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UMI
FOREST FRAGMENTATION
AND THE SOCIAL AND GENETIC STRUCTURE
OF A PERMANENT-RESIDENT BIRD

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Elena V. Pravosudova, M.S.

*****

The Ohio State University

1999

Dissertation Committee

Professor Abbot S. Gaunt, Adviser

Professor Thomas C. Grubb, Jr., Adviser

Professor Patricia G. Parker

Professor David L. Stetson

Approved by

Abbot S. Gaunt

Thomas C. Grubb, Jr.

Advisers

Department of Evolution, Ecology, and Organismal Biology
ABSTRACT

Agricultural development and suburbanization of rural America are fragmenting the habitats of wild animals, but our knowledge of the impact of these processes is limited. In this dissertation, the tufted titmouse (*Baeolophus bicolor*), a woodland songbird, is used as a representative local species to investigate the potential effects of temperate forest fragmentation. Unlike neotropical migrants, whose numbers are drastically decreasing, the presence of such non-migratory birds as this titmouse is not believed to be threatened by fragmentation. In continuous woodland, mated pairs of titmice stay on the same territory year-round and form social groups consisting of an adult pair and 1-3 young which often are their offspring. Surviving through the winter is one of the more crucial points in the annual cycle. If winter group size or composition is changed, the future reproductive success of the group members might suffer.

The objectives of this dissertation were to: 1) compare composition and genetic similarity in winter groups of titmice living in various sized forest fragments with those in continuous woodland, 2) determine if there is a nutritional cost for territorial adults to sharing resources with retained offspring, and 3) assess the effect of relatedness in winter groups on levels of aggression of adult and nutritional condition of young titmice. The rate of feather growth was used to evaluate nutritional condition, and DNA analysis was employed to determine degree of relatedness. To test the prediction that there is a
cost for territorial adults to sharing resources with retained offspring, first-year birds from
some groups were removed in early winter

I found that: 1) genetic similarity in groups from continuous forest and small
fragments was lower than in groups from large fragments, 2) nutritional condition of
adults declined in groups from which young birds had been removed, 3) levels of
aggressiveness of adults toward retained offspring were lower than toward immigrant
juveniles, yet the nutritional condition of both related and unrelated young did not differ
throughout the winter. This study furthers our understanding of how forest fragmentation
affects social and genetic structure of winter groups in a permanent-resident bird, and
reveals behavioral mechanisms involved in the formation of such groups.
TO GRANDMA AND MY OTHER GREAT TEACHERS
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VITA

December 26, 1960 .................................................... Born – St. Petersburg, Russia

1983 ............................................................................ M.S. Biology / Vertebrate Zoology
Leningrad State University

1983-1985 ................................................................. Researcher,
Magadansky Nature Preserve, Russia

1985-1991 ................................................................. Researcher,
Institute of Biological Problems of
the North, USSR Academy of
Science, Russia

1992 ........................................................................... Field Biologist,
Miles, Inc., Columbus, OH

1993-1998 ................................................................. Graduate Teaching Associate
The Ohio State University

1998-present ........................................................... Graduate Fellow
The Ohio State University

PUBLICATIONS

Rymkevich, T.A. and E.V. Pravosudova. 1986. Photoperiod as a factor in regulation of
the events in annual cycle in Spotted and Pied Flycatchers. Pp.209-210 in:
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**FIELDS OF STUDY**

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CHAPTER 1

INTRODUCTION

Although changes in a landscape can occur naturally (e.g., Wright 1974, Andrén 1994), expanding human land use is the major cause of the drastic decline of natural habitat that we see nowadays. Loss of habitat often results in subdivision of what remains into isolated fragments. Further, habitat fragmentation decreases the area of the original habitat, increases the isolation of patches, reduces habitat patch size, and increases opportunity for edge effects (Wilcox 1980, Opdam et al. 1984, Lynch 1987, Rolstad 1991). These processes provoke numerous abiotic and biotic changes. For example, forest fragmentation can alter the thermal profile of a landscape, causing climatic change (Malcolm 1998). In many avian species, increasing edge effects (Lovejoy et al. 1986) may consist of predation (Lynch 1987), brood parasitism (Harris 1988), and nest-site competition (Doherty and Grubb, unpubl. ms.). Hence, understanding the relationship between population dynamics and landscape structure has become a priority for biological conservation.

Although students of habitat fragmentation focused initially on changes in species richness and population dynamics (Lynch 1987, Rolstad 1991, Hamel 1993, Herkert 1994, McCollin 1993, Schmiegelow et al. 1997), much recent work has turned to
species-specific responses to reduction of original habitat and isolation of patches. Such work has been dominated by attention to birds and mammals under the assumption that their low population densities make them vulnerable in isolated fragments (Wilcox 1980).

The mechanisms for how fragmentation affects a species' viability often remain unclear. Some recent studies suggest that habitat loss and fragmentation may have different impacts on interior and edge species as well as on generalist and specialist species (Bender et al. 1998). For some species, habitat fragmentation appears to have little or no effect (Schmiegelow et al. 1997, Tewksbury et al. 1998).

The extent of fragmentation can be measured in both patch-scale (size, shape, ecological characteristics) and landscape-scale (distance among patches, type of habitat surrounding patches, proximity to continuous habitat) parameters. Species-specific effects of habitat fragmentation can be better understood through considering some of these parameters separately.

Patch-scale effects: patch size and relative edge length. Decreasing food availability and increasing predation rate are usually expected as fragment size decreases. Negative correlations between predation rate and fragment size were demonstrated for some species of ground-nesting Neotropical migrants (Keyser et al. 1998). An elevated predation rate was observed along forest fragment edges in an agricultural landscape in Australia (Gardner 1998). Density and pairing success seemed to be lower for territorial male North American ovenbirds (*Seiurus aurocapillus*) in small forest fragments, apparently as a consequence of decreased food abundance at the edges and lack of potential nest sites (Burke & Nol 1998). In a review of 25 studies, Bender et al. (1998) found that: 1) patch size did not affect population density of generalist species that do
equally well in edge or interior habitats; for such species, declines in population size were caused by habitat loss alone; 2) interior species decreased in number due to fragmentation itself; such declines were greater than predicted solely from habitat loss, 3) edge species did better with subdivision of habitat into patches; for them, the declines in population size were less than predicted by habitat loss alone.

_Landscape-scale effects: patch isolation and type of habitat surrounding patches._

Andrèn (1994) predicted that as the extent of habitat fragmentation within a landscape increased, patch size and patch isolation would emerge as independent causes for reductions in population sizes and species abundance. One may expect to find a difference in isolation effects for species that have different dispersal abilities (e.g., flying versus non-flying animals). However, such a difference was not found in meta-analyses (Andrèn 1994, Bender et al. 1998) comparing flying birds and insects with non-flying mammals. Nevertheless, isolation and presence of hostile habitat between patches has been shown to disrupt dispersal patterns in some avian species (Lens & Dhondt 1994, Matthysen et al. 1995, Matthysen & Currie 1996, Travis & Dytham 1999, Walters et al. 1999), but not to affect immigration/emigration rates in several species of small mammals (Rosenblatt et al. 1999, Diaz et al. 1999). Conversely, the North American white-footed mouse (*Peromyscus leucopus*) (Nupp & Swihart 1998) and eight European bird species (Berg 1997) thrived in agricultural woodlots, presumably because they could move through and forage in the farmland between wooded patches.

One of the more subtle effects of fragmentation is its possible impact on genetic diversity of plants and animals that inhabit remaining patches (Ledig 1992). If movement between patches is inhibited, members of animal and plant species can become restricted
within a fragment, acting as a 'small population.' Chronically small population size produces random gene frequency changes and fixation or loss of alleles (Frankel & Soulé 1981). Such reduced genetic variation is often associated with loss of heterozygosity through inbreeding and can lead to decreased reproductive success. Restriction of gene flow can result in pronounced genetic structure, i.e., loss of genetic variability within fragments and increased genetic differentiation between fragments. Genetic structure induced by isolation is one of the main predictions of the island biogeography model (MacArthur & Wilson 1967) and has been often applied to human-induced habitat fragments such as forest patches surrounded by an agricultural or urban landscape. The recent assertion that movement between patches in a landscape may not be as restricted as between off-shore islands (Wiens 1994) has caused researchers to look more closely into the genetic consequences of human-induced fragmentation. For example, in some species of plants from temperate zones, forest fragmentation appeared to increase, rather than decrease, gene flow, breaking down existing local genetic structure (Young et al. 1999). However, in some tropical tree species, fragmentation seemed to reduce gene flow, leading to significant inbreeding and genetic differentiation (Nason & Hamrick 1997, Aldrich et al. 1998). Among invertebrates, less mobile organisms tend to show pronounced genetic structure between fragmented populations (Davies et al. 1997). In amphibians, lower than expected loss of genetic variation was demonstrated in isolated populations even though the animals seemed unable to cross gaps between habitat patches (Gibbs 1998, Seppa & Lauria 1999).

In birds and mammals, effects of fragmentation on genetic structure are rarely observed. Fragmentation-induced loss of genetic variation has been demonstrated for
populations of ring-necked pheasant *Phasianus colchicus* (Giesel et al. 1997), Eurasian red squirrel *Sciurus vulgaris* (Wauters et al. 1994), and northern Idaho ground squirrel *Spermophilus brunneus* (Gavin et al. 1999). At the same time, many avian and mammalian species do not seem to show any significant genetic differentiation between isolated fragments. Gaines and colleagues (1997) concluded that in an experimentally fragmented landscape, all of five New World mammal species they studied showed changes in demographic processes, but not in genetic structure.

There remains some controversy about the extent to which fragmentation affects resident woodland birds. Initially, it appeared that non-migratory birds were less sensitive to fragmentation than migrants (Lynch & Whitcomb 1978). However, a recent review by Bender et al. (1998) demonstrated that as fragmentation increases within a landscape, migratory species generally decline less than resident species. This difference may result, in part, because most of the resident species are associated with interior habitat that diminishes with increasing fragmentation. Cavity-nesting birds avoid forest patches lacking trees large enough to have cavities (Berg 1997) and tend to avoid cavity sites on edges (Travis & Dytham 1999).

In populations of permanent-resident birds, changes in genetic structure are not as easily recognized as drastic changes in numbers, or changes in phenotypic patterns. The effect of fragmentation has been studied in two resident European parids, the great tit (*Parus major*) and the blue tit (*P. caeruleus*). In both species, fragmentation had no effect on reproductive success (measured by clutch size, brood size, fledging weight, and fledging success) when compared to the same parameters for the birds from continuous forest (Nour et al. 1998). However, three blue tit populations in Belgium appeared to be
significantly differentiated genetically, and in one of them consisting of subpopulations in
discrete woodlots, a slight heterozygote deficit was observed. Nevertheless, gene flow
among all three populations was high (Verheyen et al. 1995). A later study concluded that
the current degree of habitat fragmentation had no influence on genetic variation in this
species (Verheyen et al. 1997). No similar studies addressing genetic structure of
resident avian species in fragmented woodland exist for the New World.

The effects of fragmentation on bird species have typically been studied during
the breeding season, with very few works addressing wintering populations of resident
birds. Yahner (1985) reported no difference in abundance of wintering avifauna between
fragmented and non-fragmented habitat in Pennsylvania. Hamel and colleagues (1993)
found that only four of 12 species wintering in the Central Basin, Tennessee, showed any
significant response to any patch or landscape characteristic. In Canada, wintering bird
species preferred to travel through forested areas in a fragmented landscape, and as the
habitat gaps between forested patches increased, they were increasingly less likely to
cross them (St. Clair et al. 1998).

I investigated effects of forest fragmentation on the genetic composition of winter
social groups of a North American songbird, the tufted titmouse (*Baeolophus bicolor*),
and on mechanisms of formation of such groups within fragmented habitat, as well as on
some aspects of the breeding biology of this species.

The tufted titmouse is a permanent-resident cavity-nesting bird inhabiting
deciduous woodland in eastern North America where, in winter, it is a habitual member
of heterospecific flocks (Grubb & Pravosudov 1994). According to several surveys of
relatively large woodlots (10-100 ha), the density of this species does not seem to be
affected by fragmentation (Lynch & Whigham 1984, Lynch 1987, Hamel et al. 1993). On the contrary, in smaller fragments (0.5-8.0 ha), density of titmice varies with woodlot size in a rather complex fashion (Doherty and Grubb, unpubl. ms.). Tufted titmice live in pairs during the breeding season and, in winter, form small coherent groups of 2-8 individuals. Such groups usually include two adult birds (a territorial pair), one or more of their offspring and, often, one or more first-year individuals from unknown natal sites (Nice 1930, Pielou 1957, Brackbill 1970, Brawn & Samson 1983). Thus, natal dispersal of some first-year birds can be delayed for at least several months, whereas other young birds move from their natal sites to join new winter groups.

In my dissertation, I attempted to answer questions relating forest fragmentation to genetic composition of winter groups in this avian species and to understand the mechanisms of formation of such groups in fragmented and continuous woodland.

In Chapter 2, I address the problem of how of forest fragmentation influences genetic similarity of winter social groups of titmice. I determined composition of winter groups in 30 2 - 13 ha isolated fragments and at two sites in a nearly continuous 3,000-ha forest. Using DNA fingerprinting, I assessed within- and between-group genetic similarity. Multilocus minisatellite DNA fingerprinting (Jeffreys 1985a,b) is a technique that uses specific probes to detect multivariate regions of nuclear DNA and develop individual-specific patterns that can be used in estimating genetic similarity between two individuals of unknown relatedness. For the DNA analysis a small blood sample was collected from all titmice in the social groups studied.
I tested the following predictions:

(1) genetic similarity within winter groups of titmice living in forest fragments should be higher than in groups living in continuous forest; among fragments, genetic similarity should be lowest in the smallest woodlots;

(2) in fragmented habitat, patch characteristics should have more effect on within-group genetic similarity than should landscape characteristics;

(3) because of the great mobility of titmice, between-group genetic similarity should not be influenced by fragmentation.

I found that:

(1) the proportion of first-order relatives was higher in larger fragments (5 – 13 ha) than in either smaller fragments or within groups in continuous forest;

(2) fragment area, but no other fragment or landscape variable, was significantly correlated with the proportion of first-order relatives in a group;

(3) in winter groups of six titmice, within-group genetic similarity was high in forest fragments as well as in continuous forest. However, for the groups of three titmice in small fragments, genetic structure was less pronounced. My overall conclusion was that within-group genetic composition of winter flocks of tufted titmice seems to result from fragmentation effects on natal dispersal.

In Chapter 3, I examine a prediction deduced from the prolonged brood care hypothesis (Ekman and Rosander 1992, Ekman et al. 1994). In some bird species, offspring stay with their parents beyond sexual maturation rather than dispersing in search of breeding opportunities (Brown 1987). Delayed dispersal in birds is usually explained by: 1) ecological factors such as habitat saturation (Emlen 1982, Komdeur
1995); or 2) benefits of philopatry such as improved lifetime reproductive success (Emlen
1991, Rabenold et al. 1991). In temperate zones, if a permanent-resident young bird
delays dispersal for several months and stays within its natal territory, it will have to
share limited food resources with dominant adults (parents) and face a restricted food
supply throughout the winter. Such a restriction can decrease the young bird’s chances of
survival. The prolonged brood care hypothesis (Ekman and Rosander 1992, Ekman et al.
1994) predicts that territorial adults in species with delayed offspring dispersal will
monopolize resources in winter. Territorial adults may be willing to share their resources
with their own retained offspring, but less so with unrelated young that might have
dispersed from adjacent territories. A resource may be shared with non-kin members of a
winter group only if it is excessive. When a resource is shared with kin, the theory
assumes that there is a cost to the parents to allowing related young stay on their territory.
I predicted that, if a wintering adult pair is more likely to tolerate their own offspring
under conditions of lower resources, then groups of three living in isolated forest
fragments should consist of an adult pair and one of their offspring from the previous
breeding season. Thirty-six titmice (24 adults and 12 first-year birds) were captured
during the winters of 1995-96 and 1996-97 in 12 forest fragments in Crawford and Union
Counties, Ohio. Each woodland fragment was completely isolated from other woodlands
by cultivated fields and contained only three titmice in one social group. Relatedness in
groups was estimated by using a DNA fingerprinting technique. Contrary to my
prediction, only five of 12 ‘triads’ were family groups. Furthermore, pairs of adults and
their first-year offspring occupied somewhat larger fragments (> 4.5 ha), whereas in
smaller fragments (< 5.85 ha) all three birds were unrelated to each other.
In Chapter 4, I describe an experimental test of the prolonged brood care model. The model rests on the assumption that retaining an offspring through the winter months in the face of a limited food supply should have a cost for parents. I tested this idea on the tufted titmouse. Using DNA fingerprinting, I assessed the degree of relatedness between adult and juvenile birds in 17 winter groups, finding that in eight of the groups no young bird was the offspring of the territorial pair. I compared the nutritional condition of territorial adult birds in small forest fragments from which their own offspring and other young had been removed (treatment) with the nutritional condition of control birds from unmanipulated fragments. Nutritional condition was estimated by using ptilochronology (Grubb 1989). This technique is based on the fact that as a feather grows, alternating light and dark bands, pairs of which are termed growth bars, appear across the feather's vane. Each growth bar represents 24 hours' growth. Assuming that birds regenerate lost feathers as quickly as possible, individuals that are in poorer nutritional condition should have narrower growth bars than individuals in better condition (Grubb 1989). In this study, the outer tail feathers were removed from all treatment and control adults, the feathers induced during the experiment were collected after six weeks and their growth parameters compared between treatment and control individuals. The nutritional condition of adults in treatment groups (young removed) appeared to be worse, not better, than in groups where a related juvenile was present. These results suggest that the prolonged brood care model may not be universal in its application and that under some ecological conditions, retaining offspring through the winter can result in a net benefit for territorial adults despite the necessity of sharing resources.
According to Ekman and Rosander's prolonged brood care hypothesis, adults should tolerate their own kin on their winter territory over unrelated juveniles so that retained young gain better access to food during the time when resources are limited.

In Chapter 5, I test two more predictions from this hypothesis: 1) retained offspring should be in better nutritional condition throughout the winter compared to immigrant juveniles; 2) territorial adults should be more aggressive towards immigrant juveniles than towards retained offspring. I estimated relatedness within groups by using DNA fingerprinting. To test the first prediction, I calculated a fatness index and used the ptlochronology technique to assess nutritional condition of young birds. I found no difference between the nutritional condition of retained offspring and immigrant juveniles. However, a significant difference in the original feathers' parameters indicated that retained young might have been in better nutritional condition at the onset of the winter. I tested the second prediction by observing the individually marked members of five winter flocks and recording aggressiveness scores. Territorial adults were significantly more aggressive towards unrelated than towards related juveniles, which supported the second prediction. I suggest that in this species, long-term fitness benefits of retaining offspring and delaying dispersal may outweigh short-term nutritional benefits in winter.

In Chapter 6, I use DNA fingerprinting to assess the degree of genetic similarity in broods of tufted titmice from suburban habitat of central Ohio in order to address the issue of occurrence of extra-pair paternity (Parker and Burley 1998). Extra-pair copulations are a strategy by which males can increase their reproductive success and females may obtain genetic benefits. Overall, known rates of extra-pair paternity in avian
species vary considerably and range from 0% to 85% (Gowaty 1996). Until now, there
have been no reports of extra-pair behavior in tufted titmice. During 1995-1998, I used
multilocus minisatellite DNA fingerprinting to estimate paternity and degree of
relatedness in eight Central Ohio broods of this socially monogamous species. My results
indicate a rather low rate of extra-pair fertilization in the study population; three of 39
nestlings could not be attributed to the attending male. Of the three extra-pair offspring,
two were the only young in a nest attended by their mother and a non-parental male that
was a first-order relative of the mother.


Verheyen, G.R., E. Matthysen, F. Adriaensen, C. vanBroeckoven, and A.A. Dhondt. 1997. Differentiation among blue tit (Parus caeruleus) populations measured


CHAPTER 2
FOREST FRAGMENTATION DISRUPTS DISPERSAL PATTERNS IN TUFTED TITMICE: EVIDENCE FROM GENETIC SIMILARITY IN WINTER GROUPS

To be submitted to the journal CONSERVATION BIOLOGY, by E. V. Pravosudova. T. C. Grubb, Jr., P. G. Parker, and P. F. Doherty

ABSTRACT

We studied the relationship between forest fragmentation and genetic structure of winter flocks in a permanent-resident temperate-zone bird, the tufted titmouse (*Baeolophus bicolor*). Composition of winter groups was determined in 30 isolated fragments of 2 – 13 ha and at two sites in a continuous 3,000-ha forest. Using DNA fingerprinting, we tested the following predictions: (1) genetic similarity within winter groups of titmice living in forest fragments should be higher than in groups living in continuous forest, and among fragments, genetic similarity should be lowest in the smallest woodlots inhabited by titmice; (2) in fragmented habitat, patch characteristics should have more effect on within-group genetic similarity than should landscape characteristics; (3) because of the great mobility of titmice, between-group genetic
similarity should not be influenced by fragmentation. We found that: (1) the proportion of first-order relatives was higher in larger fragments (5 - 13 ha) than in either smaller fragments or within groups in continuous forest; (2) fragment area, but no other fragment or landscape variable, was significantly correlated with the proportion of first-order relatives in a group; (3) although within-group genetic similarity was higher in winter groups of six titmice from fragments, it differed from between-group similarity both in fragmented habitat and in continuous forest. However, for the groups of three titmice in small fragments genetic structure was less pronounced. Within-group genetic composition of winter flocks of tufted titmice seems to result from fragmentation effects on natal dispersal.

INTRODUCTION

Understanding the relationship between population dynamics and landscape structure has become a priority for biological conservation. Although changes in a landscape can occur naturally (e.g. Wright 1974, Andrén 1994), expanding human land use is the major cause of the drastic decline of natural habitat that we see nowadays. Loss of habitat often results in subdivision of what remains into isolated fragments. Habitat fragmentation decreases the area of the original habitat, increases the isolation of patches, reduces habitat patch size, and creates edge effects (Wilcox 1980, Opdam et al. 1984, Lynch 1987, Rolstad 1991).

The effects of fragmentation on bird species have typically been studied during the breeding season, with very few works addressing wintering populations of resident...
birds. Yahner (1985) reported no difference in abundance of wintering avifauna between fragmented and non-fragmented habitat in Pennsylvania. Hamel and colleagues (1993) found that only four of 12 species wintering in the Central Basin, Tennessee, showed any significant response to any patch or landscape characteristic. In Canada, wintering bird species preferred to travel along forested areas in a fragmented landscape, and as the distance across gaps between patches increased, they were increasingly less likely to cross them. Clair et al. 1998).

There remains some controversy about the extent to which fragmentation affects resident woodland birds. Initially, it appeared that non-migratory birds were less sensitive to fragmentation than migrants (Lynch & Whitcomb 1978). However, a recent review by Bender et al. (1998) demonstrated that as fragmentation increases within a landscape, migratory species generally decline less than resident species. This difference may result, in part, because most of the resident species are associated with interior habitat, which diminishes with increasing fragmentation. Cavity-nesting birds avoid forest patches lacking trees large enough to have cavities (Berg 1997) and tend to avoid cavity sites on edges (Travis & Dytham 1999).

One of the more subtle effects of fragmentation is its possible impact on genetic diversity of plants and animals that inhabit remaining patches (Ledig 1992). If movement between patches is inhibited, members of animal and plant species may become restricted within a fragment, acting as a ‘small population.’ Chronically small population size can produce random gene frequency changes and fixation or loss of alleles. Such reduced genetic variation is often associated with loss of heterozygosity through inbreeding and can lead to decreased reproductive success (Frankel & Soulé 1981). These processes can
result in pronounced genetic structure, i.e. loss of genetic variability within fragments and increased genetic differentiation between fragments. Genetic structure induced by isolation is one of the main predictions of the island biogeography model (MacArthur & Wilson 1967) and has been applied to human-induced habitat fragments such as forest patches surrounded by an agricultural or urban landscape. The recent assertion that movement between patches in a landscape may not be as restricted as between off-shore islands (Wiens 1994) has caused researchers to look more closely into genetic consequences of human-induced fragmentation.

Changes in genetic structure of a population are not as obvious as changes in numbers or phenotypic patterns. Considering genetic structure, three blue tit (Parus caeruleus) populations in Belgium appeared to be significantly differentiated genetically, and in one of them consisting of subpopulations in discrete woodlots, a slight heterozygote deficit was observed. Nevertheless, gene flow among all three populations was high (Verheyen et al. 1995). A later study concluded that the current degree of habitat fragmentation had no influence on genetic variation in this species (Verheyen et al. 1997). No similar studies addressing genetic structure of resident avian species in fragmented woodland exist for the New World.

The present study probed for a relationship between forest fragmentation and genetic composition of winter social groups of a North American parid, the tufted titmouse (Baeolophus bicolor). The tufted titmouse is a permanent-resident, cavity-nesting bird inhabiting deciduous woodland in eastern North America (Grubb & Pravosudov 1994). Titmice live in pairs during the breeding season and in winter form small coherent groups of 2-8 individuals. Such groups usually include two adult birds (a
territorial pair), one or more of their offspring and, often, one or more first-year individuals from unknown natal sites (Nice 1930, Pielou 1957, Brackbill 1970, Brawn & Samson 1983). Thus, natal dispersal of some first-year birds can be delayed for at least several months, while other young birds move from their natal sites to join new winter groups.

In our study area within the agricultural landscape of central Ohio, tufted titmice wintering in forest fragments form small social groups consisting of adult and first-year birds. Based on the notions that delayed dispersal of young may be common in this species and that fragmentation can inhibit dispersal movements between habitat patches (Lens and Dhondt 1994, Matthysen et al. 1995, Matthysen & Currie 1996, Travis & Dytham 1999, Walters 1999), we examined the following hypotheses and predictions:

Hypothesis 1. Since in resident bird species dispersal of young is disrupted by fragmentation, dispersal should be delayed in forest fragments more than in continuous forest. However, because very small forest fragments cannot support large winter flocks, territorial adults residing in small fragments will not be willing to share resources with any additional birds, and so will force their offspring to disperse.

Predictions from Hypothesis 1. Because fragmentation inhibits dispersal, genetic similarity within winter groups of titmice in forest patches should be higher than in groups from continuous forest. Among fragments, genetic similarity should be the lowest in the smallest woodlots inhabited by titmice, since such fragments should contain only a territorial pair of unrelated adults and no retained young.

Hypothesis 2. Because in mobile animals like birds territory quality (patch characteristics) should have greater effect on offspring retention than landscape
characteristics, patch characteristics should also have a more pronounced effect than landscape characteristics on the genetic composition of groups of resident species living in fragments.

Prediction from Hypothesis 2. Relatedness among the titmice inhabiting a fragment is correlated with the area and shape of the fragment, but not with distance to the nearest fragment, nearest corridor, or the amount of wooded area around the fragment.

Hypothesis 3. Because of the great mobility of most temperate-zone resident bird species, between-group genetic similarity should not be influenced by fragmentation.

Prediction from Hypothesis 3. There should be no difference between within-fragment and between-fragment genetic similarity. Between-group genetic similarity should be the same in fragmented landscape and continuous forest.

METHODS

STUDY AREA AND FIELD METHODS

A total of 131 titmice were captured during December-January 1995-96 and 1996-97 in 30 forest fragments within the agricultural landscape of Crawford and Union Counties, Ohio. The woodlots ranged in size between 2.38 and 13.31 ha and consisted primarily of oaks (Quercus spp.), ashes (Fraxinus spp.), shagbark hickory (Carya ovata), sugar maple (Acer saccharum), and American beech (Fagus grandifolia). Fragments were isolated from other woodlands by cultivated fields and contained 2 to 7 titmice. Average distance between sampled woodlots (measured as the minimum distance between edges) was 5.37±2.70 km for Crawford County and 5.28±2.89 km for Union
County. Nine of the woodlots had adjacent narrow fencerows of trees and shrubs, all of which were interrupted by fields or roads, so that no two woodlots were connected by a corridor.

Data from continuous habitat were obtained in Mohican State Forest, a 3,000-ha woodland in Ashland County, about 50 km east of the Crawford and Union County study area. Three groups of six titmice were captured there in January 1998 at each of 2 sites 2.20 km apart.

Birds were captured in mistnets surrounding sunflower-seed feeders, fitted with a USFWS aluminum band, and individually marked with colored streamers attached to both legs. When possible, age (first-year or adult) was determined by skull pneumatization and plumage. We considered all birds caught at the same location at the same time to belong to the same winter social group. A 50μl blood sample was taken from a brachial vein of each bird, shaken with 500μl of lysis buffer (100mm Tris, pH=8.0, 100 mM EDTA, 10mM NaCl, 5% SDS: Longmire et al. 1988), and stored at ambient temperature.

**LABORATORY METHODS**

To estimate relatedness of titmice within each social group, as well as of individuals from separate isolated groups, we used multilocus minisatellite DNA fingerprinting. Prior to extraction, 250 μg of proteinase K were added to each prepared blood sample; samples were 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 TNE$_2$ (10mM Tris, pH=7.4; 10mM NaCl; 2 mM EDTA) for 4-6 h. Two µg of DNA from each individual were digested with 7.5X excess restriction enzyme *HaeIII* at 37°C for 4 h. Resulting fragments were separated through a 0.8% agarose gel at 20V for 65 h (until all fragments smaller than 1,600 base pairs were run off the gel), and were then transferred to nylon by Southern blot (Southern 1975) in 10XSSC 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 1.5XSSC, 0.1% SDS and exposed to x-ray film at -20°C for several days.

Samples from birds from the same winter social group were positioned next to each other on a gel. Depending on the size of each group, we were able to fit samples from four to 12 winter groups on each gel. Pairs of lanes on the resulting autoradiograph were compared to examine the degree of band sharing between individuals from the same social group and/or between individuals from different groups, positioned close to each other on the same gel. The band sharing coefficient ($\chi$) reflects the degree of genetic similarity between the two individuals under comparison (Wetton et al. 1987). It can be calculated as a proportion of the total number of bands in a dyad of lanes, or

$$\chi = \frac{2S}{2S + A + B}$$

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.
To determine if any dyadic comparison indicated the presence of first-order relatives in a group (parents and offspring, siblings), we used an independent set of band-sharing values derived from nestlings and parental adults. In this second data set, seven families were sampled during the 1996-98 breeding seasons, and frequency distributions of band sharing were created based on the known band sharing coefficient values between confirmed first-order relatives and between presumably unrelated individuals (e.g., mated pairs attending nests). The two distributions overlapped at about $r=0.5$. The lower value for the 95% confidence interval of the distribution for first-order relatives and the upper value for the 95% confidence interval of the distribution for presumably unrelated individuals coincided at 0.46. This value was then assigned as a threshold so that if a band sharing coefficient between two birds of unknown relatedness was greater than 0.46, those two individuals were considered first-order relatives. Birds with band sharing values less than 0.46 were considered 'unrelated.'

**DATA ANALYSIS**

We characterized fragmented habitat with patch and landscape variables measured on USGS maps and recent aerial photographs. As the woodlots were very similar in vegetation type, we included only two variables to characterize each fragment, area and shape. Shape was indexed as $P_m/P_c$, where $P_m$ was measured woodlot perimeter (edge length) and $P_c$ was the perimeter of a circle of the same area (Hinsley et al. 1995). Landscape characteristics noted around each focal woodlot included proximity to the nearest fragment measured as the shortest distance between the two fragment edges,
distance to the nearest corridor, number of woodlots within 0.5 km or 1km measured from the geometric center of each woodlot, and area of woodland within radii of 0.5 km and 1km from a woodlot’s geometric center.

Pairwise band-sharing values obtained from DNA fingerprinting analysis may not be considered statistically independent because the proportion of the bands shared between two individuals is calculated for all possible pairwise comparisons. Therefore, using parametric statistical procedures on these data is problematic (Danforth & Freeman-Gallant 1996). We adopted two approaches to avoid interdependence among band-sharing coefficients (1) parametric tests using single estimates of relatedness per woodlot and, (2) permutation tests using all possible pairwise comparisons.

**Single estimates.**

Two methods were used to estimate within-group relatedness, the average band-sharing coefficient, and the proportion of band-sharing coefficients within a group greater than 0.46 (the ‘threshold’ for first-order relatives). For statistical analysis, proportions were arc-sin square root transformed.

**Permutation tests.**

We used Mantel tests (Mantel 1967) of raw band-sharing values to evaluate whether genetic similarity varied within and between groups. The test compared two symmetrical similarity matrices, one of which contained band-sharing values, and the other, codes for within- and between-group comparisons. The two matrices were then compared and a similarity statistic \((Z)\) calculated. The significance of the relationship between the elements of the two matrices was evaluated through 1000 rounds of a Monte Carlo procedure (e.g. Sokal et al. 1993) that randomly permuted the order of the elements
in one of the matrices and calculated $Z$ for each permutation. We considered a correlation to be highly significant if the observed $Z$-value was greater than any of the 1000 iterated $Z$-values. We used the MANTEL-STRUCT program (Miller 1999) to perform these tests.

To test the first two predictions, that average relatedness within groups of titmice from larger forest patches is higher than in groups from small patches or from continuous forest, we performed 1-way analyses of variance (ANOVA). The median area of the woodlots in our sample was 4.72 ha. For analysis, we considered woodlots less than or greater than 4.72 ha to be ‘small’ or ‘large,’ respectively. The third level of this size factor was ‘continuous forest.’ Size ranges for small and large woodlots were 2.0 – 4.7 ha and 4.74 – 13.4 ha, respectively. The continuous forest was 3,000 ha. Planned comparisons (LSD tests) were used for post-hoc pairwise comparisons.

To test the prediction concerning the influence of patch and landscape characteristics on relatedness of titmice, we performed regression analyses using average band-sharing values per group and proportion of first-order relatives in a group as dependent variables in separate tests. Area and shape of woodlots, and all the landscape characteristics mentioned earlier were used as independent variables.

To test the two predictions comparing within-group and between-group genetic similarity, we used matrix comparisons to assess genetic structure in three subsets of our data: (1) three groups of six birds each from continuous forest, (2) three groups of six birds each from fragments, and (3) six groups of three birds each from fragments. We used a subset of our samples, because between-group band-sharing coefficients can be calculated only for samples positioned on the same digestion gel.
RESULTS

WITHIN-GROUP COMPARISONS IN FRAGMENTS OF DIFFERENT SIZE AND CONTINUOUS WOODLAND.

Average group sizes in small woodlots, large woodlots, and continuous forest were $3.85\pm1.51$ ($n=15$), $4.81\pm1.60$ ($n=15$), and $6.00\pm0.00$ ($n=3$), respectively. Although average group size in the three categories was somewhat different, there was no significant relationship between either of the two estimates of within-group relatedness and group size (ANOVA, $df=5,24; F=1.01; p=0.43$ for the average band-sharing coefficient, Fig. 2.1a; $F=0.99; p=0.44$ for the proportion of first-order relatives, Fig. 2.1b). There was no significant difference in average within-group band-sharing coefficient among small fragments, large fragments, and continuous woodland (ANOVA, $p=0.34; df=2,30; F=1.11$; Fig. 2.2a). However, another estimate of within-group relatedness, the proportion of band-sharing values greater than 0.46, differed significantly among the three habitat categories (ANOVA, $p=0.04; df=2,30; F=3.51$; Fig. 2.2b). The proportion of first-order relatives in large woodlots was significantly greater than in either small woodlots (LSD test, $p=0.048$) or continuous forest (LSD test, $p=0.039$). The same comparison between small woodlots and continuous forest was not significant (LSD test, $p=0.348$).
Figure 2.1  Mean ± SE average band-sharing coefficients (A) and proportion of band-sharing coefficients > 0.46 (B) within winter groups of 2 (n=1), 3 (n=12), 4 (n=6), 5 (n=2), 6 (n=4), and 7 (n=5) tufted titmice from fragmented woodland.
Figure 2.2. Mean ± SE average band-sharing coefficients (A) and proportion of band-sharing coefficients > 0.46 (B) within winter groups of tufted titmice from ‘small’ (n=15) or ‘large’ (n=15) forest fragments, or from continuous forest (n=3).
RELATIONSHIPS WITH PATCH AND LANDSCAPE CHARACTERISTICS.

None of the patch or landscape characteristics was correlated significantly with the average within-group band-sharing coefficient. The proportion of first-order relatives was significantly and positively correlated with the woodlot area (Fig. 2.3), but not woodlot shape. None of the landscape characteristics was significantly correlated with the proportion of the first-order relatives within a woodlot. However, the correlation between proportion of first-order relatives and total area of woodland within 0.5 km of a fragment did approach significance (p=0.08, Fig. 2.4).

GENETIC STRUCTURE OF THE FLOCKS.

Matrix comparisons of average within-group and between-group band-sharing values revealed a significant genetic structure among groups of six titmice in Crawford County, average distance between patches 2.83±1.49km (for all between-group comparisons p<0.005, Table 2.1). In the three groups from continuous forest (average distance 1.46±1.27 km) average band-sharing differed significantly in two comparisons (p<0.003), but not in the third comparison (p=0.860, Table 2.2). Genetic structure was not as pronounced for the subset of six woodlots (average patch area 5.54±3.15ha) containing three titmice each (Table 2.3). Although some estimates of between-group genetic similarity in this subset were quite high, there was no correlation of the parameter with inter-patch distance (regression analysis $r^2=0.009; F=0.11; df=1,13; p=0.74$; Fig. 2.5). However, permutational tests revealed the presence of significant genetic structure in all three subsets of data (p=0.001 for groups of six from fragments habitat; p=0.002 for groups of six from continuous forest; p=0.04 for groups of three from fragments).
Figure 2.3. Proportion of band-sharing coefficients > 0.46 among the titmice in a forest fragment in relation to the area of that fragment.
Figure 2.4. Proportion of band-sharing coefficients $>0.46$ among the titmice in a forest fragment in relation to the total area of woodland within 0.5 km of that fragment.
Figure 2.5. Between-patch genetic similarity (average band-sharing coefficient) as a function of distance between forest fragments.
Table 2.1 Matrix of average band-sharing values ± SD (N) between all pairwise combinations of three groups of six titmice each from fragmented woodland. Values on the diagonal are within-group averages and off-diagonal values are between-group averages.

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>1</td>
<td>0.36±0.12 (15)</td>
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<tr>
<td>2</td>
<td>0.22±0.10 (36)</td>
<td>0.36±0.12 (15)</td>
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<tr>
<td>3</td>
<td>0.28±0.11 (36)</td>
<td>0.17±0.11 (36)</td>
<td>0.49±0.21 (15)</td>
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Table 2.2 Matrix of average band-sharing values ± SD (N) between all pairwise combinations of three groups of six titmice each from continuous woodland. Values on the diagonal are within-group averages and off-diagonal values are between-group averages.

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<th>Group</th>
<th>1</th>
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<tbody>
<tr>
<td>1</td>
<td>0.30±0.10 (15)</td>
<td></td>
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<tr>
<td>2</td>
<td>0.32±0.14 (36)</td>
<td>0.27±0.09 (15)</td>
<td></td>
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<tr>
<td>3</td>
<td>0.14±0.08 (36)</td>
<td>0.17±0.10 (36)</td>
<td>0.26±0.09 (15)</td>
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Table 2.3 Matrix of average band-sharing values ± SD (N) between all pairwise combinations of six groups of three titmice each from fragmented woodland. Values on the diagonal are within-group averages and off-diagonal values are between-group averages.

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<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>1</td>
<td>0.30±0.10 (3)</td>
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<tr>
<td>2</td>
<td>0.22±0.08 (9)</td>
<td>0.52±0.20 (3)</td>
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<tr>
<td>3</td>
<td>0.35±0.12 (9)</td>
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<td>0.25±0.05 (3)</td>
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<tr>
<td>4</td>
<td>0.40±0.13 (9)</td>
<td>0.37±0.10 (9)</td>
<td>0.17±0.08 (9)</td>
<td>0.39±0.20 (3)</td>
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<tr>
<td>5</td>
<td>0.27±0.18 (9)</td>
<td>0.32±0.10 (9)</td>
<td>0.28±0.11 (9)</td>
<td>0.17±0.11 (9)</td>
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<tr>
<td>6</td>
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<td>0.27±0.13 (9)</td>
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<td>0.32±0.15 (3)</td>
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DISCUSSION

Our study of winter social groups of tufted titmice assessed the consequences of delayed dispersal for relatedness among group members. Many of the parameters traditionally used in population genetics to estimate heterozygosity or levels of gene flow (e.g., Wright's F-statistics) could not be employed as we were not sampling breeding adults (e.g., Lynch 1991). We do know that, although territorial pairs usually remain in place between breeding seasons, some birds, particularly first-year individuals, may move among flocks during and at the close of the winter (PFD and TCG, unpubl. ms.). Thus, the composition of the groups we studied was not exactly the same as the composition of groups present in the woodlots or forest sites during the preceding or following breeding season.

WITHIN-GROUP COMPARISONS IN FRAGMENTS OF DIFFERENT SIZE AND CONTINUOUS WOODLAND.

Although the average band-sharing coefficient within a group did not differ significantly among the three habitat categories, it was somewhat higher in large woodlots. This estimate of within-group relatedness might not be a good indicator of genetic similarity, because band-sharing coefficients for first-order relatives in this species rarely exceed 0.7 (EVP, pers. obs.), and the presence of first-order relatives in some groups can be obscured by the overall mean value of band-sharing. By contrast, the proportion of first-order relatives is an exact indication of how many parent-offspring and/or sibling comparisons have been revealed by analysis.
The proportion of first-order relatives was greater in large fragments than in either small fragments or continuous forest. However, contrary to our prediction, small woodlots did not always contain just a territorial pair. Although the average group size was somewhat lower in small woodlots, it did not have a significant effect on relatedness.

The low proportion of first-order relatives in small woodlots could have been caused by several different factors. Where groups of three titmice were present in the smallest patches, they consisted of an adult pair and an unrelated first-year bird. The absence of related young in such groups, as well as in larger groups from this patch size category, could have been due to failed reproduction. For cases where reproduction might have been successful, absence of retained offspring could have been due to high levels of aggressiveness of the territorial adults towards their own young (Ekman & Rosander 1992).

The low proportion of related individuals in groups from continuous forest may have been caused by high dispersal rates in that habitat as availability of vacant breeding territories could have been easily assessed there. In fragmented habitat, dispersal may have been disrupted by reluctance of young birds to cross habitat gaps. Therefore, in general, delayed dispersal may be more pronounced in large isolated woodlots, where young can ‘afford’ to spend the first winter at their natal site, than in either small isolated woodlots where resources are not sufficient for them to survive the winter, or in continuous forest, where there are fewer environmental constraints to dispersal.

Since our study was conducted only during winter months, we cannot be sure for how long dispersal may be delayed in this species. Young tufted titmice are known to remain in their natal territory into the next breeding season, and helping at the nest has
been observed (Pielou 1957). Such helping is relatively rare in temperate species and is known to exist in only two other parids, the South African black tit (*Parus niger*) (Tarboton 1981) and the New World bridled titmouse (*Baeolophus wollweberi*) (Christman & Gaulin 1998, Nocedal & Ficken 1998). In our study, in some cases when determining the age of birds was difficult in the field, we could confirm it through relatedness. However, in several cases, there was no way of being sure if a bird related to both territorial adults was their first-year or older offspring. Such an ambiguity is irrelevant for analyzing relatedness, but having the ability to determine the age of all birds could better explain the social structure of such groups.

**Effects of Patch and Landscape Characteristics.**

In winter groups residing in fragments the average within-group band-sharing coefficient did not have any significant correlation with any woodlot or landscape parameters. However, *p*-values were the smallest with respect to patch area (*p* = 0.17) and the area of woodland within 0.5 km (*p* = 0.18). The proportion of first-order relatives was significantly correlated with woodlot area. The other patch characteristic, shape of the woodlot, did not seem to have any effect on this estimate of within-group relatedness. One may expect to see an effect of woodlot shape on size and composition of a resident social group since long, thin fragments, or irregularly-shaped patches of very low area/perimeter ratio have relatively less interior habitat, and therefore, might not be perceived as optimal by an interior, cavity-nesting species like the tufted titmouse (Travis & Dytham 1999). Since breeding success in irregularly-shaped patches with little interior may be low, few large resident ‘family groups’ are likely to be found in such habitats in
winter. The absence of a habitat shape effect in our analysis could have been due to the fact that most of our fragments were square or rectangular, with the majority of shape coefficients between 1.0 and 1.5. Only two of the 30 woodlots had a shape coefficient greater than 2.0. These two, incidentally, were our biggest fragments (11.4 and 13.3 ha), so that their interior area was large even though they varied considerably from the circular shape that maximizes the area/perimeter ratio.

Landscape characteristics were not significantly correlated with the proportion of the first-order relatives in a group. The only parameter close to significance was the total area of woodland within 0.5 km of a fragment. This result is especially interesting when contrasted with the result that the total area of woodland within 1 km of a fragment was clearly not correlated with the proportion of first-order relatives in a patch (p=0.86). Such a contrast could result from limitations to exploratory behavior of young birds at the onset of natal dispersal (Koenig et al. 1996). The behavioral mechanisms of natal dispersal are poorly understood and can, perhaps, be studied only with the help of radiotelemetry. It remains possible that dispersal in young titmice is facilitated by large areas of woodland that are close enough for exploratory trips from an isolated natal site.

That neither estimate of relatedness was significantly correlated with landscape characteristics was not surprising. Although almost all of our fragments were completely isolated from other woods and/or corridors, the distances between patches were not extremely large. It is hard to imagine such a landscape constricting gene flow in almost any temperate avian species.
**Genetic Structure of the Flocks.**

Although, as mentioned above, it was impossible to assess most of the conventional estimates of population genetic structure with our data, some inferences can be made about the structure of winter social groups. Estimates of within-group and between-group genetic similarity were examined across three groups of six birds from fragmented habitat and in three groups of six birds from two locations in continuous woodland. Within-group similarity was clearly greater than between-group similarity for fragmented habitat (Table 2.1). Such a difference was also detected between groups within the continuous forest. However, in the two groups caught there at the same site (groups 1 and 2 of Table 2.2), no between-group difference was detected. Although we were certain that the 12 titmice caught at this site in Mohican State Forest traveled in two distinct social groups of six, we decided to reanalyze the data, considering the two locations rather than social groups as the primary sampling units. The matrix comparison of genetic structure revealed that the average genetic similarity was significantly lower between locations, than within locations ($p=0.001$), even though the locations were separated by only about 2 km of continuous forest.

Although it was difficult to demonstrate significance with the very small sample sizes available, for groups of six birds, average genetic similarity within a group was somewhat lower in continuous habitat (average band-sharing $0.28\pm0.02$) than in the fragments ($0.40\pm0.07$) ($t$-test, $df=2; p=0.13$). Between-group genetic similarity of sextets did not differ in these two habitat types ($t$-test, $df=2, p=0.86$). By contrast, the genetic structure revealed by the similar analysis of six groups of three titmice from Crawford County was not as pronounced ($p=0.04$ after 1000 permutations). Here, the average
distance between woodlots (4.74±2.48 km) was higher than between the woodlots containing groups of six or between the locations in continuous forest. Although two of the groups contained an offspring of the territorial pair (groups 2 and 4, Table 2.3), genetic similarity was not as different within and between groups as in comparisons of sextets. It is of interest that none of the p-values obtained in this subset of data for between-woodlot pairwise comparisons appeared to be significant (Table 2.3).

Overall, fragmentation appears to affect winter group composition in tufted titmice by increasing the proportion of non-dispersing offspring of territorial adults in larger forest patches. Dispersal rates may be higher in the small woodlots due to poor quality winter territories there. In continuous woodland, overall relatedness within groups appears to be lower than in similar-sized groups from fragments, indicating less restricted dispersal in continuous habitat. However, the significant difference in genetic similarity within and between groups indicates philopatric tendencies in the species and may be associated with long-term offspring retention. Whether similar findings characterize permanent-resident birds lacking retained offspring remains to be determined.
LITERATURE CITED


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CHAPTER 3

PATCH SIZE AND COMPOSITION OF SOCIAL GROUPS IN WINTERING TUFTED TITMICE

Published in the journal, THE AUK, by E.V. Pravosudova, T.C. Grubb, Jr., P.G. Parker, and P.F. Doherty, Jr.

INTRODUCTION

Formation of small, non-kin winter flocks is typical in many permanently 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 the flock members (Thorpe 1963, Pulliam 1973). If a kin-based aggregation in winter is 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 might be the main factor determining size and composition of winter groups. According to their model, in the face of limited resources, a territorial mated pair will increase levels of aggressiveness towards 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 also Ekman et al. 1994). This model has been supported by studies on 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, permanently resident, temperate-zone passerines in which offspring are known to spend the winter with their parents (reviewed in Grubb and Pravosudov 1994). As coherent winter units of titmice may include up to eight members (Nice 1930) that may or may not be related, it remains unclear how often offspring are retained in this species and what factors determine size and composition of a winter group. In our study sites within the agricultural landscape of central Ohio, titmice are permanent residents of forest fragments and often form rather small wintering groups of three or four birds. We predicted from Ekman and Rosander’s (1992) model for offspring retention that a wintering adult pair of titmice would be more likely to tolerate an offspring over a non-offspring flock member under conditions of lower resources. 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 adult pair. We tested this prediction using multilocus minisatellite DNA fingerprinting (Jeffreys et al. 1985a,b) to estimate levels of relatedness among the members of the triads of titmice wintering in small forest fragments.
METHODS

For this study we selected only fragments sufficiently small in area that they contained only one winter group of three titmice. Thirty-six titmice (24 adults and 12 first-year) were captured during the winters of 1995-96 and 1996-97 in 12 forest fragments in Crawford and Union Counties, Ohio. The woodlots ranged in size between 2 and 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 woodlands by cultivated fields and contained only three titmice in one social group. The titmice were confined to these fragments; we never saw them leave their woodlot. Each woodlot and each social group were studied during only one of the two winters.

We captured the birds early in winter, using feeders filled with sunflower seeds and surrounded by mistnets. To keep to a minimum any effects of supplementary food on group composition, we kept feeders in woodlots for only 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 50μl blood sample was taken from a brachial vein, shaken with 500μl of lysis buffer (100mm Tris, pH=8.0, 100mM EDTA, 10mM 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, 250μg of proteinase K were added to each sample; samples then were 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 TNE$_2$ (10mM Tris, pH=7.4; 10mM NaCl; 2 mM EDTA) for 4-6 h. Two µg of DNA from each individual were digested with 7.5 X excess restriction enzyme HaeIII at 37°C for 4 h. Resulting fragments were separated through a 0.8% agarose gel at 20V for 65 h (until all fragments smaller than 1,600 base pairs were run off the gel), and were then transferred to nylon by Southern blot (Southern 1975) in 10 X 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 X 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). It can be calculated as a proportion of the total number of bands in a dyad of lanes, or $x=2S/(2S+A+B)$, where $S$= the number of fragments of indistinguishable mobility and intensity in the two lanes under comparison, $A$= the number of the 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, $18.58\pm4.05$ bands were scored per lane with the range of band-sharing values from $x=0.11$ to $x=0.72$. 56
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 spring of 1996 and 1997, and frequency distributions of band sharing were created based on the known band sharing coefficient values between confirmed first-order relatives and between presumably 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 first-order relatives. Similarly, birds with band sharing values less than 0.45 were conservatively considered '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 (1.20±0.84, n=5), a range of values attributable to random mutations (e.g., 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) as 1.2 X 10⁻⁴. By contrast, in cases where band-sharing coefficient values 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 (11.86±2.19, n=7), confirming our assumption that all such young were unrelated to adults in their groups. Since in all such cases band sharing values between an adult female and a juvenile (0.27±0.05, n=7) and between an adult male and a juvenile
(0.23±0.07, n=7) were equally low, extra-pair paternity need not be considered a confounding factor in this analysis. While these results do not rule out a possibility of intra-specific brood parasitism, we have not found any evidence of this phenomenon occurring naturally in parids (e.g., Kempenaers et al. 1995).

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

RESULTS

In a plot of pair-wise band-sharing values (Fig. 3.1), 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 the members of the mated pair). By contrast, in groups where the young titmouse was not related to either adult, all three points were 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 ha), whereas in smaller fragments (< 5.85 ha) all three birds were unrelated to each other. Logistic regression analysis confirmed this relationship between adult/juvenile relatedness and woodlot size (n=12, p=0.003; Fig. 3.2).
Figure 3.1. Pair-wise band-sharing coefficients among triads of tufted titmice in woodland patches of different size. The dashed line indicates the 'threshold' value of the band-sharing coefficient 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 coefficients between each adult and the juvenile in a group; triangles represent band-sharing coefficients between adults.
Figure 3.2. Logistic regression analysis of presence/absence of related juveniles in winter flocks of tufted titmice on woodlot size. Whether consisting of related or unrelated individuals, group size in all woodland patches was three.
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 sharable. Instead, smaller fragments (less than 5.85 ha) contained three unrelated titmice, two adults and one first-year bird. Since the data were collected during two winter seasons, a possibility exists that the results may be confounded by the difference between the years. However, even when we used only the data for 1995-96 (10 woodlots were studied in 1995-96 and two in 1996-97) our results remained significant, confirming that presence of related young was associated with larger woodlot size (n=10, p=0.007).

Several reasons seem possible for the disparity between our prediction from the model and our results. First, in very small woodland patches such as those we used, young titmice may tend to disperse voluntarily rather than remain with their parents. Dispersing young might then be replaced by non-kin young immigrating from other woodland 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 parents' willingness 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. As 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 (e.g., Lynch and Whigham 1984, Lynch 1987, Rolstad 1991, Andrén 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 themselves. The question of why adults tolerated unrelated young as a third group member still remains. Clearly, issues such as mechanisms inducing juvenile dispersal, aggression levels in family and 'non-family' groups, nutritional condition of young and adult birds composing these groups, and reproductive success in fragments of different size should be addressed in future research.
LITERATURE CITED


CHAPTER 4

AN EXPERIMENTAL TEST OF THE PROLONGED BROOD CARE MODEL IN THE TUFTED TITMOUSE (BAEOLOPHUS BICOLOR)

In press in the journal, Behavioral Ecology, by E.V. Pravosudova and T.C. Grubb, Jr.

ABSTRACT

The prolonged brood care model (Ekman and Rosander 1992, Ekman et al. 1994) rests on the assumption that retaining an offspring through the winter months in the face of a limited food supply should have a non-zero cost for parents. We tested this idea with a New World permanent-resident bird, the tufted titmouse (Baeolophus bicolor). Using DNA fingerprinting, we assessed the degree of relatedness between adult and juvenile birds in 17 winter groups, finding that in 8 of the groups no young bird was the offspring of the territorial pair. We compared the nutritional condition of territorial adult birds in small forest fragments from which their own offspring and other young had been removed with the nutritional condition of control birds from unmanipulated fragments. The nutritional condition of adults in treatment groups (young removed) appeared to be worse, not better, than in groups where a related juvenile was present. These results
suggest that the prolonged brood care model may not be universal in its application and that, under some ecological conditions, retaining offspring through the winter can result in a net benefit for territorial adults despite the necessity of sharing resources.

INTRODUCTION

Although factors contributing to the formation of social groups in animals have been frequently addressed (e.g., Axelrod and Hamilton 1981, Emlen 1982, 1997, Brown 1987), most studies have focused on social interactions during the breeding season. The adaptiveness of non-breeding aggregations has not received as much attention. In temperate climates, some permanent-resident birds tend to form conspecific and/or heterospecific social groups during the non-breeding season. The adaptive significance of such flocking is usually explained in terms of predator detection and/or improved foraging success (Thorpe 1963, Pulliam 1973, Dolby and Grubb 1998, in press).

Among theories explaining formation of winter social aggregations of related conspecifics, the prolonged brood care model (Ekman and Rosander 1992, Ekman et al. 1994) suggests that parental control of natal dispersal can be an important factor determining the size and composition of such groups. According to this model, in the face of low resource abundance, dominant parents wintering on their former breeding site do best by being competitive and retaining all resources. In a situation where food competition is more relaxed, the model predicts that sharing resources with independent offspring would be favored by selection. Underlying such reasoning are the assumptions that delayed dispersal is linked to relaxed winter competition and that offspring have a
higher probability of surviving their first winter in their parents' territory than if they
disperse. Even with the existence of this kin bias in territorial pairs' behavior, the model
assumes that there will be a cost to adults from sharing the limited resources in their
territory with their young. That is, in order to retain their offspring through the winter,
adult birds should have to sacrifice some resources, resulting in lower levels of nutrition
for themselves. By contrast, territorial adults are expected to increase their level of
aggressiveness towards non-kin flock members and force them to leave. The model
suggests that non-kin young will be allowed to stay only if resources are sufficient to be
shared with more than one additional flock member. Descriptive studies of Siberian
(Perisoreus infans: Ekman et al. 1994, 1996) and gray (Perisoreus canadensis; Waite
and Strickland 1997) jays were consistent with the model in demonstrating that these
corvid species favored their own retained offspring over immigrants in winter groups.
Here, we report the first controlled, manipulative test of the model.

The tufted titmouse (Baeolophus bicolor; formerly Parus bicolor) is a common
permanent resident of deciduous woodland in North America, where it is a habitual
member of heterospecific flocks (Grubb and Pravosudov 1994). In winter, titmice form
coherent conspecific groups of 2-8 individuals. Such groups usually include two adult
birds (a territorial pair) and, often, one or more of their offspring and/or first-year
individuals from unknown natal sites (Nice 1930, Pielou 1957, Brackbill 1970, Brawn
and Samson 1983). Family groups extend into the breeding season, and helping at the
nest has been reported (Pielou 1957). Such helping is relatively rare in temperate species
and is known to exist in only two other parids, the South African black tit (Parus niger)
(Tarboton 1981) and the New World bridled titmouse (*Baeolophus wollweberi*) (Nocedal and Ficken 1998).

In our study area within the agricultural landscape of central Ohio, tufted titmice wintering in forest fragments form small conspecific groups of two to five birds. Based on Ekman and Rosander's model, we predicted that if all young birds were removed from a winter group residing in a small woodland fragment, the remaining adults would not have to share the non-renewing food supply through the winter and, therefore, would be in better nutritional condition compared to territorial adults in an unmanipulated control group containing at least one retained offspring of the pair.

**MATERIALS AND METHODS**

A total of 65 tufted titmice (34 adults and 31 juveniles) from 17 different groups were captured during the winters of 1995/96 and 1996/97 in 10 forest fragments in Union County, Ohio. All woodlots were approximately the same size and were very similar in topography and vegetation, consisting primarily of oaks (*Quercus* spp.), ashes (*Fraxinus* spp.), shagbark hickory (*Carya ovata*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*). Each woodlot was completely isolated from other woodlands by cultivated fields. During 1995/96, two woodlots contained two groups of titmice each; all other woodlots contained only one social group. At the beginning of the experiment in late November, each titmouse group consisted of an adult pair and 1-3 first-year birds.

At the beginning of each winter, each woodlot was randomly assigned to the treatment or control group. Five of the woodlots were used during both years. Treatment
(5.18±0.69SD ha) and control (5.13±0.71SD ha) woodlots had similar areas (t-test, df=13; t=0.13; p=0.89). During early winter, we mist-netted titmice at sunflower-seed feeders. To minimize any effect of food supplementation on the nutritional condition of the adult titmice, we maintained feeders in woodlots for only the time required to attract and catch the birds, less than a week in every case. Each captured titmouse was fitted with a USFWS aluminum band and with colored plastic leg streamers for individual identification. Age (first-year or adult) was determined by skull pneumatization and plumage, and sex by wing length and behavior. A 50µl blood sample was taken from a brachial vein of each bird, shaken with 500µl of lysis buffer (100mM Tris, pH=8.0, 100mM EDTA, 10mM NaCl, 5% SDS; Longmire et al. 1988), and stored at ambient temperature.

While capturing adults, we banded and then removed all the young birds from the treatment woodlots and released them approximately 50 km away in suburban Columbus, Ohio. None of these young birds was seen again in the study area.

We used ptilochronology (Grubb 1989, 1995) to compare the nutritional condition of adult titmice in treatment and control fragments. This method is based on the fact that, as a feather grows, alternating light and dark bands appear across its vane. A combination of one light and one dark band is termed a growth bar and represents 24 hours' growth of the feather (Brodin 1993). The width of growth bars reflects the nutritional condition of a bird during the period when a feather was grown; birds in better nutritional condition have wider growth bars than birds in poorer nutritional condition. It has been previously shown that free-ranging tufted titmice grow feathers with wider growth bars in response to a continuously present artificially enhanced food supply (Grubb and Cimprich 1990).
To assess nutritional condition, we removed the outermost left and right tail feathers from all the adult titmice in both treatment and control woodlots at the time of capture and allowed the birds to regenerate the feathers over the course of the next six weeks. We then recaptured the birds, removed the induced feathers, and stored the feathers individually in paper envelopes.

To determine if the removal of young had an effect on the nutritional condition of adults, we measured growth bar width, feather mass, and total length of both original and induced feathers from all recaptured adult birds. Original feathers had been grown during the normal molting period the previous autumn. To avoid bias, each feather was placed in a separate coded envelope prior to being measured so that its identity was not known to the person performing the measurements. To calculate the total length and average growth bar width of a feather, we fixed it to an index card covered with a piece of dark cloth. The dark background provided by the cloth increased the visibility of growth bars. A size 0 insect-mounting pin was then pushed perpendicularly down through the cloth and card at the proximal and distal ends of the feather, and at the margins of growth bars. Using the pin pricks on the card, we measured total length of each feather to the nearest 0.1 mm and calculated the mean value of the 10 daily growth bars centered on a point two-thirds of the distance from the proximal end (Grubb 1989). The mass of each feather was determined on an electronic scale to the nearest 0.1 mg. To avoid any confounding effects of humidity or temperature, we measured mass of all feathers on the same day. As both left and right outermost tail feathers were measured for each bird, we used the average of the left and right values for all three dependent variables. For each of the three
measurements, the correlation between a bird's left and right feathers was high (all p-values < 0.005).

In preparation for statistical analysis, we took several measures to avoid any bias due to pseudoreplication. In the two cases where a woodlot contained two social groups of titmice, we randomly selected only one group for analysis. In cases, where we collected induced feathers from both members of an adult pair, we randomly selected the feathers from either the male or the female for analysis. Three of the five woodlots that were used twice (in 1995/96 and in 1996/97) had new territorial adults during the second winter. For the two woodlots that had the same adult birds both winters, we randomly selected only one year's data for analysis. Thus, the primary sampling unit was one member of one territorial pair per woodlot-year with all birds used only once.

We used ANCOVA to determine the effect of removal of young on feather growth in adults. While treatment and sex were entered as factors, to control for effects of bird size, we used dimensions of original feathers as covariates.

To determine relatedness among members of each social group, we used multilocus minisatellite DNA fingerprinting. Prior to extraction, 250 µg of proteinase K were added to each prepared blood sample; samples were 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 (10mM Tris, pH=7.4; 10mM NaCl; 2 mM EDTA) for 4-6 h. Two µg of DNA from each individual were digested with 7.5X excess restriction enzyme HaeIII at 37°C for 4 h. Resulting fragments were separated through a 0.8%
agarose gel at 20V for 65 h (until all fragments smaller than 1,600 base pairs were run off the gel), and were then transferred to nylon by Southern blot (Southern 1975) in 10XSSC 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 1.5XSSC, 0.1% SDS and exposed to x-ray film at -20°C for several days.

Samples from birds from the same winter social group 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 degree of genetic similarity between the two individuals under comparison (Wetton et al. 1987). It can be calculated as a proportion of the total number of bands in a dyad of lanes, or \( x = \frac{2S}{2S + A + B} \), 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.

To determine if first-year birds in a group were offspring of the territorial adults, we used an independent set of band-sharing values derived from nestlings and their parents. In this second data set, seven families were sampled during the 1996, 1997 and 1998 breeding seasons, and frequency distributions of band sharing were created based on the known band sharing coefficient values between confirmed first-order relatives and
Figure 4.1. Distributions of tufted titmouse band-sharing coefficients for known first-order relatives (hatched bars, \( n = 84, \text{ mean} = 0.65, \text{ SD} = 0.096 \)) and presumably unrelated individuals (open bars, \( n = 60, \text{ mean} = 0.28, \text{ SD} = 0.092 \)).
between presumably unrelated individuals (e.g., mated pairs attending nests) (Fig 4.1). The two distributions overlapped at about $x=0.5$. The lower value for the 95% confidence interval of the distribution for first-order relatives and the upper value for the 95% confidence interval of the distribution for presumably unrelated individuals coincided at 0.46. This value was then assigned as a threshold, so that if a band sharing coefficient between two birds of unknown relatedness was greater than 0.46, those two individuals were considered first-order relatives. Birds with band sharing values less than 0.46 were considered ‘unrelated.’ In all cases where a young bird in the experiment was highly related to both territorial adults ($x>0.46$), we counted the number of novel bands in its profile to confirm the presumed parentage. The number of novel bands in all such cases ranged from zero to two, a range of values attributable to random mutation (e.g., Rabenold et al. 1990, Haydock et al. 1996).

RESULTS

In 8 of 17 groups, no young bird was related to either territorial adult. Four of these groups had one first-year bird, and four had two first-year birds. The other nine groups had the following composition: in three of the groups the only young bird present appeared to be an offspring of both adults; two groups had one offspring and one unrelated young; one group had one offspring and two unrelated young; and one group contained two offspring and one unrelated young. In the remaining two winter groups some young were related to only one member of the territorial pair. In one of these groups two of the three young birds were first-order relatives of the territorial female, and
Table 4.1. Size and composition of groups of tufted titmice wintering in control and experimental woodlots.

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<td>5.72</td>
<td>96/97</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>m</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Band-sharing coefficients (the degree of genetic similarity) are given for pair-wise comparisons between the adults and young originally present in the groups. Values for first-order relatives are shown in boldface.

*f = female, m = male
in the other, one of the three young was highly related to the male (Table 4.1). In these two cases data from only the related adult were used in statistical analyses.

In statistical analyses, no measurement of original feathers differed significantly either between treatment and control birds or between males and females (2-way ANOVA, p>0.1 in all cases).

Because the molecular analysis was performed only after the field manipulation, we could not be aware of the degree of relatedness between adult and first-year members of the various groups at the time we removed juveniles. Therefore, to test the model’s main assumption, that parents incur a cost by permitting their young to remain with them through the winter, we initially used only adults that at the beginning of the experiment had had at least one retained offspring on their winter territory ('kin-group' adults). Thus, we first compared nutritional condition of control adults that shared their territory with at least one retained offspring with nutritional condition of treatment adults from whose territories such offspring (along with unrelated young, if present) had been removed. A total of eight recaptured birds (two from treatment and six from control groups) were available for this analysis. The differences between treatment and control adults were non-significant for all three parameters of induced feathers [ANCOVA; F(1,1)=1.13, p=0.48 for growth bar width; F(1,1)=45.63, p=0.094 for mass; F(1,1)=0.55, p=0.543 for total length] although all three measures of feather growth were somewhat greater in control birds.

To see if this trend persisted with larger sample sizes, we added records from treatment adults that initially had lacked retained offspring in their social group ('non-kin’ adults). For several reasons, combining the two kinds of treatment adults seemed
justified. First, because of the removal, neither kin-group nor non-kin treatment adults were required to share resources with any first-year birds. Second, the original tail feathers of kin-group and non-kin adults did not differ significantly (ANOVA; F(1,6) = 0.04, p = 0.839 for growth bar width; F(1,6) = 0.21, p = 0.658 for mass; F(1,6) = 0.03, p = 0.850 for total length), indicating that the two groups of adults were of similar quality. Third, we could not detect any difference in induced feather measurements between the two kin-group and five non-kin treatment adults [F(1,4) = 3.15, p = 0.450 for growth bar width; F(1,4) = 0.001, p = 0.972 for mass; F(1,4) = 0.93, p = 0.380 for total length]. The large p values reinforce the conclusion that non-kin and kin-group adults reacted similarly to the removal of young birds.

The comparison of treatment and control groups performed after lumping records for the two kinds of treatment adults verified the trend existing in the earlier comparison. Contrary to our prediction, average growth bar width of induced feathers in treatment-group adults was significantly narrower, not wider, than average growth bar width of controls [ANCOVA; F(1,6) = 7.44, p = 0.034 for seven treatment and six control adults; Fig. 4.2a]. Induced feathers from treatment birds were also significantly lighter than those from controls [ANCOVA; F(1,6) = 6.42, p = 0.044; Fig. 4.2b]; but the difference between treatment and control feathers was not significant for total length [ANCOVA; F(1,6) = 1.61, p = 0.251; Fig. 4.2c].

In kin-group adults, no difference could be detected between the induced feathers of males and females standardized for average values of original feathers. Four females and four males were compared [ANCOVA; F(1,1) = 0.05, p = 0.860 for the growth bar width; F(1,1) = 17.24, p = 0.150 for mass; F(1,1) = 0.72, p = 0.553 for the total length].

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Figure 4.2. Mean ± SE growth bar width (A), mass (B), and total length (C) of original (open bars) and induced (hatched bars) outermost pairs of tail feathers of adult tufted titmice wintering in the presence (control, n = 6) or absence (treatment, n = 7) of first-year titmice including one of their own offspring.
After increasing the sample size by adding values from ‘non-kin’ treatment birds, we had available records from eight females and five males. Average induced growth bar width standardized for average growth bar width of original feathers was significantly greater in males [ANCOVA; F(1,6) = 6.28, p = 0.046; Fig. 4.3a], as was standardized average total feather length [ANCOVA; F(1,6) = 8.49, p = 0.027; Fig. 4.3c]. Standardized average induced feather mass was not significantly different between males and females [ANCOVA; F(1,6) = 1.50, p=0.267; Fig. 4.3b].

There was no significant interaction (all p values >0.1) between treatment and sex for any dependent variable in any 2-factor ANCOVA analysis.

DISCUSSION

According to the prolonged brood care model, during the winter, adults should tolerate their own young more than non-kin young. In this experiment, all of the wintering groups of tufted titmice initially contained a pair of territorial adults and at least one first-year bird. However, not all the groups included an offspring of the adult pair. That the only first-year birds in 8 of 17 groups were unrelated to both adults suggests that in this species in this particular habitat, one or more mechanisms in addition to adult aggression towards young may be important in determining group composition. First, reproductive success may vary even in fragments of the same size (e.g., Lynch and Whigham 1984). As our study commenced in the winter, we do not know whether those adults lacking related young in their groups had reproduced successfully, yet it seems that
Figure 4.3. Mean ± SE growth bar width (A), mass (B), and total length (C) of original (open bars) and induced (hatched bars) outermost pairs of tail feathers of male (n = 5) and female (n = 8) adult tufted titmice.
the portion of non-kin winter groups in our study is too high to be a result of reproductive failure alone. Second, while it has been shown that fragmentation can reduce dispersal rates in avian species in some habitats (e.g., Lens and Dhondt 1994), in agricultural woodland fragments, the tendency for young to disperse voluntarily, rather than stay with their parents, may be stronger (e.g., Berg 1997). Also, during the onset of dispersal, parents might be less willing to share resources with their young in fragmented habitat (because the resource base might be limited in a fragment) and may force their offspring to leave the territories that are poor in resources. In such cases, it is quite possible that adults may be joined later on by unrelated dispersing juveniles. It is also possible that such immigrant first-year birds persist in non-kin winter groups even in the face of substantial aggression from the resident adults (J. Ekman, pers. comm.). Finally, adults could have tolerated the presence of any young, kin or non-kin, in order to gain advantages of living in a larger flock (Thorpe 1963, Pulliam 1973).

Unfortunately, we do not have quantitative data on the availability of titmouse food in our study woodlots. Thus, it remains possible that the food supply in some woodlots was sufficiently low that territorial adults forced out offspring to potentially spend the winter in a better territory. It is also possible that young birds decided on their own to disperse in search of a better territory. Immigrant young that join adults on worse territories may have been low-ranking juveniles from other woodlots that failed to establish on better grounds. It is known that in some species dominant juveniles actively force subordinate siblings from the natal territory (Strickland 1991). Furthermore, for those adults whose young had left or been expelled, it may still have paid to accept
additional flock members with whom they could share minimal amounts of food without potentially suffering inclusive fitness costs.

The model assumes that sharing limited winter resources with kin young has a cost for adult birds. Based on this assumption, we predicted that adults from manipulated groups (young removed) would do better nutritionally than adults sharing food with their offspring. The results of our experiment suggest that this was not the case: instead such adults seemed to have done worse. There might be several reasons for this disparity between prediction and result. First, even under a limited food supply, a larger group size may confer a fitness advantage. For a territorial adult, the benefits of improved foraging efficiency and better predator detection may outweigh the costs of sharing resources. Second, a deciduous forest habitat such as our study area may provide a relatively high winter supply of resources. In deciduous habitats territorial adults might not have been selected to be as despotic towards subordinate flock members as would be the case in species living in more harsh northern coniferous woodlands (Ekman et al 1994, Waite and Strickland 1997). The adaptiveness of maintaining a larger flock size may outweigh that of monopolizing scarce resources. Thus, resident adult tufted titmice may tolerate one or more juveniles in their winter flock without significantly sacrificing their food supply.

Although, because of the model’s assumptions, control groups in which all young were unrelated to adults had to be excluded from the test of the prediction, we did use adults from treatment groups that had contained only non-kin young prior to manipulation in order to increase our sample size. We have shown that kin-group and non-kin treatment adults did not differ significantly in nutritional condition during the
experiment, a result unlikely due to small sample sizes because for the same group of
treatment adults, the differences between males and females for all three feather
parameters were highly significant with all the values being higher in males [ANOVA,
F(1,7)=478.66, p=0.0002 for growth bar width; F(1,7)=14.93, p=0.006 for mass;
F(1,7)=7.15, p=0.032 for total length].

Regardless of the reasons why some of the groups lacked kin young in early
winter (e.g., adults' reproductive attempt had failed or all the resident young had
dispersed), analysis of original feathers indicated that the nutritional condition of adult
titmice in all the woodlots was about the same at the start of the manipulation. The results
of our experiment thus suggest that the significantly lower average growth bar width and
mass of induced feathers in the treatment group birds can be attributed to the
experimental manipulation. Whether adults spending the winter with retained offspring
differ in nutritional condition from adults spending the winter in non-kin groups is
beyond the scope of our original question, but all induced feather growth parameters were
not significantly different between the seven kin-group and four non-kin control adults
(p=0.365 for the growth bar width, p=0.136 for mass, and p=0.826 for total length of
induced tail feathers).

Male titmice grew their induced feathers faster and to a greater total length than
did females, a result holding even after standardization to values of original feathers.
Grubb and Cimprich (1990) found a similar sex difference in feather regeneration in this
species. As adult female tufted titmice are socially subordinate to adult males in winter
flocks (Grubb and Pravosudov 1994), it is not surprising that females in our sample were
in comparatively poorer nutritional condition. However, since no significant interaction
was shown between treatment and sex, there is no evidence that the manipulation affected sexes differently.

In conclusion, our results do not support the key assumption of the prolonged brood care model. Our experiment demonstrated that the nutritional condition of territorial adults improved, rather than suffered, in the presence of related conspecific first-year members of a winter flock. Therefore, in some systems, the nutritional benefits for adults of associating with young in winter may outweigh the costs of sharing food. However, many other factors contributing to the formation of social groups in this species remain unclear. For example, it will be important to determine how adults share resources with relatives as compared to unrelated flock members (J. Ekman, pers. comm.).

Nutritional condition of retained offspring and levels of adult aggressive behavior towards them could be compared with the same parameters in immigrant young residing in similar-sized forest fragments. However, while such an analysis could show whether adults differentiate between kin and non-kin in sharing ‘extra’ resources, it would not diminish the fact that adults benefited nutritionally from having conspecific young in their territories rather than suffering a cost from sharing resources, as the prolonged brood care model suggests.
LITERATURE CITED


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CHAPTER 5

THE INFLUENCE OF KINSHIP ON NUTRITIONAL CONDITION OF SUBORDINATES AND AGGRESSIVE BEHAVIOR OF DOMINANTS IN WINTER SOCIAL GROUPS OF TUFTED TITMICE

ABSTRACT

Permanent-resident birds of the temperate zones often form conspecific foraging groups during the winter. Such groups usually consist of a territorial pair and several first-year birds, some of which may be offspring retained from the previous breeding season. According to Ekman and Rosander's prolonged brood care hypothesis, adults should be more tolerant of their own young than unrelated juveniles on their winter territory, so that retained young should gain better access to food during the time when resources are limited. We tested two predictions from this hypothesis using a New World permanent-resident parid, the tufted titmouse: 1) retained offspring should be in better nutritional condition throughout the winter compared to immigrant juveniles, 2) territorial adults should be more aggressive toward immigrant juveniles than toward retained offspring. We estimated relatedness in groups by using DNA fingerprinting. To test the first prediction, we used a fatness index and the ptilochronology technique to assess
nutritional condition of young birds. We found no difference between the nutritional condition of retained offspring and immigrant juveniles. However, the significant difference in growth rates of original feathers grown during the autumn (pre-basic) molt, indicated that retained young might have been in better nutritional condition at the onset of the winter. We tested the second prediction by recording aggressiveness scores among the individually-marked members of winter flocks. Supporting the second prediction, territorial adults were significantly more aggressive toward unrelated than related juveniles. In tufted titmice, long-term fitness benefits of retaining offspring and delaying dispersal may be more important for adult and first-year birds than short-term nutritional benefits in winter.

INTRODUCTION

In seasonal environments winter is known to be a critical interval for survival of permanent-resident bird species, primarily due to food scarcity and low ambient temperatures (Lack 1954). During winter, birds require energy reserves to survive long nights, inclement weather, and reduced foraging success due to food unpredictability. To understand how birds survive the winter season, it is necessary to know how they manage their energy reserves. Minimizing weight is important for a flying animal, therefore birds are believed to carry less fat than the maximum possible (Pravosudov and Grubb 1997). Heavier birds are less maneuverable when escaping a predator and must spend less time on vigilance while foraging in order to maintain a higher body weight. Therefore, avian body mass is believed to represent a trade-off between risk of starvation and risk of
predation (e.g., McNamara and Houston 1990). The advantages of reducing predation risk should cause birds to lower their body mass whenever it is possible to do so without increasing risk of starvation. Such an option is more likely for birds that have comparatively high access to food resources.

Animals living in social groups benefit from improved foraging efficiency and reduced predation risk (Morse 1977). However, in winter, social dominance among group-living birds determines priority of access to resources (Hogstad 1987, Koivula and Orell 1988). In birds of family Paridae, dominance status in winter flocks is usually achieved by means of aggressive interactions (e.g., De Laet 1985) and has the following order: adult males > juvenile males > adult females > juvenile females (Gosier 1996, Lahti et al. 1998). As dominants always have first access to food, their food supply appears to be more predictable than that of subordinates. Thus, under predation risk in winter, dominants should be able to carry comparatively less weight without increasing risk of starvation, whereas subordinates would need to be heavier to cope with a higher risk of starvation (Clark and Ekman 1995).

According to Ekman and Rosander’s (1992) prolonged brood care hypothesis, during winter months when food is limited, dominant territorial adults monopolize resources, but they should be more willing to share them with retained offspring than with unrelated first-year immigrants from foreign territories. In social winter groups, adult Siberian jays (Perisoreus infaustus) were not aggressive toward kin young, but actively prevented immigrant juveniles from sharing food (Ekman et al. 1994). Additionally, retained juvenal jays obtained greater food loads and spent more time at an artificial food source than did immigrant first-year birds (Sklepkovych 1997).
Using a North American parid, the tufted titmouse (*Baeolophus bicolor*), we tested two predictions derived from Ekman and Rosander’s hypothesis. The tufted titmouse is a permanent-resident cavity-nesting bird inhabiting deciduous woodland in eastern North America where it is a habitual member of heterospecific flocks (Grubb & Pravosudov 1994). In winter, tufted titmice form small coherent conspecific groups of 2-8 individuals. Such groups usually include two adult birds (a territorial pair), one or more of their offspring and, often, one or more first-year individuals from unknown natal sites (Nice 1930, Pielou 1957, Brackbill 1970, Brawn & Samson 1983). Thus, natal dispersal of some first-year birds can be delayed for at least several months, whereas other young birds move rather promptly from their natal sites to join new winter groups. Conspecific groups of titmice exhibit a linear dominance hierarchy typical of most parids (Gosler 1996, Lahti et al. 1998). We predicted that in winter groups of tufted titmice: 1) first-year birds related to the dominant adult pair should remain in better nutritional condition than unrelated, immigrant juveniles, and 2) territorial adults should be more aggressive toward unrelated, immigrant juveniles than toward retained offspring.

**METHODS**

*Study site and field methods*

We tested our predictions in isolated forest fragments and connected patches of woodland in Crawford, Union, and Morrow Counties, Ohio. All study sites were similar in vegetation and topography, consisting of oaks (*Quercus* spp.), ashes (*Fraxinus* spp.), shagbark hickory (*Carya ovata*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*) on flat ground.
During early winter, we mist-netted titmice at sunflower-seed feeders. Group size was stable throughout the winter and ranged from 3 to 7 birds. Each captured titmouse was fitted with a USFWS aluminum band and with colored plastic leg streamers for individual identification. Age (first-year or adult) was determined by skull pneumatization and plumage, and sex by wing length and behavior, when possible. A 50μl blood sample was taken from each bird’s brachial vein, shaken with 500μl of lysis buffer (100mM Tris, pH=8.0, 100mM EDTA, 10mM NaCl, 5% SDS; Longmire et al. 1988), and stored at ambient temperature.

To test the first prediction, that juveniles related to a territorial adult pair should be in better nutritional condition compared to unrelated juveniles, we collected records during December-January of 1995-96 in Crawford and Union Counties. To minimize any effect of food supplementation on the nutritional condition of the birds in this part of the study, we maintained feeders in woodlots for only the time required to attract and catch the birds, less than a week in every case. For each of 46 captured juveniles, we measured wing-chord to the nearest mm with a ruler, tarsometatarsus length to the nearest 0.1mm with calipers, and body mass to the nearest 0.1 g with a spring Pesola balance.

At the time of capture, the two outermost tail feathers were collected from all first-year birds. Regenerated feathers were collected from the same birds at the end of the winter at least six weeks after the removal of the original feathers.

Because birds can increase their mass over the course of the day by as much as 10% (Haftom 1992), we recorded the time we captured each bird. We also noted mean daily temperature, because temperature is an important determinant of avian energy reserves (Pravosudov and Grubb 1997).
To test the second prediction, that adults should be more aggressive to immigrant juveniles than to retained young, we used birds from Morrow County during December-February of 1997-98. After capturing and marking birds in a territory, we replaced the sunflower-seed feeder with a new bait, a nylon fine mesh bag filled with shelled whole-kernel unroasted peanuts. The peanuts could not be easily extracted through the mesh, so titmice had to stay on a bag and peck for several minutes, a necessity which provoked interactions between members of a group. We recorded dyadic interactions at the bait using the four-category system of Ekman et al. (1994): 0, feeding tolerance – dominant and subordinate both stay on a bag and consume food alternately or simultaneously; birds do not exhibit antagonistic behaviors; 1, non-feeding tolerance – subordinate allowed to remain on feeding site without feeding, but not directly on a bag; 2 displacement – subordinate forced from a bag; 3, chase – after displacement, dominant chases subordinate for a few meters off the feeding site. A total of 113 interactions were recorded at 5 different territories. Because analysis of relatedness was performed after the field season, our observations were not biased by knowledge of genetic similarity among the members of each group.

**Laboratory methods**

To estimate kinship among members of each social group, we used multilocus minisatellite DNA fingerprinting (Jeffreys 1985a, b). Prior to extraction, 250 μg of proteinase K were added to each prepared blood sample; samples were 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 TNE$_2$ (10mM Tris, pH=7.4; 10mM NaCl; 2 mM EDTA) for 4-6 h. Two µg of DNA from each individual were digested with 7.5X excess restriction enzyme *HaeIII* at 37°C for 4 h. 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 been run off the gel), and were then transferred to nylon by Southern blot (Southern 1975) in 10XSSC 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 1.5XSSC, 0.1% SDS and exposed to x-ray film at -20°C for several days.

Samples from birds from the same winter social group 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 degree of genetic similarity between the two individuals under comparison (Wetton et al. 1987). It can be calculated as a proportion of the total number of bands in a dyad of lanes, or $x=2S/(2S+A+B)$, where $S$ is the number of fragments of indistinguishable mobility and intensity in the two lanes under comparison, $A$ is the number of bands unique to the first member of the dyad, and $B$ is the number of bands unique to the second member of the dyad.

To determine if first-year birds in a group were offspring of the territorial adults, we used an independent set of band-sharing values derived from nestlings and their parents. In this second data set, seven families were sampled during the 1996, 1997, and 1998 breeding seasons, and frequency distributions of band sharing were created based
on the known band sharing coefficient values between confirmed first-order relatives and
between presumably 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 and the upper value for the 95% confidence
interval of the distribution for presumably unrelated individuals coincided at 0.46. This
value was then assigned as a threshold so that if a band sharing coefficient between two
birds of unknown relatedness was greater than 0.46, those two individuals were
considered first-order relatives. Birds with band sharing values less than 0.46 were
considered ‘unrelated.’ In all cases where a young bird was highly related to both
territorial adults (x>0.46), we counted the number of novel bands in its profile to confirm
the presumed parentage. The number of novel bands in all such cases ranged from zero to
two, a range of values attributable to random mutation (e.g., Rabenold et al. 1990,
Haydock et al. 1996).

We evaluated nutritional condition of first-year birds in two different ways:
calculating a fatness index and using ptilochronology. The fatness index is computed by
dividing body mass by wing length raised to the third power (Ekman and Lilliendahl
1993, Pravosudov and Grubb 1997). For convenience in analysis, we multiplied the
resulting value by 10^4. Besides quantity of stored fat, such an index could be affected by
variation in muscle mass and amount of food in the digestive tract. However, changes in
winter body mass are believed to be associated mostly with changes in fat reserves (Blem
1990).

Ptilochronology (Grubb 1989, 1995) is based on the fact that as a feather grows,
alternating light and dark bands appear across its vane. A combination of one light and
one dark band is termed a growth bar and represents 24 hours' growth of the feather (Brodin 1993). The width of growth bars reflects the nutritional condition of a bird during the period when a feather was grown; birds in better nutritional condition have wider growth bars than birds in poorer nutritional condition. It has been previously shown that free-ranging tufted titmice grow feathers with wider growth bars in response to a continuously present artificially enhanced food supply (Grubb and Cimprich 1990).

To determine if kinship had an effect on the nutritional condition of young, we measured growth bar width and total length of both original and induced feathers from the 13 young birds we recaptured. Original feathers had been grown during the normal molting period the previous autumn. To avoid bias, each feather was placed in a separate coded envelope prior to being measured so that its identity was not known to the person performing the measurements. To calculate the total length and average growth bar width of a feather, we fixed it to an index card covered with a piece of dark cloth. The dark background provided by the cloth increased the visibility of growth bars. A size 0 insect-mounting pin was then pushed perpendicularly down through the cloth and card at the proximal and distal ends of the feather, and at the margins of growth bars. Using the pin pricks on the card, we measured total length of each feather to the nearest 0.1 mm and calculated the mean value of the 10 daily growth bars centered on a point two-thirds of the distance from the proximal end (Grubb 1989). As both left and right outermost tail feathers were measured for each bird, we used the average of the left and right values for both dependent variables.
Statistical analysis

To test the first prediction that kin juveniles should be in better nutritional condition during the winter, we performed an analysis of covariance (ANCOVA) in which fatness index was entered as the dependent variable, kinship (‘related’ or ‘unrelated’) as a factor, and group size, mean daily temperature, day of winter (counted from December 1), and time of day as covariates. Other ANCOVAs were performed on feather parameters. In four separate tests, we used average growth bar width of original tail feathers, average growth bar width of induced feathers, total length of original feathers, and total length of induced feathers. In all four tests, kinship status (‘related’ or ‘unrelated’) was used as a factor, and tarsus length was used as a covariate to standardize for the size of each bird.

To test the second prediction that territorial adults should be more aggressive towards immigrant juveniles than towards retained offspring, we performed a t-test to compare average aggression scores for each pair of dyadic interactions within five winter groups. Only interactions between territorial adults and first-year birds were included in the analysis. In all of these interactions, adult birds were dominant. We also performed a regression analysis to test for the overall relationship between genetic similarity (represented by band-sharing coefficient) and average aggression score in dyadic interactions between adult and first-year birds.
RESULTS

Fatness indices from 31 juvenile birds in 18 winter groups were available for analysis. We compared only first-year birds with no related adults in their winter groups (all band-sharing coefficients < 0.46) and first-year birds with both parental adults present (band-sharing coefficients >0.46, no more than two unattributable bands). Twenty birds did not have any related adults in their winter groups, whereas the remaining 11 were accompanied by both parents. Analysis of covariance indicated that there was no significant difference between the fatness indices of kin and non-kin young (df=1.25; F=0.85; p=0.365; Fig. 5.1). As some of the birds used in this analysis were members of the same winter group, we randomly selected only one bird per group and performed the same test using the resulting 18 birds (6 related and 12 unrelated). With these restrictions, there was still no significant difference between the two types of first-year birds (df=1.12; F=0.09; p=0.771).

In a comparison of feather growth, we found no significant difference between kin and non-kin young for either induced growth bar width (df=1.9; F=0.18; p=0.680, Fig. 5.2) or induced total feather length (df=1.9; F=0.60; p=0.457). However, standardized average growth bar width (df=1.9; F=14.73; p=0.004; Fig. 5.2) and standardized total length (df=1.9; F=11.11; p=0.009) of original feathers were both significantly greater in kin young.
Figure 5.1. Mean ± SE fatness index of retained offspring with both parental adults present in a winter group (n=11), and of immigrant juveniles with no related adults in a winter group (n=20).
Figure 5.2. Mean ± SE growth bar width of induced (closed circles) and original (open circles) tail feathers in retained offspring (n=8) and immigrant juveniles (n=5) during December – January of 1995-96.
Figure 5.3. Mean ± SE aggression score in interactions between territorial adult tufted titmice and retained offspring (n=4), and territorial adults and immigrant juveniles (n=7).
Average aggression scores were available for four kin and seven non-kin dyads. Adults were more aggressive toward immigrant juveniles than toward their own retained offspring (t-test; \( p=0.018; \) Fig. 5.3). There was a significant negative correlation between the band-sharing coefficient and the aggression score for adults and juveniles interacting at the bait (df=1.9; \( R^2=0.456; \beta=-0.68; \) \( p=0.022; \) Fig. 5.4).

**DISCUSSION**

Contrary to our first prediction, both the fatness index and feather growth rates of induced feathers indicated that kinship had no effect on the nutritional condition of first-year tufted titmice during December – January. This finding is at odds with the behavioral observations on Siberian Jays (Sklepkovych 1997) which suggested better access to food by retained offspring.

Analysis of original titmouse feathers suggested that retained offspring were in better nutritional condition at the time of their pre-basic molt than were immigrant young. These significant differences in growth bar width and total feather length were not due to an overall difference in size; the analysis controlled for the size of a bird. In species with pronounced delayed dispersal, not only do juvenile males tend to stay on the natal territory beyond fledging more often than juvenile females (Tarboton 1981, Stacey and Ligon 1987), but, also, in some species, dominant juveniles force subordinate siblings from the natal territory (Strickland 1991). Our data support the idea that socially dominant birds may be the fledglings making the decision to delay dispersal and stay in the same winter group with their parents.
Figure 5.4. Relationship between band-sharing coefficient and aggression score in dyadic comparisons between territorial adult and juvenile tufted titmice in groups.
The decision to delay dispersal can provide long-term fitness benefits. Male Siberian Jays delaying natal dispersal had higher lifetime reproductive success than males dispersing during their first year (Ekman et al. 1999). In some species with delayed dispersal, young can also gain indirect fitness benefits by helping parents at the nest during the next breeding season. Such behavior has been observed in the tufted titmouse (Pielou 1957), but the sex and relatedness of the first-year helpers at the nest in this species have not been well studied.

Aggression levels in winter groups supported our second prediction from the prolonged brood care hypothesis, that adults would be less aggressive toward retained offspring than toward unrelated immigrant juveniles. More interesting, levels of aggression decreased quite steadily with increasing degree of genetic similarity. To our knowledge, such a relationship has not been demonstrated previously for any bird species. Observations of aggressiveness levels were made during the unusually mild El Niño winter of 1997-98. It is notable, that such a progression in the tolerance of territorial adults could be detected even in the face of relaxed food competition and relatively mild ambient temperatures.

Unfortunately, we could not determine the sex of all of the juvenile birds in our study. Some aggressive interactions could have been associated with intrasexual conflicts between resident adults and immigrant juveniles (e.g., Goldstein et al. 1998). In one case, when we inferred from her very small size that the immigrant juvenile in a group of four was a first-year female, the territorial female tended to displace and chase her from the feeder, while the territorial male tolerated her presence.
Overall, our results partially supported the prolonged brood care hypothesis. As predicted, kin young were tolerated more by territorial adults. However, despite such differential tolerance, kin young did not gain nutritional benefits compared to non-kin immigrant juveniles. Thus, one of the predictions derived from Ekman and Rosander's hypothesis was supported, while another was not. The lack of complete support may have occurred because in this species and in this particular study area, factors other than kinship partially determine nutritional status and social interactions between members of winter groups. For example, unlike many corvid and woodpecker species that typically exhibit delayed dispersal, tufted titmice occur in mixed-species flocks where they are socially dominant to such other species as Carolina (Poecile carolinensis) and black-capped (P. atricapillus) chickadees (e.g., Dolby and Grubb 1998). Therefore, even the most subordinate bird in a conspecific group of titmice is still dominant over several heterospecific birds foraging in the same flock. It is possible that parental tolerance in this titmouse during winter has more to do with long-term inclusive fitness benefits for territorial adults and with direct and indirect fitness benefits for retained offspring than with short-term nutritional costs, especially in deciduous forests where food competition during winter may not be so extreme as in boreal forests occupied by Siberian jays.


CHAPTER 6

GENETIC EVIDENCE FOR EXTRA-PAIR PATERNITY IN THE TUFTED TITMOUSE

To be submitted to the journal, THE CONDOR, by E. V. Pravosudova and P. G. Parker

ABSTRACT

Until now, there have been no reports of extra-pair behavior in the tufted titmouse (Baeolophus bicolor). During 1995-1998, we used multilocus minisatellite DNA fingerprinting to estimate paternity and degree of relatedness in eight Central Ohio broods of this socially monogamous species. Our results indicate a rather low rate of extra-pair fertilization in the study population; three of 34 nestlings could not be attributed to the attending male. Of the three extra-pair offspring, two were only young in a nest attended by their mother and a non-parental male that was a first-order relative of the mother.

INTRODUCTION

In recent years, molecular techniques have revealed instances of extra-pair parentage in socially monogamous birds (e.g., Parker and Burley 1998). Extra-pair copulations are a strategy by which both males and females can increase their
reproductive success. Overall, known rates of extra-pair paternity in avian species vary from 0 to 85% (Gowaty 1996). Even variation between different populations of the same species may be very pronounced (Petrie and Kempenaers 1998, Yezerinac et al. 1999). In addition to extra-pair copulations, extra-pair offspring may be a result of intraspecific brood parasitism in the form of egg dumping or mate switching (e.g., Kempenaers et al. 1995).

Because social monogamy and biparental care are typical of passerines, this group is well represented in studies of genetic similarity within broods. The rate of extra-pair parentage (EPP) in passerines has been shown to depend on such population parameters as breeding density (Tarof et al. 1998, Yezerinac et al. 1999, but see Lubjuhn et al. 1999) and breeding synchrony (Kempenaers 1997, Yezerinac et al. 1999). EPP has also been demonstrated to vary with parameters associated with mate choice and intrasexual competition. Degree of genetic variability of males and male age have been shown to influence EPP rates (Petrie et al. 1998), particularly in highly sexually dimorphic species (Owens and Hartley 1998) and if the social father is younger (Bjornstad and Lifjeld 1997). Even such subtle factors as extent of knowledge of male quality by females (Slagsvold and Lifjeld 1997) are believed to have an effect on frequency of EPP. However, while experimental male replacement changed the rate of extra-pair paternity in broods of pied flycatchers (*Ficedula hypoleuca*) (Lifjeld et al. 1997), it did not change the proportion of females obtaining extra-pair fertilizations in tree swallows (*Tachycineta bicolor*) (Barber et al. 1998).

The tufted titmouse (*Baeolophus bicolor*) is a permanent-resident socially monogamous cavity-nesting passerine common in the eastern deciduous woodlands of
North America (Grubb and Pravosudov 1994). During the non-breeding season, titmice often live in social groups that may include one or more retained offspring of a territorial pair (Nice, 1930, Pielou 1957, Brackbill 1970, Brawn and Samson 1983). Family groups sometimes extend into the breeding season, and helping at the nest has been reported (Pielou 1957). Such helping, relatively rare in temperate species, is known to exist in only two other parids, the South African black tit (Parus niger) (Tarboton 1981) and the New World bridled titmouse (Baeolophus wollweberi) (Nocedal and Ficken 1998).

We employed DNA fingerprinting to assess extra-pair parentage in Central Ohio broods of Tufted Titmice. In our study area within a suburban landscape, titmice breed in bluebird (Sialia sialis) nestboxes in edge-type habitat near small forest fragments and golf courses. Since titmice seem to prefer natural cavities over nestboxes, and since availability of trees with cavities is low in suburban habitat, their breeding density in our study area is considered low even in places where nestbox availability is high (D. Sillick and R. Tuttle, pers. comm.).

METHODS

Field work

Nine broods of tufted titmice from nestboxes in suburban Franklin and Delaware Counties, Ohio were studied in May-June of 1995-98. Average brood size was 4.2±1.1 nestlings. We mistnetted adults at the nests when nestlings were 5 – 10 days old. Adults and nestlings were fitted with a USFWS aluminum band, and adults with plastic color bands. Sex of adults was determined using size dimorphism and presence of brood patch. On the day adults were captured, a 50-μl blood sample was taken from the brachial vein of each nestling and attending adult, shaken with 500μl of lysis buffer (100mm Tris,
pH=8.0, 100mM EDTA, 10mM NaCl, 5% SDS; Longmire et al. 1988), and stored at ambient temperature. All the families were the result of the first nesting attempt of the season, and although some nestboxes were used twice in consecutive years, all adults were used only once. At one of the nests, we captured the attending female, but failed to catch the male, so this brood had to be excluded from analysis of paternity. However, since our primary concern was to determine paternity rates, and since intra-specific brood parasitism does not seem to occur naturally in parids (Kempenaers et al. 1995), one family where only the attending male was captured was included in the analysis.

**Laboratory methods**

Prior to extraction, 250 µg of proteinase K were added to each prepared blood sample; samples were 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 (10mM Tris, pH=7.4; 10mM NaCl; 2 mM EDTA) for 4-6 h. Two µg of DNA from each individual were digested with 7.5X excess restriction enzyme HaeIII at 37°C for 4 h. Resulting fragments were separated through a 0.8% agarose gel at 20V for 65 h (until all fragments smaller than 1,600 base pairs were run off the gel), and were then transferred to nylon by Southern blot (Southern 1975) in 10XSSC 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 1.5XSSC, 0.1% SDS and exposed to x-ray film at -20°C for several days.

Assignment of parentage

Individual samples from the same family 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 degree of genetic similarity between the two individuals under comparison (Wetton et al. 1987). It can be calculated as a proportion of the total number of bands in a dyad of lanes, or 

\[ x = \frac{2S}{2S + A + B} \]

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. The number of novel bands in the profile of each nestling was counted.

An attending adult was considered to be non-excluded as a parent of a particular nestling if the band-sharing value between the two birds was high (>0.46) and the number of novel bands in the profile of the nestling did not exceed two. The 'borderline' values of band-sharing were evaluated using values from presumably unrelated birds that were members of separate winter flocks of titmice, and from known first-order relatives (offspring and non-excluded parents) (Fig. 6.1). The two distributions overlapped at about x=0.5. The lower value for the 95% confidence interval of the distribution for first-order relatives and the upper value for the 95% confidence interval of the distribution for presumably unrelated individuals coincided at 0.46. In cases, where parentage could not be confirmed otherwise, x=0.46 was used as a conservative 'threshold' for assigning first-
order relatives. 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) as $1.2 \times 10^{-4}$.

RESULTS

On average, $21.07 \pm 3.30$ bands were scored per lane, with dyadic band-sharing values ranging from $x=0.18$ to $x=0.92$. In eight of the broods used in the analysis, 31 of 34 nestlings could be attributed to both attending adults. In the ninth brood, where the male was the only adult attending the nest, band-sharing values between each of the five nestlings and him fell above the 0.46 'threshold' ($x=0.53 \pm 0.05$, $n=5$). The number of novel bands in the profiles of the remaining 26 young, apparently related to both attending adults, ranged from 0 to 2 (average $0.65 \pm 0.74$, $n=26$) (Fig. 6.2).

Three of 34 nestlings from the families used for parentage analysis could not be assigned to the attending male (average number of novel bands $5.00 \pm 1.00$, $n=3$). One of these nestlings was apparently an extra-pair offspring (band-sharing with putative father $x=0.35$; 5 novel bands, Fig. 6.2) in a nest with three other young, all of which were highly related to both members of the territorial pair. The remaining two unattributed nestlings were the only chicks in a nest attended by their mother and a non-parental male (Fig. 6.3). While the band-sharing values between this attending male and the nestlings were quite high ($x=0.41$ and $x=0.49$), the comparatively large number of unattributable bands (4 and 6) in the profiles of the young made us exclude him as the genetic father. Band-sharing analysis ($x=0.58$) suggested that the attending male was a first-order relative of the attending female.
DISCUSSION

Only three of 34 nestlings from eight broods could not be attributed to the attending male. Such a rate of extra-pair fertilizations, 8.8%, is relatively low. Reported EPP rates for other parids range from 0.9% in willow tits Parus montanus (Orell et al. 1997) to 25.3% in coal tits Parus ater (Lubjuhn et al. 1999). Variation also exists among populations of the same parid species. For example, in the great tit Parus major, EPP rates from 3.5% (Verboven and Mateman 1997) to 15% (Gullberg et al. 1991 in Orell et al. 1997) have been reported. It is unclear how typical our estimate of EPP is for the tufted titmouse as a species as the low rate in our study could have been due to low population density (but see Lubjuhn et al. 1999).

Only one of the three unattributed nestlings could be considered a 'truly' extra-pair offspring (5 unattributable bands, Fig. 6.2). The other two chicks (Fig. 6.3), although not fathered by the attending male, appeared to be his full siblings, half-siblings, or some form of second-order relatives, since the genetic similarity estimates between these nestlings and the male were quite high (r=0.49, x=0.41, Fig. 6.3). Possibly, the attending male was a helper rather than a cuckolded male. It remains unclear whether the genetic father at this nest ever participated in caring for the young. It is also of interest that the possible helper male in question was highly related (r=0.55) to a male attending a nest located 0.7 km away. Furthermore, both of the males were highly related (r=0.50, x=0.53) to the female from the first nest, the genetic mother of the two unattributable
Figure 6.1. Distributions of tufted titmouse band-sharing coefficients for first-order relatives (hatched bars, $n=84$, mean=0.65, SD=0.10) and presumably unrelated individuals (open bars, $n=60$, mean=0.28, SD=0.09)
nestlings. It is possible, that the two males were her offspring from a previous season, or that all three birds were siblings. There was no indication that the adult females from the two nests were related.

The low rate of extra-pair fertilizations and possibly high rate of male philopatry observed in our study may not be typical of tufted titmice under more natural conditions where nest site availability is higher and dispersal is not interrupted by unsuitable habitat. At the same time, with the food of resident species in suburban areas now being supplemented virtually year round, one might expect young to be more likely to delay natal dispersal with the result that occurrence of helpers at nests might be higher. However, we have never observed more than two adult birds attending any nestbox.
Figure 6.2. Band-sharing coefficients (x) and number of novel DNA fragments between each nestling tufted titmouse and its putative mother (triangle) and father (square). The dashed line indicates the ‘threshold’ band-sharing value for first-order relatives.
Figure 6.3. Band-sharing coefficients within one particular titmouse family as determined by pairwise comparisons between the attending adults (f-m), the two nestlings (n-n), the female and each of the nestlings (f-n), and the male and each of the nestlings (m-n). The dashed line indicates the 'threshold' band-sharing value for first-order relatives.
LITERATURE CITED


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CHAPTER 7

CONSPECTUS

In the previous chapters, I investigated several questions concerning the behavioral ecology of an avian species that stays in social groups during the winter, and how certain aspects of its ecology are affected by habitat fragmentation. I will now summarize the results of this study into a conspectus. It is my thesis that the conclusions of earlier investigators of this topic were over-generalized, as would be expected of pioneering studies. Specifically, the organization of conspecific winter social groups is influenced by decision-making of all the potential members of such a group and by more factors than just winter resource availability.

Human activity has altered natural woodland, changing it into a matrix of isolated and connected forest patches. In general, it is thought that many woodland bird species are not affected by habitat fragmentation because their population densities are not reduced in whatever habitat remains. However, in some woodland species, the presence of unsuitable habitat between forest patches and reduced territory availability have been shown to affect dispersal patterns of young individuals. I examined the prediction that forest fragmentation alters dispersal patterns of the first-year birds in a social species. If dispersal patterns are altered, normal social and genetic composition of social units may
I used genetic markers to determine the structure of winter social groups in tufted titmice. Because food availability on a territory is presumed to determine the size of a social group residing there, and the size of a forest fragment is presumed to be positively correlated with resource availability, I expected to find only unrelated individuals (a territorial pair) in the smallest patches inhabited by titmice. In larger fragments, as well as in continuous woodland areas, I expected to find a high proportion of retained offspring in winter social groups. However, because of the great mobility of titmice, as would be expected of an avian species, I did not expect to see significant genetic structure among winter groups in either fragmented habitat or continuous forest.

My study revealed that there indeed was a difference between genetic composition of titmouse groups in small vs. large fragments and continuous forest. Genetic similarity, measured by proportion of first-order relatives in each group, was lowest both in the smallest fragments inhabited by titmice and in continuous forest; it was the highest in large fragments. Surprisingly, the low genetic similarity in very small fragments was due not to the absence of first-year birds, as I expected, but rather to the presence of young, unrelated to the territorial adults. In fact, the group size, despite being somewhat lower in small fragments, did not differ significantly among small fragments, large fragments, and continuous forest. Groups from large fragments and continuous forest often included retained offspring of a territorial pair, although unrelated juveniles were almost always present in such groups as well. Also contrary to my predictions, genetic structure of winter social groups was pronounced in continuous as well as in fragmented habitat.
To further study mechanisms of formation of winter social groups in this species, I performed several tests of the prolonged brood care hypothesis, proposed by Jan Ekman and colleagues in 1992 and 1994 (Chapters 3-5). These researchers suggested that although adults, controlling natal dispersal patterns, gain long-term inclusive fitness benefits from retaining offspring on their territory, they still suffer short-term nutritional costs from having to share winter resources with such offspring. Based on this hypothesis, I predicted that: 1) if a related juvenile is removed from a winter social group, the nutritional condition of remaining adults will be better compared to the condition of adults from unmanipulated larger groups; 2) adults will always be more aggressive to immigrant young than to the retained offspring; 3) retained offspring will always be in better nutritional condition over winter compared to immigrant juveniles. I found, however, that 1) adults' nutritional condition declined, rather than improved, when related juveniles were removed from a group, 2) territorial adults were consistently more aggressive toward unrelated juveniles, and 3) retained offspring and immigrant young did not differ in nutritional condition over the winter, although retained young seemed to have been in better nutritional condition in the fall.

Overall, I have found that fragmentation affects genetic composition of winter flocks of tufted titmice by possibly inhibiting natal dispersal. This, however, is true only if the size of a fragment, and therefore the quality of the territory, is sufficient for young to delay dispersal. Very small fragments do not seem to be suitable for family groups during winter. The fact that territorial adults in such fragments almost never have retained offspring, but still have immigrant juveniles present in a group, suggests that, for this species, the benefits of staying in a larger group outweigh the cost of sharing resources.
with additional flock members. This conclusion was also supported by removal experiment results, in which adults seemed to benefit from, rather than pay for, retaining their offspring.

I have shown that Ekman's prolonged brood care hypothesis is not universal in its application, or at least that it must take a more complex form. Tufted titmice in Ohio and Siberian jays (Ekman's study species) in northern Europe do not respond the same way to evidently similar situations. Hence, different mechanisms affect the formation of non-breeding social groups. For tufted titmice, it pays to stay in a larger group, even if resources are limited.

My understanding of how winter social groups are formed by tufted titmice living in a patchy forest habitat is as follows. In low-quality territories, when adults fail to reproduce, they can benefit from allowing a strange dispersing juvenile to join them in a winter group. If adults have successfully reproduced on a low-quality territory, they may choose to force their own young to disperse in search of a better wintering grounds, but allow strange juveniles to enter, thereby benefiting from being in a larger group without potentially suffering inclusive fitness costs. This way, their own young may have a chance to reproduce the following season, having dispersed early, while adults can maintain high levels of aggression towards immigrant young, retaining most of the winter resources to themselves. For adults reproducing on a high-quality territory, it may pay to allow their own young to delay dispersal. Thus, they can secure their offspring's survival through the first winter and simultaneously enjoy the benefits of staying in a larger winter group.
Also contrary to Ekman, the decisions of adults are apparently not the only mechanism determining winter group composition. Dominant young from a brood can decide whether to leave a natal territory at the onset of the dispersal period or to stay at home over winter. This decision should depend primarily on territory quality. In a low-quality territory, the chances are high that dominant young will leave in search of better grounds. On better natal territories, such young will be more likely to delay dispersal. On the other hand, subordinate young will probably have no chance to make decisions to delay dispersal, but will be forced out by parents or dominant siblings, regardless of the territory quality.

Many aspects of tufted titmouse social group formation and dynamics remain to be addressed in future studies. For example, the effect of fragmentation on breeding biology of this species remains completely unstudied. The question is still open if young, who delay dispersal through the winter, extend their stay into the breeding season and help at the nest. The question of how common this phenomenon is in titmice and whether it is affected by fragmentation is important in further understanding of their social life.
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