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Patch Size and Composition of Social Groups in Wintering Tufted Titmice

ELENA V. PRAVOSUDOVA,¹ THOMAS C. GRUBB, JR., PATRICIA G. PARKER, AND PAUL F. DOHERTY, JR.
Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 1735 Neil Avenue,
Columbus, Ohio 43210, USA

Formation of small, non-kin flocks in winter is typical of many resident passerines of the temperate zone (e.g. Ekman 1989). The adaptive significance of such aggregations is usually explained in terms of better predator detection and improved foraging success for flock members (Thorpe 1963, Pulliam 1973). If kin-based aggregations in winter are formed by means of delayed dispersal of young, additional benefits may arise for both adult and first-year group members (Emlen 1997).

Ekman and Rosander (1992) suggested that parental control of natal dispersal could be the main factor determining the size and composition of winter groups. According to their model, in the face of limited resources, a territorial mated pair will increase their level of aggression toward non-kin flock members, forcing them to leave. By contrast, subordinate kin (e.g. offspring) will be tolerated to a greater extent by the territorial pair as long as resources are sufficient. When adults cannot "afford" to share food even with their own offspring, they will exclude such offspring from the flock as well (see Ekman et al. 1994). This model has been supported by studies of Siberian Jays (*Perisoreus infaustus*; Ekman et al. 1994, 1996) and Gray Jays (*Perisoreus canadensis*; Waite and Strickland 1997).

The Tufted Titmouse (*Baeolophus bicolor*) is one of few non-corvid, permanent-resident, temperate-zone passerines in which offspring are known to spend the winter with their parents (Grubb and Pravosudov 1994). Because cohesive winter units of titmice may include up to eight members (Nice 1930), which may or may not be related, it remains unclear how often offspring are retained in this species and what factors determine the size and composition of winter groups. In our study sites within the agricultural landscape of central Ohio, titmice are permanent residents of forest fragments and often form small wintering groups of three or four birds. We predicted from Ekman and Rosander's (1992) model for offspring retention that under conditions of low resources, a wintering pair of adult titmice would be more likely to tolerate an offspring than a non-offspring flock member. When a pair of titmice is the only territorial pair in a very small woodlot, which presumably affords low levels of food resources, we expected that any juvenile in the same woodlot

would be an offspring of the resident pair. We tested this prediction using multilocus minisatellite DNA fingerprinting (Jeffreys et al. 1985a, b) to estimate levels of relatedness among members of triads of titmice wintering in small forest fragments.

Methods.—For this study, we selected fragments sufficiently small in area that they contained only one winter group of three titmice. Thirty-six titmice (24 adults and 12 first-year birds) were captured during the winters of 1995–1996 and 1996–1997 in 12 forest fragments in Crawford and Union counties, Ohio. The woodlots ranged in size from 2 to 10 ha and consisted primarily of oaks (*Quercus* spp.), ashes (*Fraxinus* spp.), shagbark hickory (*Carya ovata*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*). Each fragment was completely isolated from other woodlots by cultivated fields and contained only three titmice in one social group. The titmice apparently were confined to these fragments because we never saw them leave their respective woodlots. To maintain independence, we studied each woodlot and each social group during only one of the two winters.

We captured the birds early in winter using feeders filled with sunflower seeds and surrounded by mist nets. To keep to a minimum any effects of supplementary food on group composition, we kept feeders in woodlots only for the period of time necessary to capture titmice. Each titmouse was banded and individually marked with colored streamers attached to both legs. Age (first year or adult) was determined by skull pneumatization and plumage. A 50- μ L blood sample was taken from the brachial vein, shaken with 500 μ L of lysis buffer (100 mM Tris, pH = 8.0; 100 mM EDTA; 10 mM NaCl; 5% SDS; Longmire et al. 1988), and stored at ambient temperature.

We used multilocus minisatellite DNA fingerprinting to determine relatedness among members of each social group. Prior to extraction, 250 μ g of proteinase K were added to each sample, which was then incubated at 65°C overnight. Subsequently, two extractions with phenol, two extractions with 25:24:1 phenol:chloroform:isoamyl alcohol, and one extraction with 24:1 chloroform:isoamyl alcohol were performed. Following the last extraction, the aqueous phase was dialyzed extensively against TNE₂ (10 mM Tris, pH = 7.4; 10 mM NaCl; 2 mM EDTA) for 4 to 6 h. Two μ g of DNA from each individual were digested with 7.5 \times excess restriction enzyme *Hae*III

¹ E-mail: pravosudova.1@osu.edu

at 37°C for 4 h. The resulting fragments were separated through a 0.8% agarose gel at 20V for 65 h (until all fragments smaller than 1,600 base pairs had run off the gel) and were then transferred to nylon by Southern blot (Southern 1975) in 10× SSC buffer and fixed to the membrane by UV crosslinking. Jeffreys' multilocus minisatellite probe 33.15 (Jeffreys 1985a, b) was radiolabeled by primer extension. Hybridizations were run overnight, after which hybridized filters were washed at 62°C in 1.5× SSC, 0.1% SDS, and exposed to x-ray film at -20°C for several days.

Samples from birds living in the same woodland fragment were positioned next to each other on a gel. Pairs of lanes on the resulting autoradiograph were compared to examine the degree of band sharing between individuals. The band-sharing coefficient (x) reflects the genetic similarity between two individuals under comparison (Wetton et al. 1987). We calculated band sharing based on the proportion of bands in a dyad of lanes as $x = 2S / (2S + A + B)$, where S = the number of fragments of indistinguishable mobility and intensity in the two lanes under comparison, A = the number of bands unique to the first member of the dyad, and B = the number of bands unique to the second member of the dyad. On average, we scored $18.58 \pm \text{SD of } 4.05$ bands per lane; band-sharing values ranged from 0.11 to 0.72.

To determine if the first-year bird in a group was an offspring of the territorial adults, we used an independent set of band-sharing values derived from nestlings and parental adults from suburban Columbus, Ohio. In this second data set, seven families were sampled during the springs of 1996 and 1997, and frequency distributions of band sharing were created based on the known band-sharing coefficients between confirmed first-order relatives and presumed unrelated individuals (e.g. mated pairs attending nests). The two distributions overlapped at about $x = 0.5$. The lower value for the 95% confidence interval of the distribution for first-order relatives was then assigned as a threshold (0.45) so that if a band-sharing coefficient between two birds of unknown relatedness fell above it, those two individuals were considered to be first-order relatives. Similarly, birds with band-sharing values less than 0.45 were conservatively considered to be "unrelated." In all cases where a young bird was highly related to territorial adults ($x > 0.45$), the number of novel bands in its profile was counted to confirm the presumed parentage. The number of novel bands in all such cases ranged from zero to two ($\bar{x} = 1.20 \pm 0.84$, $n = 5$), a range of values attributable to random mutations (Rabenold et al. 1990, Haydock et al. 1996). Using the mean proportion of bands shared between presumably unrelated individuals ($x = 0.28$), we calculated the probability of mistakenly assigning an unrelated bird as a parent (Rabenold et al. 1991) to be 1.2×10^{-4} . By contrast, in cases where band-sharing

coefficients between a first-year bird and territorial adults fell below the threshold value ($x < 0.45$), the number of novel bands in its profile was high ($\bar{x} = 11.86 \pm 2.19$, $n = 7$), confirming our assumption that each such young was unrelated to the adults in its group. Because in all such cases band-sharing values between an adult female and a juvenile ($\bar{x} = 0.27 \pm 0.05$, $n = 7$) and between an adult male and a juvenile ($\bar{x} = 0.23 \pm 0.07$, $n = 7$) were equally low, extrapair paternity need not be considered a confounding factor in this analysis. Although these results do not rule out the possibility of intraspecific brood parasitism, we have found no evidence that this phenomenon occurs naturally in parids (see Kempenaers et al. 1995).

We used logistic regression to determine if a relationship existed between the presence/absence of an offspring in a group of three titmice and the size of the forest fragment where the group resided.

Results.—In a plot of pairwise band-sharing coefficients (Fig. 1), each nuclear family group had two points above the band-sharing threshold (comparisons between each adult and the young) and one point below the line (comparison between members of the mated pair). In contrast, in groups where the young was not related to either adult, all three points fell below the band-sharing threshold. Contrary to our prediction, only 5 of 12 triads occupying these small forest fragments were family groups. Furthermore, pairs of adults and their first-year offspring occupied somewhat larger fragments (4.5 to 10.0 ha), whereas in smaller fragments (2.5 to 5.9 ha) all three birds were unrelated. Logistic regression analysis confirmed this relationship between adult/juvenile relatedness and woodlot size ($P = 0.003$, $n = 12$; Fig. 2).

Discussion.—That the juvenile member of a triad in larger woodlots was related to the two adults supported our prediction from Ekman and Rosander's model. At the same time, according to the model, one might expect to find only two individuals, the territorial adults, in even smaller fragments if food there is unlikely to be shareable. Instead, smaller fragments (<5.9 ha) contained three unrelated titmice, two adults and one first-year bird. Because the data were collected during two winter seasons, a possibility exists that the results were confounded by the difference between years. However, even when we used only the data from 1995–1996 (10 woodlots in 1995–1996 and 2 in 1996–1997), our results remained significant, confirming that the presence of a related young was associated with larger woodlots ($P = 0.007$, $n = 10$).

Several reasons seem possible for the disparity between our prediction from the model and our results. First, in very small woodlots such as those we studied, young titmice may tend to disperse voluntarily rather than remain with their parents. Dispersing young might then be replaced by non-kin young

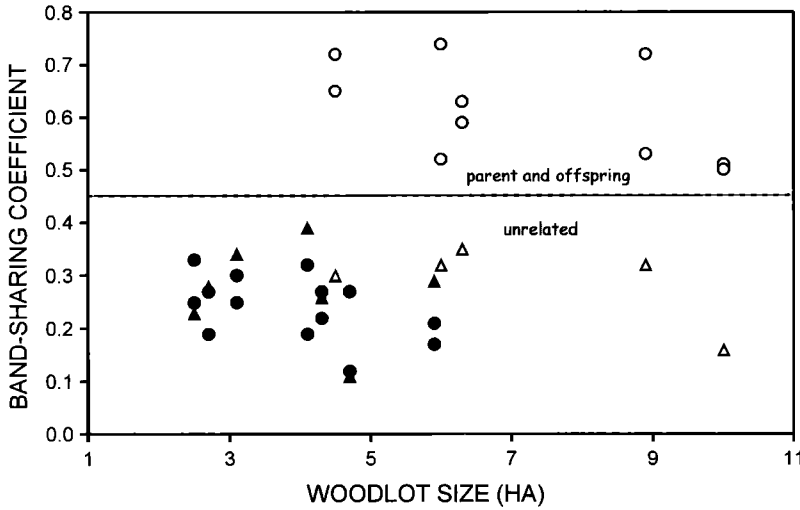


FIG. 1. Pairwise band-sharing coefficients among triads of Tufted Titmice in woodland patches of different size. The dashed line indicates the threshold value of band-sharing for first-order relatives. Closed symbols denote band-sharing coefficients within groups of three unrelated individuals, and open symbols denote band-sharing coefficients within family groups consisting of a male, a female, and one of their offspring. Circles represent band-sharing between each adult and the juvenile in a group; triangles represent band-sharing between adults.

from other patches in the landscape. The territorial pair may tolerate such strange first-year birds to gain the advantages of a larger flock size.

A second possible reason concerns the willingness of parents to share very scarce resources. In the smaller woodlots, parents may have expelled their own offspring rather than share the food supply. The unrelated juveniles, then, could have immigrated to such small woodlots and persisted there even in the face of substantial aggression from the resident adults monopolizing the food (J. Ekman pers. comm.).

A third possible explanation concerns differential reproductive success. Because our study was carried out only during the winter, we do not know whether the adults inhabiting the smallest woodlots had reproduced successfully. In addition to low food availability, tiny fragments may have high nest predation and parasitism due to pronounced edge effects (Lynch and Whigham 1984, Lynch 1987, Rolstad 1991, Andr en 1992). Thus, it remains possible that birds in the smallest woodlots may have been joined by a strange first-year bird after failing to reproduce there themselves. The question of why adults tolerated unrelated young in their group still remains. Clearly, issues such as mechanisms that induce juvenile dispersal, aggression levels in family and "non-family" groups, nutritional condition of young and adults that comprise these groups, and reproductive success in fragments of different sizes should be addressed in future research.

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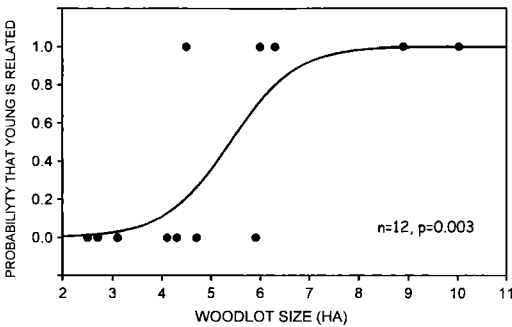


FIG. 2. Logistic regression analysis of presence/absence of related juveniles in winter flocks of Tufted Titmice based on woodlot size. Whether consisting of related or unrelated individuals, group size in all woodlots was three.

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