bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Revisiting a classic hybrid zone: rapid movement of the northern flicker hybrid zone in contemporary times

Stepfanie M. Aguillon[1,2,3,*] and Vanya G. Rohwer[1,4]

[1]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853

[2]Fuller Evolutionary Biology Program, Cornell Lab of Ornithology, Ithaca, NY 14850

[3]Current address: Department of Biology, Stanford University, Stanford, CA 94305

[4]Cornell University Museum of Vertebrates, Ithaca, NY 14850

*Corresponding author: stepfanie.aguillon@gmail.com

The authors wish to be identified to reviewers.

Abstract

Natural hybrid zones have provided important insights into the evolutionary process, and their geographic stability/instability over time can help to disentangle the underlying biological processes that maintain them. Here, we leverage replicated sampling of an identical transect across the hybrid zone between yellow-shafted and red-shafted flickers to assess its stability over ~60 years (1955-1957 to 2016-2018). Using a plumage scoring approach that we validate with independent multispectral photography, we identify a ~73 km westward shift in the hybrid zone center towards the range of the red-shafted flicker but no associated changes in width. By integrating previous work in the same geographic region, it appears likely that this movement has occurred rapidly in the years since the early 1980s, prior to which the hybrid zone had remained stable over the previous century. This recent, rapid movement may be related to changes in climate or land management in contemporary times.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Introduction

Naturally hybridizing taxa have provided unique insights on the process of speciation (Barton and Hewitt 1985; Harrison 1993; Harrison and Larson 2014). Hybrid zones, geographical regions where differentiated taxa interbreed and produce hybrids, have long-been described as "windows on evolutionary process" as they provide opportunities to assess the outcome of recombination over many generations (Harrison 1990). Additionally, the geographic locations of hybrid zones can provide important insights and movement of hybrid zones has been of particular interest in recent years (e.g., as "windows on climate change" Taylor et al. 2015). Hybrid zones may move for a number of reasons, including differences in population density of the hybridizing taxa (Barton and Hewitt 1985), competitive advantage of one taxa over the other (Buggs 2007), asymmetric hybridization (Konishi and Takata 2004), or changes in the environment (Taylor et al. 2015). Molecular methods have recently made it possible to identify signatures of historical hybrid zone movement in the genome (Wielstra et al. 2017; van Riemsdijk et al. 2019; Wielstra 2019), but this inferred evidence of movement does not always match results from direct resampling over broad temporal and spatial scales (Wang et al. 2019). Although difficult to accomplish, repeated sampling of hybrid zones over time remains the best way to definitively identify movement.

Here, we directly assess movement in the hybrid zone between yellow-shafted (*Colaptes auratus auratus*) and red-shafted (*C. a. cafer*[1]) flickers, a long-studied system in ecology and evolution (e.g., Short 1965; Moore and Buchanan 1985; Wiebe 2000)

[1] The subspecific epithet of the red-shafted flicker is based on a term that is an extreme racial slur against Black Africans, particularly in South Africa. We include the official scientific name here, but purposefully refer to the flickers elsewhere only by their common names. We have proposed changing the name to *Colaptes auratus lathami* (Aguillon and Lovette 2019), but this has not been officially accepted.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

that has intrigued naturalists since at least the mid-1800s (Audubon et al. 1897). Flickers are common woodpeckers widely distributed across wooded areas of North America—red-shafted flickers in the west and yellow-shafted flickers in the east (Wiebe and Moore 2020). These two forms come into secondary contact in an extensive hybrid zone in the Great Plains that roughly follows the Rocky Mountains from northern Texas to southern Alaska (Figure 1). Hybridization between the flickers is clearly visible due to differences across six distinct plumage traits (Figure 1, Table S1), yet there is only mixed evidence of assortative mating based on these traits—with no evidence in the US portion of the hybrid zone (Bock 1971; Moore 1987) and weak but significant evidence in the Canadian portion of the hybrid zone (Wiebe 2000; Flockhart and Wiebe 2007; Wiebe and Vitousek 2015).

We assess movement of the flicker hybrid zone by comparing the spatial transition of plumage characters between two sampling periods of an identical transect separated by ~60 years (Figure 1 inset). Using geographic cline analyses, we estimate the center and width of the plumage clines in the historic (1955-1957) and contemporary (2016-2018) sampling periods. We then use approaches following Wang et al. (2019) to evaluate changes in the cline center and width against neutral expectations. Finally, we validate our plumage scoring approach using multispectral photography. Our previous work has demonstrated the extremely low levels of genomic divergence between allopatric red-shafted and yellow-shafted flickers (Aguillon et al. 2018) and we used their extensive admixture to link the few differentiated regions of the genome that exist with differences in plumage coloration (Aguillon et al. 2021). Thus, assessing patterns in the

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

plumage traits of flickers is a straightforward approach to understand hybrid zone dynamics at both the phenotypic and genotypic level.

Material and Methods

Sampling

The most extensive study of hybridization and phenotypic variation in flickers was undertaken by Short (1965), for which he collected specimens intensively along the Platte River in Nebraska and Colorado from 1955-1957 (Figure 1 inset). This hybrid zone transect for flickers (and those for several other hybridizing species pairs) remains one of the most extraordinary components of the ornithological collection in the Cornell University Museum of Vertebrates (CUMV). During the spring and summer of 2016-2018, the CUMV replicated Short's sampling along the Platte River—revisiting many of his original localities—to amass a modern-day transect of the hybrid zone. This was additionally supplemented by banding individuals in 2016.

Henceforth, we will use "historic" and "contemporary" to refer to flickers sampled from 1955-1957 and 2016-2018, respectively. We focus here on adults to avoid confounding patterns due to immature plumage in juveniles. We include 252 historic flickers (all vouchered in the CUMV) and 107 contemporary flickers (91 specimens vouchered in the CUMV and 16 individuals that were banded, photographed, and released) in this study (Table S2). We group the sexes together across all analyses except for those on the malar stripe (the only sexually dimorphic character in flickers), where we include only males (138 historic and 72 contemporary).

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Plumage scoring

The flickers differ across six primary plumage characteristics (Figure 1): the eponymous "shaft" (wing and tail) color, crown color, ear covert color, throat color, malar stripe color in males, and the presence/absence of the nuchal patch (Short 1965). In brief, these birds differ vividly in the shaft color (bright yellow in the yellow-shafted flicker versus salmon red in the red-shafted flicker) and in the overall coloring of the face and head. Hybrids can exhibit various combinations of the six parental traits, as well as colors intermediate to the parental extremes.

We scored plumage characters of historic and contemporary flickers on a categorical scale from 0 (pure yellow-shafted) to 4 (pure red-shafted) for each of the six plumage traits following a protocol slightly modified from Short (1965; see Table S1 for details); a method that has been used extensively within the flicker system (e.g., Moore and Buchanan 1985; Moore 1987; Wiebe 2000; Flockhart and Wiebe 2007; Aguillon et al. 2021). The main modification from Short (1965) is differences in our scoring of the shaft color based on an increased understanding of carotenoid pigmentation, particularly around orange shaft feathers (e.g., Hudon et al. 2017). We additionally calculated an overall plumage hybrid index by summing across the trait scores and standardizing to range from 0 to 1. This standardization makes comparisons between males and females possible, as females lack the malar stripe present in males. All scoring was conducted by S.M.A. to ensure consistency.

Geographic cline analyses and hybrid zone movement

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

To evaluate the distribution of phenotypic traits across the hybrid zone, we fit a series of equilibrium sigmoidal cline models (Szymura and Barton 1986; Gay et al. 2008) using the 'nls' function in R v.3.6.2 (R Core Team 2018), where we modelled the relationship between locality (*x*) and hybrid index or trait score (*y*) to estimate cline center and width. *S* is included as a scaling factor for trait scores that do not vary from 0 to 1. Confidence intervals for center and width were calculated using the 'confint' function in R. We repeated this process for both historic and contemporary sampling periods and compared the results for the overall hybrid index (*S*=1) and individual phenotypic traits (*S*=4).

$$y = \frac{S}{1 + e^{\frac{4(x-center)}{width}}} \quad (1)$$

We grouped samples based on sampling location (Figure 1, Table S3) and set the start of the cline to the western-most locality sampled in the Rocky Mountains. To estimate the distance of each locality from the start of the cline, we determined the mean latitude across all localities and then used the 'distm' function in the geosphere R package (Hijmans 2019) to calculate the distance each locality was from the start of the cline along the mean latitude value, using the longitude of the locality and assuming an ellipsoid shape.

We additionally assessed changes in the hybrid zone between the two sampling points using the overall hybrid index in two ways following an approach taken by Wang et al. (2019). First, to assess movement of the hybrid zone center, we used AIC to compare the contemporary cline to a model using the contemporary data but with the cline center fixed on the estimated historic center. This approach accounts for uncertainty due to sampling error between the two sampling periods. If there was no

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

difference between the estimated cline centers for the two time periods, the fixed center model would be expected to have lower AIC than the true model. Second, to assess change in the width of the hybrid zone and see if selection is maintaining the cline, we followed an approach developed by Wang et al. (2019) that leverages repeated sampling of the same transect over time to test against the neutral diffusion model (Barton and Hewitt 1985).

$$w_{t2}^2 - w_{t1}^2 = 2\pi\sigma^2\Delta t \quad (2)$$

Where $w_{t2}^2 - w_{t1}^2$ is the difference in cline width squares between the two sampling points, σ is a measure of dispersal distance, and Δt is the number of generations between the sampling points. We calculated the bootstrap distribution of $w_{contemporary}^2 - w_{historic}^2$ by resampling with replacement and fitting clines over 100,000 iterations.

We used both realistic and conservative values to estimate the neutral diffusion expectations from equation (2). For more realistic values, we used a σ of 100.7 km as estimated by Moore and Buchanan (1985) using banding data and a Δt of 60 generations (i.e., 1 year/generation). For more conservative values, we used a σ of 30 km as natal dispersal is typically greater than 15 km (and likely much greater; Wiebe and Moore 2020) and a Δt of ~33.3 generations (i.e., 1.8 years/generation; Milá et al. 2007).

Comparison of plumage scoring with multispectral photography

To assess the accuracy of our plumage scoring method, we additionally collected multispectral images of the contemporary flickers, plus examples of allopatric individuals (4 yellow-shafted and 8 red-shafted; Table S2). For complete details on image collection

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

and processing, see Supplemental Text S1 and Ligon et al. 2018. In brief, we photographed each specimen from three viewing angles (ventral, dorsal, and lateral) and under two conditions (all visible light between 400-700 nm and UV light between 300-400 nm). We then created standardized multispectral image files using the micaToolbox (Troscianko and Stevens 2015) in ImageJ (Schneider et al. 2012), and outputted values for each color channel (red, green, blue, UV) and luminance for each of the six plumage traits, as well as the overall area for the nuchal patch.

We first compared the color channel and luminance values (for the crown, ear coverts, malar stripe, shaft, and throat) and area (for the nuchal patch) to the distance along the hybrid zone using linear regressions in R (Figure S1). We isolated image parameters with slopes significantly different from 0 and fit equilibrium sigmoidal cline models (Szymura and Barton 1986; Gay et al. 2008) as described above. *S* was calculated as the average of the last three localities along the transect, and the sign of the exponential function varied depending on the direction of the cline. Allopatric individuals were included in this analysis by placing them 100 km past the start or end of the cline. We calculated confidence intervals for the cline center and width for the image parameters with well-formed clines and compared these estimates to those obtained from the categorical plumage scoring approach.

Finally, we performed multiple regressions to directly compare the plumage score with the image parameter values obtained from the multispectral photography. For the crown, ear coverts, malar stripe, shaft, and throat we compared the plumage score to the values for the four color channels and luminance. For the nuchal patch we compared the plumage score to the area.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Results

Westward movement of the flicker hybrid zone

We detected a significant westward shift of the hybrid zone cline between the historic and contemporary sampling periods for the plumage hybrid index of ~73 km (Figure 2A, Table S4). The individual clines for the six plumage traits are broadly overlapping within both the historic (Figure S2A) and contemporary (Figure S2B) periods, and we identified a significant contemporary westward movement—similar to that seen in the hybrid index—for three of the six traits (Figure 2B, Table S4). The cline center from the historic sampling period did not fit the contemporary data (Figure 3A; AIC = -32.7 for the true model, AIC = -6.6 for the model with the historic center), further supporting the movement of the hybrid zone between the two sampling periods. However, the bootstrap distribution of $w^2_{contemporary} - w^2_{historic}$ (95% CI: -37,441, 90,116) was significantly less than predicted by the neutral diffusion model under both realistic ($w^2_c - w^2_h$ = 3,822,875 km^2) and more conservative ($w^2_c - w^2_h$ = 188,496 km^2) dispersal distance and generation time values (Figure 3B), suggesting that selection has maintained the narrow width of the hybrid zone.

Concordance between multispectral photography and plumage scoring

Geographic clines for the area of the nuchal patch and the red channel of the malar stripe were the only image parameters that resulted in well-formed clines (Figure S3, S4). Comparing the clines from these two image parameters with those from the categorical plumage scoring approach resulted in a broad overlap in estimates for both

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

cline center and width (Figure S5, Table S5). Moreover, multiple regressions comparing the plumage scores with the image parameter values were strongly significant for all six plumage traits (Table S6).

Discussion

The hybrid zone between red-shafted and yellow-shafted flickers in the Great Plains of North America was an important study system in the early development of ideas about hybrid zone dynamics (e.g., Moore and Buchanan 1985; Moore and Price 1993). In this study, we compared historic (1955-1957) and contemporary (2015-2018) samplings of an identical transect across the flicker hybrid zone using a plumage scoring approach (Table S1) that we validated with independent multispectral photography (Table S5, S6) to assess changes over the past ~60 years. We detected a significant westward shift of the hybrid zone center of ~73 km towards the range of the red-shafted flicker (Figure 2A, Figure 3A). In the historic sampling period, the cline center was ~208 km east of the start of the transect (between localities 11 and 12 in Figure 1), while in the contemporary sampling period, the center shifted to ~135 km east of the start of the transect (near localities 5 and 6). Similar westward shifts were repeated separately across all six phenotypic traits, although confidence intervals were non-overlapping for only three (Table S4, Figure 2B, S2). This movement in the flicker hybrid zone differs greatly from previous work done in the same region that instead found stability when comparing samples from 1889-1968 and 1981-1982 (Moore and Buchanan 1985), though they did not resample an identical transect. Although the range of years in the early sampling period of Moore and Buchanan (1985) is quite large, 69% of their

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

samples are from the 1950s and later (see their Table 1). This broadly overlaps with our historical sampling, but in contrast to our results, they did not find evidence for hybrid zone movement. Thus, it seems likely that the movement we detected occurred in the latter ~35 years between our historic and contemporary sampling points (i.e., after the study by Moore and Buchanan 1985), which suggests the rate of movement has been quite rapid: ~2.1 km/year since the early 1980s.

Similar to the findings in Moore and Buchanan (1985), we did not find evidence of significant changes in the width of the flicker hybrid zone over our ~60-year sampling period (Table S4). Using an approach that takes advantage of repeat sampling of the same transect (Wang et al. 2019), we additionally were able to directly refute the neutral diffusion model (Figure 3B; Barton and Hewitt 1985). Although these results support some selective force preventing changes in the width of the hybrid zone, there is no clear evidence for endogenous selection acting against flicker hybridization as would be expected under a tension zone model (Barton and Hewitt 1985, 1989). To date, no fitness consequences of hybridization have been identified in any part of the hybrid zone (Moore and Koenig 1986; Wiebe and Bortolotti 2002; Flockhart and Wiebe 2009) and there is no evidence for assortative mating in this part of the hybrid zone (Bock 1971; Moore 1987).

The flicker hybrid zone may instead be better described by an environmental selection gradient model (May et al. 1975; Endler 1977), and in fact, has previously been hypothesized as an example of hybrid superiority (Moore 1977; Moore and Price 1993). Under this model, if there is ecological change that moves the geographic area where hybrids have higher fitness than parentals, there can be hybrid zone movement

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

to track this change. This movement could occur without associated changes in width as long as the geographic area moves without expanding or narrowing. Thus, the rapid movement of the flicker hybrid zone since the early 1980s may be tied to changes in the environment (land management and/or climate changes). Environmental factors are known to affect the locations of the Great Plains avian hybrid zones (Swenson 2006), and intriguingly, similar westward movements have previously been documented in two of these: the hybrid zone between the lazuli (*Passerina amoena*) and indigo (*P. cyanea*) buntings (Carling and Zuckerberg 2011) and the hybrid zone between the Baltimore (*Icterus galbula*) and Bullock's (*I. bullockii*) orioles (Corbin and Sibley 1977; Walsh et al. 2020). Similar westward shifts across three hybrid zones in the same ecotone is suggestive of a shared driver, although it is difficult to disentangle the potential role of different environmental factors (e.g., climate change from changes in land management).

Our results underscore the importance of biological collections. We identify a significant westward movement in the long-studied flicker hybrid zone that may have gone unnoticed without repeat sampling efforts—something that is difficult to accomplish without the long memory of collections. We hope that in another 60 years additional sampling of this transect along the flicker hybrid zone will be undertaken and provide further discoveries about the evolutionary process. In the meantime, future work should investigate the cause of this westward movement and its relationship to environmental changes.

Acknowledgments

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

We greatly appreciate the insights, guidance, and resources provided by I. J. Lovette for this project. We thank R. A. Ligon for generously providing the photography equipment (and the know-how) necessary to take the multispectral images and for providing helpful feedback on data processing. M. Bishop provided illustrations of the flickers. We thank B. Mims, N. A. Kramer, and T. Brooks for providing assistance in the field and G.-F. Siegmund for input on data analysis. J. Walsh, J. B. Searle, L. Campagna, and M. N. Vitousek provided helpful comments on earlier versions of this manuscript. This work was supported by the Cornell Lab of Ornithology Athena Fund, the Garden Club of America Frances M. Peacock Scholarship, and the Cornell University EEB Betty Miller Francis and Paul P. Feeny Graduate Student Research Funds (all to S.M.A.). S.M.A. was supported by the US National Science Foundation Graduate Research Fellowship Program (DGE-1144153) and the AAUW American Dissertation Fellowship. Science is only moved forward by standing on the shoulders of those who came before, so we would like to acknowledge the ground-breaking efforts undertaken by Lester L. Short to collect and study flickers in the 1950s.

Statement of Authorship

S.M.A. conceived the study, analyzed the data, and wrote the original draft of the manuscript. S.M.A. and V.G.R. collected the data and revised the final manuscript.

Data and Code Availability

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Datafiles will be made available on the Dryad Digital Repository upon publication. Scripts for all analyses are available at https://github.com/stepfanie-aguillon/flicker-HZ-movement.

Literature Cited

Aguillon, S. M., L. Campagna, R. G. Harrison, and I. J. Lovette. 2018. A flicker of hope: genomic data distinguish northern flicker taxa despite low levels of divergence. The Auk: Ornithological Advances 135:748–766.

Aguillon, S. M., and I. J. Lovette. 2019. Change the specific/subspecific/morphological group name of the red-shafted flicker from *cafer* to *lathami*. AOS North American Classification Committee 2019-A-10:66–71.

Aguillon, S. M., J. Walsh, and I. J. Lovette. 2021. Extensive hybridization reveals multiple coloration genes underlying a complex plumage phenotype. Proceedings of the Royal Society B 288:20201805.

Audubon, J. J., M. R. Audubon, and E. Coues. 1897. Audubon and His Journals. Scribner, New York.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113–148.

———. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.

Bock, C. E. 1971. Pairing in hybrid flicker populations in eastern Colorado. The Auk 88:921–924.

Buggs, R. J. 2007. Empirical study of hybrid zone movement. Heredity 99:301–12.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Carling, M. D., and B. Zuckerberg. 2011. Spatio-temporal changes in the genetic structure of the *Passerina* bunting hybrid zone. Molecular Ecology 20:1166–1175.

Corbin, K. W., and C. G. Sibley. 1977. Rapid Evolution in Orioles of the Genus *Icterus*. The Condor 79:335.

Endler, J. A. 1977. Geographic Variation, Speciation and Clines. Princeton University Press, Princeton, New Jersey.

Flockhart, D. T. T., and K. L. Wiebe. 2007. The role of weather and migration in assortative pairing within the northern flicker (*Colaptes auratus*) hybrid zone. Evolutionary Ecology Research 9:887–903.

———. 2009. Absence of reproductive consequences of hybridization in the northern flicker (*Colaptes auratus*) hybrid zone. The Auk 126:351–358.

Gay, L., P.-A. Crochet, D. A. Bell, and T. Lenormand. 2008. Comparing clines on molecular and phenotypic traits in hybrid zones: a window on tension zone models. Evolution 62:2789–2806.

Harrison, R. G. 1990. Hybrid zones: windows on evolutionary process. Oxford Surveys in Evolutionary Biology 7:69–128.

Harrison, R. G. 1993. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York.

Harrison, R. G., and E. L. Larson. 2014. Hybridization, introgression, and the nature of species boundaries. Journal of Heredity 105:795–809.

Hijmans, R. C. 2019. *geosphere: spherical trigonometry. R package version 1.5-10*.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Hudon, J., R. J. Driver, N. H. Rice, T. L. Lloyd-Evans, J. A. Craves, and D. P. Shustack. 2017. Diet explains red flight feathers in yellow-shafted flickers in eastern North America. The Auk: Ornithological Advances 134:22–33.

Konishi, M., and K. Takata. 2004. Impact of asymmetrical hybridization followed by sterile F1 hybrids on species replacement in *Pseudorasbora*. Conservation Genetics 5:463–474.

May, R. M., J. A. Endler, and R. E. McMurtrie. 1975. Gene frequency clines in the presence of selection opposed by gene flow. The American Naturalist 109:659–676.

Milá, B., J. E. McCormack, G. Castaneda, R. K. Wayne, and T. B. Smith. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus Junco. Proceedings of the Royal Society B 274:2653–60.

Moore, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. The Quarterly Review of Biology 52:263–277.

———. 1987. Random mating in the northern flicker hybrid zone: implications for the evolution of bright and contrasting plumage patterns in birds. Evolution 41:539–546.

Moore, W. S., and D. B. Buchanan. 1985. Stability of the northern flicker hybrid zone in historical times: implications for adaptive speciation theory. Evolution 39:135–151.

Moore, W. S., and W. D. Koenig. 1986. Comparative reproductive success of yellow-shafted, red-shafted, and hybrid flickers across a hybrid zone. The Auk 103:42–51.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

Moore, W. S., and J. T. Price. 1993. The nature of selection in the northern flicker hybrid zone and its implications for speciation theory. Pages 196–225 *in* R. G. Harrison, ed. Hybrid Zones and the Evolutionary Process. Oxford University Press, Oxford, UK.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Short, L. L. 1965. Hybridization in the flickers (*Colaptes*) of North America. Bulletin of the American Museum of Natural History, New York 129:307–428.

Swenson, N. G. 2006. Gis-based niche models reveal unifying climatic mechanisms that maintain the location of avian hybrid zones in a North American suture zone. Journal of Evolutionary Biology 19:717–25.

Szymura, J. M., and N. H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40:1141–1159.

Taylor, S. A., E. L. Larson, and R. G. Harrison. 2015. Hybrid zones: windows on climate change. Trends in Ecology and Evolution 30:398–406.

Troscianko, J., and M. Stevens. 2015. Image calibration and analysis toolbox—a free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution 6:1320–1331.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

van Riemsdijk, I., R. K. Butlin, B. Wielstra, and J. W. Arntzen. 2019. Testing an hypothesis of hybrid zone movement for toads in France. Molecular Ecology 28:1070–1083.

Walsh, J., S. M. Billerman, V. G. Rohwer, B. G. Butcher, and I. J. Lovette. 2020. Genomic and plumage variation across the controversial Baltimore and Bullock's oriole hybrid zone. The Auk: Ornithological Advances 137:ukaa044.

Wang, S., S. Rohwer, K. Delmore, and D. E. Irwin. 2019. Cross-decades stability of an avian hybrid zone. Journal of Evolutionary Biology 32:1242–1251.

Wiebe, K. L. 2000. Assortative mating by color in a population of hybrid northern flickers. The Auk 117:525–529.

Wiebe, K. L., and G. R. Bortolotti. 2002. Variation in carotenoid-based color in northern flickers in a hybrid zone. The Wilson Bulletin 114:393–400.

Wiebe, K. L., and W. S. Moore. 2020. Northern Flicker (*Colaptes auratus*), version 1.0. *in* P. G. Rodewald, ed. Birds of the World. Cornell Lab of Ornithology, Ithaca, New York, USA.

Wiebe, K. L., and M. N. Vitousek. 2015. Melanin plumage ornaments in both sexes of northern flicker are associated with body condition and predict reproductive output independent of age. The Auk: Ornithological Advances 132:507–517.

Wielstra, B. 2019. Historical hybrid zone movement: more pervasive than appreciated. Journal of Biogeography 46:1300–1305.

Wielstra, B., T. Burke, R. K. Butlin, A. Avcı, N. Üzüm, E. Bozkurt, K. Olgun, et al. 2017. A genomic footprint of hybrid zone movement in crested newts. Evolution Letters 1:93–101.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

404

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

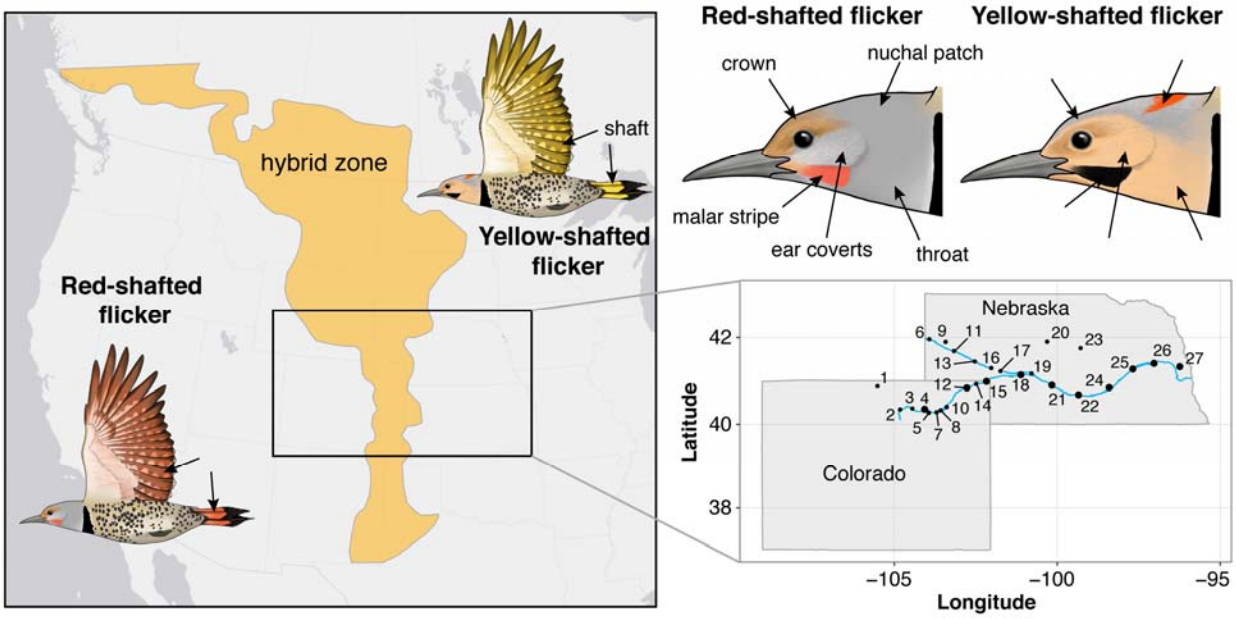


Figure 1. The geographic extent of the southern portion of the hybrid zone between red-shafted and yellow-shafted flickers as estimated in Moore and Price (1993). The inset map of Colorado and Nebraska depicts the repeated sampling conducted along the Platte River with numbered sampling localities (Table S3). Larger points on the inset map indicate localities that were sampled during both the historic (1955-1957) and contemporary (2016-2018) sampling periods. The six plumage differences are shown with arrows on the flicker illustrations (created by M. Bishop) and details are described in Table S1.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

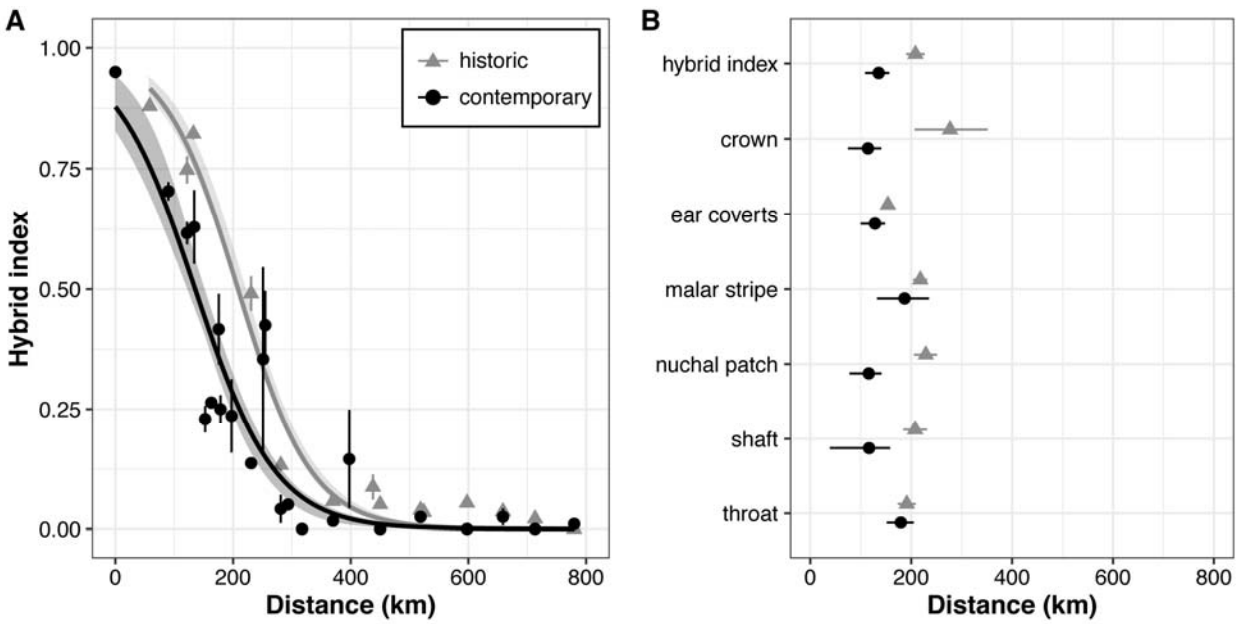
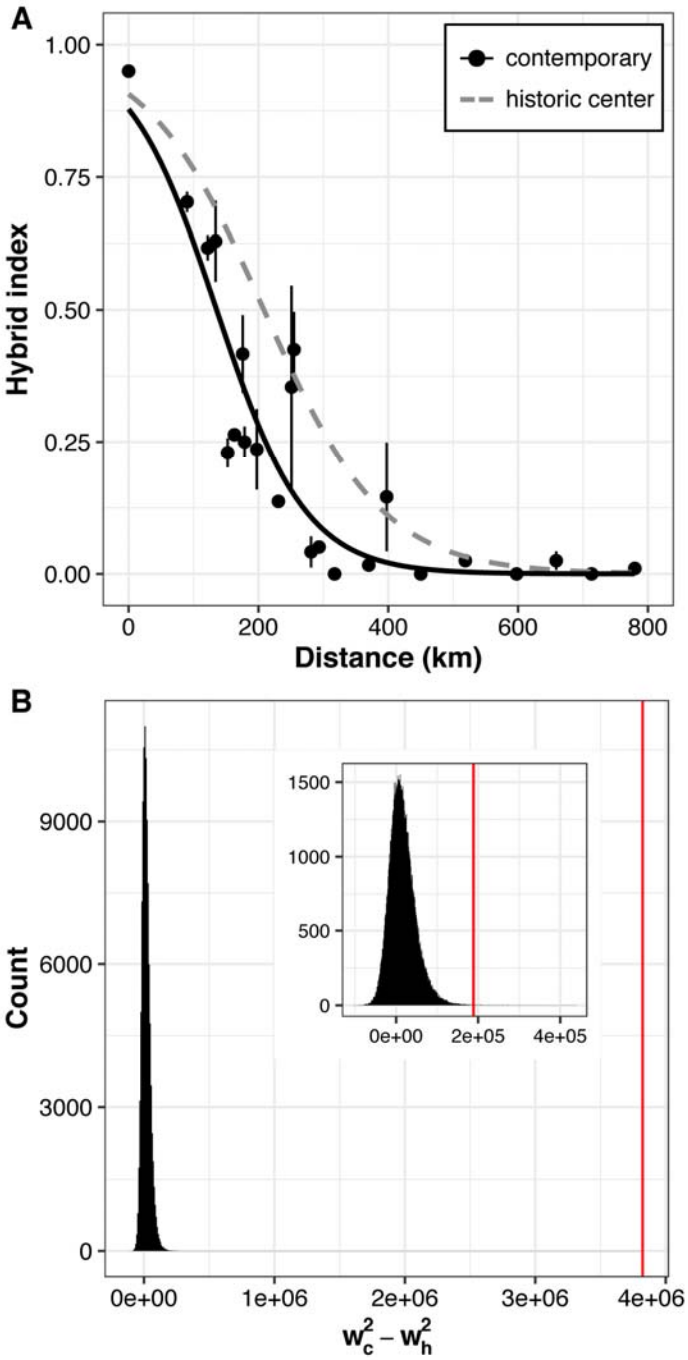


Figure 2. (**A**) Geographic clines of the overall hybrid index as estimated for historic (gray, triangles) and contemporary (black, circles) flickers demonstrate the ~73 km westward movement of the hybrid zone in the ~60 years between the two sampling periods. Points indicate the mean and standard error of the hybrid index at each sampling locality and shading represents the 95% bootstrap confidence interval. (**B**) Cline centers with 95% confidence intervals for the geographic clines estimated from the hybrid index and separately for six plumage traits. Full model details are available in Table S4.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

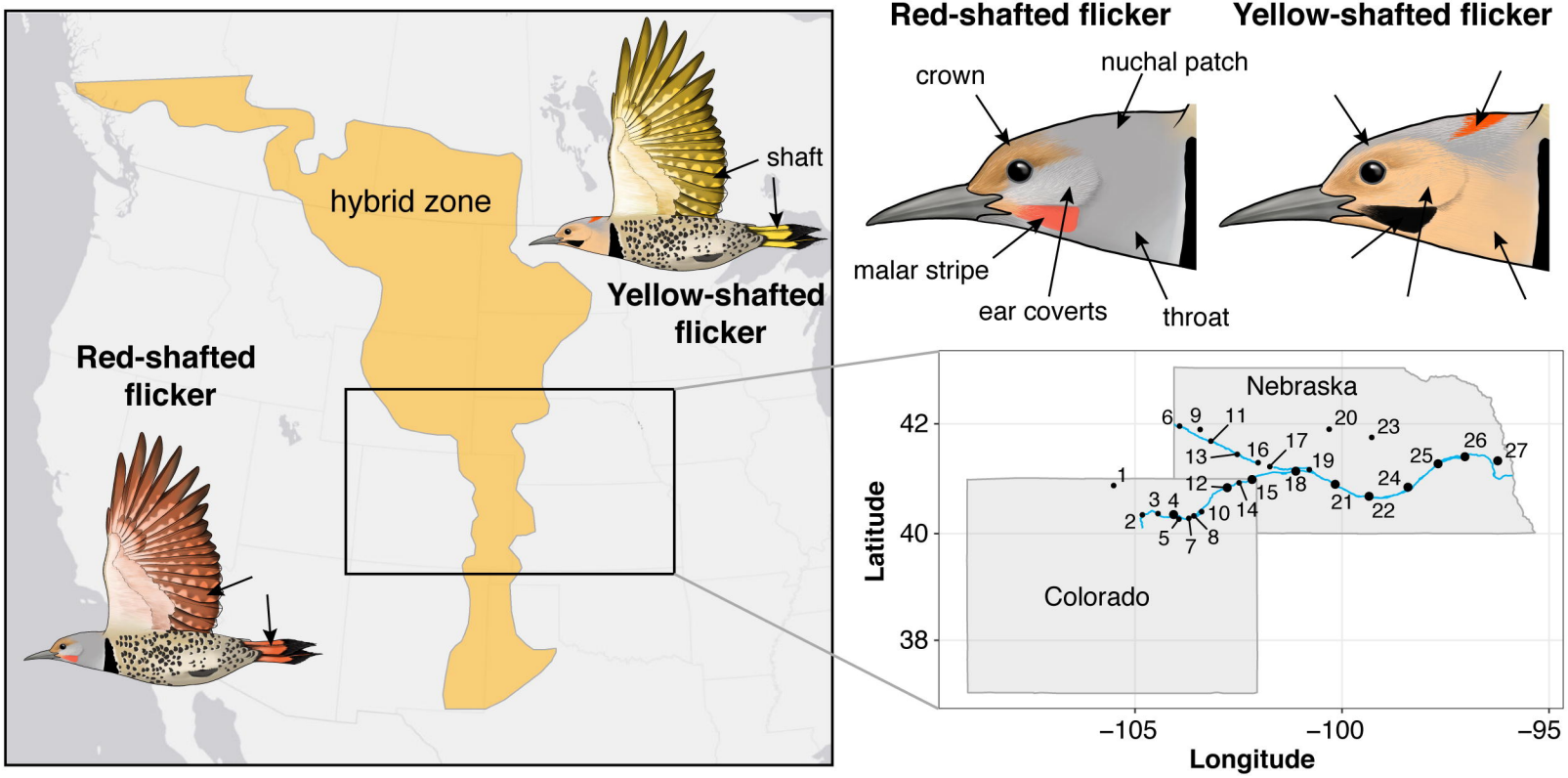


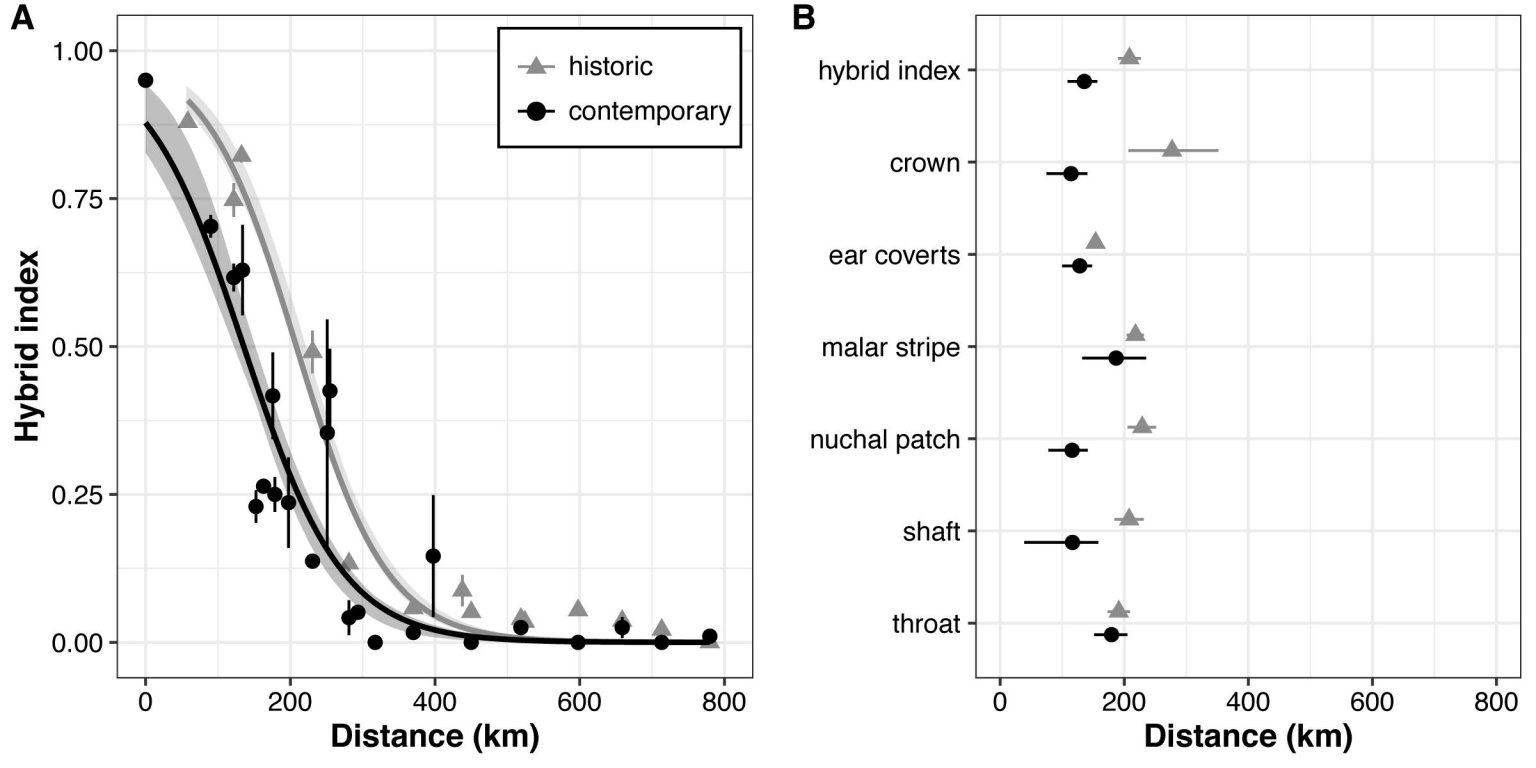
425

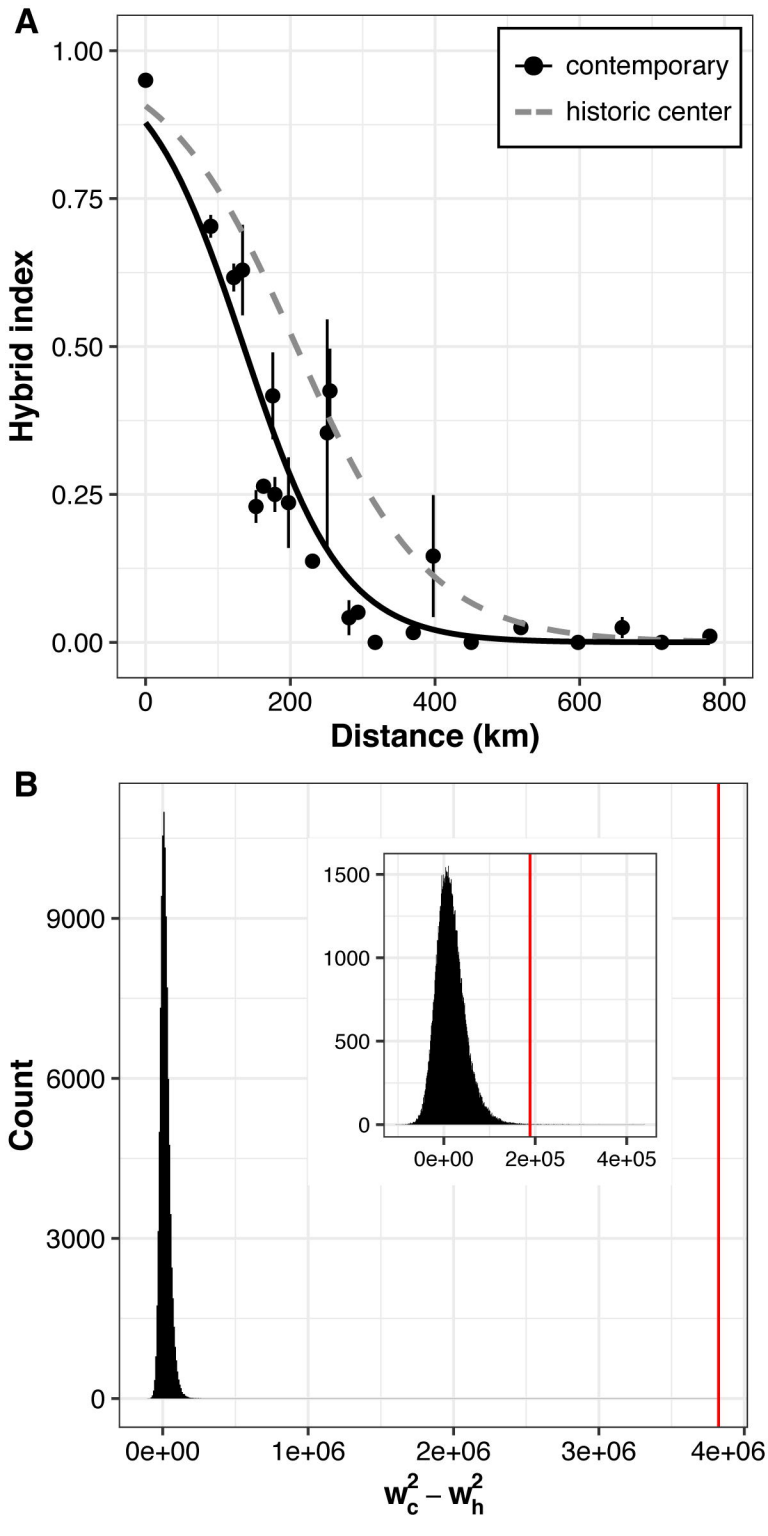
426 **Figure 3.** (**A**) Comparison of the true contemporary geographic cline for hybrid index
427 (black line, circles) to a contemporary cline with the center fixed to the estimate of the
428 historic center (gray dashed line). ΔAIC indicates the true cline is significantly better,
429 further supporting the difference in cline centers between the historic and contemporary

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.16.456504; this version posted August 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

sampling periods. (**B**) The bootstrap distribution of $w^2_{contemporary} - w^2_{historic}$ of the hybrid index cline is significantly different from the expectation (red line) under the neutral diffusion model for both realistic (main plot) and conservative (inset plot) values of dispersal and generation time, suggesting the hybrid zone has not widened to the extent expected under neutral diffusion.


hybrid zone
shaft
Yellow-shafted flicker
Red-shafted flicker
Red-shafted flicker
Yellow-shafted flicker
crown
nuchal patch
malar stripe
ear coverts
throat
Nebraska
Colorado
Latitude
Longitude
42
40
38
−105
−100
−95
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27


A
historic
contemporary
Hybrid index
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Distance (km)
B
hybrid index
crown
ear coverts
malar stripe
nuchal patch
shaft
throat
0
200
400
600
800
Distance (km)


A
contemporary
historic center
Hybrid index
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
Distance (km)
B
Count
9000
6000
3000
0
0e+00
1e+06
2e+06
3e+06
4e+06
$w_c^2 - w_h^2$
1500
1000
500
0
0e+00
2e+05
4e+05