IBIS
BOU international journal of avian science
Ibis (2018)
doi: 10.1111/ibi.12674

Characterizing patterns of introgressive hybridization between two species of *Tyrannus* following concurrent range expansion

ALEXANDER J. WORM,[1]* DIANE V. ROEDER,[2] MICHAEL S. HUSAK,[2] BROOK L. FLUKER[1] & THAN J. BOVES[1]

[1]*Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72467, USA*

[2]*Department of Agriculture, Biology, and Health Sciences, Cameron University, 2800 West Grove Boulevard, Lawton, OK 73505, USA*

Hybridization between two species typically occurs when allopatric or ecologically dissimilar species expand into areas of secondary contact or habitat transitions. However, as species' ranges shift rapidly in response to environmental change, the potential for novel types of ephemeral hybrid zones exists. Here, we document and describe the occurrence, prevalence and symmetry of a previously undocumented hybrid zone involving two sympatric, ecologically similar sister species that have been expanding their ranges eastward in the central USA: Scissor-tailed Flycatchers *Tyrannus forficatus* and Western Kingbirds *Tyrannus verticalis*. We identified cases of hybridization and introgression using analyses of eight microsatellite DNA loci and a single mitochondrial gene. We also evaluated short-term reproductive consequences of hybridization for both species by surveying for both species and potential hybrids at the periphery of their ranges in northeastern Arkansas and western Tennessee, USA. Genetic data revealed bi-directional backcrossing at the periphery of the species' ranges, including a cryptic hybrid. We also analysed DNA of putative 'pure' individuals from other parts of their ranges and detected two cryptic admixed individuals, suggesting backcrossed individuals from the periphery may be dispersing to breed or that hybridization events have occurred in the core. Finally, our results suggest that there are no short-term reproductive consequences of hybridization for the two species. In total, hybrid zones that occur at the edges of expanding, sympatric ranges may be ephemeral; we suggest they play an important role in introgression and may have long-standing impacts for sympatric sister species. Exploring the extent of hybrid zones such as this for other range-expanding taxa will elucidate whether this type of hybrid zone is unique or a common occurrence.

Keywords: hybrid fitness, hybrid zone, Scissor-tailed Flycatcher, Tyrannidae, Western Kingbird.

Hybridization and subsequent genetic introgression occur frequently in nature (Mallet 2005). For example, 10% of bird species are currently known to hybridize and produce offspring (Grant & Grant 1992, Mallet 2005). Depending on its prevalence, hybridization can have evolutionary, ecological and conservation consequences. Hybridization events may have positive individual and population level effects by increasing genetic variation and introducing novel alleles, which can facilitate rapid adaptation and even species formation (Mavarez *et al.* 2006, Abbott *et al.* 2010, Lamichhaney *et al.* 2018). Alternatively, these events can have negative consequences on individuals or populations, such as reduced fitness (i.e. hybrid inviability, sterility, loss of adaptive alleles; Rhymer & Simberloff 1996, Arnold 1997, Muhlfeld *et al.* 2009), displacement and extinction of one or both parental groups (Rhymer & Simberloff 1996) or convergence of distinct taxa (Grant *et al.* 2004, Seehausen 2006). Whether beneficial or detrimental, the extent of introgressive

*Corresponding author.
Email: alexanderj.worm@gmail.com

hybridization can alter species' evolutionary trajectories.

In addition to our understanding of their consequences, our knowledge of the spatial and temporal contexts of hybridization and introgression has recently changed. Traditionally, natural hybridization events were primarily thought to occur and impact individuals/populations in secondary contact zones of closely related allopatric species, often following range expansion of one or both species (e.g. tension hybrid zones; Mayr 1963, Barton & Hewitt 1985, Arnold 1997, Mallet 2005). However, advancements in genetic techniques have now shown that even localized, ephemeral hybridization events can result in introgression across population ranges (Mallet *et al.* 2016). For example, mosaic hybrid zones are ephemeral areas of habitat transitions (e.g. during ecological succession) found throughout a species' range where hybridization occurs between two ecologically dissimilar species (Rand & Harrison 1989, Larson *et al.* 2013). This type of hybridization has been shown to lead to introgression in portions of the species' ranges (Vallender *et al.* 2009, Duckworth & Semenov 2017). Despite the potential impact of these ephemeral zones, it is likely that their occurrence is underestimated because they exist over a much shorter time frame than stable hybrid zones, thereby reducing the likelihood that they will be documented or explored (Moore 1977, Barton & Hewitt 1985).

Although hybridization events and ephemeral zones are probably underestimated, the conditions that lead to their occurrence are broadly recognized. One proximate cause of hybridization is a lack of conspecific mates available for reproduction; hybridization often occurs when at least one species is relatively rare (Hubbs principle: Hubbs 1955). For example, at the peripheries of species' ranges, especially those that are expanding, densities are often low and greater hybridization rates exist (Rhymer & Simberloff 1996, Allendorf *et al.* 2001). As species' ranges continue to shift in response to modern environmental changes (Walther *et al.* 2002, Hitch & Leberg 2007), novel hybrid zones, either stable or ephemeral, may be expected to form (Chunco 2014, Taylor *et al.* 2015) and may differ spatially or temporally from previously described patterns.

One such novel hybrid zone may occur when species in sympatry simultaneously undergoing range expansion hybridize at the periphery of their ranges. Anecdotal evidence for this type of hybrid zone has been accumulating over the past decade for Scissor-tailed Flycatchers *Tyrannus forficatus* and Western Kingbirds *Tyrannus verticalis* in the central USA. These species of Tyrannidae are broadly sympatric sister species (MacPherson 2017) that have been simultaneously expanding their breeding ranges eastward over the past 50 years (Gamble & Bergin 2012, Regosin 2013). Based on field observations by birdwatchers at the easternmost periphery (eastern Arkansas and western Tennessee, USA), the birds appear to be regularly hybridizing in this region (eBird 2012; J. Wilson pers. comm.). However, despite observations suggesting the occurrence of hybridization and backcrossing, no systematic investigation of the presence, extent or consequences of the presumed hybridization in the region yet exists. Additionally, if hybridization and backcrossing have occurred, it is unknown whether genetic introgression into other portions of the range has also occurred. Thus, the aims of this study were to: (1) use mitochondrial (mt) and nuclear DNA to describe the occurrence, prevalence and symmetry of hybridization and introgression for each species at both the periphery and throughout their ranges, and (2) assess short-term fitness consequences of hybridization and introgression at the periphery of each species' range. By evaluating this new, potentially ephemeral hybrid zone, we hope to elucidate the context and consequences of hybridization for species expanding their ranges.

METHODS

Study species and study area

Tyrannus forficatus and *T. veritcalis* are Nearctic-Neotropical migrant sister species (MacPherson 2017) that are largely sympatric and ecologically similar. During the breeding seasons, *T. forficatus* are found throughout the southern Great Plains of the USA in: Texas, Oklahoma, Kansas, Nebraska, Missouri, Arkansas and Louisiana (Regosin 2013) and *T. verticalis* are found throughout the same region, as well as farther west in the USA and into southwestern Canada (Gamble & Bergin 2012). Over the past two decades, both species have simultaneously expanded their breeding ranges eastward into eastern Arkansas and western Tennessee, USA (eBird 2012, Sauer *et al.* 2014, Fig. 1). Across their ranges, both species breed in

open and semi-open grasslands, agricultural fields and urban areas (Gamble & Bergin 2012, Regosin 2013). However, at the peripheries of their expanded ranges, *T. verticalis* appear to prefer breeding in highly urbanized settings, while *T. forficatus* use a range of open habitats including urban environments (eBird 2012, A. J. Worm pers. obs.).

Despite broad sympatry and ecological similarity, hybridization between the two species is thought to be very rare. Previously, only four putative hybrids (based on morphology alone) have been documented in the scientific literature, none of which were known to reproduce successfully. These occurred in Texas (Davis & Webster 1970), Oklahoma (Tyler & Parkes 1992), Colorado (Rosenberg *et al.* 1991) and California (Rottenborn & Morlan 2000). Interspecific pairings between *T. verticalis* and vagrant *T. forficatus* have also been reported in California (Bevier 1990) and Minnesota (Fall 1998), but neither of these previous reports resulted in successful reproduction.

Field methods

In northeastern Arkansas and western Tennessee, USA (the eastern periphery of both species ranges),

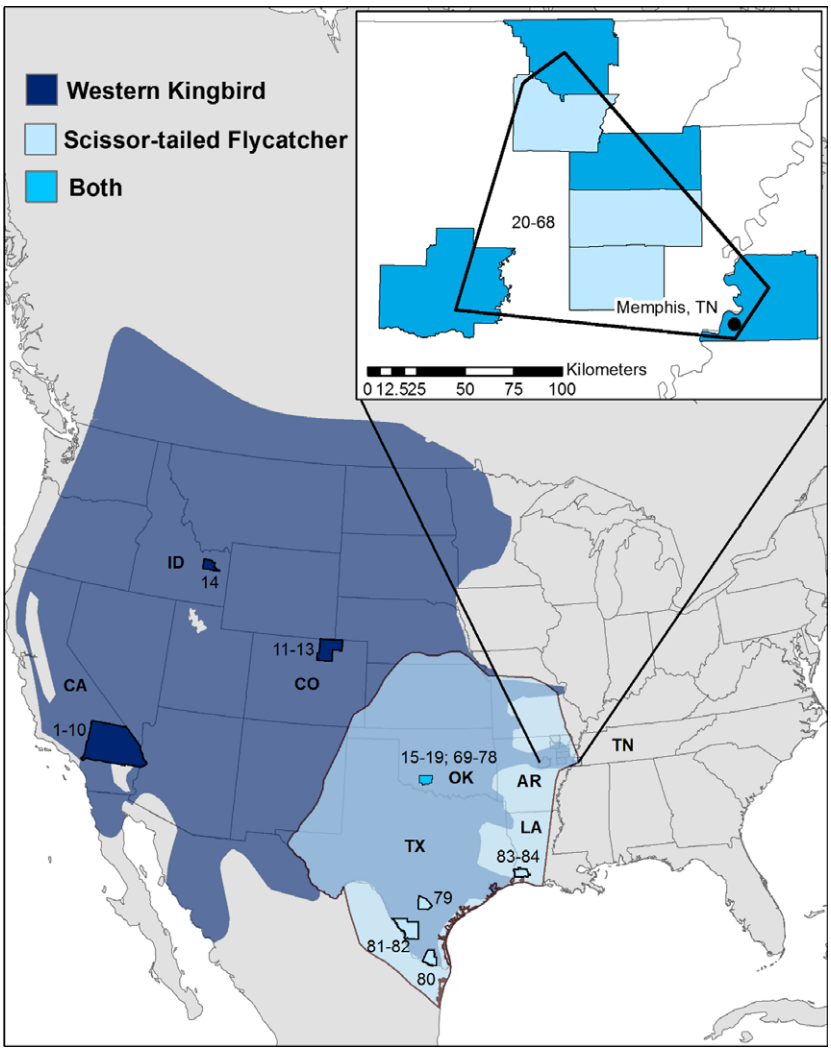

Figure 1. Breeding distribution of Western Kingbirds (*Tyrannus verticalis*; dark blue) and Scissor-tailed Flycatchers (*Tyrannus forficatus*; light blue) modified from Bird Life International (2017). Counties highlighted in dark blue depict locations where Western Kingbird samples were collected, light blue where Scissor-tailed Flycatchers were collected, and intermediate blue where samples from both species where collected. Individual sample numbers correspond to column numbers in STRUCTURE output (Fig. 2).

we located breeding *T. forficatus*, *T. verticalis* and putative hybrids (based on morphology in the field) by conducting systematic surveys from April the end of July in 2014 and 2015. Surveys were centred at reported locations for both species from previous citizen science efforts (e.g. eBird 2012, Sauer *et al.* 2014). At each location, we drove the majority of roads within a 5-km radius, scanning the roadside for both species and walking into concealed areas that made scanning from the road difficult. While driving to and from these locations, we also conducted opportunistic surveys for both species. Once birds were located, we determined pairing status and located nests by following behavioural cues (i.e. parent incubating, feeding young, vocalizations) and scanning suitable nesting structures. Once found, nests were monitored every 3–5 days to determine number of nestlings and nest fate. Nests were considered successful if one or more of the young fledged.

In this same region, we captured as many adult birds as possible using mist-nets in conjunction with conspecific and predator (e.g. American Crow *Corvus brachyrhynchos*) decoys and recorded vocalizations during the 2015 and 2016 breeding seasons. Because nestlings were unable to be sampled from the nests, juvenile birds were opportunistically captured immediately after they fledged. For all individuals captured, we determined age as hatch year if recently fledged, or second-year or after-second-year based on feather moult patterns (following Pyle *et al.* 1997) and extracted ~50 μL of brachial blood that was stored in Queen's lysis buffer until processed (Seutin *et al.* 1991). Putative hybrids were identified in the field based on a combination of plumage coloration and tail morphology. *Tyrannus forficatus* have light grey heads and breasts, pinkish underwings, and long forked tails, whereas *T. verticalis* have darker heads and breasts, yellow bellies, and a square tail bordered with white outer rectrices. We assumed hybrids would have intermediate phenotypes. A U.S. Geological Survey numbered aluminium band and a unique combination of plastic colour bands were fitted to each bird's legs for resighting without recapture.

To provide putative pure parental samples as molecular references and to explore the possibility of introgression across the species' ranges, we also acquired six *T. forficatus* and 14 *T. verticalis* tissue samples from the Louisiana State University Museum of Natural Science (LSUMNS) tissue repository. To limit the likelihood of historical hybridization in these samples, we chose specimens that, as often as possible, came from allopatric portions of the species' ranges (see Fig. 1 for locations of museum specimens). We also obtained 10 *T. forficatus* and five *T. verticalis* blood samples from the historical core breeding range in Oklahoma, USA, where species are sympatric (Fig. 1) but have no apparent hybridization (Gamble & Bergin 2012, Regosin 2013).

Data analysis

Microsatellite genotyping

We determined hybrid status and assessed genetic introgression by comparing individual genotypes for one mitochondrial gene and eight polymorphic microsatellite DNA loci. DNA was extracted from blood and tissue samples using DNeasy Blood and Tissue kit (Qiagen, Germantown, MD, USA; #69504). Fluorescently labelled forward primers and unlabelled reverse primers were used from previous studies (ACG5, ASE9, DPU16, EMIC23, EMID46, EMIZ27, GATA5, SAP22; Roeder *et al.* 2016; Table S1) to amplify eight microsatellite DNA loci for each sample using PCR. DNA reactions were performed in a 10-μL total volume containing 10–50 ng template DNA, 0.2 μM of each primer, 0.2 μM of each dNTP, 0.1 U of Taq DNA polymerase (Promega, Madison, WI, USA) and 1.5 mM $MgCl_2$, 5× reaction buffer (colourless); the final reaction volume was brought to 10 μL with ddH_2O. The complete thermal profile for PCR was 94 °C for 2 min, then 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s for 35 cycles, followed by a 72 °C extension for 7 min. Fluorescently labelled PCR fragments (HEX and 6-FAM) were mixed with GeneScan 500 ROX Size Standard and visualized on an Applied Biosystems 3730xL DNA Analyzer (Yale School of Medicine Keck DNA sequencing lab). Allele fragments were sized using PEAKSCANNER2 (Life Technologies, Carlsbad, CA, USA).

Genetic diversity of microsatellite DNA

We assessed deviations from Hardy–Weinberg equilibrium (HWE) expectations for *T. forficatus*, *T. verticalis* and putative hybrids using exact tests in GENEPOP v4.2 (Rousset 2008). Linkage disequilibrium (LD) was tested across all pairs of loci in GENEPOP. ARLEQUIN v3.5 (Excoffier & Lischer 2010) was used to calculate the mean number of alleles per locus (A), observed heterozygosity (H_o)

and unbiased expected heterozygosity (H_e) for *T. forficatus*, putative hybrids and *T. verticalis* (Nei 1978).

Hybrid identification

We detected hybrids using the Bayesian clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard *et al.* 2000), which uses allele frequency data to probabilistically assign individuals into clusters (K) without *a priori* information. The STRUCTURE runs were performed under the admixture model with allele frequencies correlated, for $K = 2$ and 10 independent replicates with a burn-in period of 100 000 followed by 1 000 000 Markov chain Monte Carlo (MCMC) iterations. The 10 STRUCTURE replicates for $K = 2$ were averaged through the pipeline CLUMPAK (Kopelman *et al.* 2015) and the figure created in DISTRUCT v1.1 (Rosenberg 2004). In our study, the threshold q_i values were set so $q_i \geq 0.90$ were cluster I (*T. verticalis*) and $q_i \leq 0.1$ were cluster II (*T. forficatus*), and hybrids set as $0.1 < q_i < 0.90$ (Vähä & Primmer 2006).

Amplification and sequencing of mtDNA

We amplified a 1007-bp fragment of the mtDNA NADH dehydrogenase subunit 2 (ND2) gene using primers developed by Sorenson (2003; forward L5216 5′-GGCCCATACCCCGRAA AT-3′ and reverse H6313 5′-ACTCTTRTT TAAGGCTTTGAAGGC-3′). PCR amplifications were performed in 25-μL total volume containing 10–50 ng of template DNA, 0.2 μM of each primer, 0.2 μM of each dNTP, 0.2 U of Taq DNA polymerase (Promega), 1.5 mM $MgCl_2$, 5× reaction buffer (colourless); the final reaction volume was brought to 25 μL with ddH_2O. The PCR thermal profile was as follows: 94 °C for 3 min, then 94 °C for 30 s, 58 °C for 45 s and 60 s at 72 °C for 35 cycles, followed by a 72 °C extension for 7 min. Amplified products were visualized on 1% agarose gels stained with GelRed, and purified using QIAquick PCR purification (Qiagen; #28106). PCR products were sequenced in both directions with the amplification primers and visualized using a 3100 Genetic Analyzer (Applied Biosystems). Sequences were aligned manually and visually confirmed with the chromatograms using CHROMASPRO v2.1.3 (Technelysium Pty Ltd, South Brisbane, Qld, Australia). All sequences are deposited with GenBank (accession numbers MH747665–MH747745).

Maximum likelihood tree and haplotypes: mtDNA

Aligned ND2 sequences were used to construct a maximum-likelihood tree for *T. forficatus*, *T. verticalis* and hybrids using R (R Developer v3.1.3) with the packages 'ape' v4.0 (Paradis *et al.* 2004) and 'phangorn' v2.1.1 (Schliep 2011). We used the function modelTest within the package phangorn to test for our best nucleotide substitution model (based on the lowest Akaike information criterion, AIC_c), which was GTR + I. A published ND2 sequence of a Forked-tailed Flycatcher (*Tyrannus savanna*) was used as the out-group (GenBank accession number: GU816828, Fjeldså *et al.* 2010). The resulting tree was viewed in FIGTREE v1.4.3 (Rambaut & Drummond 2009). The number of unique mtDNA haplotypes was determined using DNASP v5.10 (Librado & Rozas 2009).

Reproductive consequences of hybridization

To assess the short-term consequences of hybridization and introgression on nesting success, we constructed a logistic exposure model that included year (to account for seasonal variability) and species/hybrid status in the nest survival module of MARK v8.0 (White & Burnham 1999) and compared it with a null model of year alone based on AIC_c. Individuals were considered to be hybrids when $0.1 < q_i < 0.90$ (from STRUCTURE assignment) or there was a mismatch of STRUCTURE assignment with mtDNA haplotype. If $\Delta AIC_c \leq 2$, we considered models to have equal support. Using the model that included hybrid status, we also estimated and compared daily nest survival rate (DSR) for *T. forficatus*, *T. verticalis* and hybrids. Additionally, we compared the average number of fledglings produced at the periphery by successful *T. forficatus*, *T. verticalis* and hybrids.

RESULTS

We captured 49 birds at the periphery of the expanding *T. verticalis* and *T. forficatus* ranges. Based on morphology, we putatitively assigned these birds as 10 adult and one fledgling *T. forficatus*, 17 adults and 12 fledgling *T. verticalis*, two putative adult hybrids and seven hybrid fledglings (backcrossed). We also observed, but were unable to capture, two more putative adult hybrids. Combined with the museum specimens and those from Oklahoma, USA, we included 84 individuals in our sample for genetic analysis.

Microsatellite analysis

All eight microsatellite loci were amplified and scored across all samples of *T. forficatus*, *T. verticalis* and hybrids (Tables S1 & S2). We did not find significant deviations from HWE (*P*-values > 0.05) and did not observe linkage between any pair of loci after Bonferroni correction. Overall comparison between *T. forficatus* and *T. verticalis* showed significant differentiation (AMOVA; overall $F_{ST} = 0.06$; $P < 0.001$). In general, when compared with *T. forficatus* ($A = 5.87$, $H_o = 0.62$, $H_e = 0.62$), *T. verticalis* had lower number of alleles ($A = 3.75$), observed heterozygosity ($H_o = 0.41$) and expected heterozygosity ($H_e = 0.42$) based on microsatellites.

For the assignment test, all 10 independent replicates yielded consistent results, and individual's assignment probabilities (q_i) were largely assigned to cluster I (*T. verticalis*; $q_i \geq 0.90$) or cluster II (*T. forficatus*; $q_i \leq 0.1$), with individuals not assigning to either cluster ($0.1 < q_i < 0.90$) as admixed. All putative pure *T. verticalis* based on morphology in the field were assigned to cluster I (*T. verticalis*) with probabilities ≥ 0.90, except one cryptic hybrid individual from the core (Oklahoma, USA), which had an assignment probability of 0.75. All putative pure *T. forficatus* based on morphology in the field were assigned to cluster II (*T. forficatus*) with probabilities of ≤ 0.1, except one cryptic hybrid individual from the core (Texas, USA), which had an assignment probability of 0.16 (Fig. 2). At the periphery, the two adult putative hybrids and five of the seven putative hybrid fledglings were not assigned to either cluster I or cluster II, indicating that these individuals were hybrids (assignment probabilities of 0.12–0.89). The remaining two putative hybrids were assigned to cluster I (*T. forficatus*; assignment probabilities 0.02 and 0.07) but these two individuals possessed *T. verticalis* mtDNA, indicating introgression (Fig. 2).

Mitochondrial DNA analysis

We successfully sequenced mtDNA of 81 birds. One that failed to amplify was a fledgling and its mtDNA haplotype was inferred based on the mother's haplotype (Fig. S1). For the individuals sequenced, a total of 23 haplotypes were detected, nine for *T. forficatus*, six for hybrids, of which four were unique, and 10 for *T. verticalis*. We detected mitochondrial introgression (or a mismatch between our microsatellite assignment and mtDNA assignment) for three individuals at the periphery (Fig. 2). Morphologically, one appeared to be an adult male *T. forficatus* (albeit with a relatively short tail); this had an assignment probability = 0.09 but had a *T. verticalis* mtDNA haplotype. The other two were backcrossed offspring from a single hybrid mother. We detected bidirectional mtDNA introgression; at the periphery, two of the 10 hybrids possessed mtDNA of *T. forficatus*, whereas the other eight possessed *T. verticalis* mtDNA (Fig. 2). For the two cryptic hybrids from other portions of the ranges, both of their mtDNA aligned with morphology (Fig. 2).

Reproduction

The species/hybrid status model was not strongly supported when compared with the null model ($\Delta AIC_c = 0.46$), indicating species/hybrid status did not affect nest success. DSR for nests with at least one hybrid parent (determined genetically) overlapped with both pure parents rates (0.97 ± 0.018 se; $n = 7$; Fig. 3). From successful nests, the number of fledglings produced between the three groups was very similar (*T. forficatus* = 2.7 ± 0.22 se, $n = 27$; *T. verticalis* = 2.5 ± 0.19 se, $n = 23$; hybrids = 2.7 ± 0.48 se fledglings, $n = 4$). Three hybrid adults (two females and one male) that backcrossed with pure mates fledged 19 putative backcrossed offspring (seven were captured and all were genetically confirmed as hybrids). One of these adult hybrids (female) also successfully double-brooded during 2015 (which is uncommon in both species; Gamble & Bergin 2012, Regosin 2013). Finally, we observed bi-directional backcrossing; hybrid females successfully backcrossed with pure males of both species. Photographic examples of both parental and adults and fledglings can be seen in Fig. 4.

DISCUSSION

In this study, we genetically confirmed introgressive hybridization between two sympatric species at the expanded periphery of their breeding ranges and found evidence that introgressed individuals may have spread into other portions of their ranges as well. Introgression may occur via natal or breeding dispersal, as we also provided evidence that hybridization does not negatively affect short-term

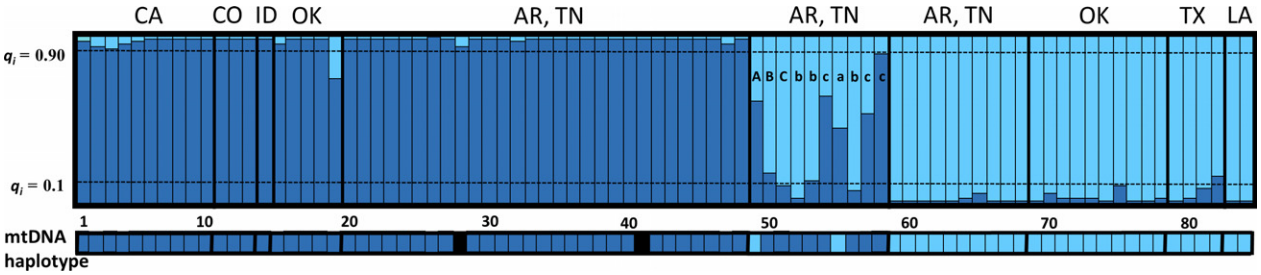

Figure 2. Bayesian admixture analysis of microsatellite data, n = 84: the y-axis represents individual proportion of membership (q_i) of Western Kingbirds in dark blue and Scissor-tailed Flycatchers in light blue, estimated using STRUCTURE with K = 2. Individuals are represented as vertical bars grouped in two clusters. Individuals were considered admixed if q_i values were $0.1 < q_i < 0.90$; mtDNA assignment is represented for each individual by a separate colour square below each bar: dark blue = Western Kingbird, light blue = Scissor-tailed Flycatcher, black = no assignment. Letters inside bars indicate parent/offspring pairs; capital letters indicate parent and corresponding lowercase letter indicates their offspring. Sampling locations (all in USA) are indicated above each group bar: AR, Arkansas; CA, California; CO, Colorado; ID, Idaho; LA, Louisiana; OK, Oklahoma; TN, Tennessee; TX, Texas. Individuals are ordered by sample number which also corresponds to sample numbers depicted in Fig. 1.

reproductive success of either parental species. This system represents, to our knowledge, a previously undocumented pattern of hybridization where broadly sympatric species simultaneously expand their breeding ranges and then hybridize at the periphery. This system is similar to other models of temporary hybridization, such as mosaic hybrid zones, in that it also involves two broadly sympatric species, but differs in that the two species are ecologically similar and do not appear to be hybridizing regularly throughout their ranges (Rand & Harrison 1989, Larson *et al.* 2013). Contrasting with classic hybrid tension zones, hybridization occurs at the periphery of *T. forficatus* and *T. verticalis* ranges but those ranges are broadly overlapping as opposed to meeting at the tension zone (Barton & Hewitt 1985, Arnold 1997).

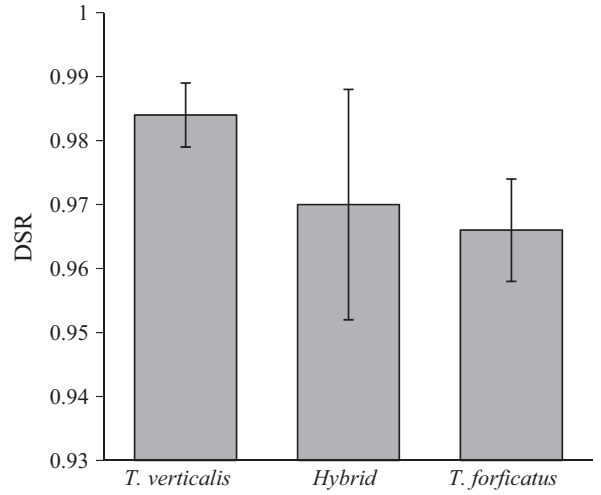

Figure 3. Daily survival rate (DSR; ± se error bars) for nests associated with Western Kingbird parents (*Tyrannus verticalis*; n = 32), at least one hybrid parent (n = 7) and Scissor-tailed Flycatcher parents (*Tyrannus forficatus*; n = 58). Nests were all located at the recently expanded periphery of both species' ranges in northeastern Arkansas and western Tennessee, USA.

In addition to this system's unique geographical and ecological conditions, we found bi-directional gene flow between *T. forficatus* and *T. verticalis*, thus increasing the likelihood of downstream genetic consequences. Although even low amounts of introgression can have implications for hybridizing species (Allendorf *et al.* 2001, Abbott *et al.* 2013), the long-term importance may depend on the stability of the system (but see Lamichhaney *et al.* 2018 for potential consequences of even a single hybridization event). Stable hybrid zones typically have pure parental mixed pairings producing offspring over many generations (Barton & Hewitt 1985). Although we did not find pure *T. forficatus* × *T. verticalis* pairings, they must occur given the number of likely F1 hybrids backcrossing with pure individuals of both species.

Even if this hybrid zone is short-lived, ephemeral hybridization events can also have individual and population consequences, both locally and beyond (Arnold 1997, Barton 2001). Locally, relatively small breeding populations at the periphery may experience greater impacts from genetic introgression, as novel combinations of alleles form and spread rapidly (similar to founder populations,

Figure 4. Photographic examples of adult Scissor-tailed Flycatchers (*Tyrannus forficatus*), adult Western Kingbirds (*Tyrannus verticalis*), and adult and fledgling hybrids captured in northeastern Arkansas and western Tennessee, USA. Photos depict the following birds: (a) an adult male *T. forficatus* (associated with column 67 in Fig. 2); (b) an adult male *T. verticalis* (column 48); (c) an adult female hybrid (column 50); (d) a social pair of an adult male hybrid (1; column 51) and an adult female *T. verticalis* (2; column 23); (e) a social pair of an adult hybrid (1; column 49), an adult male *T. forficatus* (2; column 59) and one of their hybrid fledglings (3; column 55).

Clegg *et al.* 2002). These alleles could either be beneficial, making individuals better adapted to new environments and enabling further expansion (Arnold 1997, Barton 2001, Grant *et al.* 2004), or detrimental, leading to reduced fitness of individuals at the periphery and a contraction of recent expansion (Rhymer & Simberloff 1996, Arnold 1997, Muhlfeld *et al.* 2009).

Beyond local effects, introgression has the potential to be widespread in these species because of low natal and breeding philopatry in both species (Gamble & Bergin 2012, Regosin 2013, Becker *et al.* 2018). Ephemeral hybridization events could lead to the spread of introgressed alleles as hybrids disperse into other portions of the species' ranges to breed in subsequent years. Our results support this possibility as we detected two admixed individuals in the core range. These could represent cases of hybrids dispersing from their natal regions along the periphery and eventually backcrossing, but may also be signatures of past hybridization events from the core range. In either case, consistent backcrossing could eventually result in cryptic hybrids:

admixed individuals that resemble a pure parental species morphologically (e.g. Vallender *et al.* 2009). Provided there are no post-zygotic barriers at play, these cryptic individuals may be readily selected as mates even in the core of the population, leading to further introgression. In this case, backcrossing already appears to have resulted in some cryptic hybrids (at least from a human perspective) at both the periphery and within the core range, and it is possible that even more will be produced throughout the ranges of these species as hybrid individuals continue to disperse. It is also possible that cryptic hybrids may already be more numerous than realized, as molecular analyses are required for detection. If this is the case, long-term effects of such range-wide introgression are unclear and should continue to be monitored. Finally, ephemeral hybridization events may also act as footholds for expanding species, especially at the underpopulated peripheries, where mating with conspecifics can maintain recent expansion until species-specific densities can increase (Hubbs 1955, Canestrelli *et al.* 2016).

Despite the seemingly idiosyncratic nature of this system, we believe our results may in fact be observable in other species, even within the same genus (*Tyrannus*). Several species of *Tyrannus* seem to be undergoing range expansions (eBird 2012, Sauer *et al.* 2014) and there have been multiple reports of hybridization among different species pairs based solely on morphology (Kale 1977, Traylor 1979, Binford 1989, Brewer *et al.* 1991, McGowan & Spahn 2004). Furthermore, a number of these species are broadly sympatric; there are at least four regions throughout North, Central and South America where four or more *Tyrannus* species co-occur (eBird 2012, Bird Life International 2017). Thus, the conditions appear ripe for more cases of range expansion-induced sympatric hybridization

In conclusion, we provided evidence that introgressive hybridization has occurred at the periphery of the expanding ranges of two sympatric, ecologically similar, songbird species and that introgressed alleles may be spreading into individuals in other parts of their ranges. Furthermore, these hybridization events seem to have no short-term reproductive effects on either parental species. Further research on this system should include genomic analysis to assess which portion of the genomes are being introgressed and how they may be related to the (de)urbanization of these species, a range-wide genetic survey to more fully detect hybridization and the impacts of introgression elsewhere, behavioural studies at the periphery to better understand the proximate factors involved in hybridization events, and continued monitoring to uncover the long-term effects of hybridization at the periphery. Additionally, assessing hybridization at the periphery of expanding ranges for other sympatric sister species in other groups will allow us to determine whether this model of hybridization is unique to our current system or is a widespread consequence of low population densities at the expanding periphery of species ranges.

We thank our field technician S. Wilcox, B. McCall and A. Matthews for lab assistance, and Louisiana State University Museum of Nature Science (LSUMNS) for lending tissue samples. We also thank reviewers for their feedback. This work was funded by the Department of Biological Sciences at Arkansas State University, an Arkansas State University Faculty Research grant awarded to TJB, and grants from the Arkansas Audubon Society Trust awarded to AW. Banding was conducted under the auspices of USGS federal banding permit #23877 and research followed IACUC guidance, permit 745496-1 issued by Arkansas State University.

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Received 19 February 2018;
revision accepted 11 September 2018.
Associate Editor: Frank Rheindt.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Microsatellite primers used for detection of Scissor-tailed Flycatchers (*Tyrannus forficatus*), hybrids, and Western Kingbirds (*Tyrannus verticalis*).

Table S2. Microsatellite DNA loci scores for all individuals in the current study with SRT# corresponding to the column number of the STRUCTURE output in Fig. 2.

Figure S1. Maximum-Likelihood tree built using the mtDNA ND2 gene for Western Kingbirds (*Tyrannus verticalis*) and Scissor-tailed Flycatchers (*Tyrannus forficatus*) with Forked-tailed Flycatcher (*Tyrannus savanna*) as outgroup, constructed in Program R, $n = 81$.