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Hybridization drives genetic erosion in sympatric desert fishes of western North America

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Abstract(241/250)

Many species have evolved or currently coexist in sympatry due to differential adaptation in a heterogeneous environment. However, anthropogenic habitat modifications can either disrupt reproductive barriers or obscure environmental conditions which underlie fitness gradients. In this study, we evaluated the potential for an anthropogenically-mediated shift in reproductive boundaries that separate two historically sympatric fish species (*Gila cypha* and *G. robusta*) endemic to the Colorado River Basin using ddRAD sequencing of 368 individuals. We first examined the integrity of reproductive isolation while in sympatry and allopatry, then characterized hybrid ancestries using genealogical assignment tests. We tested for localized erosion of reproductive isolation by comparing site-wise genomic clines against global patterns and identified a breakdown in the drainage-wide pattern of selection against interspecific heterozygotes. This, in turn, allowed for the formation of a hybrid swarm in one tributary, and asymmetric introgression where species co-occur. We also detected a weak but significant relationship between genetic purity and degree of consumptive water removal, suggesting a role for anthropogenic habitat modifications in undermining species boundaries. In addition, results from basin-wide genomic clines suggested that hybrids and parental forms are adaptively non-equivalent. If so, then a failure to manage for hybridization will exacerbate the long-term extinction risk in parental populations. These results reinforce the role of anthropogenic habitat modification in promoting interspecific introgression in sympatric species by relaxing divergent selection. This, in turn, underscores a broader role for hybridization in decreasing global biodiversity within rapidly deteriorating environments.

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Introduction(926)

Many natural populations respond to anthropogenic change by either shifting geographic distributions or adjusting life histories so as to 'track' optimal conditions (Hoffmann and Sgrò 2011; Pecl *et al.* 2017). However, the ability of organisms to track changing environments is conditioned upon the rate of environmental change (Lindsey *et al.* 2013) and the rate at which adaptive machinery can act (Orr and Unckless, 2014). This evolutionary caveat creates an incentive for hybridization, in that recombinant genotypes might more rapidly establish in a dynamic adaptive landscape (Klonner *et al.* 2017). Widespread hybridization thus may provide an effective mechanism of population persistence in changing or novel conditions (Pease *et al.* 2016; Meier *et al.* 2017). Introgressed alleles which are beneficial under novel conditions can then be driven to fixation by the combined action of recombination and selection (Arnold and Martin 2010).

However, the relationship between hybridization and extinction is not well established under contemporary timescales. On one hand, hybrid lineages might facilitate adaptation by providing access to a greater pool of genetic variation (Dittrich-Reed and Fitzpatrick 2013; Schumer *et al.* 2018), whereas on the other, diversity might diminish as species boundaries dissolve (Buerkle *et al.* 2003; Kearns *et al.* 2018). Often, results are a combination of the above. Introgressed genotypes may initially compensate for erratic conditions and facilitate population persistence in the near term, but with lineages eventually merging if environmental change is prolonged (Seehausen *et al.* 2008). This presents an obvious paradox for the conservation biologist, in that the permeability of species-boundaries may be seen as promoting both persistence and extinction.

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Hybridization also represents a legacy issue for conservation policy (Allendorf *et al.* 2001), due primarily to its conflict with a species-centric management paradigm (Fitzpatrick *et al.* 2015; Hamilton and Miller 2016). Although the reticulate nature of speciation has become a contemporary research focus (e.g. Mallet *et al.* 2016), it has yet to gain consensus among managers (vonHoldt *et al.* 2018). This unanimity is required to understand the manner by which anthropogenic modifications disrupt species boundaries (Grabenstein and Taylor 2018; Ryan *et al.* 2018). However, predicting the outcome of hybridization in a changing environment requires an understanding of both the temporal and spatial stability of the mechanisms (e.g. intrinsic and extrinsic) that are responsible for maintaining species boundaries. In this sense, consistent patterns can often be obscured by local context (e.g. individual behaviors, population demographics; Klein *et al.* 2017). Hence, there remains a need to quantify the manner by which species boundaries in diverse taxa respond to rapid environmental change. We applied these perspectives to endemic, large-bodied and long-lived minnows that exist within the Colorado River, one of the most impacted riverine ecosystems of the Anthropocene (Hughes *et al.* 2007). Because of the pervasive human impacts therein, the Colorado River provides a natural laboratory within which to examine the stability of species undergoing rapid, anthropogenically-induced environmental change.

Hybridization in Gila

Hybridization has long been recognized as an evolutionary process in fishes (Hubbs, 1955), and as such, has been hypothesized as a mechanism for native fish diversification in western North America (e.g. DeMarais *et al.* 1992). External fertilization and limited predisposition for pre-mating isolation also likely promote hybridization as a common

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mechanism throughout fishes (e.g. Mayr 1963). An inseparable link also exists between fishes and their environment, such that opportunities for hybridization are controlled by characteristics of the riverscape (Hopken *et al.* 2013; Thomaz *et al.* 2016). The instability produced by modified flows may compromise boundaries between historically coexisting species, or provide ecological opportunities within which hybrid lineages might capitalize (Dowling and Secor 1997). The fact that habitats in western North America were historically subjected to tectonism and progressive aridity also provides an explanation for the high prevalence of hybridization relative to species richness in southwestern North America (e.g. Mandeville *et al.* 2017; Bangs *et al.* 2018). However, more contemporary anthropogenic modifications are also prominent and widespread, most apparent in the form of water acquisition and retention (Cayan *et al.* 2010). As a result, niche gradients that historically segregated species are now seriously perturbed. This, in turn, can promote hybridization by effectively removing selection against hybrid phenotypes, and by disrupting the phenology and reproductive cues that discourage heterospecific mating (Grabenstein and Taylor 2018).

We applied these perspectives to three species of conservation concern endemic to the Colorado River Basin: Humpback chub [*Gila cypha* (IUCN status=Endangered)], Roundtail chub [*G. robusta* (Near Threatened)], and Bonytail [*G. elegans* (Critically Endangered)]. All are hypothesized as exhibiting various levels of historic hybridization, with contemporary populations shaped by geologic processes and anthropogenic interventions. *Gila cypha* and *G. robusta*, display not only morphological intergradation (McElroy and Douglas 1995) but also taxonomic ambiguity (Douglas *et al.* 1989) and cannot be distinguished on the basis of mitochondrial (mt)DNA (Douglas and Douglas 2007; Dowling and DeMarais 1993), despite numerous lines of evidence supporting evolutionary independence [genetic structuring in nuclear

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markers (microsatellites: Douglas and Douglas 2007); discrete persistence in the fossil record (e.g. Uyeno and Miller 1963, 1965); pre-mating isolation in the form of exclusive reproductive ecology and phenology (Kaeding *et al.* 1990); and divergent phenotypic evolution (Smith *et al.* 1979; Valdez *et al.* 1990; Portz and Tyus 2004)]. Also, McElroy and Douglas (1995) and Douglas *et al.* (1998) found clear species-level differentiation in discriminant and geometric morphometric space, respectively, while the former also reported species-intermediacy at two sympatric localities (Desolation and Cataract canyons).

A parsimonious explanation for this mosaic pattern would invoke historic separation followed by contemporary hybridization. We tested this hypothesis herein and framed our results within the context of change both on geologic and contemporary timescales.

Methods(1449)

Sampling

Fin tissue was non-lethally sampled from 368 specimens across three native *Gila* of the Colorado River Basin (*G. cypha*, *G. elegans*, and *G. robusta*; Table 1), collected primarily by state/ federal agencies between 1997-2017 (see Acknowledgements). One location, at the San Rafael River, was sampled both in 2009 and 2017. Given the conservation status of these fishes, we minimized impacts on already-stressed populations by opportunistic sampling which took advantage of monitoring activities by agencies.

Gila cypha is constrained within five known aggregates associated with specific geomorphic features: Black Rocks, Cataract, Desolation, Grand, Westwater, and Yampa canyons (Fig. 1; USFWS, 2011), all of which were sampled save Cataract Canyon. Westwater and Black Rocks were treated separately, despite their potential for connectivity (Francis *et al.* 2016). Due

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to its range-wide extirpation, samples of *G. elegans* were obtained from the Southwestern Native Aquatic Resources and Recovery Center, Dexter, NM (formerly the Dexter National Fish Hatchery). Our sampling of *G. robusta* encompassed its entire range, to include pre-defined MUs (=Management Units; Douglas and Douglas 2007) and represented wild populations, with the exception the Mancos River, which was obtained from the Colorado Department of Wildlife Native Aquatic Species Restoration Facility. *Gila robusta* from the lower basin Bill Williams and Gila River drainages was not included, given its known polyphyly (Dowling and DeMarais 1993; Chafin *et al.* unpubl.).

Data collection

Genomic DNA was extracted using either PureGene® or DNeasy® kits (Qiagen Inc.), with electrophoresis (2% agarose gel) confirming presence of sufficiently high molecular weight DNA. Our ddRAD library preparations were modified from previous protocols (Peterson *et al.* 2012). Restriction enzyme pairings and size-selection ranges were optimized using an *in silico* procedure (FRAGMATIC; Chafin *et al.* 2018). Samples were digested with *Msp*I (5'-CCGG-3') and *Pst*I (5'-CTGCAG-3') following manufacturer's protocols (New England Biosciences). Fragments were then purified using Ampure XP beads (Beckman-Coulter Inc.) and concentrations standardized at 100ng per sample. Custom adapters containing in-line barcodes were ligated with T4 Ligase (New England Biosciences), pooled in sets of 48, and size-selected with the Pippin Prep (Sage Sciences) at 250-350bp prior to adjusting for adaptor length. We then utilized a 12-cycle PCR to extend adapters with indexed Tru-Seq primers and Phusion high-fidelity DNA polymerase (manufacturer protocols; New England Biosciences). Final libraries

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were visualized on the Agilent 2200 TapeStation fragment analyzer and pooled for 100bp read length single-end sequencing (Illumina HiSeq 2500; University of Wisconsin/Madison).

Assembly and filtering of genomic data

Data assembly was performed using computing resources at the Arkansas High Performance Computing Center (AHPCC), and the XSEDE-funded cloud computing resource JetStream (co-managed by the Pervasive Technology Institute/Indiana University, and the Texas Advanced Computing Center/Austin).

Raw Illumina reads were demultiplexed and filtered using the PYRAD pipeline (Eaton, 2014). Discarded reads exhibited >1 mismatch in the barcode sequence or >5 nucleotides with Phred quality <20. Loci were then clustered *de novo* within and among samples using a distance threshold of 80%. We then removed loci with: >5 ambiguous nucleotides; >10 heterozygous sites in the consensus sequence; >2 haplotypes per individual; <20X and >500X coverage per individual; >70% heterozygosity per-site among individuals; or <50% individual coverage. Individuals with >50% missing data were also discarded. Scripts for post-assembly filtering and file conversion are available as open-source (github.com/tkchafin/scripts).

Estimating population and individual ancestry

Hypotheses of admixture and hybridization were based on genetic differentiation, as visualized using Discriminate Analysis of Principal Components (DAPC; R-package *adegenet;* Jombart, 2008). Discriminant functions combine principal components (PCs) so as to maximally separate hypothesized groups. Importantly, sufficient PC axes must be retained so as to summarize the high-dimensional input, yet also avoid over-fitting. We accomplished this using

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184 the following cross-validation procedure: Stratified random sampling defined 80% of samples
185 per population as a "training set," with the remaining 20% then classified. PC retention was
186 optimized by minimizing root-mean-square error (RMSE) while maximizing classification
187 success across analyses.

188 These results were contrasted with model-based assignment tests (STRUCTURE, Pritchard
189 *et al.* 2000; ADMIXTURE, Alexander and Novembre 2009). A shared assumption is that
190 populations can be divided into *K*-clusters identified by permuting membership so as to
191 minimize linkage disequilibrium and departure from Hardy-Weinberg expectations. Given
192 excessive runtimes in STRUCTURE, we first applied ADMIXTURE to evaluate a broader range of
193 models (i.e., *K*=1-20, using 20 replicates), followed by STRUCTURE on a reduced range (*K*=1-10,
194 using 10 replicates with 500,000 MCMC iterations following a burn-in period of 200,000).

195 Model selection followed a cross-validation procedure in ADMIXTURE where assignment
196 error was minimized by optimal choice of *K*, with results parsed using available pipelines
197 (github.com/mussmann82/admixturePipeline). We used the delta *K* method (Evanno *et al.* 2005)
198 to define the proper model in STRUCTURE (CLUMPAK; Kopelman *et al.* 2015).

199 We identified putative admixed individuals using Bayesian genealogical assignment
200 (NEWHYBRIDS, Anderson and Thompson 2002) that assessed the posterior probability of
201 assignment to genealogical classes (e.g. F_1, F_2), as defined by expected genotype frequency
202 distributions. This component is vital, in that mixed probability of assignment in STRUCTURE and
203 ADMIXTURE can stem from weakly differentiated gene pools. The MCMC procedure in
204 NEWHYBRIDS was run for 4,000,000 iterations following 1,000,000 burn-in, using a panel of 200
205 loci containing the highest among-population differentiation (F_{ST}) and lowest linkage
206 disequilibrium ($r^2 < 0.2$), as calculated in GENEPOPEDIT (Stanley *et al.* 2017). To ensure

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accuracy of this method as applied to our data, we performed a power analysis using the HYBRIDDETECTIVE workflow (Wringe *et al.* 2017). We first generated simulated multi-generational hybrids using 50% as a training dataset, and then analyzed classification success across replicated simulations using the remaining 50% of samples as a validation set. To examine convergence, simulations were run across three replicates, each with three independent MCMC chains.

Spatial and genomic heterogeneity in introgression

We tested for signatures of reproductive isolation by examining clinal patterns in locus-specific ancestry across hybrid genomes, using multinomial regression to predict genotypes as a function of genome-wide ancestry. Analyses were performed in the R-package *introgress* (Gompert and Buerkle 2010). Putatively 'pure' populations of *G. robusta* and *G. cypha* were diagnosed from results generated by NEWHYBRIDS. We first filtered loci to include those with allele frequencies that differed in the reference populations (as defined by $\delta > 0.8$, where δ is the allele frequency differential at a given locus; Gregorius and Roberds 1986). We generated a null distribution by randomly re-assigning genotypes across 1,000 permutations, so as to test for deviations from neutral expectations. The significance of locus-specific clines (fit via multinomial regression) was then determined by computing a log-likelihood ratio of inferred clinal models versus the null model (at $P<0.001$).

To test for localized breakdown in reproductive barriers, we examined congruence of locus-specific introgression among sampling localities. We did so by deriving site-wise genomic clines within species, then subsequently contrasting the fit of site-wise regression models to the global pattern for each locus. This was accomplished by estimating probabilities of the observed

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genotypes for each site ($X_{i,j}$ where X=genotypic data over i sites for each locus j) given the site-specific models ($M_{i,j}$) versus the range-wide model ($M_{global,j}$). Concordance was reported as the log-likelihood ratio of $L(M_{global,j} \mid X_{i,j})$ to $L(M_{i,j} \mid X_{i,j})$ computed per-locus (Gompert and Buerkle 2009).

Testing effects of anthropogenic pressures

To test correlations between anthropogenic pressures on rates of hybridization, we parsed pressure indices per river reach for four dimensions of human impact from the global stream classifications of Grill *et al.* (2019). These were: 1) River fragmentation (=degree of fragmentation; DOF); 2) Flow regulation (=degree of regulation; DOR); 3) Sediment trapping (=SED); and 4) Water consumption (=USE), from the global stream classifications of Grill *et al.* (2019). We also tested predictive capacity of an integrated multi-criterion connectivity status index (=CSI), also from the free-flowing river assessments of Grill *et al.* (2019). Briefly, the DOF index (from 0 to 100) represents the flow disruption on a reach from dams, while also considering natural barriers such as waterfalls. The DOR index is derived from the relationship between storage volumes of reservoirs and annual river flows and is expressed as the percentage of total river flow that can be withheld in the reservoirs of a river reach. SED and USE quantify the potential sediment load trapped by dams, and the long-term average anthropogenic water consumption as a percentage of natural flow, respectively. The CSI index is a weighted average of these pressure indicators, while also considering road densities and degrees of urbanization [see Grill *et al.* (2019) for details regarding derivation of these indices and their underlying data sources].

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252 We assigned pressure index values for all sites containing at least 1 hybrid (as classified
253 using a 0.90 posterior probability threshold), and tested the predictive power of each pressure
254 dimension on 'genetic purity' (calculated via linear regression as the proportion of individuals
255 per population assigned to either P_0 or P_1).
256
257 **Results(1345)**
258 A mean of 106,061 loci were assembled per sample (σ=42,689). Following quality/depth
259 filtering, and with mean coverage of 88X, this yielded 16,001 per sample (σ=6427). Loci were
260 removed if absent in <50% of individuals, with paralog filtering performed on the basis of allele
261 count and excess heterozygosity. This resulted in 13,538 loci (μ=10,202; σ=3601), and
262 1,257,356 nucleotides. Putative orthologs contained 62,552 SNPs, of which 38,750 were
263 parsimony-informative, corresponding to 4.9% and 3% of sampled nucleotides. We retained one
264 SNP per locus, with a final dataset comprising12,478 unlinked SNPs.
265
266 *Population structure*
267 Choice of *K* varied by assignment test, with *K*=8 (ADMIXTURE; Fig. S1, S2, and *K*=5
268 (STRUCTURE; Fig. S2). We thus retained *K*-values from 5-8.
269 The discriminant function axis with the greatest differentiation (DF1) primarily
270 segregated *G. robusta* in the Little Colorado River (=RECC) from the remaining *G. robusta* and
271 *G. cypha*, with DA2 differentiating Upper Colorado *G. robusta* from *G. cypha* (Fig. 2A). Both
272 assignment tests (Fig. 3) differentiated Little Colorado *G. robusta* from conspecifics, with *G.*
273 *elegans* forming a discrete cluster. Interestingly, DA3 (Fig. 2B) seemingly identified structure
274 within *G. cypha* as well as potentially admixed populations of *G. robusta*. STRUCTURE models

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with *K*>8 and ADMIXTURE *K*>5 showed similar restrictions in gene flow between Grand Canyon *G. cypha versus* upper basin sites. Desolation Canyon showed the highest probability of assignment to an 'upper basin' *G. cypha* cluster. Within *G. robusta*, a weak signal of reduced intraspecific gene flow was apparent when Green River tributaries were compared with the mainstem Colorado River (i.e. *K*=8, both models).

DAPC and assignment tests each indicated potential hybridization among *G. cypha* and *G. robusta*, most prominently in regions of sympatry (i.e. Black Rocks, Westwater, and Yampa canyons; Fig. 3). Those *G. robusta* sites most 'distant' in multivariate space (Fig. 2B) generally showed least probability of interspecific assignment (Fig. 3), presumably due to low or nonexistent admixture with *G. cypha.* Signals of asymmetric introgression were apparent when sympatric localities were examined, with *G. cypha* generally having higher levels of heterospecific assignment.

One exception was Desolation Canyon, where all specimens phenotypically identified as "*G. robusta*" were genetically indistinguishable from those designated as *G. cypha*. Misidentifications at time of capture is a likely cause, owing to the morphological intermediacy of *Gila spp.* at this site (i.e. McElroy and Douglas 1995).

Allopatric populations of *G. robusta* showed less interspecific ancestry, with the exception being the San Rafael River, where samples had 30-50% assignment to *G. cypha* ancestry (Fig. 3), a pattern supported by the weak differentiation of San Rafael in DAPC analyses. Allopatric *G. robusta* from the San Juan River also showed mixed probability of assignment to *G. cypha*, albeit with low probability and consistency.

Hybrid detection and genealogical assignment

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298 Genealogical assignment in NEWHYBRIDS was used to parse STRUCTURE and ADMIXTURE
299 results for contemporary hybridization. We first defined a prior probability of genetic purity for
300 *G. robusta* as being the upper-most Little Snake River tributaries, and for *G. cypha* as the Little
301 Colorado River confluence in Grand Canyon. Both were chosen based on STRUCTURE and
302 ADMIXTURE results (Fig. 3), and additionally informed by prior studies of natural recruitment
303 (Douglas and Douglas 2010; Kaeding and Zimmerman 1983). As hypothesized, introgressive
304 hybridization at sympatric locations was found to be asymmetric (Fig. 4). Individuals were
305 'assigned' to genealogical class when posterior probabilities exceeded a critical threshold of 0.90
306 (based upon a <0.025% classification error in power analyses Fig. S4). In cases of mixed
307 assignment, individuals were classified as either "late-generation hybrid," or "of uncertain status"
308 (Table 2). *Gila robusta* were largely classified as pure in both sympatric and allopatric sites, with
309 a few exceptions (outlined below). The minority of samples assigned to hybrid classes tended to
310 be *robusta*-backcrossed. In contrast, *G. cypha* at sympatric localities had comparatively low
311 purity (0–61%), with most hybrids categorized as either F_2, *cypha*-backcrossed, or unclassifiable
312 (Table 2). The genetic effects of hybridization are thus inferred as asymmetric, with a greater
313 penetration of introgressed alleles into *G. cypha* populations. For both species, Desolation
314 Canyon samples were mostly classified as either late-generation or unassignable. F_1 hybrids were
315 notably absent at all localities, suggesting hybridization occurred over multiple generations and
316 ongoing introgression (i.e., hybrids fertile and reproductively successful).
317 Both species showed little signal of contemporary hybridization (i.e., F_1 hybrids) at
318 allopatric locations, but with notable exceptions being the San Rafael River and, to a lesser
319 extent, the Mancos River. Nearly all San Rafael *G. robusta* were assigned with high probability
320 as either F_2 or *G. robusta*-backcrossed hybrids, a pattern consistent across years (2009 *versus*

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2017), and regardless of priors used. Samples from 2009 were mostly classified as F_2 (45%) or *robusta*-backcrosses (45%). However, the greatest proportion of 2017 samples were *robusta*-backcrosses (67%) or late-generation hybrids (25%), suggesting an increase of admixture over time (although increased sampling is needed to verify this trend; two-tailed Fisher's exact test p=0.0967; Table 2). The Mancos River samples, composed of 20% hybrids (Table 2), were derived from hatchery stock, not a natural population. Thus, we cannot say if our results represent natural or accidental hybridization that coincided with, or was subsequent to, stock establishment.

Genomic clines

We also examined how introgression varied across significantly differentiated genomic SNPs and species-diagnostic markers. Here, we considered locus-specific ancestry as the probability of sampling a homozygous *G. cypha* genotype [i.e. P(AA)] as a function of genome-wide ancestry, with the expectation that scant bias should occur if fitness is independent of hybrid ancestry. All loci exhibited clinal patterns that deviated significantly from neutral expectations (p>0.001, estimated via permutation; Fig. 5A). The majority displayed coincident sigmoidal relationships between genome-wide ancestry (hybrid index; h) and locus-specific ancestry (ϕ), although with some divergence. The sigmoidal pattern suggested a deficiency in interspecific heterozygosity, presumably reflecting heterozygote disadvantage (Fitzpatrick, 2013). Notably, some locus-specific clines showed alternative forms (Fig. 5A), indicating that gene flow may be driven by other processes in a minority of genomic regions.

We also examined the observed genotypes at each locus, given expectations from the range-wide model and site-specific regression models. These were reported as a log-likelihood

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ratio *per* locus and *within* each sampling locality (Fig. 5B). We found the 'fit' of the range-wide clinal models was rather variable, although with most loci showing little deviation. One notable exception was San Rafael *G. robusta*, where an exceptionally flattened distribution of the locus-specific log-likelihood ratios was apparent. This in turn suggested that the global expectation was a poor predictor of within-site genotypes. Thus, while most sites reflected patterns consistent with selection against hybrids, the same cannot be said for the San Rafael River population. It also displayed a strong signal of interspecific admixture in the Bayesian and ML assignment tests (Fig. 3), and variable assignment to >2nd generation hybrid classes in NEWHYBRIDS (Fig. 4), as well as greater intermediacy in multivariate genotypic clustering (Fig. 2). Several other sites also showed a 'flattened' distribution of clinal fit among loci (e.g., HWWC, HBKR, HDES, RDES, RUGR, RMCO; see Fig. 5B). This could be a response to elevated introgression and a relative breakdown of heterozygote disadvantage, especially where previous analyses indicated admixture (Fig. 2-4), or as an artefact of reduced sampling (RUGR, RMCO).

Our hypothesis of the San Rafael River as being the most notable discrepancy across all analyses was extended by evaluating samples across different time periods: 2009 (N=11) and 2017 (N=12). We then fitted locus-specific clines among years (Fig. 5C) and compared those to range-wide expectations (Fig. 5B). We found little change in the overall distribution, save for four outliers in 2017, suggesting further breakdown of clinal expectations over time.

Testing dimensions of anthropogenic pressure

Among sites showing varying levels of hybridization (N=10; Table 2), only the consumptive water use (=USE) was significantly corelated with a decline in genetic purity (r^2=0.375; p=0.035; see Fig. S5). The connectivity status index (CSI) showed a weak but non-

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367 significant positive relationship (i.e. increased connectivity = increased genetic purity; r^2=-0.103;
368 p=0.7), although we caution that sample sizes were notably low (N=10) after sites were reduced
369 to those containing hybrids and which could be assigned pressure indices (see Fig. S6 for reach
370 assignments).
371
372 **Discussion(2211+246=2457)**
373 We found strong evidence for contemporary hybridization among *G. cypha* and *G.*
374 *robusta* extending beyond their regions of sympatry. These results refine rather than conflict with
375 previous studies employing 'legacy' genetic markers (Douglas and Douglas 2007; Dowling and
376 DeMarais 1993; Gerber *et al.* 2001). In addition, these results broaden our understanding of each
377 species and their evolutionary histories, as well as the trajectory of their ongoing evolutionary
378 change in the face of extensive anthropogenic modifications.
379
380 *Species boundaries and reproductive isolation in* Gila
381 Our survey of the nuclear genome suggested that hybridization between our study species
382 is contemporary in nature, as interpreted from several lines of evidence: The coincidence and
383 shape of our genomic clines; the pervasive signal of genealogical assignment to early-generation
384 hybrid classes; and signatures of selection antagonistic to interspecific heterozygous genotypes.
385 We interpret these results as remarkable, particularly given the coexistence of study
386 species since at least the mid-Pliocene (Uyeno and Miller, 1965; Spencer *et al.* 2008). In
387 addition, continuing hybridization also stands in stark contrast to the sustained morphological
388 divergence displayed in sympatry (Douglas et al. 1989; McElroy and Douglas 1995). This

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389 suggests that genetic exchange is ongoing despite, rather than in the absence of, reproductive
390 isolation.

391 Dowling and DeMarais (1993) suggested that hybridization between *G. cypha* and *G.*
392 *robusta* may have contributed to the evolutionary persistence of each species as a distinct entity
393 in the face of historic environmental fluctuations. We concur, and further note that this exchange
394 is ongoing, with a substantial risk that contemporary habitat change will outpace the rate at
395 which introgressed alleles are adaptively "filtered." If so, then continued habitat alteration could
396 lead to a scenario in which genetic/demographic swamping contributes to local extirpations, or to
397 eventual genetic homogenization of one species by the other (Todesco *et al.* 2016). This pattern
398 is particularly evident in the strong asymmetric signal of gene flow in *G. cypha*, a species of
399 particular concern given its fragmentary distribution and reduced densities within the upper
400 Colorado River Basin (e.g. Badame 2008; Franci *et al.* 2016; USFWS 2017).

401 To consider the plausibility of such a scenario in which environmental change leads to
402 the dissolution of a species boundary, we must first consider how this boundary is itself
403 structured. We do so by combining the results of our genetic data with those derived from
404 species-specific morphology and life history. Several morphological evaluations have
405 demonstrated that reproductive isolation in extant populations is incomplete (Douglas and
406 Douglas 2007; Douglas *et al.* 2001; McElroy and Douglas 1995). This is likely the result of
407 secondary admixture, rather than a prolonged (i.e., primary) divergence that lead to only weakly-
408 differentiated species. Pliocene fossils that are morphologically consistent with *G. robusta* and
409 *G. cypha* predate major geomorphic and tectonic events that could have triggered vicariant
410 events. For example, the Upper Colorado River was segregated from the contemporary lower
411 basin prior to the mid-Pliocene, (McKee *et al.* 1967), with the uplifting of the Colorado Plateau

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412 diverting its flow into one or more Colorado Plateau lakes (Spencer *et al.* 2008). Flows were
413 subsequently diverted by headwater erosion though the Grand Canyon, forming the modern
414 course of the river. Fossil evidence implies that ecological divergence occurred during, or prior
415 to this time, and was sufficient in strength to generate both morphological forms (Uyeno and
416 Miller, 1965). This suggests the existence of ecological conditions that reflect those to which the
417 species are now adapted. Additionally, numerous perturbations [i.e., tectonism, extreme drought
418 (Meko *et al.* 2007)] also occurred during the interval between divergence and present, yet both
419 species not only persisted but did so with some semblance of morphological continuity. Given
420 this, one must again assume that an extant blurring of species-boundaries is a more contemporary
421 occurrence. To test this hypothesis, we considered the ecological dimensions underlying adaptive
422 differentiation in these species.
423
424 *Reproductive barriers in* Gila
425 Phenotypic and ecological specializations of each species provide potential insights into
426 the mechanisms promoting assortative mating. *Gila cypha* displays phenotypic characteristics
427 interpreted as adaptations to the torrential flows of canyon-bound reaches (McElroy and Douglas
428 1995; Miller 1946; Valdez and Clemmer 1982). These include a prominent nuchal hump,
429 dorsoventrally flattened head, embedded scales, terete body shape, and a very narrow caudal
430 peduncle that terminates in a caudal fin with a high aspect ratio, indicative of a hydrodynamic
431 shape and powerful propulsion. Its current distribution also reflects association with this type of
432 habitat.
433 In contrast, *G. robusta* has a comparatively more generalized phenotype, characterized
434 by a deeper and less streamlined body with non-imbedded scales and larger, more falcate fins

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(Miller 1946). It is found in the upper tributaries of larger rivers (Vanicek and Kramer 1969) with moderate flows. It fails to maintain position within the current when subjected to the extreme flows associated with *G. cypha*, and instead becomes benthic so as to avoid being swept away (Moran *et al.* 2018). This suggests a natural history diametrically opposed to that of *G. cypha,* where dynamic flow regimes clearly predominate. Accordingly, radiotelemetric studies verified habitat preferences for each species, with *G. cypha* seldom straying from the deep eddies and turbulent flows of canyon-bound reaches (Douglas and Marsh 1996; Gerig *et al.* 2014; Kaeding *et al.* 1990). These observations underscore the role that functional morphology plays with regards to species boundaries, in that intermediate morphologies would be maladaptive in either habitat.

However, barriers that sustain reproductive isolation are unclear, in that both species are broadcast-spawners (Johnston and Page 1992), with a temporal overlap in spawning period (Kaeding *et al.* 1990). The latter is likely a consequence of shared environmental cues triggering reproduction, namely seasonal changes in flow rate and temperature, with spatial segregation driven by subsequent alterations in microhabitat and substrate preference (Douglas and Douglas 2000; Minckley 1996). Widespread movements by *G. robusta* during the spawning season contrast with the relative localized focus found in *G. cypha* (Kaeding *et al.* 1990; Tyus *et al.* 1982), and again reinforce the restricted habitat requirements of the latter. Additionally, there is a stronger 'homing' component in the microhabitat preferences of *G. cypha* (Valdez and Clemmer 1982). These ecological differences, combined with overall higher abundance of *G. robusta* in most areas (e.g. Francis *et al.* 2015) likely contribute to the observed asymmetric introgression between the two species (Edelaar *et al.* 2008). Intraspecific recognition as a mate-choice mechanism is also an observed behavior that promotes reproductive isolation. Despite congruent

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458 reproductive condition and the presence of suitable substrate in a brood stock tank, natural
459 spawning did not occur between *G. robusta* x *G. elegans* and *G. elegans* x *G. cypha* (Hamman
460 1981).

461 Thus, we contend that reproductive isolation in *G. robusta* and *G. cypha* is driven by
462 extrinsic factors, with pre-mating isolation primarily in the form of microhabitat selection and
463 post-mating isolation driven by functional morphological differences. Our data point to selection
464 against hybrids as represented by their relatively poor performance in the environment, or to a
465 diminished success in mating. However, we noted a possible breakdown of this expectation at
466 some localities when genomic clines were fitted to within-site patterns. The San Rafael River, for
467 example, is one such exception. Fortney (2015) quantified anthropogenic changes in this river
468 over the last 100 years, with the channel being extensively canalized and diverted, and flows
469 diminished by 83% due to water withdrawals. These manipulations yielded a narrower, relatively
470 deeper channel that stands in sharp contrast to an historically wider and slower river whose flow
471 regime was governed by geomorphology and dominated by flooding. Anthropogenic alterations
472 apparently provided an opportunity for adaptive hybridization, an hypothesis consistent with the
473 exclusive presence of late-generation hybrids in the San Rafael population (Fig. 4). Under this
474 scenario, selective advantage would similarly drive outlier loci and the reduced-fit seen in our
475 clinal models (Fig. 5). The origin of *G. cypha* alleles in this population is unclear, although they
476 may possible be derived from a remnant population in the upper reaches (e.g., Black Box Gorge;
477 Badame, pers. comm).

478 An examination of the degree to which anthropogenic pressures drive basin-wide
479 hybridization point to a role for consumptive water use in driving a decline in overall genetic
480 purity. Consumptive water usage, and the impact of the associated infrastructures (such as

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481 diversions and reservoirs), are often implicated as detrimental to freshwater fish diversity (e.g.
482 Xenopoulos *et al*. 2005). Insofar as river discharge is one dimension of ecological heterogeneity,
483 and given the trend of decreasing species richness as flow declines (Oberdorff *et al.* 1995), we
484 posit that a coincidental relationship is rather extreme in the Colorado River when anthropogenic
485 manipulations and extensive hybridization are contrasted. Yet, a test of this hypothesis is
486 difficult without further experimental work (i.e. leveraging hatchery-produced interspecific
487 hybrids to test for viability in varying habitats, represented within a series of mesocosms). While
488 increased sampling is also necessary, it would be difficult given that we have already sampled 4
489 of the 5 extant *G. cypha* populations.
490
491 *Modified environments and genetic swamping*
492 Grabenstein and Taylor (2018) defined mechanisms that drive anthropogenically-
493 mediated hybridization in coexisting species: 1) Interspecific contact promoted by habitat
494 homogenization or altered phenology; 2) Disruption of mate selection/ choice; and 3) Habitat
495 alteration, such that hybrid genotypes are favored (Anderson 1948). All three are plausible for
496 *Gila*, with the 'hybrid swarm' of the San Rafael River an extreme case. Asymmetric
497 hybridization was implicated in all extant sympatric *G. cypha* populations, save the Cataract
498 Canyon aggregate not evaluated in this study. The latter reflects a more '*robusta*-like'
499 morphology (McElroy *et al.* 1997), with low population numbers and a slower growth rate
500 relative to other extant populations (Badame 2008). Taken together, these suggest an elevated
501 risk for genetic or demographic swamping in Cataract Canyon *G. cypha* (Todesco *et al.* 2016),
502 and lend urgency to their inclusion in future genetic surveys.

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503 Such a scenario may also be invoked for *G. cypha* in the Yampa River, recognized even
504 prior to our sampling as being of reduced and declining numbers (Tyus 1998). The ubiquity of
505 highly-admixed genomes in our sampling (from 1999-2001), coupled with the absence of
506 genetically pure individuals in more recent surveys (USFWS 2017), suggest the potential for
507 local extirpation. Given the prevalence of asymmetric hybridization in other sympatric *G. cypha*,
508 it is possible that genetic swamping may have also played a role in the decline of the Yampa
509 Canyon population (although we cannot test that hypothesis). Of note are recent surveys that
510 have documented diminished catch ratios for *G. cypha* at these sympatric localities (Fig. 4;
511 Francis *et al.* 2016; USFWS 2017). Thus, an elevated risk of genetic swamping appears as a
512 strong potential for all *G. cypha* populations sympatric with *G. robusta*.
513
514 *Genetic swamping and Allee effects*
515 The capacity for populations to track changing conditions is constrained not only by
516 standing genetic variation but also complex demographic processes that feed back to
517 reproductive fitness (Kokko *et al*. 2018). As the effective population size (N_e) of a population
518 decreases, so also do beneficial variants, primarily due to reduced efficacy of selection relative to
519 genetic drift and associated inbreeding depression (i.e., Allee effects; Kramer *et al.* 2009). This
520 in turn can induce a negative feedback that drives local extirpation (Polechová and Barton 2015).
521 Using a similar logic, we posit that maladaptive introgression within diminishing populations
522 could also synergistically trigger a "runaway" process of genetic swamping (Fig. S7).
523 In this conceptual model, demographically-driven Allee effects weakens purifying
524 selection against maladaptive introgressed alleles, whereas their continued influx further reduces
525 fitness via outbreeding depression. In this way, maladaptive gene flow can continually depreciate

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526 N_e and effectively promote an "extinction vortex" (Gilpin and Soulé 1986), and we posit this
527 mechanism may contribute to the decline of those *G. cypha* populations sympatric with *G.*
528 *robusta*. Although some signal of selection against heterospecific alleles was apparent, another
529 manifestation of shrinking N_e is the expansion of genomic linkage disequilibrium (Nachman
530 2002). As a result, purifying selection can actually be counterproductive, wherein beneficial
531 genetic variation is lost via selection against linked regions (Nachman and Payseur 2012).
532 Under this paradigm, the risk of swamping in *G. cypha* is elevated by the numerous
533 factors that increase the relative impact of genetic drift. These are: Reduced population sizes in
534 extant populations (Douglas and Marsh 1996; Tyus 1998); A fragmented distribution (Fagan,
535 2002); and a "slow" life history (i.e., long generation time and extended lifespans; Olden *et al.*
536 2008), and higher vulnerability to regulated and reduced flows given its habitat preference of
537 turbulent rivers (as above). The hybrid swarm in the San Rafael River, and the suspected genetic
538 swamping of *G. cypha* in the Yampa River, are potential harbingers of this erosion. Genetic
539 integrity may be preserved in the short term by cultivating "pure" progeny via hatchery
540 production, so as to potentially extend existing pure populations, although a propogation
541 program risks further reducing N_e (Allendorf *et al.* 2001). The development of pure stock for *G.*
542 *robusta* should be relatively easy, whereas upper basin *G. cypha* are more problematic in that
543 they display various levels of hybridization (i.e., Figs. 3-4). In this regard, we echo the
544 “producer’s gambit” philosophy (McElroy *et al.* 1997) where hybrid populations fall under an
545 expanded conservation paradigm when genetic purity cannot otherwise be maintained (Lind-
546 Riehl *et al.* 2016). Given apparent ecological non-equivalency of hybrids, we suggest that habitat
547 restoration is the only long-term means to resurrect genetic purity in these populations (Wayne
548 and Shaffer 2016).

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549

550 *Conclusions*(246)

551 A reduced-representation assay of nuclear genomes in *G. robusta* and *G. cypha* provided
552 evidence of asymmetrical hybridization that is range-wide and spatially heterogeneous (Fig. 3,
553 4). We interpreted this as reflecting secondary contact, rather than a persistent echo reverberating
554 throughout their respective histories, particularly given the pervasive selection we found with
555 regard to genomic clines operating against interspecific heterozygotes (Fig. 5). Although we
556 lacked appropriate sampling to adequately test for temporal changes in hybridization rates, we
557 did observe the expansion of a hybrid swarm in the San Rafael River over an eight-year period,
558 as well as high levels of asymmetric hybridization in all sympatric populations of *G. cypha*
559 (Table 2). This underscores the potential for genetic/demographic swamping by *G. robusta*, as
560 well as exacerbating the extirpation risk for extant populations of *G. cypha*. We argue that
561 conservation plans for *G. cypha* must consider this possibility. We also suspect the species-
562 boundary for *G. cypha* is largely maintained by extrinsic factors (i.e., lower fitness of hybrid
563 phenotypes and differential microhabitat preferences). As such, further habitat degradation and
564 homogenization may lead to complete genetic erosion, either by contravening habitat selection
565 for pure individuals, or by promoting modified anthropogenic riverscapes that serve as habitat
566 for novel hybrid lineages/swarms. The scenario playing out in *Gila* emphasizes a philosophical
567 dilemma that conservation policy must confront: Is hybridization antagonistic to the conservation
568 of biodiversity or is it instead an adaptive tactic which species employed routinely by species in
569 their evolutionary struggle to persist.

570

571

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600

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602 The authors declare no conflict of interest.

603

604 **Literature Cited(3566)**

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822 **Data Accessibility**

823 Pending acceptance, all raw sequence files will be uploaded to the NCBI GenBank Short-read
824 Archive (SRA). Relevant curated (i.e. assembled and filtered) datasets will be archived on
825 Dryad. Codes and custom scripts developed in support of this work are available as open-source
826 under the GNU Public License via GitHub: github.com/tkchafin

827 - Raw sequence files: NCBI SRA (pending)
828 - Sampling locality metadata, curated sequence files, and alignments: Dryad (pending)
829 - Codes and scripts: github.com/tkchafin (and as cited in-text)

830

831 **Author Contributions**

832 TKC, MED, and MRD conceptualized and designed the research. MED and MRD contributed
833 sampling and coordination of agency efforts. TKC performed molecular work and analyses. TKC
834 and BTM wrote software and codes to analyze data in support of this research. All authors
835 contributed to writing the manuscript and approve of the final submission.

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837 **Figures and Tables(814)**

838 Table 1: Sampling locations for *Gila robusta*, *G. cypha* and *G. elegans*. Site=abbreviated locality
839 identifier for each species, Major Drainage=River, Location=geographic site, County, State=per
840 sampling site, and *N*=Number of samples excluding those that sequenced with sufficient
841 coverage.
842
843
844

Site	Major Drainage	Location	County, State	*N*
Gila robusta				
RBKR	Colorado	Black Rocks Canyon	Mesa, CO	11
RDES	Green	Desolation Canyon	Uintah, UT	23
RC15	Colorado	15-mile reach	Mesa, CO	10
RECC	Little Colorado	East Clear Creek	Coconino, AZ	16
RMCO	San Juan	Mancos River	Montezuma, CO	10
RMGR	Green	Middle Green R. tributaries	Sweetwater, WY	24
RNJA	San Juan	Navajo R.	Rio Arriba, NM	10
RLSR	Yampa	Little Snake R. tributaries	Carbon, WY	31
RLYC	Yampa	Little Yampa Canyon	Moffat, CO	11
RSRR	Green	San Rafael R.	Emery, UT	23
RUGR	Green	Upper Green R. tributaries	Sublette, WY	16
RWRW	White	White R. mainstem near Weaver Cn.	Uintah, UT	15
RWWC	Colorado	Colorado mainstem near Westwater Cn.	Grand, UT	11
RYAM	Yampa	Yampa R. mainstem	Moffat, CO	15
Gila cypha				
HBKR	Colorado	Black Rocks Canyon	Mesa, CO	18
HDES	Green	Desolation Canyon	Uintah, UT	24
HGCN	Colorado	Grand Canyon	Coconino, AZ	37
HLCR	Little Colorado	Atomizer Falls and Colorado confluence	Coconino, AZ	22
HWWC	Colorado	Westwater Canyon	Grand, UT	22
HYAM	Yampa	Yampa Canyon	Moffat, CO	8
Gila elegans	Hatchery stock	USFWS Hatchery at Dexter, NM	Chaves, NM	11

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847 Table 2: Proportions of *Gila robusta* and *G. cypha* assigned genealogically at each sample site.
848 Site=abbreviated locality identifier for each species; P_0=Pure *robusta*; P_1=Pure *cypha*; F_1=First
849 filial hybrid; F_2=second filial hybrid; B_0=*G. robusta*-backcrossed hybrid; B_1=*G. cypha*-
850 backcrossed hybrid; F_N=late-generation or uncertain hybrid. Samples were assigned to a
851 genealogical class per posterior probability ≥0.80, as assessed using 250,000 post burn-in
852 MCMC generations in NEWHYBRIDS.
853

Site	P_0	P_1	F_1	F_2	B_0	B_1	F_N
HLCR	-	1.000	-	-	-	-	-
HGCN	-	1.000	-	-	-	-	-
HDES	-	0.083	-	0.083	-	0.542	0.292
HWWC	-	0.150	-	0.200	-	0.550	0.100
HBKR	-	0.615	-	0.231	-	0.077	0.077
HYAM	-	-	-	0.667	-	0.333	-
RDES	0.046	0.046	-	0.136	-	0.364	0.409
RSRR (’17)	-	-	-	0.083	0.667	-	0.250
RSRR (’09)	-	-	-	0.455	0.455	-	0.091
RNJA	1.000	-	-	-	-	-	-
RMCO	0.800	-	-	-	0.100	-	0.100
RWWC	0.875	-	-	-	0.125	-	0.375
RC15	1.000	-	-	-	-	-	-
RBKR	0.857	-	-	-	-	-	0.143
RECC	0.938	-	-	-	0.063	-	-
RWRW	0.875	-	-	-	0.125	-	-
RUGR	1.000	-	-	-	-	-	-
RMGR	0.955	-	-	-	-	-	0.045
RYAM	1.000	-	-	-	-	-	-
RLYC	1.000	-	-	-	-	-	-
RLSR	1.000	-	-	-	-	-	-

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Figure 1: Sampling localities for *Gila cypha* (blue) and *G. robusta* (red) within the Colorado River Basin, western North America. Locality codes are defined in Table 2. Sympatric locations (BKR, DES, WWC, YAM) are slightly offset for visibility purposes. Inset cartoons the respective morphologies of each species.

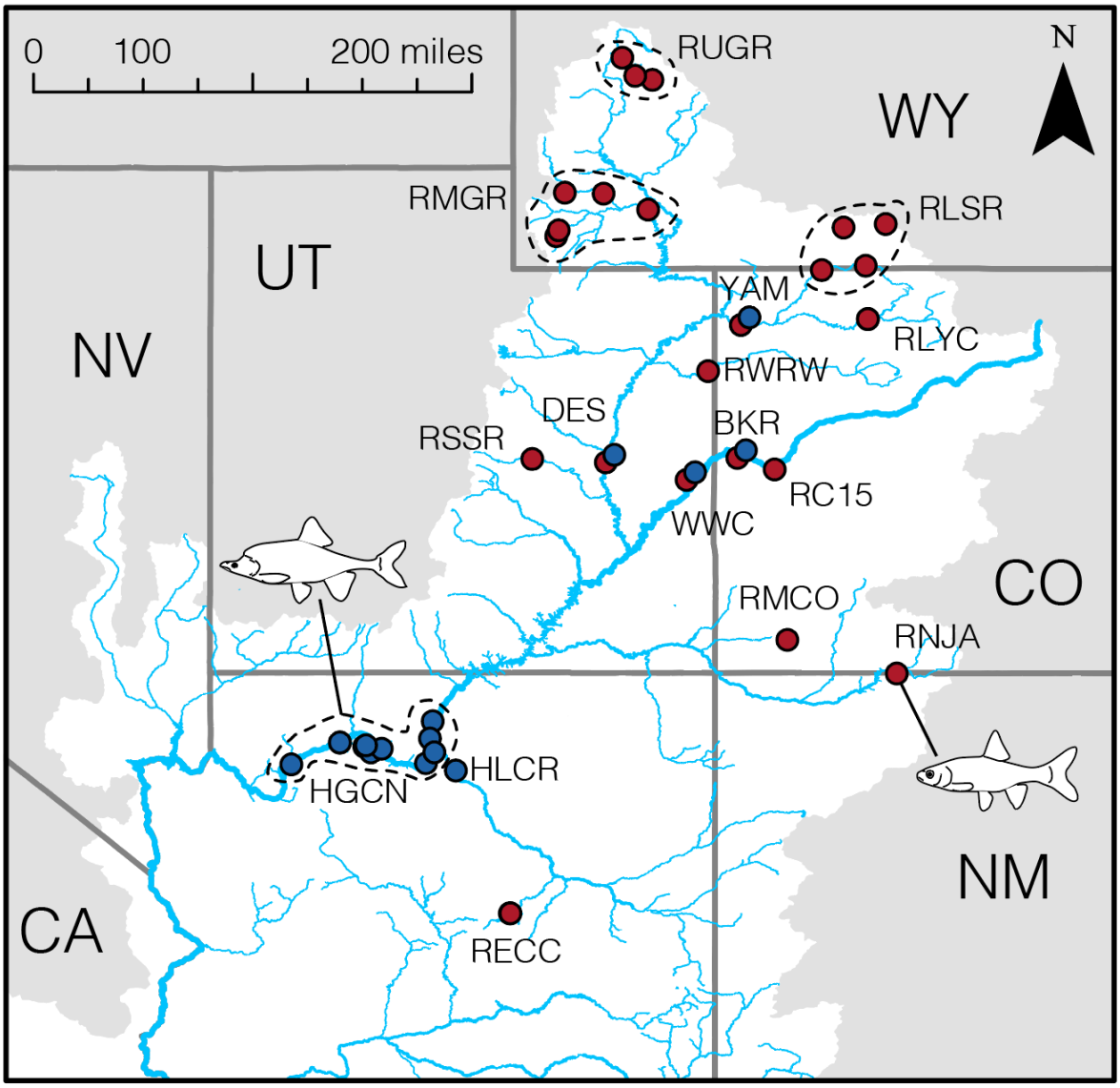

Figure 2: Results of a Discriminate Analysis of Principal Components (DAPC) analysis depicting *Gila robusta* (red), *G. cypha* (blue), and their respective populations (as colored). (A) discriminant function axes 1 and 2 (=DF1xDF2) showing discrimination among both species; (B) axes 2 and 3 (=DF2xDF3) reflecting the manner by which populations of each species (grouped within ellipses) are distributed in discriminant space. The relative percent variance captured by each discriminant function is presented in parentheses. Sample localities are defined in Table 1.

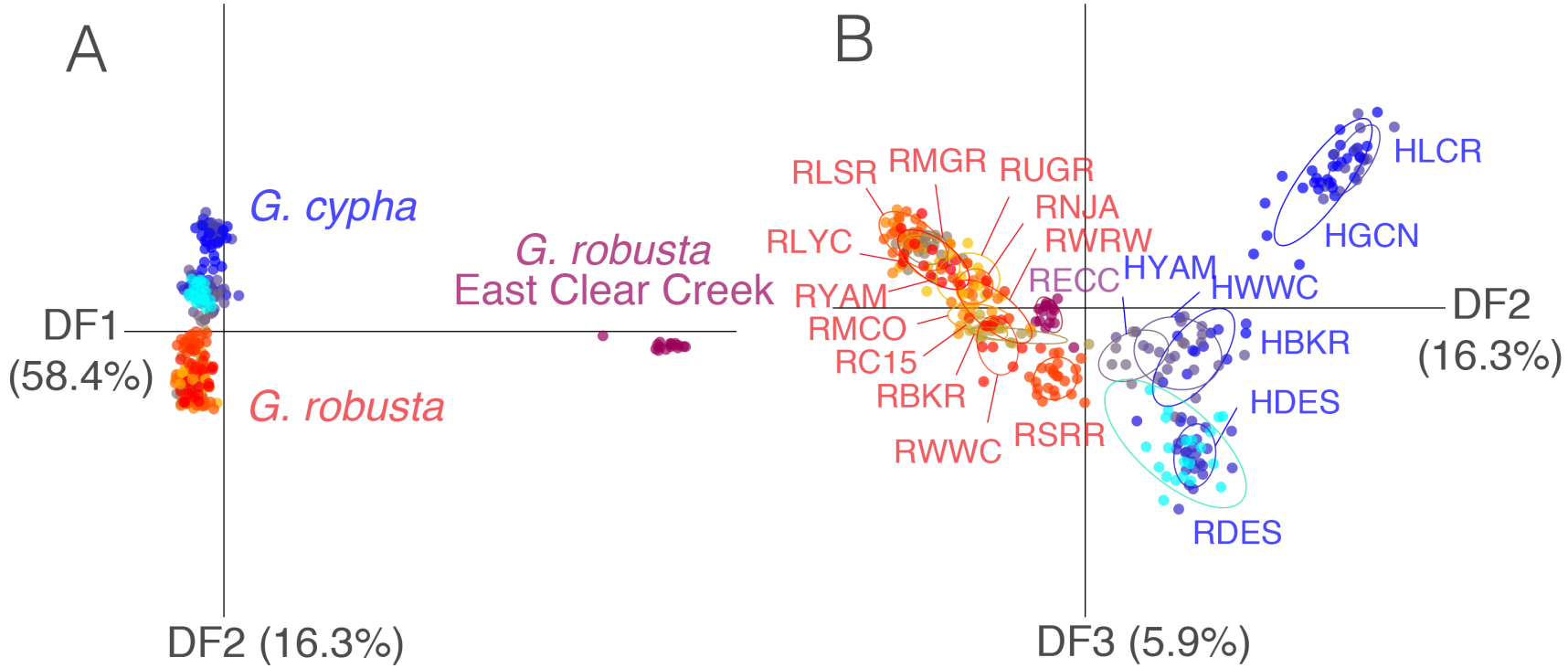

Figure 3: Assignment results for ADMIXTURE and STRUCTURE analyses involving *Gila robusta*, *G. cypha,* and *G, elegans*. *K*-values range from STRUCTURE optimum *K*=5 (see Fig. S2) to ADMIXTURE optimum *K*=8 (see Fig. S3). Locality abbreviations are as defined in Table 1.

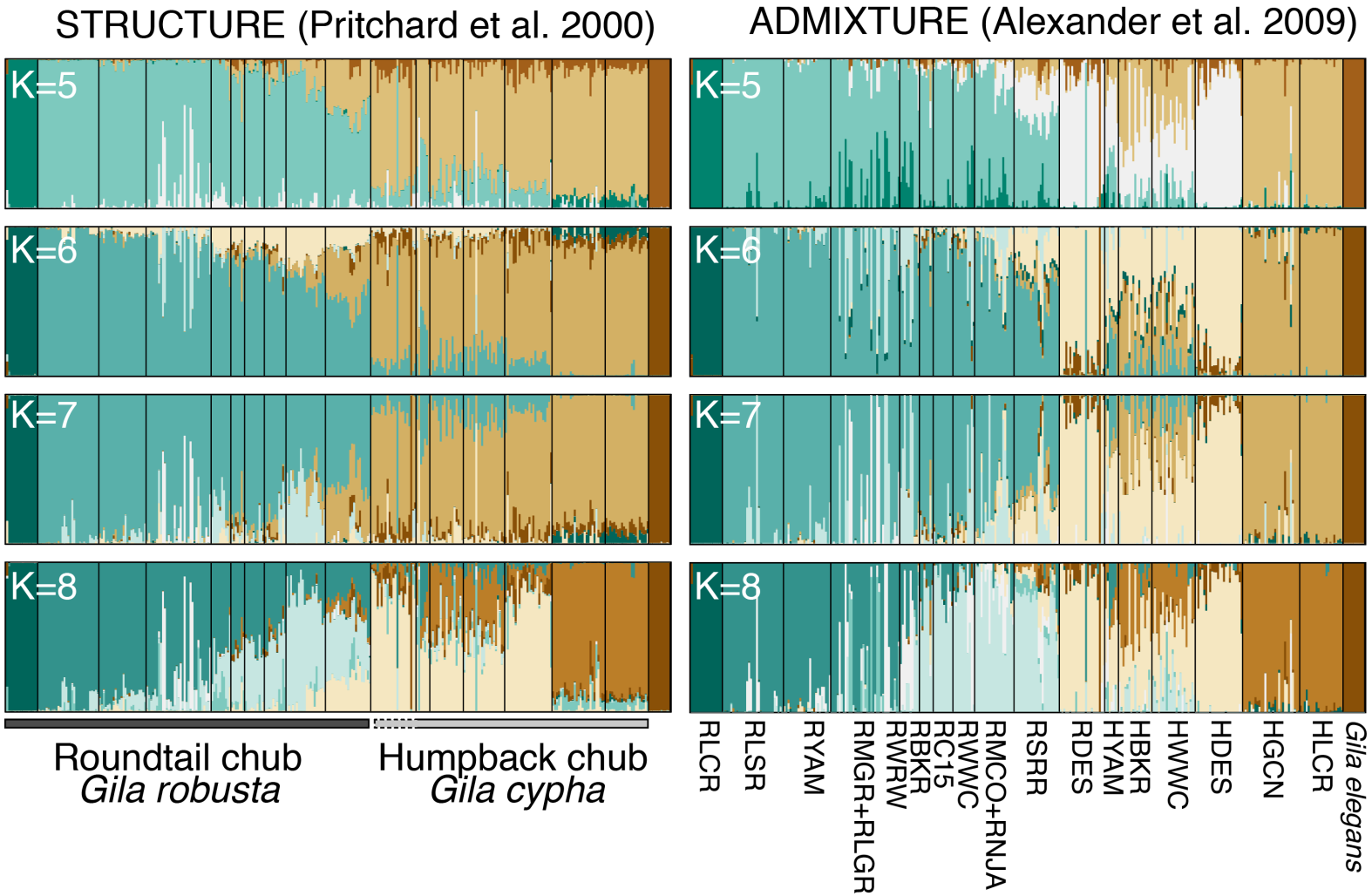

Figure 4: Genealogical assignment for individual *Gila robusta* and *G. cypha*, as compiled from NEWHYBRIDS analysis. Individuals are represented by colored bars, with proportion of color indicating posterior probability of assignment per genealogical class. Prior 'parental' allele frequencies for *G. cypha* were derived from the Little Colorado River (HLCR) and from the Little Snake River (RLSR) for *G. robusta* (alternative prior assignments had no significant affect; see Fig. S4 and S5). Colors are as follows: Red=pure *G. robusta*; Blue=pure *G. cypha*; Purple=F_1 hybrid; Light purple=F_2 hybrid; Light blue=*cypha*-backcrossed hybrid; Light red=*robusta*-backcrossed hybrid.

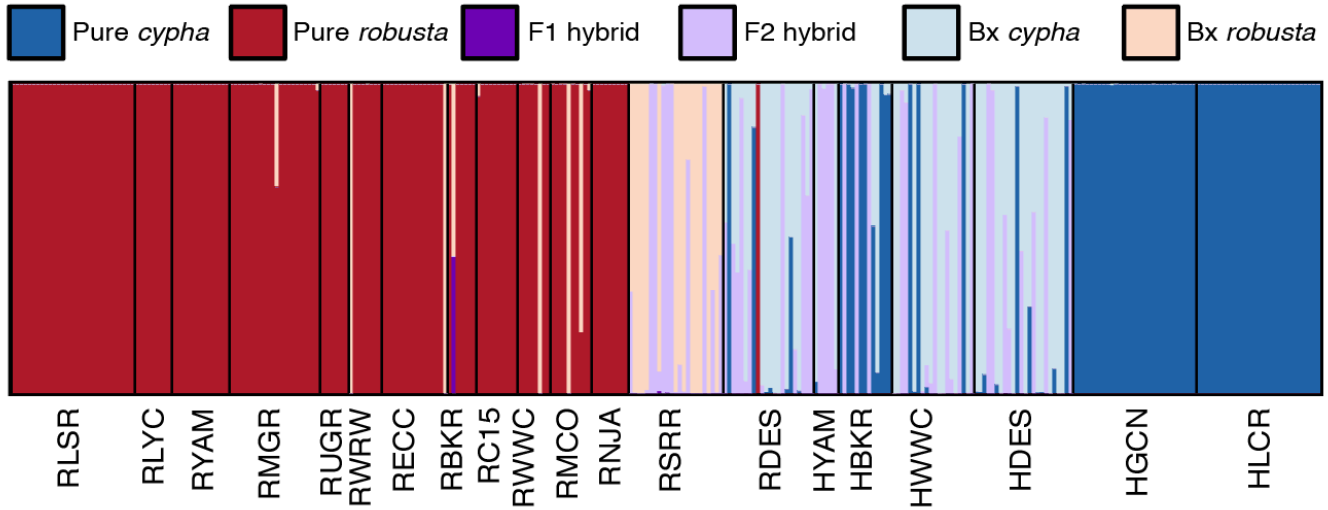

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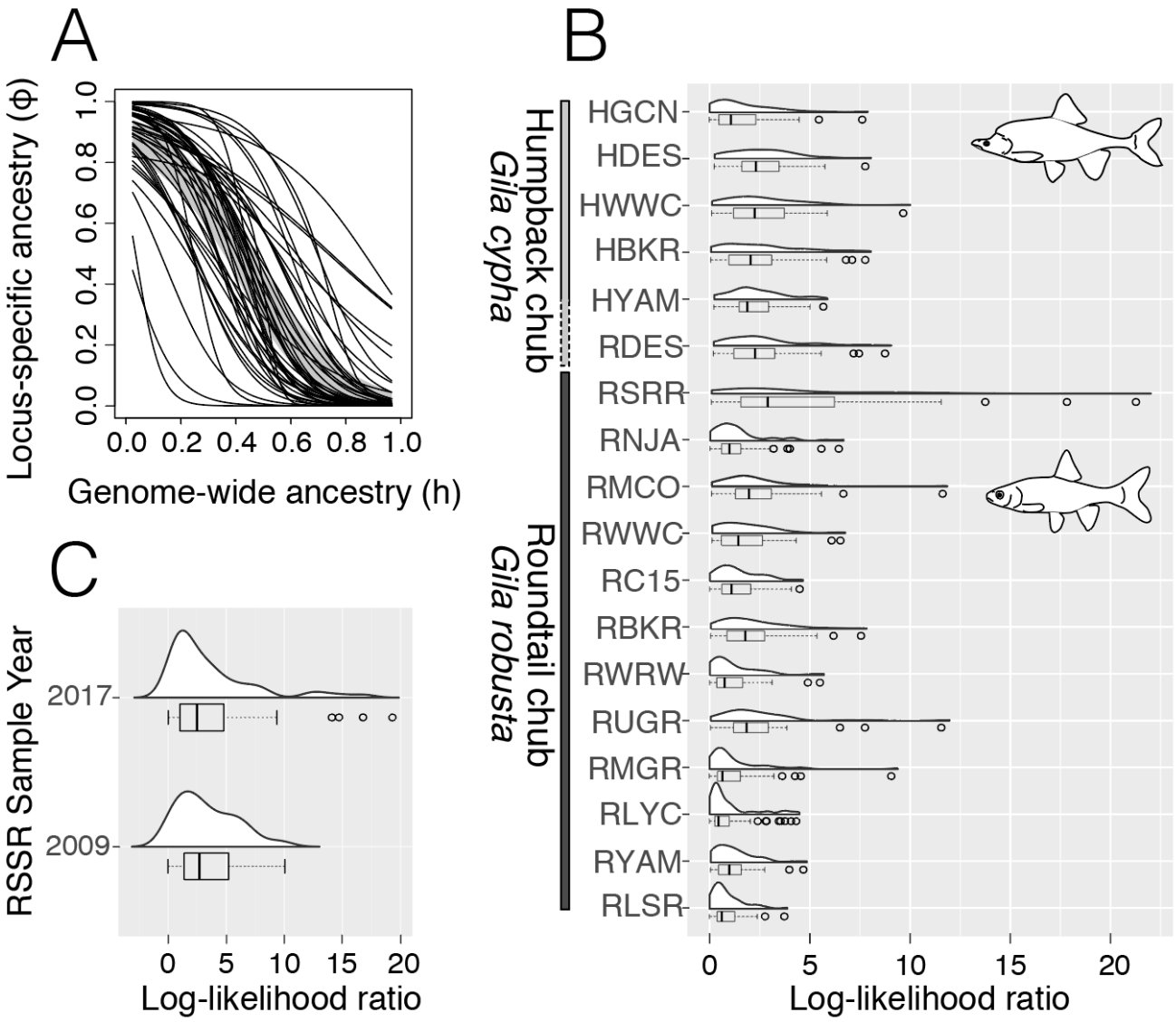
Figure 5: Genomic cline analyses for populations of *Gila robusta* and *G. cypha,* presented as: (A) Per-locus clinal relationships for 50 SNPs with δ>0.8 (all significantly non-neutral at α=0.001) compared to the neutral expectation (shaded gray region); (B) Log-likelihood ratio distribution of site-wise per-locus clines compared to the global pattern, where higher log-likelihood ratio indicates greater discordance; (C) Per-locus incongruence in genomic clines in the *Gila robusta* samples from the San Rafael River, partitioned by year (2009 versus 2017). Locality codes for populations of each species are defined in Table 1.



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Supplemental Material

Figure S1: Cross-validation error analysis for ADMIXTURE, as represented by number of clusters (*K*)

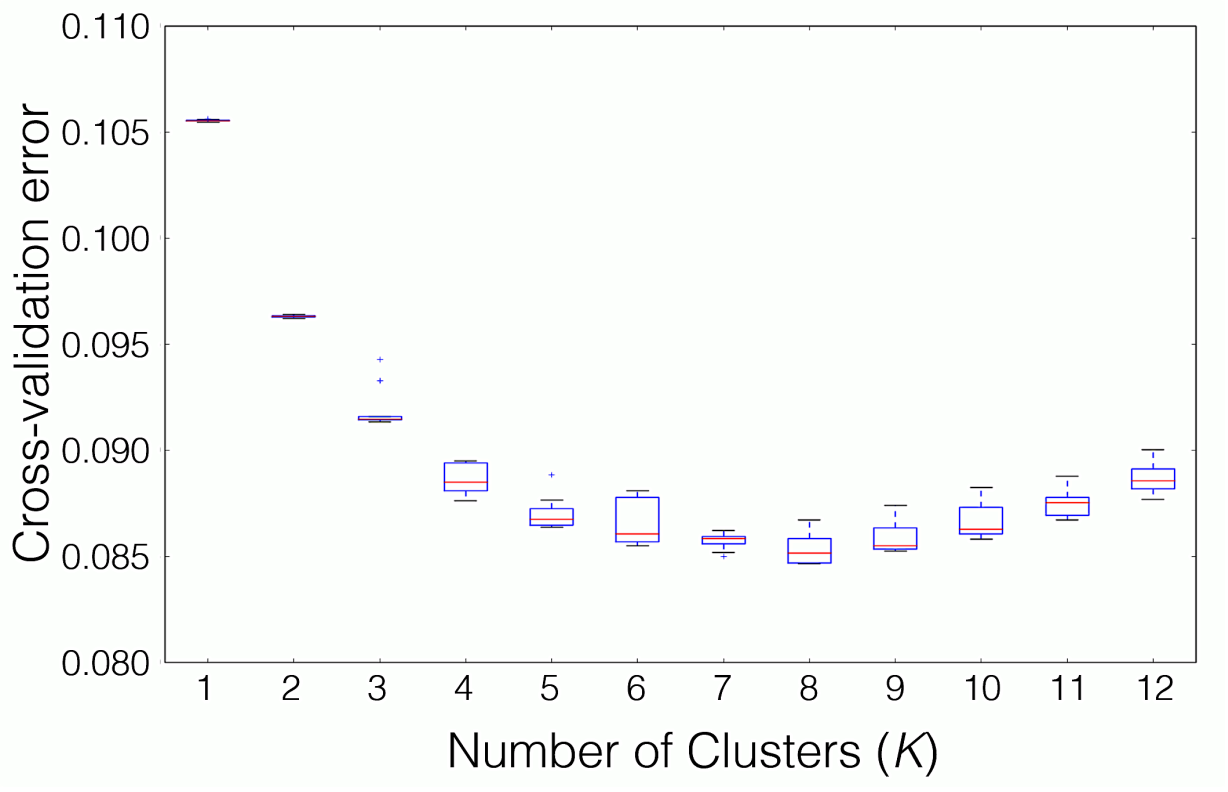

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Figure S2: Change in model likelihood by *K* derived from a STRUCTURE analysis.

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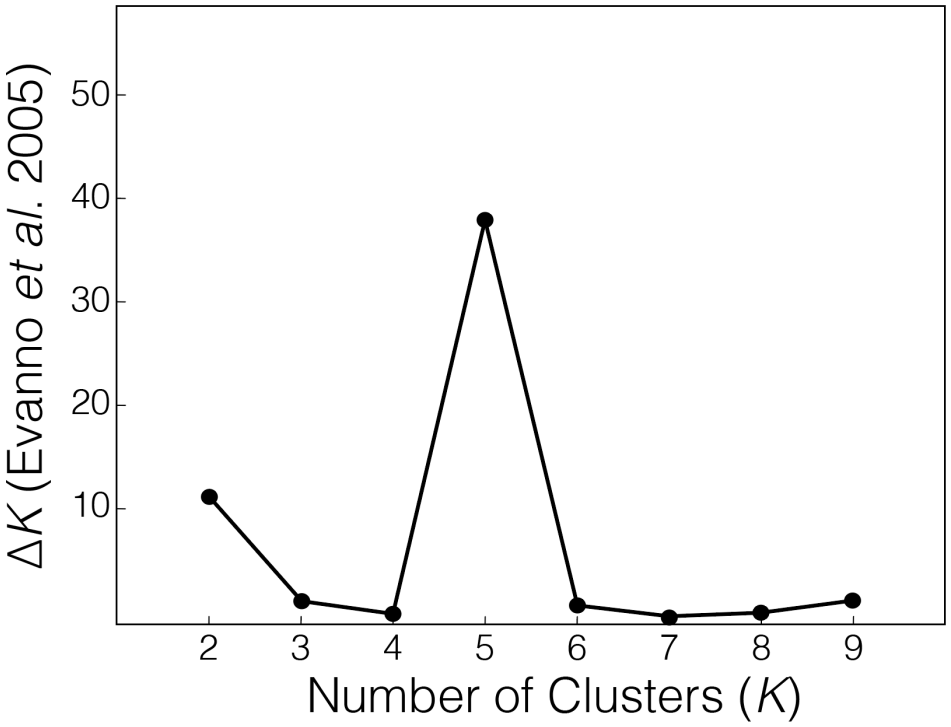
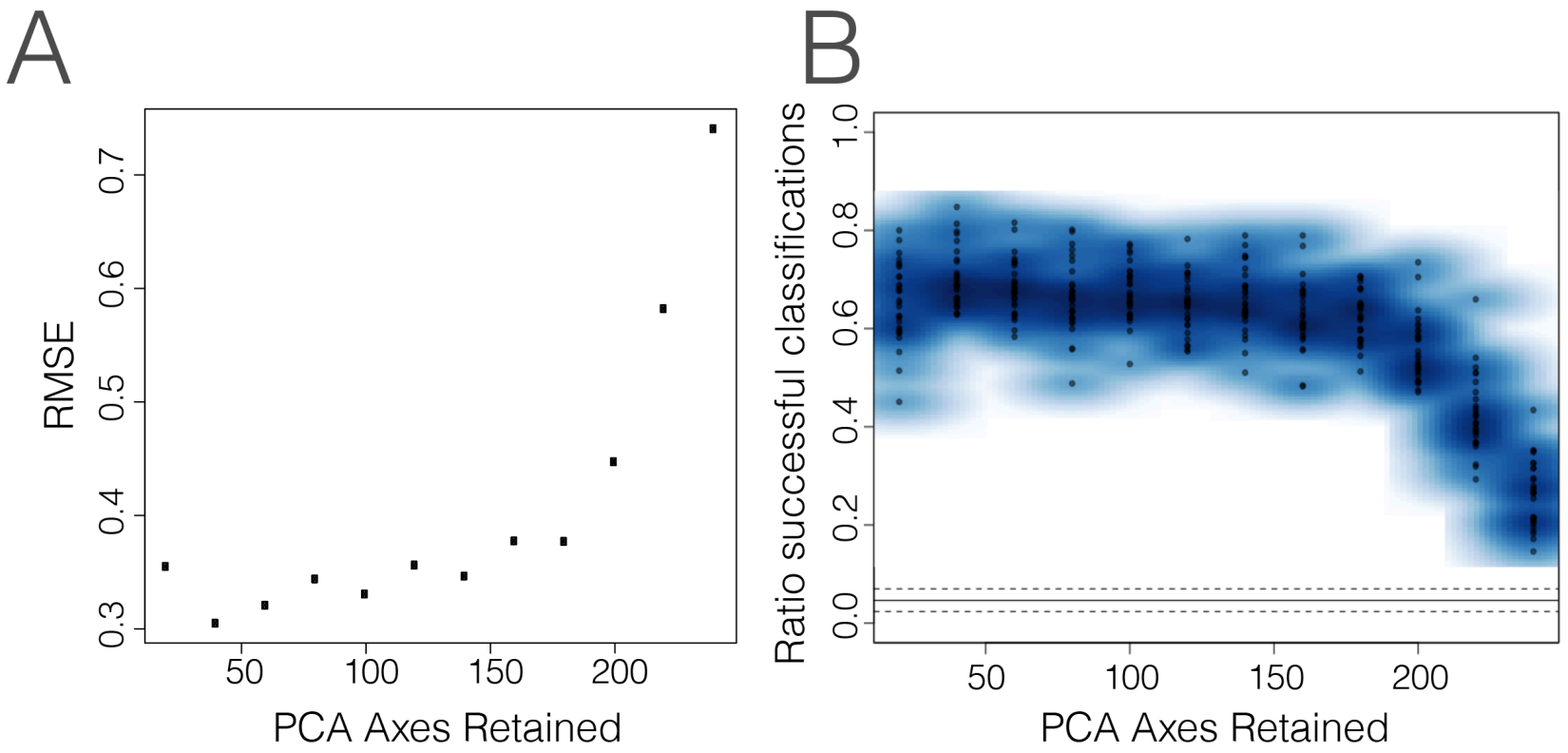


Figure S3: Results of cross-validation analysis in Discriminant Analysis of Principal Components (DAPC), depicting (A) root-mean-square error (RMSE) for classifications under varying number of Principal Component axes (PC's) retained, and (B) proportion of successful classifications for 20 replicates with varying number of PC's retained.



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911 **Figure S4:** Plot of statistical power of assignment for each of six genotype frequency classes.
912 NewHybrids of simulated hybrid genotypes in HybridDetective, using various critical thresholds
913 (from 0.5 to 1.0) for posterior assignment probabilities. Solid lines indicate mean power, while
914 dashed lines are standard deviations across replicates. Note that accuracy of assignment is 100%
915 in all cases (not shown).

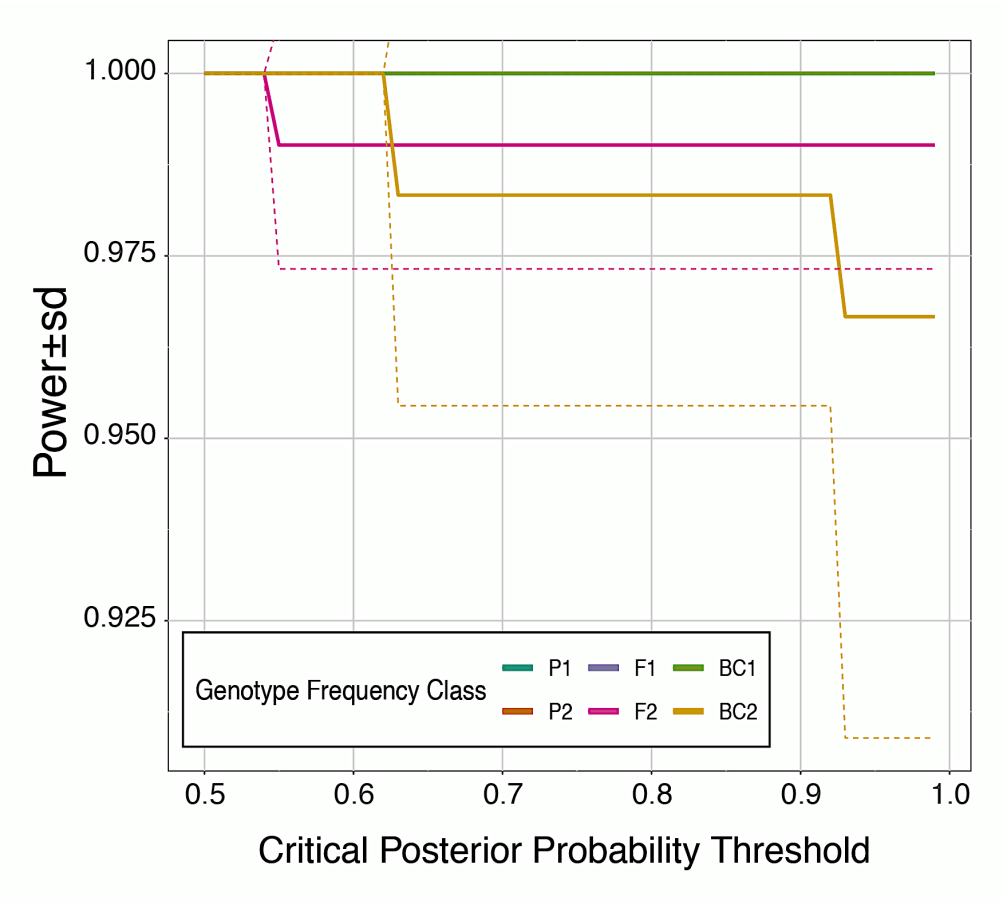

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Figure S5: Relationships between five indices of anthropogenic pressure on stream reaches, and the proportion of genetically pure individuals sampled therein. DOF=Degree of fragmentation; DOR=degree of regulation; SED=degree of sediment trapping; USE=percent consumptive water use; CSI=connectivity status index. Scales for DOF, DOR, SED, and USE reflect a proportion of effect, with 100 = 100% impact. CSI scales from 0 to 100, with 100 being full connectivity (i.e. not detectable human impact).

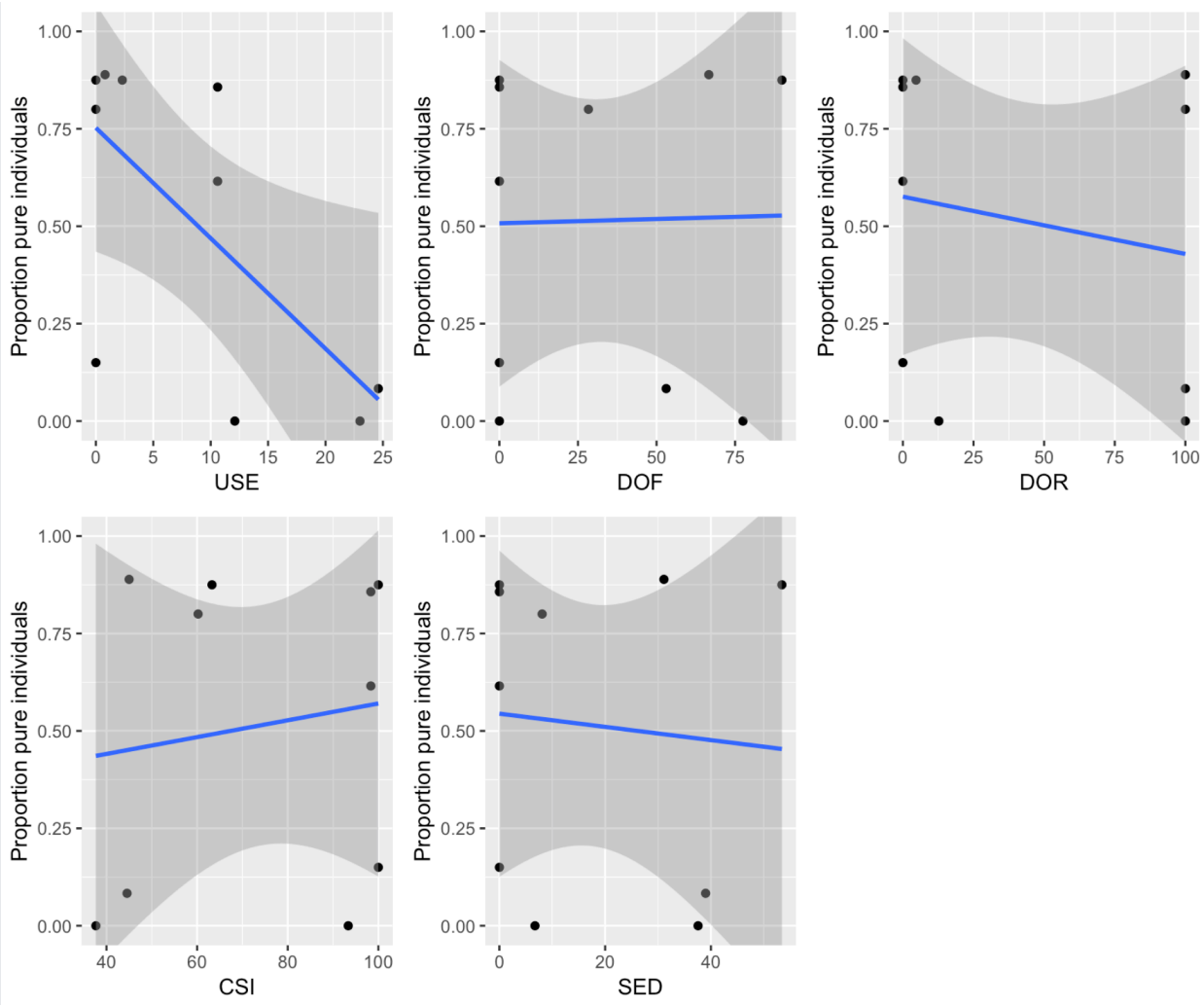

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Figure S6: Human pressure indices plotted onto stream reaches in the Colorado River. DOF=Degree of fragmentation; DOR=degree of regulation; SED=degree of sediment trapping; USE=percent consumptive water use; CSI=connectivity status index. Scales for DOF, DOR, SED, and USE reflect a proportion of effect, with 100 = 100% impact. CSI scales from 0 to 100, with 100 being full connectivity (i.e. not detectable human impact).

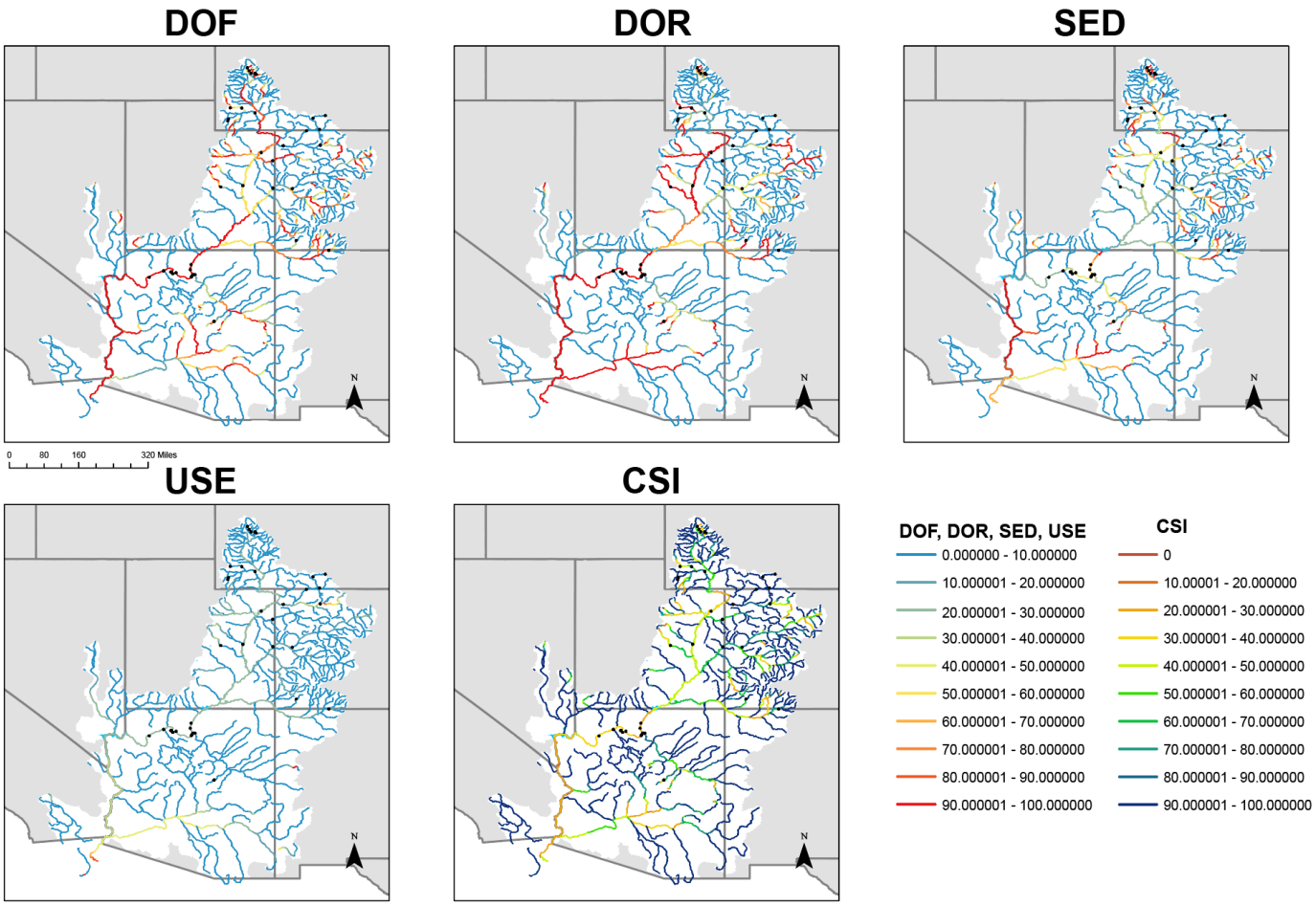

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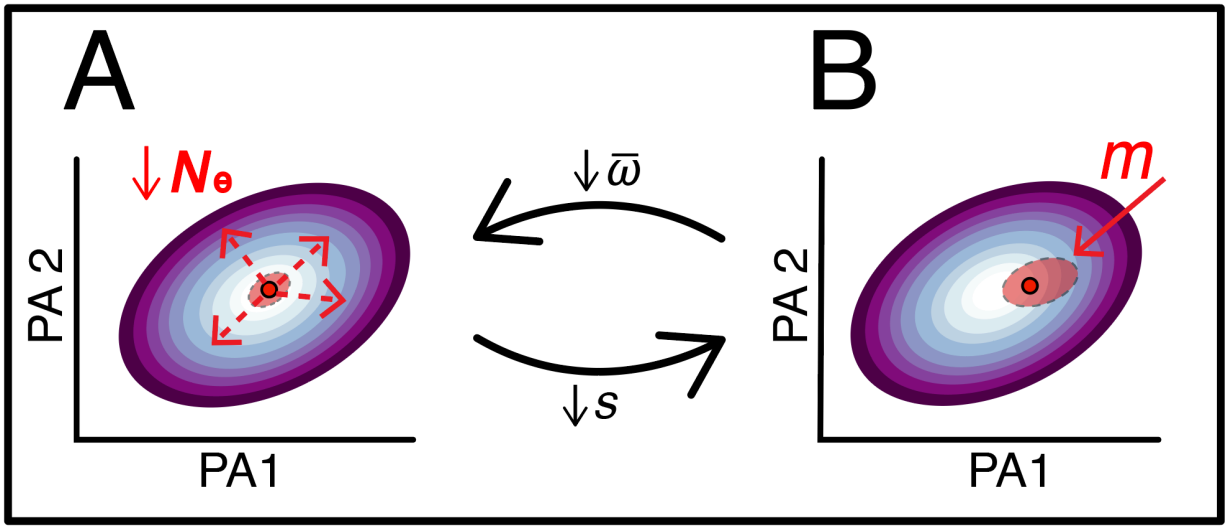
Figure S7: Extinction vortex via negative feedback of inbreeding and outbreeding depression. Shown is the population mean fitness ($\bar{\omega}$; red dot) and variance (red ellipse) within a fitness gradient from low (purple) to high (white), as a function of two arbitrary phenotypic axes (PA1, PA2). (A) Decreasing effective populations size (N_e) lowers the strength of selection (s) and reduces both $\bar{\omega}$ and genetic variance (=inbreeding depression). (B) When introgression is maladaptive, increased gene flow (m) bolsters maladaptive genetic variance while driving a further reduction in $\bar{\omega}$ (=outbreeding depression). This, in turn, prompts further depreciation in N_e, with weakened purifying selection and a relatively greater influence of maladaptive m as a consequence. Persistent coupling of these processes can then drive population extirpation, especially given extrinsic effects on $\bar{\omega}$, such as rapid environmental change.



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