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Tufted titmice, black-crested titmice, and their hybrids occupy different types of habitat within their hybrid zone

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Abstract

Tufted Titmice (*Baeolophus bicolor*) (TUTI) and Black-crested Titmice (*Baeolophus atricristatus*) (BCTI) are sister species that hybridize within a narrow east–west contact zone that extends from central Texas into southwest Oklahoma. The zone corresponds with an ecotone that transitions from closed-canopy deciduous forests in the east, occupied by TUTI, to arid and open woodlands in the west occupied by BCTI. Furthermore, the ecotone encompasses areas impacted by urban and suburban development. We tested whether landscape-level landcover characteristics found within the TUTI×BCTI hybrid zone influence the abundances of the two parent species and their hybrids. We predicted that the parent species would differ in their habitat associations and hybrids would associate with habitats intermediate between that of TUTI and BCTI and with human disturbed habitats. In ArcGIS Pro, we used a grid-based sampling design to extract estimates of six land cover variables (derived from National Landcover Database data) and TUTI, BCTI, and hybrid abundances (derived from eBird data). Partial redundancy analysis (RDA) was used to examine the habitat associations of the three titmice types. The results revealed that TUTI associated most strongly with the percentage of mixed forest cover, and BCTI with the percentage of evergreen forest cover and impervious surface cover. No single landcover variable could be identified as a definitive association for hybrids, however, hybrid individuals do appear to be more abundant in a habitat type that is intermediate between that of the two parent species. Future studies should incorporate genotypic data from titmice in the hybrid zone and analyze habitat associations at finer spatial scales to gain a more conclusive understanding of the patterns detected in this broad scale study.

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Introduction

Many hybrid zones form within ecotones, defined as narrow geographic regions where two different ecosystems or habitat types transition into one another (Meacham 1962; Zweifel 1962; Hubbard 1969; Jackson 1973; De Dios et al. 2006). Individuals of parent taxa are potentially better adapted to and therefore associated with one of the habitat types to a greater extent than the other (Endler 1977; Moore 1977; Seehausen et al. 2008). The transition and mixing of habitat characteristics within ecotones allows the species to co-occur at some level of sympatry, thus creating the opportunity for hybridization (Moore 1977; Kark and Rensberg 2006). Additionally, novel niches that hybrids are able to exploit may be created by the heterogeneous environment of the ecotone (Anderson 1948; Grant 1971). For instance, with regard to birds, some form of vegetation structure intermediate between the respective habitats of the two parent species, might be utilized more efficiently for foraging by hybrids than by individuals of either parent species (Wood et al. 2015). Such habitat intermediacy exists as increased heterogeneity (mixing) of landcover types and/or as intermediate levels of coverage of the land cover types. For example, an ecotone between grassland and forest might consist of an area that has a mosaic of scattered tall trees and grassy patches, or it might be a relatively homogeneous area of smaller trees and shrubs.

Hybridization between taxa is also sometimes correlated with disturbed habitat; that is, areas affected by human activity (e.g., urbanization and agriculture) (Wright and Lowe 1968; Urbanska 1987; Pierotti and Annet 1993). These disturbed areas break up natural habitats that the parent taxa normally associate with and in turn may create homogenized, fragmented or even novel habitat (Anderson 1948; Grabenstein and Taylor 2018; Grabenstein et al. 2023) that is structurally intermediate. In some cases, hybrid individuals appear to be better adapted to and numerically dominant in these disturbed settings (Wright and Lowe 1968; Grant 1971; Moore 1977; Pierotti and Annet 1993; Vila et al. 2000). Restriction of hybrids to anthropogenically modified environments has been documented for some plant and vertebrate species (Wright and Lowe 1968; Grant 1971; Urbanska 1987; Pierotti and Annet 1993; Wood et al. 2015).

Among vertebrates, hybridization in birds is particularly extensive with at least 16% of all bird species hybridizing in the wild (Ottenburghs et al. 2015). Tufted Titmice (*Baeolophus bicolor*) (TUTI) and Black-crested Titmice (*Baeolophus atricristatus*) (BCTI) are sister species that frequently hybridize in a narrow north–south zone extending from central Texas into southwest Oklahoma (Dixon 1955, 1978, 1990; Curry and Patten 2014). TUTI and BCTI likely began to diverge at the end of the last glaciations of the late Pleistocene, about 12,000 years ago (Dixon 1978; Avise and Zink 1988; Gill and Slikas 1992). Approximately 4000 years ago, increasing aridity and natural fragmentation of pinyon-oak-juniper woodlands led to geographic range expansion of both species and subsequent range overlap which created a secondary contact zone (Fig. 1) (Dixon 1955, 1978). An additional secondary contact zone emerged about 100 years ago in southwest Oklahoma after mesquite began spreading due to fire suppression and overgrazing (Dixon 1955; Sutton 1967; Patten and Smith-Patten 2008; Curry and Patten 2014). This habitat alteration facilitated range expansion of BCTI (Van Auken 2000; Callaghan 2002; Curry 2014). The new contact zone in Oklahoma is spatially continuous with the older contact zone in Texas (Fig. 1), therefore, in this study we consider them as a single hybrid zone. The hybrid zone corresponds with an ecotone that transitions from closed-canopy deciduous forests in the east, occupied by TUTI, to arid, open woodlands comprised of primarily evergreen vegetation in the west, occupied by BCTI (Fig. 2) (Curry and Patten 2014). Furthermore, the ecotone
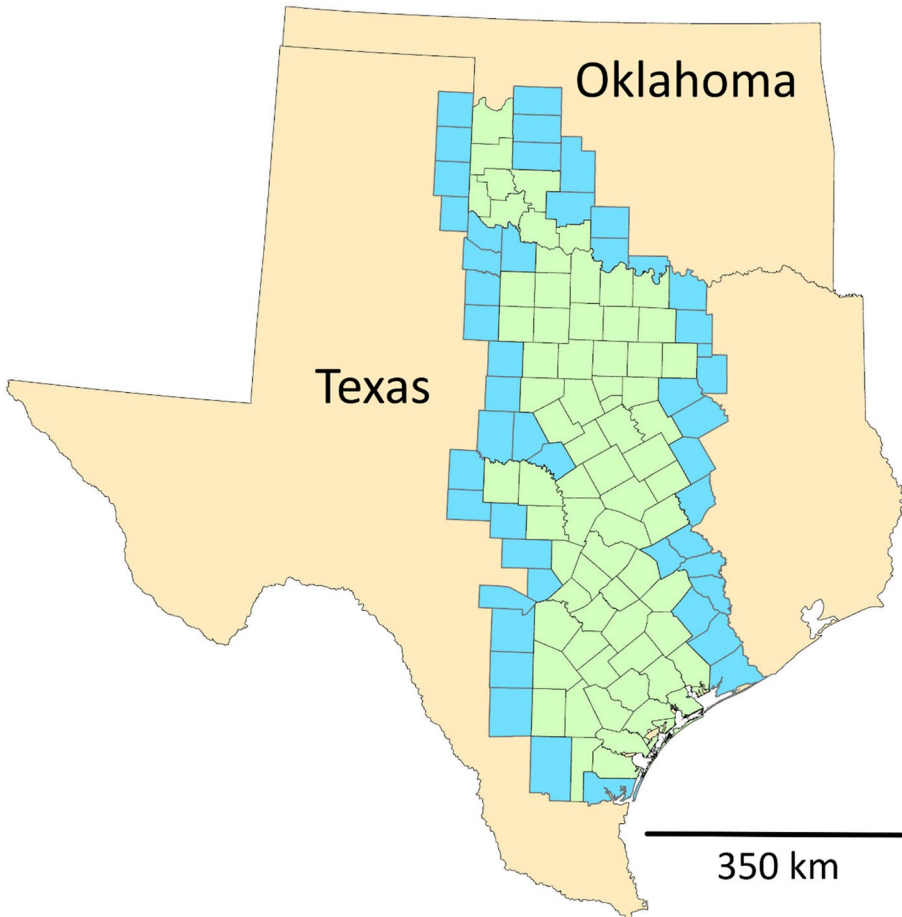
Fig. 1 The contact (hybrid) zone between the Black-crested and Tufted Titmouse (green-shaded counties). The zone was delineated as those counties that have recorded hybrid sightings in the eBird database between 2012 and 2020. The designated study region contains the contact zone along with a narrow band of surrounding counties that have recorded sightings for either parent species but not both (blue shading)

encompasses areas impacted by urban and suburban development (Smith 1910; Dixon 1955; Brauning 1992). Dixon (1955, 1978, 1990) and Curry and Patten (2014) revealed that the frequency of co-occurrence and incidence of hybridization varies across the contact zone, with pure TUTI individuals occurring more frequently along the eastern edge of the zone and pure BCTI individuals along the western edge. Throughout the contact zone, multi-generational hybridization and backcrossing events are indicated by the presence of titmice with forehead and crown plumage that is intermediate between that of the parents (Curry 2014).

Previous studies have focused on habitat associations of the two parent species separately, primarily outside of the contact zone (Smith 1910; Dixon 1961; Wauer 1971; Conner et al. 1983; Brauning 1992). Furthermore, no studies have directly tested for a correlation between human disturbance and the incidence of hybridization in TUTI and BCTI. Therefore, the TUTI×BCTI hybrid zone provides an opportunity to investigate whether
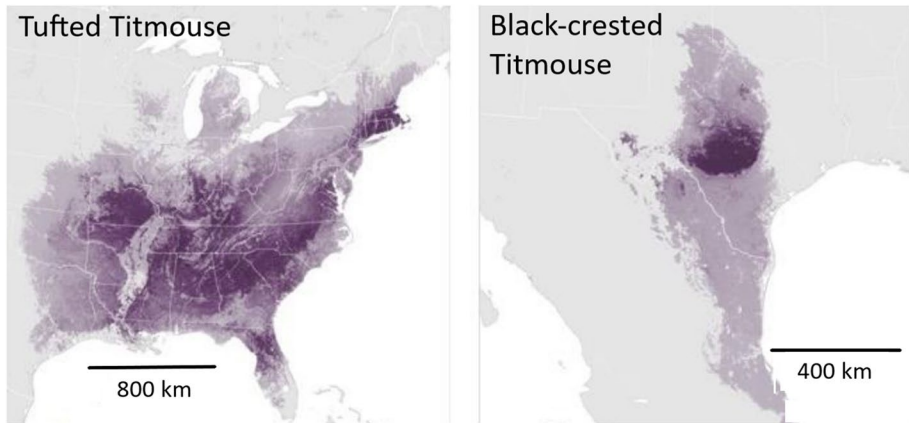
Fig. 2 The year-round geographic ranges of the Tufted Titmouse and the Black-crested Titmouse in North America. Darker shades of purple indicate higher relative abundance. Maps provided by the Cornell Lab of Ornithology with data from eBird (Fink et al. 2023)

both habitat characteristics and anthropogenic disturbance have a significant effect on the current distributions of TUTI, BCTI, and their hybrids. In this study we tested whether the distinct habitat associations of TUTI and BCTI remain in the hybrid zone or whether the two parent species overlap in habitat associations. Additionally, we aimed to identify the habitat characteristics that hybrids most strongly associate with. All analyses used in this study were conducted at a landscape level spatial resolution in order to gain greater sample sizes; therefore, we were only able to detect broad scale habitat associations that may not necessarily reflect the associations of the three titmice types at more local levels. Nonetheless, we intended this study to be a starting point for determining habitat associations within the BCTI×TUTI species complex, and a helpful reference for future studies that will aim to analyze the habitat associations implied here at finer spatial scales for more accurate depictions. With these caveats in mind, we hypothesized that the habitat associations of TUTI and BCTI identified in allopatry would be maintained within the hybrid zone such that (1) TUTI and BCTI would differ in their habitat associations and (2) hybrid individuals would associate with habitat characteristics that are intermediate between those of the parent species and/or with novel anthropogenic habitats comprised of moderate to high percentages of impervious surface cover (primarily asphalt and concrete).

Methods

eBird data and study region

eBird is a citizen science initiative administered and managed by the Cornell Lab of Ornithology which also hosts an interactive website to facilitate data acquisition and use. Participants upload checklists of bird species encountered during a given outing of birding. All survey checklists are geo-referenced to a single latitudinal/longitudinal point, often marking the starting or mid-point of a survey that may have involved some amount of walking or vehicle travel by the birder. For each checklist, the birder often tabulates a total count of each species identified during their outing. Additionally, when submitting checklists on the

website portal, survey participants may include information on the environmental conditions, detailed descriptions of the birds' appearances, and/or specific behaviors. Unusual sightings or descriptions for an area are flagged and reviewed by eBird staff for data quality control. Importantly, the hybrid between TUTI and BCTI is one of the few hybrids that eBird participants routinely report as a count separate from the two parent species, likely due to the ease of identifying hybrids based on plumage morphology.

We acquired count data for TUTI, BCTI, and their hybrids from checklists submitted within Texas and Oklahoma between 2012 and 2020. This nine-year time period bracketed the 2016 land cover data (see next section) used in the habitat analysis. In a grid-based analysis (see below) the count data across all years were combined to represent a total count for each titmouse type over the 9-year time period within a grid cell. Pooling the count data across years provided a larger sample size and accounted for variation in sampling effort across survey (eBirding) locations from year to year. To further focus our analysis, we identified all Texas and Oklahoma counties that had checklists reporting hybrids along with one or both of the parent species. These counties thus encompassed the hybrid zone. We also included counties bordering the hybrid zone that had either species appearing on checklists. These counties allowed us to include and examine habitat associations of the parent species outside of the zone (Fig. 1). To ensure spatial accuracy of the eBird data, we eliminated checklists in which the observer (eBirder) reported travelling more than 10 km. Most checklists reported traveling less than 1 km. The data was further filtered to guard against pseudoreplication by removing all duplicate observations such as when two or more eBird participants submit the same checklist. A final caveat: some back-crossed hybrids may resemble one or the other parent species (although F1 hybrids are very distinctive). Thus, the counts of hybrids in our final dataset may be slightly underestimated to the extent that backcrossed individuals may have been occasionally identified as "pure" individuals of either parent species.

Landscape-level habitat data

The National Land Cover Database (NLCD), provided by the United States Geological Survey and other agencies, was used to obtain landscape-level landcover data. The NLCD consists of several different GIS raster layers derived from remote-sensing imagery from the Landsat satellites. NLCD data are publicly available at a 30×30 m pixel resolution on the website of the Multi-Resolution Land Characteristics Consortium (https://www.mrlc.gov/). For our habitat analysis, we used the Land Cover layer, Tree Canopy Cover layer, and the Developed Imperviousness layer of the 2016 NLCD. The Land Cover layer assigns each 30×30 m pixel located within the continental United States to one of 16 different land cover categories. However, in our habitat analysis, we examined only four categories (deciduous forest, evergreen forest, mixed forest, and scrub/shrub) given that the other 12 land cover types were known a priori as being unsuitable for titmice.

The Tree Canopy Cover layer was used to assess canopy structure (separate from tree species being either deciduous or evergreen) given that we suspected differences in the associations of TUTI and BCTI with canopy density. This layer assigns a percent canopy cover value in 1% increments to each 30×30 m pixel. Similarly, the Developed Imperviousness layer assigns the percentage of pavement and building cover (mainly asphalt and concrete) within a 30×30 m pixel in 1% increment values. This GIS layer was incorporated because it more directly measures levels of anthropogenic disturbance than the developed land cover categories of the Land Cover layer. Including the

Developed Imperviousness layer was important considering that the two titmice species and hybrids have geographic ranges that overlap areas impacted by human development and have been noted to occur in developed areas (Smith 1910; Dixon 1955; Brauning 1992). To summarize, we analyzed six variables from the NLCD raster layers to examine habitat affinities of TUTI, BCTI, and hybrids: deciduous forest (Dec), evergreen forest (Eve), mixed forest (Mix), scrub/shrub (Shr), canopy cover (Can), and impervious surface cover (Imp).

GIS processing of data

We used ArcGIS Pro 2.8.2. (ESRI Software) to derive grid-based estimates of the six land cover variables and TUTI, BCTI, and hybrid abundances. A shapefile of counties in Texas and Oklahoma (as described previously) was uploaded to visualize and define the boundaries of the study region (Fig. 1). The three NLCD raster layers were then uploaded and the 'Clip Raster' tool was used to clip each layer to the extent of the study region (i.e., the selected counties). The three eBird datasets (TUTI, BCTI, and hybrid counts from 2012 to 2020) were imported as three separate point feature class layers and displayed over the NLCD raster layers. Each "point" corresponded to the latitude/longitude coordinates of an eBird checklist with the associated total count of the given species. We then used the 'Create Fishnet' tool to overlay a grid of 10×10 km cells across the study region. The 'Aggregate Points' tool was used to sum the total count (over the 9 years) each of TUTI, BCTI, and hybrids within each 10×10 km grid cell across the study region. These total counts were log-transformed ($+1$) given that the raw counts spanned a range of 0 to over 10,000.

We obtained estimates of the six landcover variables within each grid cell through the use of various spatial analysis tools in ArcGIS Pro. For the NLCD Land Cover layer, we used the 'Tabulate Area' tool to calculate the total area and percentage of each land cover type within each 10×10 km grid cell. For the NLCD Tree Canopy and Developed Imperviousness layers, we used the 'Zonal Statistics as Table' tool to calculate the mean percentages of canopy cover and impervious cover across all 30×30 m pixels within each grid cell (i.e., a 10×10 km grid cell has 111,111 pixels). These data extraction processes for both the eBird and NLCD data were repeated utilizing fishnets with 5×5 km and 2.5×2.5 km grid cells. The data extracted from the three different spatial scales were treated as three separate data sets and analyzed separately. The more highly resolved grids (smaller cell size) were intended to examine differences in habitat associations at finer (more local) spatial scales. Also, comparison among the three grid-cell sizes allowed for an assessment of whether habitat associations varied with spatial scale. By reducing the grid cell size, some error in the total counts of the three titmice types may have been introduced if some of the eBird data were spatially imprecise. However, again, most checklists were from eBird outings spanning less than 1 km, therefore error was likely minimal. Other studies have assumed a similar level of spatial precision of eBird data in their analyses (Muller et al. 2018; Huang et al. 2021; Brooks et al. 2022; Echeverría-Caro et al. 2022). Lastly, we removed (from further consideration) all grid cells that had a total titmouse count of less than 50. This removal step was done at all spatial scales to ensure that we only included grid cells that had been adequately sampled. After removal, the final datasets had the following number of grid cells: 10×10 km (N = 498), 5×5 km (N = 784), and 2.5×2.5 km (N = 1041).

Statistical analysis

We used partial redundancy analysis (RDA) to examine the habitat associations of TUTI, BCTI, and their hybrids. A separate RDA was conducted on the 10×10, 5×5, and 2.5×2.5 km datasets in RStudio 4.0.5 using the 'rda' function of the 'vegan' package. RDA was chosen because it combines multiple linear regression with principal components analysis (PCA) and thus is appropriate for analyzing data in which there are multiple response variables (e.g., species abundances) and multiple environmental predictor variables (Borcard et al. 2018). Multivariate multiple linear regression allowed us to test for significant relationships between the landcover variables and abundances of the three titmice types simultaneously, while PCA helped us visualize the direction and magnitude of those relationships. Partial RDA (rather than standard RDA) was used because we wanted to determine if the habitat variables significantly explained the distributions of the three titmice types when the effect of the geographic location of a grid cell (i.e., latitude and longitude) was held constant. Therefore, latitude and longitude were included as conditioned variables within the RDA model at each spatial grid-cell size. Furthermore, the quadratic terms of each of the six landcover variables, along with latitude and longitude, were included in the RDA models. We hypothesized that hybrids would associate with intermediate habitat types; that is, habitats comprised of a mixture of landcover types that either parent species associates with in allopatry and/or habitats that have an intermediate form of landcover variables deemed important in allopatry. Therefore, the quadratic terms were included so as to identify any relationships that would involve the species responses (logged raw abundances) being at a maximum (or minimum) at intermediate values of each landcover variable. Including the quadratic terms for latitude and longitude as conditioned variables accounted for the possibility that titmouse abundance would be greatest within the middle of the hybrid zone. Mathematically, raw quadratic terms are always highly correlated ($r > 0.97$) with their respective non-quadratic variables, therefore, it is best to compute orthogonal polynomials before conducting analyses (Borcard et al. 2018). We used the 'polyvars' R function (Borcard et al. 2018) to create a matrix of the landcover and spatial variables and their respective orthogonal quadratic terms. Thus, each RDA model included a total of twelve predictor variables (each landcover variable and its squared term) and four conditioned spatial variables (latitude, longitude, and their squared terms).

As a preliminary check for correlation among landcover variables, variance inflation factor (VIF) scores and correlation coefficients between all landcover variables were examined before creating the RDA model for each spatial scale. At the 5×5 km and 2.5×2.5 km spatial scales, all landcover variables were included due to their low VIF scores (most were < 4 and all were < 10). VIF < 10 is generally considered sufficiently low to alleviate any concerns about multicollinearity (Neter et al. 1989; Chatterjee et al. 2000). At the 10×10 km spatial scale, percent evergreen forest had a high VIF score (> 10) due to its strong correlation with mean canopy cover ($r = 0.86$). Therefore, mean canopy cover was removed from the model, which reduced the VIF score to 2.03 for percent evergreen forest. A permutational ANOVA was then used to determine the significance of all partial RDA models with the 'anova.cca' function in 'vegan'. If the model was significant, additional permutational ANOVAs were used to identify significant RDA axes and landcover variables (Borcard et al. 2018).

Results

For all three spatial grid cell sizes (10×10 km, 5×5 km, and 2.5×2.5 km), grid cells with exclusively BCTI individuals were more common across the study region than those with exclusively TUTI individuals, and only a few grid cells (N = 2 for 10×10 km, 3 for 5×5 km, and 5 for 2.5×2.5 km) contained hybrids alone (Table 1). TUTI were absent from grid cells more often than BCTI or hybrids. TUTI were recorded in 211, 274, and 291 grid cells (10, 5, and 2.5 km respectively) whereas BCTI were recorded in 343, 560, and 793, followed by hybrids recorded in 272, 393, and 528 grid cells. Grid cells that contained both BCTI and hybrids occurred most frequently across the study region at all spatial scales (Table 1). Plots of log-transformed data revealed grid cells with varying abundances of the three titmouse types (Fig. S1).

Means of the six landcover variables were calculated across grid cells for each titmice category (BCTI only, TUTI only, and BCTI/TUTI/hybrids all present) at all three spatial scales (Fig. 3). Although these mean values are only a preliminary examination, some apparent differences in habitat associations were revealed between the two parent species, and as predicted, hybrids were found to have some habitat associations intermediate between the two parent species, although some associations were found to be similar to that of BCTI. For all three grid cell sizes, cells with only BCTI had substantially higher mean percent shrub coverage than those with only TUTI (Fig. 3). Furthermore, grid cells with only BCTI individuals and grid cells with both parents and/or hybrids present had similar means for percent evergreen and canopy cover, which were both considerably higher than grid cells with only TUTI (Fig. 3). Grid cells with only TUTI had slightly greater mean percent imperviousness and deciduous cover than did BCTI-only cells and cells containing both species and/or hybrids (Fig. 3). Grid cells with both parents and/or hybrids present had intermediate percentages for shrub cover at all three spatial scales, and intermediate values for mean percent imperviousness at the 10×10 and 5×5 km scales (Fig. 3).

At all three spatial grid cell sizes, partial RDA models were significant ($P < 0.001$) as revealed by the permutational ANOVAs. For each partial RDA, only the first two RDA axes were statistically significant ($P < 0.001$) (Table 2). However, the eigenvalues of the RDA axes were considerably lower than the eigenvalues of the unconstrained axes (e.g., PC1, PC2, and PC3) at all spatial scales. PCA axes explained much more of the variation in titmice abundances than did RDA axes (Table 2). This finding verified our initial presumption that geographic position (latitude and longitude as included in the PCA) of

Table 1 The number of grid cells that contain different combinations of BCTI, TUTI, and/or hybrids at each spatial grid cell size as based on eBird data (2012–2021)

Combination	Grid cell size (km)		
	10×10	5×5	2.5×2.5
BCTI only	128	234	337
TUTI only	94	154	170
Hybrid only	2	3	5
BCTI, TUTI	4	6	6
BCTI, hybrid	157	276	408
TUTI, hybrid	59	70	73
BCTI, TUTI, hybrid	54	44	42

a grid cell would greatly influence the recorded abundances of the three titmice types; hence our use of partial RDA models to control for the effect of geographic location.

Despite the strong effect of geography on titmice abundances, many of the landscape variables were found to be statistically significant ($P < 0.05$) in the partial RDA models (Table 3 and Fig. 4) thus revealing habitat associations of the two titmouse parent species and hybrids. At all three spatial scales, mixed forest was the only variable in which the linear and quadratic (squared) terms were both statistically significant ($P < 0.05$) (Table 3). For the other five landcover variables, either the linear or quadratic term was statistically significant ($P < 0.05$) (Table 3).

The biplots (Fig. 4) revealed the habitat associations of each titmouse species and hybrids. In the biplots, a species vector near to the vector of a landcover variable indicates a correlation (i.e., habitat association) and the length of the landcover variable is proportional to the amount of variance in titmouse abundance explained by that variable (Table 3). At all three grid-cell sizes, TUTI associated most strongly with the percentage of mixed forest cover. BCTI was most strongly associated with the percentage of evergreen forest cover and impervious surface cover at all grid-cell sizes (Fig. 4). Hybrid abundance did not correlate with any single landcover variable, except possibly the squared term for shrub cover at the 2.5×2.5 km cell size. Although no single landcover variable could be identified as a definitive characteristic of the habitat of hybrids, hybrid individuals do appear to be more frequent in a habitat type that is intermediate between that of the two parent species. This result is shown in the biplots wherein the vector for hybrids is exactly intermediate between the vectors for TUTI and BCTI. Contrary to our prediction, there was no apparent correlation between hybrid abundance and disturbed habitat (percent impervious cover) (Fig. 4.)

Discussion

The primary goal of our study was to evaluate the influence of habitat characteristics on the abundances of TUTI, BCTI, and their hybrids across their hybrid zone, and more specifically examine whether hybrids tend to occupy an intermediate habitat type. The role of habitat in either inhibiting or influencing hybridization and thus eventual speciation (or lack thereof) continues to be studied by evolutionary ecologists (Schluter 2001; Wolf et al. 2001; Seehausen et al. 2008; Pierce et al. 2017). Considering TUTI and BCTI share similar foraging and nesting strategies (Dixon 1961), and that habitat characteristics vary throughout the study region, we reasoned that habitat associations within the contact zone might differ between the parent species and thus presumably reduce interspecific competition, which in turn would decrease the probability of hybridization and promote the maintenance of species boundaries (Endler 1977; Seehausen et al. 2008). Specifically, we hypothesized that the different habitat associations of TUTI and BCTI known to exist outside of the hybrid zone would be maintained within the hybrid zone and that hybrids would associate with a habitat type considered to be intermediate between those habitats of the two parent species and with more disturbed landscapes. This hypothesis was partially supported by the results obtained in our study.

Preliminary inspection revealed that grid cells containing only BCTI individuals had higher percentages of shrub, evergreen forest, and canopy cover and lower percentages of mean impervious cover and mixed forest than did cells containing only TUTI (Fig. 3). Grid cells with observed or probable hybrids present had means for percent shrub cover and

Fig. 3 Mean values of the habitat variables across grid cells at the three different spatial scales (top panel ▶ − 10 km, middle panel − 5 km, bottom panel − 2.5 km) categorized as only BCTI present, only TUTI present, or both parents and/or hybrids present as based on the eBird data. The standard deviation (SD) bars are set to 0.5 × SD for display purposes

imperviousness that were intermediate between the two parent species, but were also found to have means for percent evergreen forest and canopy cover that were similar to those for BCTI-only cells (Fig. 3). This similarity may have been due to the hybrid grid cells also containing substantial proportions of BCTI (more so than TUTI). That is, results for "hybrid" cells likely reflect the habitat associations of BCTI given that it was the most abundant titmouse type within the hybrid zone (Table 1). BCTI habitat associations may have skewed the results for hybrids. Nonetheless, the redundancy analyses (which were not based on categorizing grid cells) revealed some of the same habitat associations. According to the biplots (Fig. 4), BCTI are more likely to be found in landscapes with higher proportions of evergreen forest and lower proportions of mixed forest, whereas TUTI are the opposite. Hybrids were not strongly correlated with any of the landcover types, which implies that hybrids might exist most frequently in landscapes that have a mixture (mosaic) of the landcover types that are preferred by either parent species. The quadratic term for shrub cover was the one landcover variable that hybrids strongly associated with, but only at the 2.5 × 2.5 km spatial scale. This suggests that hybrids are more likely to be found in landscapes with intermediate levels of shrub cover.

In general, our results were in line with the habitat associations exhibited by BCTI and TUTI throughout the allopatric portions of their geographic ranges. BCTI tend to associate with higher proportions of evergreen and mixed forest, and TUTI with higher proportions of deciduous forest (Smith 1910; Dixon 1955; Miller 1955; Conner et al. 1983; Carter 1986; Brauning 1992). Regarding mixed forest, we found the opposite for the two parent species within the contact zone. That is, TUTI was more strongly associated with mixed forest than was BCTI. However, this finding is not entirely contrary to the known habitat associations of each species. The mixed forest cover type is a mixture of evergreen and deciduous vegetation. Therefore, our result may reflect that BCTI has a strong aversion to the deciduous component of mixed forest whereas TUTI has a stronger preference for such vegetation.

Associations or differences in mean canopy cover were not detected among the grid cells occupied by the three titmice types contrary to the reported preference of TUTI for denser canopy cover than BCTI (Dixon 1955; Conner et al. 1983; Brauning 1992), and hybrids were not found to be associated with higher levels of impervious cover (disturbance) as we predicted. These unexpected results may be due to the broad scale of the habitat analysis. Perhaps analysis at the territory or neighborhood scale (e.g., a few hectares surrounding a nest site) would better reveal the importance of canopy cover and impervious surfaces in defining differences in the habitats of the three titmice types. However, it is possible that land cover types are more important in determining BCTI and TUTI abundance than is overall canopy cover and imperviousness. In either case, further examination of the habitat associations of the parent species within and immediately outside of the hybrid zone should be conducted, especially at spatial scales that involve the territories of titmice, to draw stronger conclusions.

Our results were mostly in line with theory that emphasizes the importance of ecological variables in contributing to the formation and maintenance of hybrid zones that arise through secondary contact (Endler 1977; Moore 1977; Seehausen et al. 2008). Taxa that
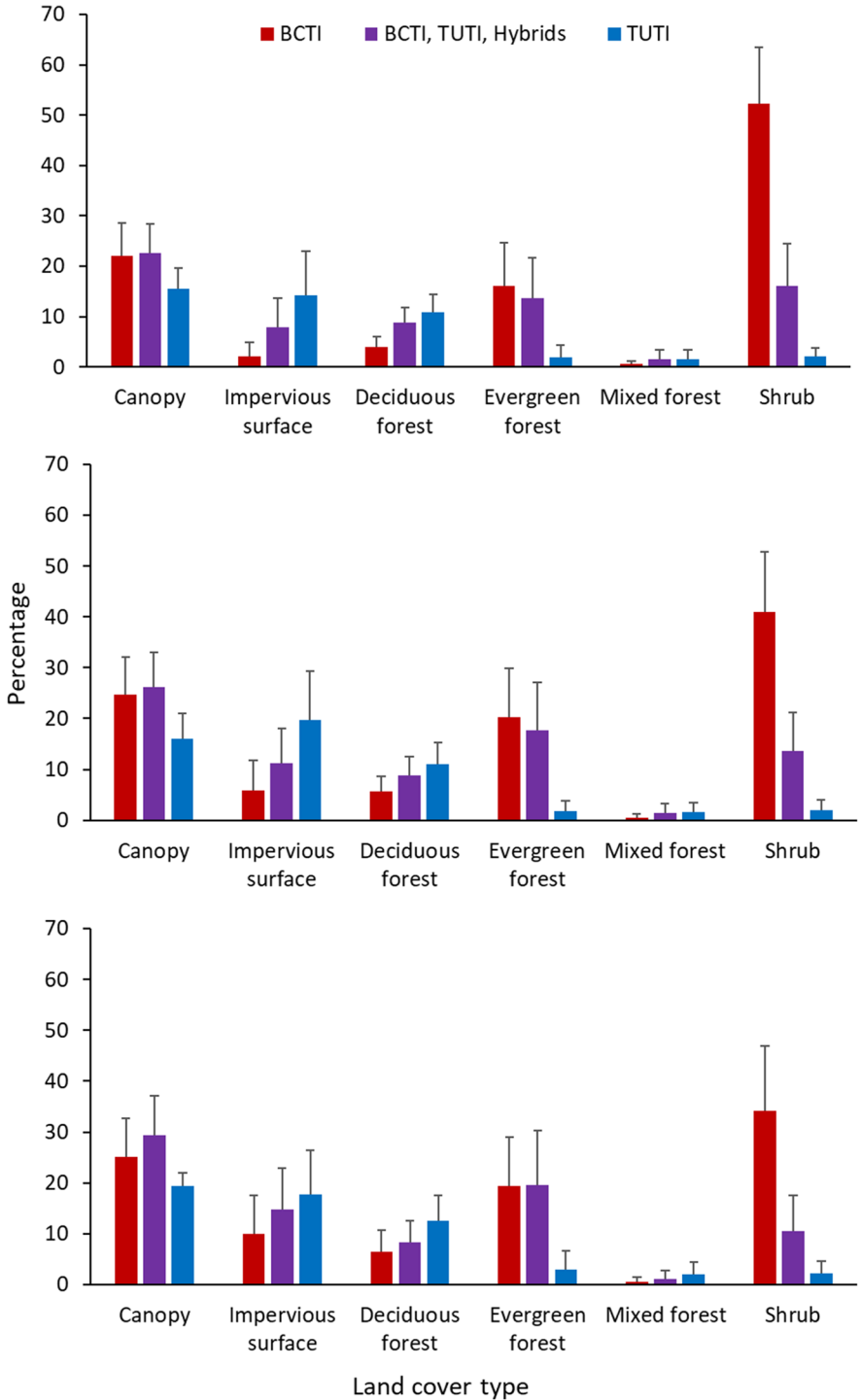
Table 2 Results of the partial RDA models compared to PCA for each spatial grid cell size. F- and P-values for the RDA axes were obtained from permutational ANOVAs

| Model (scale)	Axis	Variance (eigenvalue)	F	P	RDA cum. var. explained	PCA cum. var. explained*
10×10 km	RDA1	0.129	60.01	<0.001	0.098	0.480
	RDA2	0.117	54.46	<0.001	0.186	0.695
	RDA3	0.022	10.37	0.201	0.203	0.797
	Residual	1.054	–	–	–	–
5×5 km	RDA1	0.125	92.56	<0.001	0.097	0.493
	RDA2	0.112	82.48	<0.001	0.183	0.703
	RDA3	0.010	7.19	0.860	0.191	0.809
	Residual	1.051	–	–	–	–
2.5×2.5 km	RDA1	0.103	96.29	<0.001	0.079	0.504
	RDA2	0.090	84.20	<0.001	0.148	0.732
	RDA3	0.007	6.70	0.866	0.153	0.847
	Residual	1.108	–	–	–	–

*Last column gives the cumulative variance explained by each successive PCA axis

Table 3 Variance in titmouse abundances explained by each landcover variable at each grid cell size as revealed by the permutational ANOVAs

Landcover variable	10×10 km		5×5 km		2.5×2.5 km		Significant at all scales?
	Variance explained	P	Variance explained	P	Variance explained	P	
Deciduous	0.017	<0.001	0.036	<0.001	0.032	<0.001	Yes
Deciduous^2	0.005	0.085	0.013	<0.001	0.019	<0.001	No
Evergreen	0.037	<0.001	0.045	<0.001	0.031	<0.001	Yes
Evergreen^2	0.011	0.005	0.003	0.089	0.002	0.158	No
Mixed	0.060	<0.001	0.071	<0.001	0.060	<0.001	Yes
Mixed^2	0.009	0.015	0.010	0.003	0.012	<0.001	Yes
Shrub	0.068	<0.001	0.028	<0.001	0.009	0.053	No
Shrub^2	0.025	<0.001	0.008	0.002	0.003	<0.001	Yes
Canopy	–	–	0.011	<0.001	0.008	0.004	Yes
Canopy^2	–	–	0.002	0.226	0.002	0.174	No
Impervious	0.016	<0.001	0.018	<0.001	0.021	<0.001	Yes
Impervious^2	0.019	<0.001	0.002	0.236	0.001	0.347	No

genetically diverged in allopatry might have diverged in their ecological requirements due to local adaptation. These differing requirements therefore limit the distributions of the parent species in their current sympatric ranges where hybridization occurs and helps maintain some degree of reproductive isolation. Hybrids are thus thought to be restricted, or perhaps even better adapted, to areas where ecological characteristics are intermediate or transitional between those preferred by either parent species (Rising 1969; Moore 1977; Hewitt 1988). Bullock's Orioles (*Icterus bullokii*) are physiologically adapted (i.e., high

Fig. 4 Biplot of species scores (TUTI, BCTI, and hybrids shown as red vectors) and scores of the landcover (i.e., constraining variables; blue vectors) as obtained from a partial RDA at each of the grid cell sizes. Vectors are only shown for those landcover variables that were highly significant ($P<0.001$, Table 3). In the partial RDA, latitude, longitude, and their respective quadratic terms were the conditioned variables. The "scaling 2" option was used in the plot () command of the 'vegan' package which means that the angle between two vectors represents correlation strength with smaller angles representing stronger correlation. Length of vector corresponds to eigenvalue and proportion of explained variance in titmice abundances. See text for variable abbreviations and species acronyms
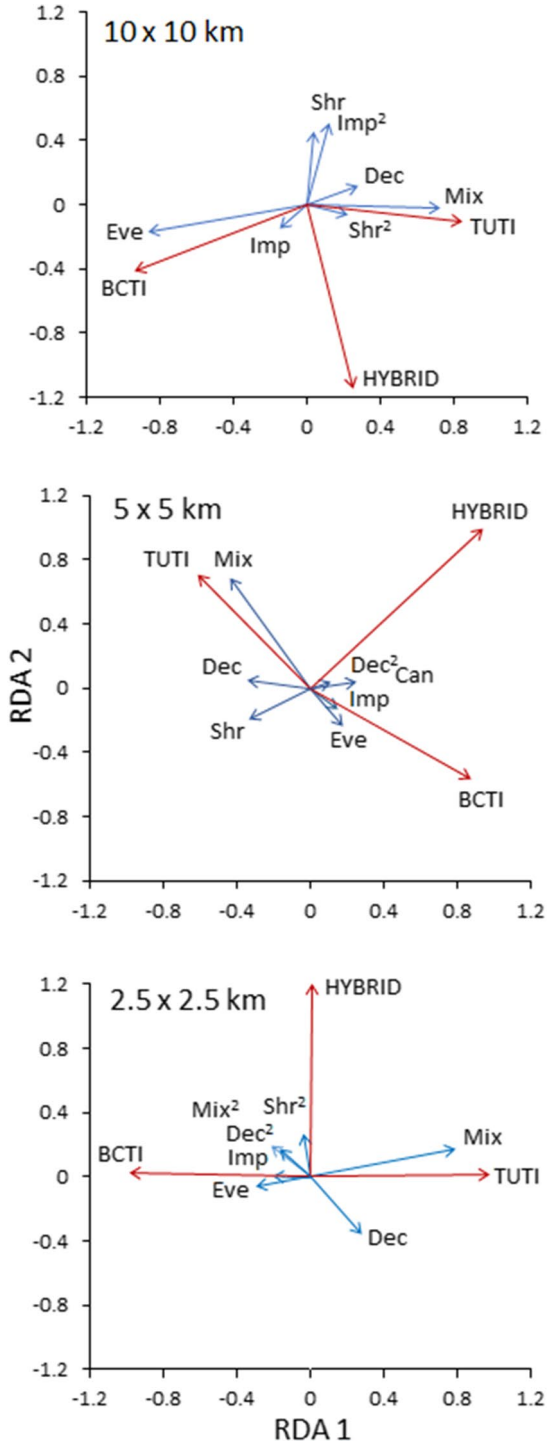
tolerance to hyperthermia and water restrictions), to the relatively xeric environment of the Rocky Mountains, whereas Baltimore Orioles (*Icterus galbula*) are adapted (i.e., low tolerance to hyperthermia and water restrictions) to the mesic environment of eastern deciduous forests. Hybridization between these two wide-ranging species is therefore narrowly restricted to the Rocky Mountains/Great Plains ecotone where habitat features from both environments meet and become heterogeneous (Rising 1969). Within Colorado, hybrids of Steller's Jays (*Cyanocitta stelleri*), birds of primarily montane coniferous forests, and Blue Jays (*Cyanocitta cristata*), birds primarily of deciduous wooded prairies, have been found along ecotonal areas of pine and deciduous forests (Rising 1983). Furthermore, the Spotted Towhee (*Pipilo erythrophthalmus arcticus*) (western subspecies) and Unspotted towhee (*P. e. erythrophthalmus*) (eastern subspecies) hybridize where the open chapparal environment of the western plains meets deciduous forests in northern Colorado, Nebraska, and South Dakota (Sibley and West 1959; Rising 1983).

Although our analysis revealed hybrid individuals occupying a type of landscape intermediate between that of the parent species, we are not certain that this interpretation would also apply at a finer spatial scale. It would be useful to have detailed descriptions of habitat within territories in order to determine whether hybrid individuals are associated with intermediate habitat types (e.g., mixed tree cover) at that fine spatial scale. At the territory level, it is possible that hybrids are not associated with an intermediate type of habitat but rather associate with either of the habitat types (e.g., deciduous and evergreen tree cover) also occupied by the parent species. At the landscape level, the ecotone consists of a mosaic of these cover types such that hybrids associate with an intermediate habitat type when it is defined as a broad mosaic. The importance of different habitat preferences and selection in maintaining species boundaries within the hybrid zone could be deduced with more detailed information on the composition of the habitat types within the territories held by BCTI, TUTI, and hybrid individuals. Future studies should incorporate neighborhood and territory level habitat analyses to confirm the habitat association patterns detected within our broad scale study, and to discover potential habitat associations our analysis was unable to detect.

Our analysis did not find the predicted association between hybrid individuals and human disturbed environments. However, the effect that urbanization has on hybridization within the BCTI×TUTI species complex should also be further investigated at the neighborhood and territory level considering recent studies found increased hybridization rates in or near urbanized areas where habitats can become more heterogenous or fragmented. Carpenter et al. (2022) found that the hybrids between the eastern and western subspecies of Warbling Vireo (*Vireo gilvus*) were most common in areas representing a transition between forest and non-forested (human altered) areas. Graham et al. (2021) revealed admixture rates of four North American chickadee species (*Poecile*) to be highest in urban parkland and mixed/ intermediate habitats. Admixture rates were highest within species pairs that included Black-capped Chickadees (*P. atricapillus*), which are considered to be habitat generalists. Similar to Black-capped Chickadees, BCTI are believed to have less specialized habitat preferences than their sister species (Smith 1910; Dixon 1955; Miller 1955; Carter 1986), and therefore may be more tolerant to habitat changes produced by urbanization. Indeed, in our study, we found BCTI to be more strongly associated with impervious surface cover than was TUTI (Fig. 4). Further study is needed to determine whether urban expansion in central Texas plays a partial role in the rates of hybridization between BCTI and TUTI. Previous studies of hybridization in birds have utilized some form of genetic testing on captured birds to determine their ancestry and degree of genetic admixture. Citizen science data (e.g.,

eBird) can be beneficial in obtaining large sample sizes for more powerful statistical analyses and for illustrating large scale patterns, but the risk of identification errors is larger, and it is difficult to measure the extent or direction of geneflow given that all observations are strictly phenotypic. Although phenotype is a fairly reliable determinant of genotype in titmice (Curry 2104), it is still possible for a bird that physically looks like either a pure BCTI or TUTI, to have alleles from the other parent species (cryptic hybrids). Therefore, hybrid individuals are likely more prominent within the hybrid zone than what our study detected. Future studies of this system could use genotyping of captured birds to confirm the classification of titmice and produce more accurate estimates of their abundances across the hybrid zone although this would entail much effort and time.

Further examination of the magnitude and direction of the relationships between titmice ancestry and specific habitat characteristics may help determine whether habitat acts as a selective force on genotypes within the BCTI×TUTI species complex. Such findings could help resolve questions over the initial formation of the hybrid zone and whether we could expect distributions of the parent species and hybrids to change as habitat continues to evolve within Texas and Oklahoma. In a greater context, a study combining detailed genetic data and habitat analyses at more local spatial scales could provide further insight into the role of habitat, anthropogenic disturbance, directionality of gene flow, and species range expansion in facilitating hybridization in birds and perhaps other species.

Declarations

Conflict of interest The authors have not disclosed any competing interests.

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