Comparative riverscape genetics reveals reservoirs of genetic diversity for conservation and restoration of Great Plains fishes

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Abstract

We used comparative landscape genetics to examine the relative roles of historical events, intrinsic traits and landscape factors in determining the distribution of genetic diversity of river fishes across the North American Great Plains. Spatial patterns of diversity were overlaid on a patch-based graphical model and then compared within and among three species that co-occurred across five Great Plains watersheds. Species differing in reproductive strategy (benthic vs. pelagic-spawning) were hypothesized to have different patterns of genetic diversity, but the overriding factor shaping contemporary patterns of diversity was the signature of past climates and geological history. Allelic diversity was significantly higher at southern latitudes for *Cyprinella lutrensis* and *Hybognathus placitus*, consistent with northward expansion from southern Pleistocene refugia. Within the historical context, all species exhibited lowered occupancy and abundance in heavily fragmented and drier upstream reaches, particularly *H. placitus*; a pelagic-spawning species, suggesting rates of extirpation have outpaced losses of genetic diversity in this species. Within most tributary basins, genetically diverse populations of each species persisted. Hence, reconnecting genetically diverse populations with those characterized by reduced diversity (regardless of their position within the riverine network) would provide populations with greater genetic and demographic resilience. We discuss cases where cross-basin transfer may be appropriate to enhance genetic diversity and mitigate negative effects of climate change. Overall, striking similarities in genetic patterns and in response to fragmentation and dewatering suggest a common strategy for genetic resource management in this unique riverine fish assemblage.

Keywords: dendritic landscapes, dispersal constraints, graph theory, habitat fragmentation, landscape genetics, river networks

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Introduction

Over the last 150 years, fish communities in rivers of the Great Plains of North America have been radically altered by habitat fragmentation, dewatering and land-use changes that negatively affect fish habitat connectivity, heterogeneity, quality and extent (Hoagstrom *et al.* 2011; Perkin & Gido 2011; Perkin *et al.* 2014a). Remnant

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populations of once vastly abundant and widely distributed riverine fishes now occur in drier, fragmented habitats that have resulted from impoundment and diversion of surface waters and groundwater pumping (Hoagstrom *et al.* 2011; Perkin *et al.* 2014a). In response, fish species richness and diversity have declined at local and regional (i.e. within major drainages) scales. Nonetheless, even though many fish species in Great Plains rivers are imperilled and there have been numerous local extirpations (Hubert and Gordon 2007; Jelks *et al.* 2008; Hoagstrom *et al.* 2011; Perkin & Gido 2011),

relatively few extinctions have been recorded (the Rio Grande Basin is a notable exception; Platania 1991; Hoagstrom *et al.* 2010). Previous studies have identified locations spread across the Great Plains that are apparent refuges for ecologically (Hoagstrom *et al.* 2011) and genetically diverse endemic taxa (Osborne *et al.* 2010, 2013). As long as fishes and refuges persist, management actions could potentially restore preimpact community characteristics in suitable or restored reaches of the Great Plains.

A logical course of management action would be to first address environmental causes of fish imperilment, followed by repopulation of key species in sufficient densities to sustain them in newly restored habitats (Perkin *et al.* 2014a). This strategy necessitates identification and protection of appropriate reservoirs of genetic diversity because reintroduction efforts are more successful and persist longer when repatriates are genetically diverse (Williams 2001). Specifically, genetically diverse repatriates may be more likely to adapt to local conditions. When possible, maintenance of historical patterns of population structure is also important. For these reasons, it is vital to understand how genetic diversity is distributed on the landscape and the causal mechanisms explaining these patterns. With this knowledge, it may be possible to make general predictions regarding the distribution of genetic diversity that are applicable across species, which in turn could be used to guide reintroduction efforts for whole assemblages.

The distribution of genetic diversity on the landscape is determined by at least three broad classes of factors, and these include geological processes, intrinsic traits and landscape features that include human-mediated habitat changes. Geological processes cause drainage rearrangements that isolate or connect river systems, and persistent climatic gradients can shape diversity patterns similarly across codistributed species over evolutionary time (Smith 1981; Richardson & Gold 1995; Waters & Nordt 1995). For example, a divide between previously connected drainages could produce a similar pattern of genetic structure among co-occurring species (Waters *et al.* 2001). In contrast, intrinsic factors such as body size, fecundity and vagility are expected to relate to genetic diversity through their influence on genetic effective population size and migration (gene flow) (Crispo *et al.* 2006; Faulks *et al.* 2010). Finally, more recent alterations to the landscape itself, such as human-mediated fragmentation and river dewatering, may also affect patterns of genetic diversity (Jager *et al.* 2001; Turner *et al.* 2006; Junge *et al.* 2014). For example, impediments to migration can deplete genetic diversity within riverine populations (Jager *et al.* 2001). Such alterations can influence genetic diversity through an interaction with intrinsic features in a manner somewhat akin to ecological filtering, where constraints of landscape features depend on traits related to reproduction and migration that vary among species (e.g. Turner *et al.* 2006; Junge *et al.* 2014).

In this study, we characterized the relative roles of geological and landscape factors in shaping diversity across species with different life-history strategies. We quantified genetic diversity at microsatellite loci for three co-occurring Great Plains river fishes within and among five major tributary basins of the Great Plains. Two of three target species are members of a reproductive guild of pelagic-spawning fishes (eggs and larvae drift in the water column) that are highly sensitive to fragmentation and dewatering because of downstream displacement of propagules (Dudley & Platania 2007; Perkin & Gido 2011). The other species is a physiologically tolerant (Matthews & Maness 1979) and abundant fish that employs benthic spawning (deposits eggs in or on substrate). If, despite their ecological differences, all three species exhibit similar patterns of genetic diversity across watersheds, we can infer a strong role for historical factors or similar response to landscape modification that have uniformly acted upon the riverine fish assemblage. Alternatively, species with differing ecological traits may respond differently to these factors (McGill *et al.* 2006). As such, a comparative demographic and population genetic approach, where multiple, codistributed species are studied, may establish whether general or species-specific patterns best describe overall diversity and improve our understanding of where and when reintroductions are likely to be successful in the Great Plains and other systems.

Methods

Study area

The focal area of this study encompassed five major tributary basins of the North American Great Plains listed from north to south: (i) the Platte between the Wyoming–Nebraska border and the confluence with the Missouri River; (ii) the Kansas upstream of the confluence with the Missouri River; (iii) the Arkansas between Larkin, Kansas and Keystone Reservoir in Oklahoma; (iv) the Canadian between the panhandle of Texas and Eufaula Reservoir in Oklahoma; and (v) the Red upstream of Lake Texoma in Oklahoma and Texas (Fig. 1). Together, these cover a large extent of the south-central Great Plains. Riverscapes in this region were historically connected longitudinally and laterally, characterized by periods of flashy high flows that inundated expansive floodplains as well as stochastic droughts that desiccated broad extents of stream (Dodds *et al.* 2004). This natural hydrologic expansion
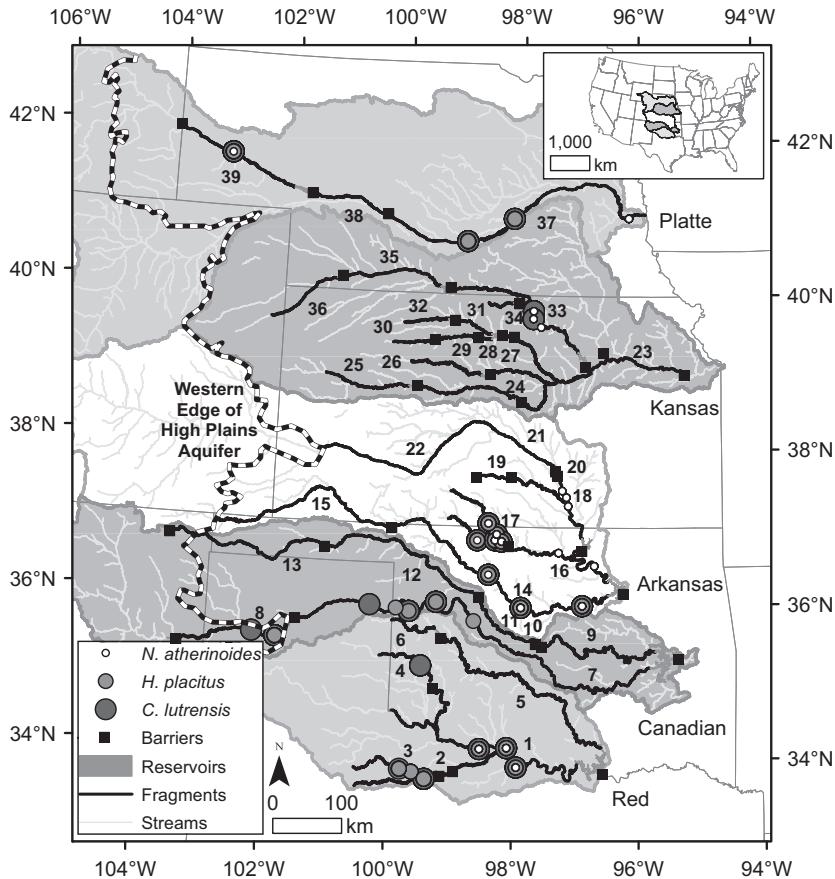
Fig. 1 Map of the study area and sampling localities for *H. placitus*, *N. atherinoides* and *C. lutrensis*. Numbers refer to stream fragments and fragment details are provided in Table S1.

and contraction drove unique adaptations among Great Plains stream biota, which consequently evolved ecological characteristics that are intimately linked to the natural variation in hydrology of Great Plains streams (Fausch & Bestgen 1997; Hoagstrom & Turner 2013). During the past century, major forms of landscape alteration included conversion of drainage basins into agricultural land, construction of instream barriers, and the diversion and extraction of surface and subsurface water (Costigan & Daniels 2012), all of which have contributed to alterations of stream fish communities in synergistic fashion (Gido *et al.* 2010; Hoagstrom *et al.* 2011).

Study species and life-history contrasts

We selected one representative fish from three predominant reproductive guilds. These fishes (and associated guild) included plains minnow *Hybognathus placitus* (pelagic broadcast spawning: releases nonadhesive semi-buoyant eggs into the pelagic-zone; Taylor & Miller 1990; Platania & Altenbach 1998), emerald shiner *Notropis atherinoides* (pelagic-substrate spawning: release nonadhesive demersal or sinking eggs into pelagic zones; Flittner 1964; Campbell & MacCrimmon 1970)

and red shiner *Cyprinella lutrensis* (substrate spawning: deposit adhesive demersal eggs over a variety of substrata; Gale 1986; Vives 1993). Although these three fish species were historically codistributed across much of the Great Plains, their distributions have shifted because of broad-scale landscape changes that have occurred in the region, including massive declines for *H. placitus* (Perkin & Gido 2011), declines in some areas but concurrent expansion in others for *N. atherinoides* (Pflieger & Grace 1987; Perkin *et al.* 2014b) and widespread persistence and expansion of *C. lutrensis* (Poulos *et al.* 2012). We selected the once broadly distributed *H. placitus* as a focal species to guide sampling across the landscape because this species represented the most severe declines.

Key landscape features and sampling site selection

The two major forms of landscape alteration affecting native and endemic fishes in the Great Plains involve dewatering and habitat fragmentation and associated habitat changes (Hoagstrom *et al.* 2011). Although these drivers are implicated in the decline of a number of species (Gido *et al.* 2010), they are also directly related to changes in the distribution of stream fish reproduc-

tive guilds selected for inclusion in this study (Perkin *et al.* 2014a). Consequently, we selected sampling site locations that incorporated the maximum variation in dewatering (measured as the number of days with zero flow using U.S. Geological Survey stream flow gages) and stream fragment length (measured as the longitudinal length of stream between two barriers). As these alterations have constrained the distribution of *H. placitus*, our sampling site selection was limited to stream fragments in which *H. placitus* persists and where *C. lutrensis* and *N. atherinoides* also co-occur. We prioritized sampling among 45 large stream fragments across the five tributary basins using data from collections taken between 1993 and 2013 (Perkin *et al.* 2014a) that described the probability of occurrence for these fish species. We began by targeting stream fragments that were most likely to be inhabited by *H. placitus* across all five tributary basins. During the summer of 2013, we visited fragments and sampled fishes using seines by targeting all available habitats at a site for at least 2 h or until a minimum of 30 individuals of each species could be collected. Caudal fin clips were collected from *H. placitus*, *N. atherinoides* and *C. lutrensis,* and fin clips were stored in 100% ethanol for subsequent DNA isolation.

Molecular methods

Genomic DNA was extracted from air-dried fin clips using standard proteinase-K digestion and standard phenol/chloroform methods (Hillis *et al.* 1996). *Hybognathus placitus*, *N. atherinoides* and *C. lutrensis* were assayed for variation at nine, seven and eight microsatellites, respectively. Microsatellites were amplified as 10 μL reactions, containing one μL diluted DNA, 1X Colorless GoTaq® Flexi Buffer, 2 mM MgCl$_2$ solution, 125 μM dinucleotide triphosphates (dNTPs), 0.4 μM of both forward and reverse primers, and 0.375 units of GoTaq® DNA Polymerase. For *H. placitus,* PCRs were initially denatured at 90 °C for 2 m, followed by 30 cycles of denaturing at 90 °C for 30 s, with annealing of 56 °C (*Ca3/Ca12*) or 58 °C (*Ppro126/Ppro132, Nme93/232*) or 49 °C (*Lco3/Lco6* and *Lco7*) for 30 s, and extension at 72 °C for 45 s and ending with a final extension at 72 °C for 30 m. For *N. atherinoides,* PCRs were the same but with annealing at 59 °C [*Nme232, Nme93, Nme208* (Gold *et al.* 2004), *Ppro126, Ppro132* (Bessert & Ortí 2003) and (*Ca12*) (Dimsoski *et al.* 2000)] or 49 °C *Ca6* (Dimsoski *et al.* 2000) for 30 s, and extension at 72 °C for 45 s and ending with a final extension at 72 °C for 30 m. For *C. lutrensis,* PCR conditions were the same with annealing temperatures of 58 °C (*Nme232, Nme93*) or 52 °C for *Nme208,* or 59 °C (*Ppro126/Ppro132, Nme174, Ca12*) or 50 °C for *Lco6* (Turner *et al.* 2004). For

each sample, one microlitre of PCR product was mixed with 10 μL of formamide and 0.4 μL of HD400 size standard and then denatured at 90 °C for 5 min. All samples were run on an automated ABI 3130 DNA sequencer and analysed with GENEMAPPER software (ABI).

Data analyses

Genetic variability microsatellites. GENEPOP version 3.1 (Raymond & Rousset 1995) was used to conduct modified exact tests to determine whether the observed genotype frequencies conformed to Hardy–Weinberg expectations within each collection locality. This program was also used to conduct the global test for linkage disequilibrium among loci. Sequential Bonferroni correction (Rice 1989) was applied to account for multiple comparisons. As the number of alleles and expected heterozygosity are dependent on sample size, we used a resampling procedure to calculate diversity measures. The minimum cut-off size was determined by the minimum sample size shared across species; hence, sites with fewer than 20 samples were excluded. Briefly, 1000 random subsamples were drawn without replacement from each temporal sample. Diversity and 95% CIs were calculated for each locus across subsamples and a mean was obtained across loci for each statistic [corrected number of alleles (N_{ac}), gene diversity (H_{ec}) and heterozygosity (H_{oc})]. This analysis was conducted in the R statistical package (R script available on request) (R Development Core Team 2008). Diversity statistics were calculated for each species and stream fragment (Table 1). One-way analysis of variance on ranks was conducted in SIGMA PLOT version 11.4 to examine whether genetic diversity statistics differed significantly between tributary basins for each species.

Genetic response to historical features. Hoagstrom & Berry (2006) suggested that *H. placitus*, *C. lutrensis* and *N. atherinoides* invaded streams of the northern plains from Gulf of Mexico drainages following glacial periods. If this was the case, higher diversity at southern latitudes is consistent with the prediction that stable populations survived *in situ* during Pleistocene climatic oscillations. Hence, to test the prediction that past environments and geologic events are the overriding factors shaping how diversity is distributed across the landscape, we used ordinary least-squares linear regression of allelic diversity and latitude conducted in SIGMA PLOT version 11.4.

We evaluated population structure for each species using Weir & Cockerham's (1984) hierarchical *F*-statistics calculated in ARLEQUIN version 3.11 (Excoffier *et al.* 2005). We conducted two AMOVAs, the first to determine

Table 1 Fragment length (river kms), sample size (n) and genetic diversity statistics (N_{ac} — allelic diversity, H_{ec} — gene diversity and H_{oc} — observed heterozygosity) obtained using a resampling approach for each species sampled from river fragments in the Great Plains shown in Fig. 1

Fragment	Fragment length	*Hybognathus placitus*				*Notropis atherinoides*				*Cyprinella lutrensis*			
		n	N_{ac}	H_{ec}	H_{oc}	n	N_{ac}	H_{ec}	H_{oc}	n	N_{ac}	H_{ec}	H_{oc}
Platte 37	504	29	8.339	0.730	0.519	26	10.190	0.770	0.701	24	7.867	0.694	0.592
Platte 39	198	12	—	0.804*	0.612*	2	—	—	—	22	8.172	0.692	0.536
Kansas 33	332	—	—	—	—	30	9.133	0.711	0.643	32	9.302	0.758	0.678
Arkansas 14	528	35	10.380	0.745	0.565	30	11.682	0.842	0.715	30	10.115	0.740	0.659
Arkansas 16	292	—	—	—	—	30	10.504	0.839	0.784	—	—	—	—
Arkansas 17E	186	20	8.444	0.658	0.528	20	11.286	0.827	0.738	20	8.767	0.765	0.597
Arkansas 17W	186	30	9.453	0.684	0.556	21	10.791	0.848	0.794	30	10.289	0.796	0.973
Arkansas 18	251	—	—	—	—	30	11.958	0.849	0.806	—	—	—	—
Canadian 7	793	6	—	—	—	—	—	—	—	51	10.701	0.768	0.649
Canadian 8	220	32	9.712	0.711	0.555	—	—	—	—	30	10.846	0.834	0.654
Red 1	793	24	10.049	0.803	0.508	28	11.201	0.850	0.749	30	12.441	0.843	0.635
Red 3	203	30	10.129	0.795	0.540	—	—	—	—	18	10.375	0.835	0.576
Red 4	162	—	—	—	—	—	—	—	—	12	—	0.818*	0.740*

*Collections that were excluded from resampling analysis (due to small sample size) for diversity metrics, raw values for H_e and H_o are provided.

whether or not genetic variance could be attributed to differences between major basins. For this analysis, samples were grouped according to drainage basins (i) Missouri [Platte and Kansas] and (ii) Arkansas-Red [Red, Canadian and Arkansas]). The second AMOVA was used to determine whether there were significant differences in population structure among the five sampled tributary basins (F_{CT}), among fragments within tributary basins (F_{SC}) and among fragments irrespective of tributary basin (F_{ST}). In this case, fragments were grouped according to tributary basin of origin (i) Platte, (ii) Kansas, (iii) Arkansas, (iv) Canadian and (iii) Red River fragments. Significance was assessed by 1000 bootstrap replicates.

In addition to AMOVA, we assessed the level and nature of population structure for each species in a spatial context with the GENELAND software package executed in the R environment (Guillot *et al.* 2005). This program uses a geographically constrained Bayesian model that accounts for the spatial position of sampled individuals and their multilocus genotypes. Five replicate runs were conducted for each species with 500 000 Markov chain Monte Carlo (MCMC) iterations, the Dirichlet model of allele frequencies, no uncertainty in the spatial coordinates, maximum rate of the Poisson process was 100 and the maximum number of nuclei for the Poisson–Voronoi tessellation was 300. We assessed plots of MCMC runs for convergence. The range of Ks (number of genetic clusters) to test was set from one to the maximum number of sampled fragments for each species

and hence ranged from 9 to 10. The run with the highest log posterior density was selected as the best representation of the data. An additional five runs were conducted at the optimal value of K determined in the previous analyses with a burn-in of 5000 to trim the posterior distribution in the post processing step. For each species, a heat map was produced of the posterior probabilities of locations belonging to specific genetic clusters. Tributary basin boundaries were superimposed on the heat maps to illustrate that population groups fall clearly within the riverine network.

Genetic response to contemporary landscape features. To examine the genetic response to contemporary landscape features, we used an information-theoretic approach (Akaike 1973). The response variables were gene diversity, observed heterozygosity and allelic diversity adjusted to account for differences in sample size among collections (as described above). The following determinant (landscape features) variables were included for each river fragment: fragment length, discharge and percentage of days with zero flow. This analysis was repeated using the residual values for the response variables (H_{ec}, H_{oc} and N_{ac}) after accounting for the effect of latitude. Fragment length represented the maximum length (river kilometres) of uninterrupted stream available to fishes between two barriers. Discharge represented the average daily discharge value for the period 1970–2013 based on U.S. Geological Survey stream flow gages distributed among fragments.

Finally, percentage of days with zero flow represented the proportion of days that stream flow gages logged zero discharge for the period 1970–2013.

We used graph theory to illustrate landscape-scale patterns in population genetic diversity metrics. For genetic-level graphics, nodes were sized according to allelic diversity and coded to illustrate gene diversity for each fragment (Erős *et al.* 2012). Links were sized to illustrate pairwise F_{ST} values between patches arranged immediately adjacent to each other such that thick lines represented small F_{ST} values (i.e. high connectivity) and thin lines represented larger F_{ST} values near to one (i.e. low connectivity). For fragments where *H. placitus* or *N. atherinoides* were not encountered during sampling, or where *C. lutrensis* were collected but not analysed, we used default and standardized node and link sizes to reflect lacking data whilst still maintaining the architecture of the network. We created separate graphics for each target species and visually inspected them for similarities and differences among species.

Genetic effective size. The linkage disequilibrium method (Hill 1981) was used to estimate the effective number of breeders (N_{eD}) from microsatellite DNA data for each species using the program NEESTIMATOR version. 2.0 (Do *et al.* 2014). Fragments were grouped by river for this analysis to achieve sample sizes that were sufficient to maximize accuracy and precision of N_{eD} estimates. NEESTI-MATOR implements a correction factor to account for bias that may occur when the sample size is less than the real (unknown) effective size (England *et al.* 2006). Highly polymorphic loci with many rare alleles, typical of microsatellites, can cause biased estimates of N_{eD} (Hedrick 1999; Waples 2006). For this reason, NEESTIMATOR calculates estimates after excluding all alleles with frequencies of less than a specified critical value. Here, we used $P_{crit} = 0.02$ as suggested where the number of individuals sampled is >25 (Waples & Do 2010). This value of P_{crit} generally provides a good balance between precision and bias (Waples & Do 2010). Upper- and lower-bound 95% confidence intervals for N_{eD} were calculated using the jackknife approach implemented in LDNE.

Results

Sample site selection and genetic variability metrics

The patch-graph theoretic approach to visualizing species probability of occurrences between 1993 and 2013 illustrated spatially explicit patterns across tributary basins that were associated with fragment lengths (Fig. 2). Despite the historically wide distribution of *H. placitus*, truncated occurrences were evident in each of the five tributary basins. Generally, *H. placitus* occur-

rences were high in longer fragments (larger circles) that were wet 90% of the time (open circles in Fig. 2). *Notropis atherinoides* followed a similar pattern, whereas *C. lutrensis* was typically persistent in all fragments and indicated no apparent pattern of reduced occurrence in any type of habitat. Based on these data, we sampled 24 fragments during the summer of 2013. Fragment numbers from which we encountered targeted species and obtained genetic samples are indicated by bolded and underlined numbers in Fig. 2 (see Table S1 for details of each fragment).

Linkage disequilibrium occurred between *Nme232* and *Nme174* (in the Platte) in *C. lutrensis* and between *Ppro126* and *Lco3* in *H. placitus*. For *N. atherinoides,* there was no evidence of linkage disequilibrium and departures from Hardy–Weinberg expectations at loci *Nme93* and *Ca6* were observed after adjusting for multiple comparisons. For *C. lutrensis,* there were eight departures from HWE from 161 comparisons after adjusting for multiple comparisons. For *H. placitus,* there were seven departures from HWE from 189 tests after Bonferroni adjustment. Diversity metrics did not differ significantly among tributary basins for either *H. placitus* or *N. atherinoides* (Table 1), but N_{ac} differed significantly among tributary basins for *C. lutrensis* ($P = 0.037$); specifically, N_{ac} was higher in southern tributary basins (Canadian and Red) when compared to populations in the Platte tributary basin.

Genetic response to historical factors

For *C. lutrensis* and *H. placitus*, there was a strong latitudinal gradient in N_{ac} which increased in a southerly direction, with latitude describing 73% ($P = 0.002$) and 63% ($P = 0.032$) of the variation, respectively (Fig. 3). The same trend was apparent for *N. atherinoides,* but the association was weaker and not statistically significant ($r^2 = 0.38$, $P = 0.101$).

The first AMOVA, testing for differences between major basins (Missouri and Arkansas-Red-Canadian), showed that F_{CT} was significant for *C. lutrensis* and *N. atherinoides* (Table 2A). For *H. placitus*, F_{CT} was not significantly different from zero indicating that variance could not be attributed to differences between Missouri and Arkansas-Red-Canadian drainages. For the second AMOVA (Table 2B), a significant portion of variance was explained by differences among tributary basins for *C. lutrensis* and *H. placitus* but not for *N. atherinoides*. For all species, there was significant divergence among fragments within tributary basins and among fragments irrespective of tributary basins.

Results from the Bayesian assignment of individuals conducted using GENELAND grouped populations into either two (*N. atherinoides* and *C. lutrensis*) or three (*H. placitus*) major clusters (Fig. 4A–C). *Notropis atheri-*
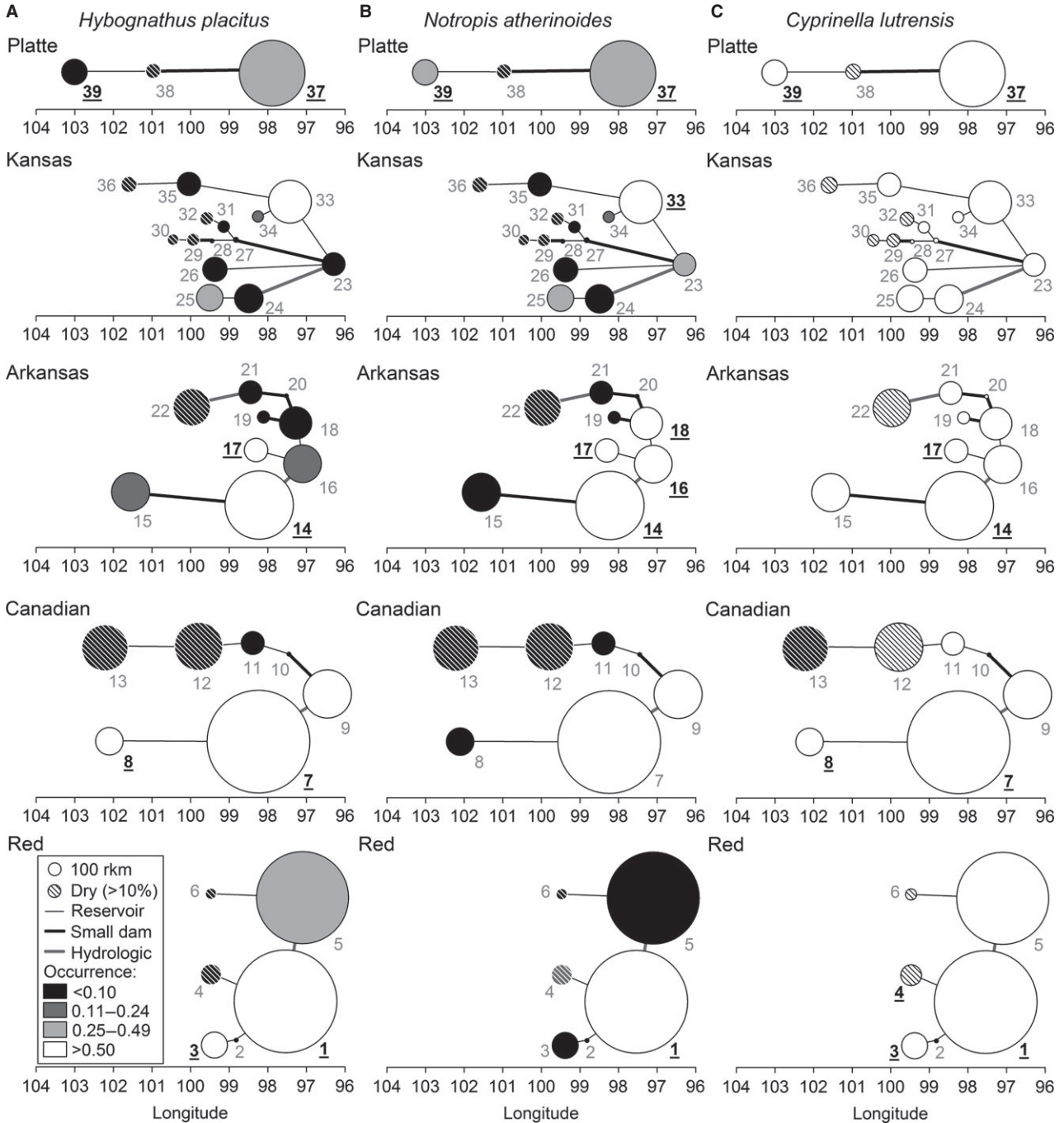
Fig. 2 Graph theory plots illustrating spatial patterns in population-level responses to fragmentation and dewatering in three species (A) *H. placitus*, (B) *N. atherinoides* and (C) *C. lutrensis*. Population-level patch-based graphics were constructed by sizing nodes (circles) in relation to the longitudinal length of stream between barriers as well as coding nodes by percent of days with zero flow (cross-hashing) and species probability of occurrence during 1993–2013 (colours; data from Perkin et al. 2014a). Links (lines) represent the height of barriers that isolate fragments. Upstream (shorter, drier) fragments generally occur to the left side of each graph.

noides clustered into northern (Platte and Kansas tributary basin populations) and southern (Arkansas and Red River tributary basin populations) groups. *Cyprinella lutrensis* population clusters were (i) Platte-Kansas and (ii) Arkansas, Red and Canadian River fragments, consistent with AMOVA analysis. Finally, for *H. placitus*, clusters were (i) Platte fragments, (ii) Arkansas-Canadian River fragments and (iii) Red River fragments.
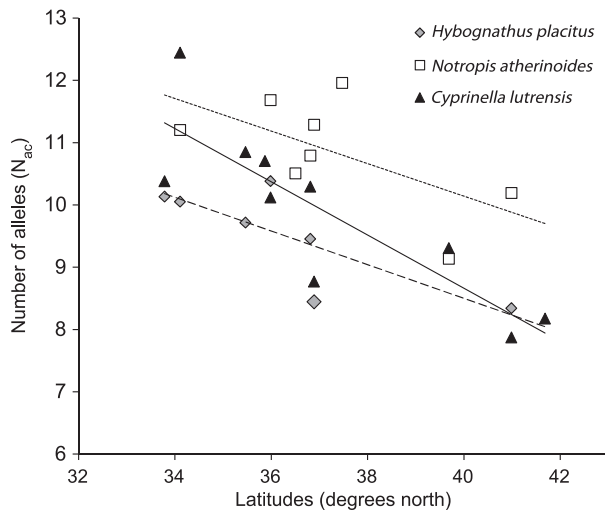
Fig. 3 Least square regression for number of alleles (N_{ac}) and latitude for *H. placitus* (dashed line), *N. atherinoides* (dotted line) and *C. lutrensis* (solid line).

Genetic response to landscape features

Reducing the available sampling sites to those with high probability of occurrence for *H. placitus* truncated environmental gradients regarding fragment size and extent of dewatering (Fig. 5). Stream fragments <200 rkm in length accounted for 36% of availability, but could not be included among those sampled for genetic analysis because *H. placitus* did not persist in these fragments (Fig. 3A). Similarly, only 50% of available intermediate sized fragments (200–400 rkm) were included in analysis because of *H. placitus* extirpations from these fragments. The majority (80%) of those >400 rkm were analysed because *H. placitus* persisted and was collected within these fragments. *H. placitus* had very low probabilities of occurrence in stream fragments that dried more than 20% of the time and these stream fragments were not included in sampling. Higher probabilities of occurrence were estimated in fragments that never dried or dried

between 10 and 20% of the time, and these were included in sampling (Fig. 5).

Regression analyses using an information-theoretic approach to model selection suggested uniformly that differences in genetic diversity metrics were not attributable to sampled gradients of contemporary human alterations involving fragmentation and dewatering of rivers (Table S2 A–C). Results were essentially the same using the residual values for the response variables (H_{ec}, H_{oc} and N_{ac}) after accounting for the effect of latitude. In the context of river basin networks, these findings illustrate strongholds for each species in all tributary basins except the Kansas for *H. placitus* and the Canadian for *N. atherinoides* (Fig. 6), where neither species were collected. In some instances, spatially explicit variation in gene diversity illustrated reduced diversity in upstream sites for *H. placitus* in the Red (fragment 3) and Arkansas (fragment 17) tributary basins (i.e. darker shading in upstream nodes). In the Arkansas tributary basin, allelic diversity was also lower in an upstream fragment (17) for *H. placitus* (i.e. small nodes upstream). For *C. lutrensis*, allelic diversity was higher in the downstream fragment (fragment 1) in the Red, but the reverse was true for the Canadian tributary basin fragments. No clear pattern was observed for *N. atherinoides*.

Genetic effective size

Estimates of N_{eD} were highly variable across species and tributary basins (Table 3). N_{eD} for *H. placitus* was very small for the Platte tributary basin (N_{eD} = 35, 95% CIs 21 to 71) and ranged from 1249 (Arkansas tributary basin [95% CIs 252 to infinity]) to 6970 (209 to infinity) in the Canadian tributary basin. N_{eD} estimates for *N. atherinoides* were small in the Kansas (N_{eD} 85, 95% CIs 34 to infinity) and Platte (N_{eD} 96, 95% CIs 40 to infinity) tributary basins. N_{eD} estimates were negative for the Arkansas and the Red tributary basin populations of *N. atherinoides*; hence, we cannot reject the possibility of very large popu-

Table 2 Results of AMOVA testing for genetic structure across basins with associated *P*-values. (A) Samples were grouped into major drainage basins belonging to either the Missouri (Platte-Kansas) or Arkansas-Red basins. (B) Samples were grouped according to the five sampled tributary basins (Platte, Kansas, Arkansas, Canadian and Red River tributary basins)

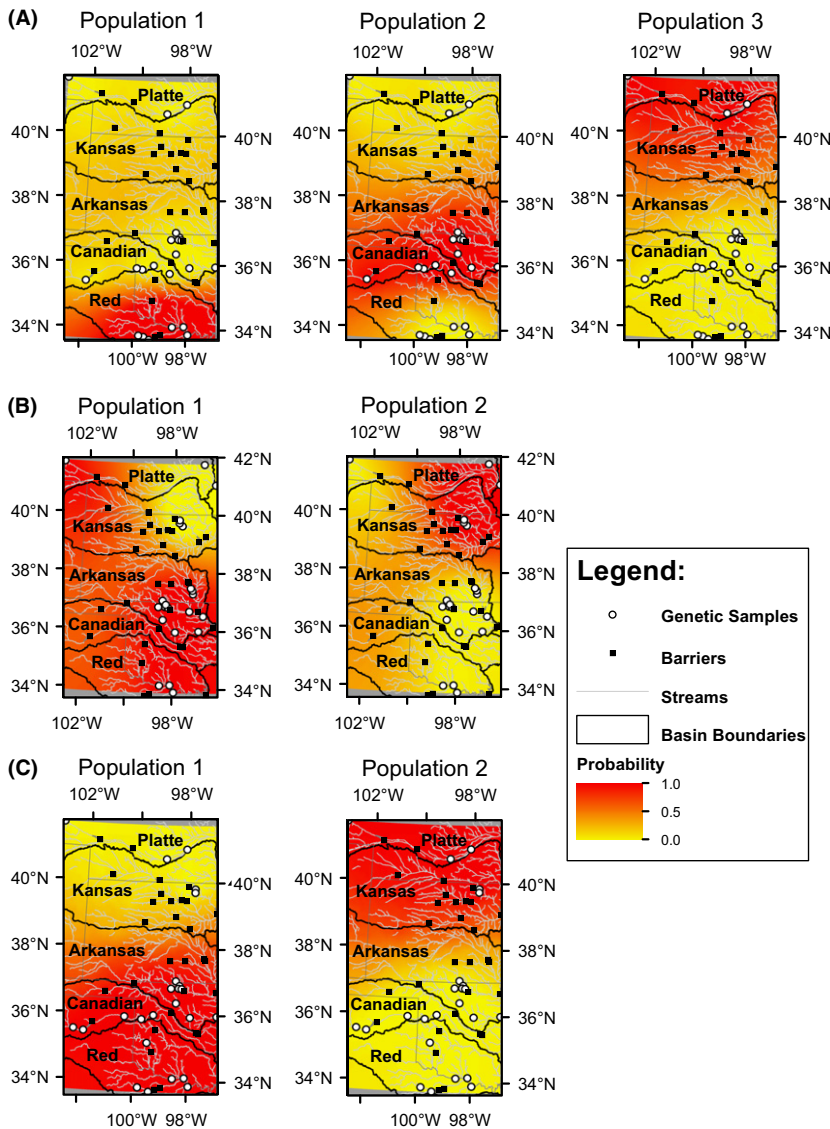|	*Hybognathus placitus*	*P*	*Notropis atherinoides*	*P*	*Cyprinella lutrensis*	*P*
A. Missouri/Arkansas-Red						
F_{CT}	−0.018	0.727	0.053	0.008	0.031	0.032
F_{SC}	0.1	<0.0001	0.014	<0.0001	0.029	<0.0001
F_{ST}	0.084	<0.0001	0.066	<0.0001	0.0582	<0.0001
B. Tributary Basin						
$F_{CT \ (Tributary \ Basin)}$	0.099	0.006	0.032	0.073	0.041	0.0001
$F_{SC \ (FRAGMENTS)}$	0.014	0.002	0.017	0.0001	0.006	0.0136
F_{ST}	0.111	0.0001	0.049	0.0001	0.046	0.0001

Fig. 4 Heat maps of posterior probabilities of population membership (A) *H. placitus*, (B) *N. atherinoides* and (C) *C. lutrensis*. Red indicates regions with a high probability (>0.70) of population membership. The axes are latitude and longitude and open dots indicate genetic sampling localities shown in Figure 1. Filled squares indicate the location of in-stream barriers. Tributary basin boundaries are also shown.

lation sizes for this species in these tributary basins. N_{eD} of *C. lutrensis* was small in the Canadian tributary basin (179, 95% CIs 102 to 530) and moderate in the Arkansas (357, 95% CIs 91 to infinity), Platte (447, 95% CI 38 to infinity) and Kansas tributary basins (788, 95% CIs 78 to infinity) and indistinguishable from infinity (95% CI: 288 to infinity) in the Red tributary basin.

Discussion

A comparative landscape genetic framework was employed to elucidate the relative roles of geological processes (e.g. drainage rearrangements), intrinsic differences in species traits and contemporary landscape changes for shaping diversity across species, and these results were used to identify genetically diverse populations that could be used in conservation efforts for

aquatic communities in Great Plains streams. The overriding factor shaping contemporary landscape-scale patterns of genetic diversity in *C. lutrensis* and *H. placitus* was the historical signature of past climates and geology. Specifically, allelic diversity was higher at more southern latitudes in these species consistent with northward expansion from southern Pleistocene refugia. Most, but not all, remnant populations exhibited high levels of genetic diversity regardless of species.

Population diversity and structure across the landscape

Gradients in genetic diversity have been explained by several processes (Fisher 1930, 1937; Huxley 1938; Haldane 1948) including adaptation to local conditions along an environmental gradient. Alternatively, contemporary haplotypic/genotypic distributions may reflect the
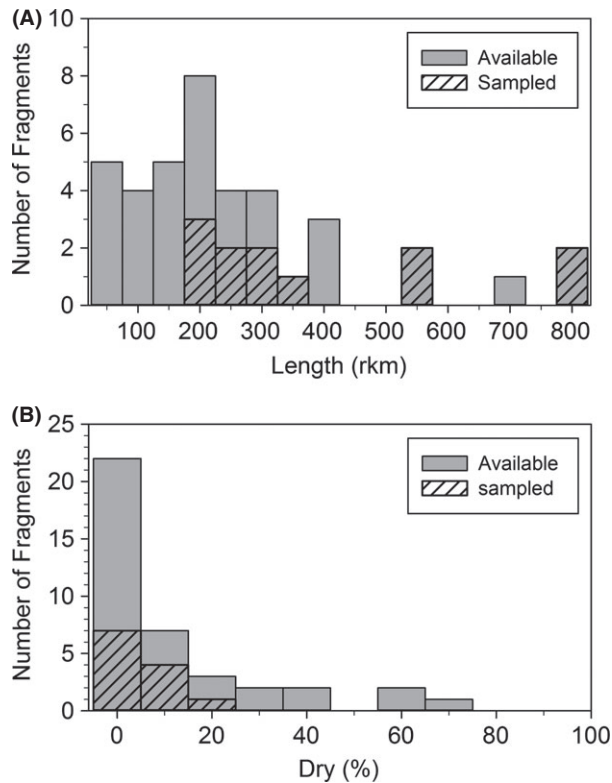
Fig. 5 Distributions of available (gray bars) and sampled (hashed bars) stream fragments illustrating the truncated variability in (A) fragment length and (B) days with zero flow for samples involved with molecular-level responses to landscape features. Samples included in this study tended to be from fragments with longer length and fewer days with zero flow.

response of a species (range expansion and contraction) to multiple demographic events determined by past geological and climatic forces (Koizumi *et al.* 2012). Latitudinal clines are not surprising as climatic factors including temperatures, rainfall and evaporation vary from north to south (Huxley 1942). For example, latitudinal gradients in spawning season have been observed in minnows, with shorter spawning seasons at northern latitudes (Gotelli & Pyron 1991). Many European and North American fish species have high genetic diversity and older lineages at southern latitudes, whilst diversity is lower at northern latitudes, indicative of colonization from the south following glacial periods (Hewitt 1999).

In two of the three species we examined (*H. placitus* and *C. lutrensis*), similar patterns of decreasing allelic diversity with increasing latitude were consistent with findings of Hoagstrom & Berry (2006), who suggested that *H. placitus* and *C. lutrensis* (as well as *N. atherinoides*) invaded streams of the northern plains from Gulf of Mexico drainages following glacial periods. Higher diversity in populations at southern latitudes suggests that populations survived *in situ* where habitats were likely more benign and harboured stable rear-edge

populations (Channel & Lomolino 2000; Alberto *et al.* 2008) that were less affected by climatic oscillations and river rearrangements compared to northern populations. Although microsatellites may evolve too rapidly to make inferences that far back into evolutionary time, patterns revealed here are consistent with other studies. For example, results presented for *C. lutrensis* reiterate findings of Richardson & Gold (1995) who identified latitudinal differences in phylogeographic structuring in *C. lutrensis* and of Pittman (2011) who presented similar results for *Notropis stramineus* (sand shiner). Specifically, populations far from glaciers have more phylogeographic diversity, deeper clades and clear geographic structuring. Previous authors attributed this finding to glacial influences, which impacted populations beyond the ice margin (Bernatchez & Wilson 1998). Also, the widespread northern clade of *N. stramineus* shows higher genetic diversity in populations further from the Pleistocene glacial fronts (Pittman 2011) as does northern plains killifish, *Fundulus kansae* (Brown 1986).

Hierarchical patterns of population genetic structure differed somewhat between the three species examined. *Hybognathus placitus* fell into three genetically distinct clusters corresponding to the (i) Platte tributary basin, (ii) Arkansas-Canadian tributary basins and (iii) Red tributary basin fragments. Previously, Al-Rawi & Cross (1964) identified clinal differences in the number of scales above and below the lateral line with high scale counts for individuals from northern populations (upper Missouri) to low scale counts in central population individuals (Platte, Kansas and Arkansas) populations of *H. placitus*. They observed a break in the cline separating these populations (northern and central) from those in the south (Red, Brazos and Colorado) where individuals had a high number of scales above and below the lateral line. Other species with similar geographic clustering include the plains killifish with distinct (i) northern group (Platte-Republican-Smoky Hill), (ii) central group (Arkansas-Canadian-Cimarron) and (iii) Red-Brazos-Pecos populations (Kreiser *et al.* 2001). This suggests these species and *H. placitus* were likely affected similarly by geologic and climatic events. Geneland heatmaps and significant F_{SC} values for *N. atherinoides* and *C. lutrensis* supported clustering of fragments into northern (Platte-Kansas) and southern groups (Arkansas-Red). Consistent with the finding of a Platte-Kansas cluster, these drainages were connected as recently as the Pleistocene, which likely provided a dispersal corridor facilitating movement into the Platte River. The paleo-Red River was much more extensive in the Miocene and included all of the modern Arkansas tributary basin in the Plains (Galloway *et al.* 2011). The lower Arkansas formed relatively recently (late Pliocene–early Pleistocene) and captured much of the upper Red including the Canadian. Glacial advances
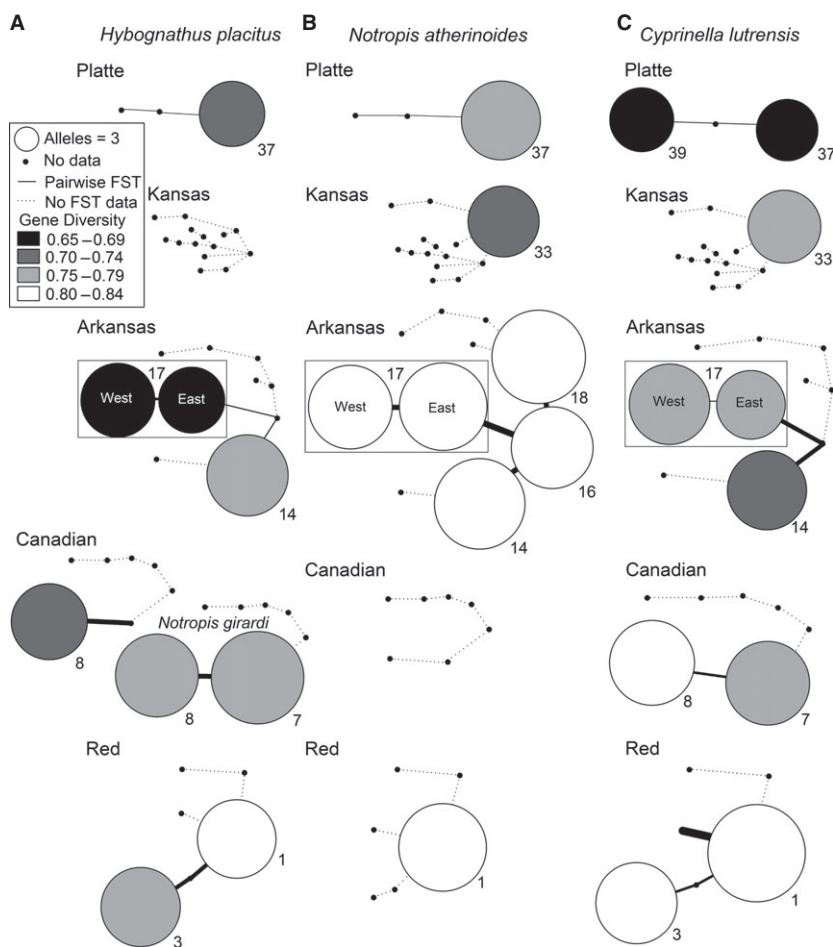
Fig. 6 Graph theory plots illustrating spatial patterns in molecular-level responses to fragmentation and dewatering for three species (A) *H. placitus*, (B) *N. atherinoides* and (C) *C. lutrensis*. Molecular-level patch-based graphics were constructed by sizing nodes in relation to allelic diversity and coding nodes by gene diversity. Lines represent pairwise F_{ST} values such that thick lines indicate relatively lower F_{ST} values. We also included a graph theory plot for *Notropis girardi* Arkansas River shiner (a pelagic-spawning species) (inset in the *H. placitus* plot for the Canadian) based on previous work (Osborne et al. 2013). Upstream (shorter, drier) fragments generally occur to the left side of each graph.

Table 3 Genetic effective size estimates (N_{eD}) and associated 95% confidence intervals by species and tributary basin. Dashes indicate that no samples were taken in that basin

Tributary Basin	*Hybognathus placitus*			*Notropis atherinoides*			*Cyprinella lutrensis*		
	$N_{eD(Pcrit = 0.02)}$	−95%	95%	$N_{eD(Pcrit = 0.02)}$	−95%	95%	$N_{eD(Pcrit = 0.02)}$	−95%	95%
Platte	35	21	71	96	40	∞	447	38	∞
Kansas	—	—	—	86	34	∞	789	78	∞
Arkansas	1249	253	∞	∞	1375	∞	357	91	∞
Canadian	6969	209	∞	—	—	—	179	102	269
Red	1867	196	∞	∞	70	∞	∞	288	∞

and retreats characterized the Pleistocene, causing cycles of river incision and valley aggradation along the upper Mississippi causing this lowland valley to repeatedly trap flanking rivers, including the Red and Arkansas, facilitating movement between these systems.

Genetic response to contemporary landscape features

We did not detect a relationship between contemporary landscape alterations and genetic diversity. We anticipated, based on population genetics theory, that shorter (e.g. Jager *et al.* 2001) and drier river fragments should harbour less genetic diversity than those in larger fragments in the absence of gene flow and roughly constant fish densities (Gido *et al.* In Press). There are several possible reasons for either the absence of, or a failure to detect a relationship between fragment length and genetic diversity, two of these are (i) populations sampled for genetic analysis did not include the shortest fragments (and hence, smallest populations) and (ii)

stochastic events cause local extirpations before negative genetic effects occur. Truncated sampling across key gradients (Fig. 5) resulted in a reduced power to document genetic responses at extreme endpoints (i.e. shortest fragments or driest streams). This is because focal species (*H. placitus*) was extirpated or had declined to the point of nondetection, rendering tissue samples unavailable for genetic analysis. This was particularly true of pelagic-spawning species which were rare or absent in short fragments with a greater number of days with zero flow (Fig. 5; Dudley & Platania 2007; Perkin *et al.* 2014a). Hence, populations sampled for genetic analysis are a subset of previously occupied fragments and do not include the smallest fragments or smallest populations where genetic deterioration is most likely. This is also illustrated by the population-level plots that show low probability of occurrence for *H. placitus* and *N. atherinoides* in the majority of fragments; particularly short ones, hence genetic diversity could not be measured. Also absent from these fragments were other pelagic-spawning species including *N. girardi* (Perkin *et al.* 2014a).

Fragmentation and dewatering differentially affected persistence of fishes included in this study. The shortest reach where enough samples could be obtained for genetic analysis for *H. placitus* was 180 rkms. This reach may be large enough to support relatively large population sizes of *H. placitus* if other habitat features (i.e. backwaters, sand bars and other essential habitat) are present. Such habitat heterogeneity and complexity could maintain larger populations and slow rates of genetic drift. Large populations are suggested by the effective size estimates for *H. placitus* in the Red, Canadian and Arkansas rivers. The absence of pelagic broadcast spawning species in small fragments is consistent with previous findings (Luttrell *et al.* 1999; Dudley & Platania 2007; Perkin & Gido 2011) that examined the relationship between river fragmentation and extirpation of native pelagic-spawning species. These studies found that almost all reaches <100 rkms failed to retain pelagic-spawning species, whilst reaches exceeding 100 rkm retained some percentage of them. Pelagic-substrate-spawning fishes such as *N. atherinoides* also respond to fragmentation of riverscapes and can be missing from shorter stream fragments (Perkin *et al.* 2014a). A major difference between fishes belonging to this guild and most pelagic-spawning fishes is the ability to inhabit and reproduce within reservoirs, which has likely helped to buffer pelagic-substrate-spawning fishes against the effects of fragmentation and dewatering throughout the Great Plains (Perkin *et al.* 2014a). In fact, reservoirs are implicated in the expansion of *N. atherinoides* upstream of reservoirs (Pflieger & Grace 1987; Hoagstrom & Turner 2013), which provides some

clue as to the mechanism behind the enigmatic pattern by which *N. atherinoides* is vulnerable to the effects of fragmentation but has also shown long-term increases in abundance for some Great Plains streams (Perkin *et al.* 2014a,b). Finally, *C. lutrensis* was widely persistent even among the most highly fragmented and dewatered stream fragments included in this study and might not be expected to respond to fragmentation at such broad spatial extents, although there is evidence that the species is vulnerable to the effects of isolation at smaller scales (Matthews & Marsh-Matthews 2007).

Stochastic demographic and environmental events generally appear to cause local population extirpation before genetic effects are apparent in Great Plains rivers. For example, available genetic data for the endangered Rio Grande silvery minnow (*Hybognathus amarus*) (U.S. Department of the Interior 1994) (Alò & Turner 2005; Osborne *et al.* 2012) showed that diversity at microsatellites was not alarmingly low even when the population was teetering on the edge of extinction in the wild (Osborne *et al.* 2012). However, in this species, high variance in reproductive success among spawning aggregations caused by the interaction of fragmentation and pelagic early life-history is likely responsible for the small effective to census size ratio observed in *H. amarus* (Alò & Turner 2005; Osborne *et al.* 2005; Turner *et al.* 2006). In the most vulnerable species considered here (*H. placitus*) and those that share its pelagic life-history (but that were not detected in short fragments; Perkin *et al.* 2014a), it is plausible that stochastic forces such as drought ultimately determine the fate of populations rather than negative genetic impacts (Kelsch 1994; Perkin *et al.* 2014b). Numerous studies have demonstrated that factors including short lifespan combined with increased isolation from other populations and occasional absence of reproduction or recruitment (e.g. caused by absence of spawning cues such as spring run-off, Bonner & Wilde 2000) cause strong fluctuations in population size and thereby increase extinction risk (Leigh 1981; Pimm *et al.* 1988; Sjögren 1991). Historical and contemporary periods of drought (Matthews & Marsh-Matthews 2007; Perkin *et al.* 2013, 2014b) combined with negative effects of fragmentation (Winston *et al.* 1991; Luttrell *et al.* 1999; Wilde & Ostrand 1999) are thought to have eliminated populations of endemic fish, particularly pelagic-spawning species (Kelsch 1994). Specifically, Perkin *et al.* (2014b) hypothesize that the disappearance of three pelagic-spawning species (*N. girardi*, *H. placitus* and *Macrhybopsis tetranema* [peppered chub]) from the Arkansas and Ninnescah Rivers were related to the effects of fragmentation and periodic droughts over the past few decades. They further suggested that drought may be the final 'nail in the coffin' for pelagic-spawning species that have already

suffered losses of habitat and connectivity caused by fragmentation (Luttrell *et al.* 1999) and stream desiccation (Durham & Wilde 2008). However, our results are only a snapshot of genetic processes and we do not know if diversity has changed in these fragments over time.

Implications for identifying conservation priorities

Patch-based graphs of capture probabilities show that longer and wetter river fragments, mainly located in downstream areas, retain higher fish community diversity (Fig. 2; Perkin *et al.* 2014a). This pattern is repeated across species and Great Plains drainages and has also been described elsewhere (Schlosser 1990). In contrast, genetic diversity within remnant populations is typically high – particularly within southern tributary basins – and there is no detectable relationship between fragment length or stream dryness and genetic diversity (this study). However, we reiterate that the shortest and driest fragments were not included for genetic analysis because the most sensitive species (*H. placitus*) was no longer detected in them (Fig. 5). Stream flow is directional, thus fragmentation effects riverine populations differently than other ecosystems and colonization/ extinction probabilities adhere to a longitudinal gradient (Jager *et al.* 2001). Hence, shorter and drier fragments, usually located upstream, exhibit higher rates of extirpation (Gotelli & Taylor 1999) and cannot be naturally recolonized because of impoundments (Winston *et al.* 1991) or impassable barriers. Based on similar genetic studies in less fragmented systems (Turner *et al.* 2006; Osborne *et al.* 2010, 2013), it is likely that gene flow and metapopulation dynamics linked downstream and upstream sites for many species before fragmentation in Great Plains rivers. This set of observations justifies a conservation focus on reconnection of fragment reaches regardless of their position in the riverine network to restore both community and genetic diversity (Perkin *et al.* 2014a). Natural colonization may occur rapidly once habitat connectivity and quality is restored (Catalano *et al.* 2007; Archdeacon & Remshardt 2012; Hitt *et al.* 2012), but if not, it is appropriate for managers to translocate fishes to speed local recovery. As an example, reconnection of fragments within the Platte tributary basin would increase the length of available habitat and may increase the distribution of pelagic-spawning species. Although there is considerable drying in one of these fragments, provision of access to stretches that remain wet would likely be beneficial for most, if not all fish species (Perkin *et al.* 2014a). Genetic effective size is <50 for *H. placitus* in the Platte tributary basin, suggesting that this population is already at risk from genetic factors. Although not considered here, the population in the Kansas tributary basin is also likely to be at risk from stochastic impacts because of dwindling occurrences and shrinking populations throughout the Smoky Hill and lower Kansas tributary basins (Gido *et al.* 2010). Loss of genetic diversity is inversely related to effective size, such that small N_e increases that rate of diversity loss through genetic drift. Increased connectivity within tributary basins would facilitate gene flow among fragments and would provide populations with greater genetic resilience.

Genetic divergence across major drainage basins was observed for all three species. Furthermore, within-tributary basin diversity decreased with increasing latitude for *H. placitus* and *C. lutrensis*, which may be a general evolutionary pattern in North American freshwater fishes (Bernatchez & Wilson 1998). This raises important questions regarding whether cross-tributary basin stocking is detrimental to adaptive potential of restored populations, or alternatively, should cross-tributary basin stocking be employed to rescue populations with critically low diversity? For example, there are three genetically distinct clusters for *H. placitus* (Platte; Arkansas-Canadian; Red) and the population in the Platte harbours the least genetic diversity of the populations considered here (but the Kansas tributary basin population may be the most imperilled). Introduction of *H. placitus* from the Arkansas River could re-cover local diversity in the Platte, but perhaps at the expense of disrupting locally adapted gene complexes and lowering fitness. This is a complicated issue that requires further study, but we can offer two possible advantages of cross-basin introductions, particularly from genetically diverse southern populations as donors to relatively depauperate northern drainages as recipients. Genetic diversity in some populations may be so low that inbreeding effects may compromise their ability to naturally populate restored river reaches (e.g. *H. placitus* in the Platte River). More generally, introduction of genetic diversity from southern basins may provide genetic 'raw material' to allow for an evolutionary response to global warming effects in northern tributary basin populations (but see Matthews & Zimmerman 1990).

Conclusions

Proactive restoration and conservation strategies must be employed to stem predicted losses of freshwater biodiversity in the Great Plains, especially in the face of global warming. It may be possible to restore river habitat connectivity and quality to portions of the Great Plains without jeopardizing water security for human use (Perkin *et al.* 2014a). Our comparative landscape genetic study shows that underlying processes shaping diversity are similar across species, suggesting that similar restoration processes could be implemented for

these species and perhaps the entire riverine fish assemblage. In general, genetic diversity is concentrated in longer and wetter reaches that presumably harbour larger populations, such that restoration of functional connectivity with adjacent reaches will be most effective for restoring both species and genetic diversity. Once river habitat connectivity and complexity are restored, the preferred strategy would be to allow natural recolonization from occupied reaches in the same tributary basin, or in certain cases by facilitated stocking from other tributary basins (with appropriate precautions to avoid inadvertent transfer of nontarget species). Transfer between tributary basins may be warranted in cases where existing diversity is insufficient to ensure successful repopulation and long-term persistence of fishes of the Great Plains.

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Data accessibility

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Descriptions for 39 river fragments, including the United States Geological Survey (USGS) gage used to obtain flow data, longitudinal length measured in river km, average discharge during 1970–2013 (m^3/s), and percent of days with zero flow (dry).

Table S2. AICc results (A) *Hybognathus placitus* (B) *Notropis atherinoides*, (C) *Cyprinella lutrensis* unadjusted and adjusted for latitude.