is outside the 95% confidence interval for the expected distribution.

Using the ML-relate program (Kalinowski et al., 2006), we tested for heterozygote deficiency using the Hardy-Weinberg test based on 1,000 Monte Carlo re-sampling replicates (Guo and Thompson, 1992; Rousset and Raymond, 1995). In genetic studies based on microsatellite markers, the datasets are prone to involve null alleles by genotyping error (Dakin and Avise, 2004), particularly in those obtained from non-invasive samples (Gagneux et al., using the ML-relate program (Kalinowski et al., 2006), we tested for heterozygote deficiency using the Hardy-Weinberg test based on 1,000 Monte Carlo re-sampling replicates (Guo and Thompson, 1992; Rousset and Raymond, 1995). In genetic studies based on microsatellite markers, the datasets are prone to involve null alleles by genotyping error (Dakin and Avise, 2004), particularly in those obtained from non-invasive samples (Gagneux et al.,

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele size (bp)</th>
<th>No. of alleles</th>
<th>Primer sequence</th>
<th>Locus references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ase18</td>
<td>210–250</td>
<td>7</td>
<td>T: ATTCAGTCTTCGCAAAAGCC R: TGCCCCAGAGGGAGAGGAAAG</td>
<td>Richardson et al., 2000</td>
</tr>
<tr>
<td>Ppi1</td>
<td>260–290</td>
<td>5</td>
<td>T: TTACCATCATCCGAGCTC R: GGAAAAGGCTCAGAATGATTTTTC</td>
<td>Martinez et al., 1999</td>
</tr>
<tr>
<td>Ppi2</td>
<td>260–290</td>
<td>7</td>
<td>T: CAGAGGAGGAGGAGGAGAG</td>
<td>Martinez et al., 1999</td>
</tr>
<tr>
<td>Ppi3</td>
<td>240–290</td>
<td>7</td>
<td>T: CCAAAACAGAATCAGGTGCA R: TTTGTGCGGGAGGAGGAGG</td>
<td>Martinez et al., 1999</td>
</tr>
<tr>
<td>Ppi4</td>
<td>160–180</td>
<td>6</td>
<td>T: AAATCAAGGTACCAAGCCACC</td>
<td>Martinez et al., unpublished data</td>
</tr>
</tbody>
</table>

**Fig. 1.** Permutation test (1,000 replicates) of two roosting groups, Hye-Hwa (HH (A)) and Sun-Reung (SR (B)) based on mean identity value (Ixy). The arrows indicate the location of observed relatedness values in each group.
In our dataset, null alleles were detected in two loci (Ppi3 and Ppi4) in HH and in four loci (Ase18, Ppi1, Ppi2 and Ppi3) in SR. Thus, in ML-relate, we accommodated the presence of null alleles in the estimations of pairwise relatedness and relationship among individuals within each group (Kalinowski et al., 2006). ML-relate was also used to estimate pairwise relatedness ($ML_{xy}$), which is calculated by Wright's (1922) coefficient of relatedness. Based on a maximum likelihood approach, the most likely relationship among four categories — parent–offspring (PO), full-sib (FS), half-sib (HS), or unrelated (U) — was estimated. Using likelihood ratio test, we detected the presence of kin pairs, whose putative kin relationship had significantly lower log-likelihood than alternative non-kin relationship (i.e. unrelated).

### RESULTS

The number and size range of alleles varied among the two roost groups (Table 1). From the five loci we used, the combined non-exclusion probability for identity was $6 \times 10^{-6}$ in HH and $9 \times 10^{-5}$ in SR, indicating that these five loci were sufficient to achieve a low probability of identity among the individuals.

The observed values of pairwise identity indices, $I_{xy}$, at the two roosts were within 67.9% of expected $I_{xy}$ distribution in HH ($I_{xy} = 0.3004$, expected distribution ranged from 0.2696 to 0.3637) and 21.8% in SR ($I_{xy} = 0.2723$, expected distribution ranged from 0.2579 to 0.3158) as shown in Fig. 1. As the observed values of $I_{xy}$ were within the range of the permuted values, average pairwise relatedness among roosting members in both HH and SR was not significantly different from random. This means that individuals in the groups were distantly related.

On the other hand, when using absolute genetic relatedness to look at pairwise relationships in the two groups, some pairs were closely related, corresponding to relationships of full-sibling or parent-offspring (Table 2). In the two groups, five pairs (one in HH and four in SR) were estimated to be related to the level of parent-offspring or full-siblings, although the relationship between two pairs in SR (Ind 2 & 7 and 2 & 8) could not be distinguished from being unrelated (Table 2). Moreover, the coefficients of variation of $ML_{xy}$ were large in both roosting groups (1.56 in HH and 1.76 in SR), indicating the degree of relatedness among the individuals varies within a roost. These results indicate that the level of relatedness among the roost members is generally low, containing small portion of family subgroups at most.

### DISCUSSION

Our results suggest that communal roosts in magpies may be composed of kin and non-kin subgroups; average pairwise relatedness in the roosts was not higher than random, but estimates of absolute genetic relatedness detected the presence of close relatives in the roosts.

Permutation tests showed that the magpie roosts were not significantly different from a random aggregation of individuals. This would imply that simple social benefits from aggregating, such as mating or sharing information on food sources, could be sufficient for the formation of communal roosts even in the absence of enhanced benefits of inclusive fitness. The same situation was found in the common raven, where it was found that roost members were not closely related (Parker et al., 1994). Roost members may share information on the food source, or at least “scrounge” what others are eating (Barta and Giraldeau, 2001), during the winter season when the food availability is low. Communal roosts may also serve as a place where individuals can find a mate or monitor the breeding status of neighboring pairs (Møller, 1985; Blanco and Telia, 1999). In this case, roost members may reduce the risk of inbreeding if several kin and non-kin groups aggregated by random chance as shown in our results. Although the results suggest that winter roosts of Eurasian magpies mainly consist of non-kin subgroups, a small portion of kin subgroups has been detected. Hence, our findings do not exclude the possibility of altruistic interactions among the kin members to increase inclusive fitness (Hamilton, 1964). Roosting in kin-groups could also be an extension of parental care. For instance, magpie fledglings followed their parents to the roost and siblings usually remained together (Buitron, 1988). Parents might allow them to delay dispersal and form roost groups together if juveniles encounter harsh environment with thermoregulatory challenge and high predation risk. Future studies with enlarged dataset and with detailed observations of movement patterns and pair formation among the roost members, may better reveal the ecological function of the communal roosts in animals.

### ACKNOWLEDGMENTS

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**Table 2.** Average pairwise relatedness coefficients by ML-relate ($ML_{xy}$ ± standard error) in two roosting groups, the maximum likelihood relationship and the log likelihood of the relationship between the individuals, and the alternative relationships and the log likelihood difference between the maximum likelihood and the likelihood of the alternative relationship in the roosting groups (10 individuals in each group; PO: parent-offspring, FS: full sibling, HS: half-sibling, U: unrelated relationships; The alternative relationships can be rejected when the p-value was low, $P < 0.05$; $P < 0.005^*$).

<table>
<thead>
<tr>
<th>Roosting group</th>
<th>Focal pair</th>
<th>Relationship</th>
<th>Maximum likelihood</th>
<th>Alternative relationship</th>
<th>$ML_{xy}$ (± SE)</th>
<th>$I_{xy}$</th>
<th>$I_{xy}$</th>
<th>$I_{xy}$</th>
<th>$I_{xy}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>0.107 (± 0.025)</td>
<td>Ind 5 &amp; 9</td>
<td>PO</td>
<td>FS</td>
<td>0.09</td>
<td>11.00</td>
<td>0.09</td>
<td>11.00</td>
<td>0.09</td>
</tr>
<tr>
<td>SR</td>
<td>0.125 (± 0.033)</td>
<td>Ind 2 &amp; 7</td>
<td>PO</td>
<td>FS</td>
<td>0.87</td>
<td>-10.96</td>
<td>0.52</td>
<td>-10.96</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FS</td>
<td>0.09</td>
<td>11.00</td>
<td>0.09</td>
<td>11.00</td>
<td>0.09</td>
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<td></td>
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<td></td>
<td>U</td>
<td>0.09</td>
<td>11.00</td>
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<td>11.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>FS</td>
<td>0.22</td>
<td>-10.96</td>
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<td></td>
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<td></td>
<td></td>
<td>U</td>
<td>0.46</td>
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<td></td>
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<td>FS</td>
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<td>U</td>
<td>1.39</td>
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<td></td>
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<td>FS</td>
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<td>U</td>
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</tbody>
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