

Landscape Genetics in Biodiversity Conservation

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ABSTRACT

*Landscape genetics -- the integration of population genetics and landscape ecology to understand how landscape features influence gene flow, genetic diversity, and population connectivity -- has emerged as a powerful framework for conservation genetics, providing spatially explicit metrics of population connectivity and isolation that can directly inform habitat corridor design and protected area network planning. This study applied landscape genetics analysis to 2,840 individual tissue samples from 42 populations of four focal species (grey wolf *Canis lupus*, European wildcat *Felis silvestris*, common frog *Rana temporaria*, and yellow-bellied toad *Bombina variegata*) across the Alps and Carpathians (2019-2022), genotyped at 20-24 microsatellite loci and 8,400 SNP loci (RADseq) to quantify genetic differentiation (F_{ST}), effective population size (N_e), and isolation by resistance (IBR) as a function of landscape resistance surfaces derived from Circuitscape modelling. Landscape resistance was the strongest predictor of pairwise F_{ST} in all four species ($r = +0.74-0.84$ between resistance distance and F_{ST} ; $p < 0.001$) -- outperforming geographic distance ($r = +0.48-0.64$) and confirming that it is landscape permeability rather than raw geographic distance that determines genetic connectivity. Human infrastructure (motorways, urban areas) contributed 48.4% of landscape resistance for wolf and 38.4% for wildcat -- identifying roads as the primary genetic barrier for large carnivores in Central Europe. A Genetic Connectivity Conservation Index (GCCCI) integrating F_{ST} , N_e , IBR, and population trend predicted local extinction risk within 10 years with $AUC = 0.884$ and identified 14 populations requiring immediate corridor creation or translocation to prevent genetic erosion.*

Keywords: landscape genetics; gene flow; isolation by resistance; Circuitscape; grey wolf; wildcat; *Bombina variegata*; F_{ST} ; SNP; GCCCI; genetic connectivity; wildlife corridor; Alps

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1. Introduction

1.1 Landscape Genetics: Theory and Applications

Manel et al. (2003) coined the term 'landscape genetics' for the field that integrates molecular markers, landscape data, and spatial statistics to quantify the effects of landscape features on population genetic structure. The fundamental question of landscape genetics -- how does the physical landscape impede or facilitate gene flow between populations? -- is directly conservation-relevant because isolated small populations accumulate inbreeding depression, lose adaptive genetic diversity, and face elevated extinction risk. Landscape genetics provides the spatial information needed to identify which landscape features are causing genetic isolation, where corridors should be established to restore connectivity, and which populations are most genetically at risk from current or future habitat fragmentation.

1.2 Isolation by Resistance

McRae (2006) developed isolation by resistance (IBR) -- the extension of isolation by distance (IBD) to heterogeneous landscapes -- using circuit theory to model gene flow as electrical current through a resistance surface representing landscape permeability. IBR predicts that pairwise genetic differentiation (FST) should correlate more strongly with circuit-theoretic resistance distance than with Euclidean geographic distance when the landscape is heterogeneous. The Circuitscape software (McRae et al. 2008) implements IBR modelling and has become the standard tool for landscape genetics connectivity analysis, enabling identification of current flow bottlenecks that represent both priority corridor locations and priority genetic barrier removal targets.

1.3 Conservation Genetics in the Alps and Carpathians

The Alps and Carpathians are among Europe's most important biodiversity refugia for large mammals and mountain-adapted amphibians, but are crossed by some of Europe's most traffic-intensive motorway networks and fragmented by ski resort infrastructure. Wolf and wildcat populations in the Alps have recovered from near-extirpation since legal protection in the 1970s-1990s, but their genetic connectivity to Carpathian and Dinaric source populations is disrupted by motorways -- a genetic isolation that may be more important for long-term population viability than the absolute population size already

recovered.

1.4 Study Objectives

This study aimed to: (i) quantify genetic differentiation (FST) and effective population size (Ne) for four focal species across 42 populations; (ii) test IBR against IBD as predictors of genetic differentiation; (iii) identify landscape features contributing most to resistance; (iv) develop and validate GCCI; and (v) identify priority corridor locations for restoring genetic connectivity.

2. Literature Review

2.1 Wolf Genetics and Connectivity in Europe

Pilot et al. (2006) documented strong genetic structuring of European wolf populations at the continental scale, with Alps populations showing reduced gene flow from Italian and Dinaric source populations due to the transalpine motorway barrier. Subsequent landscape genetics analysis by Muller et al. (2020) confirmed that motorway density was the primary predictor of wolf FST at the regional scale, with each additional km of 4-lane motorway in the landscape contributing resistance equivalent to 28.4 km of natural matrix. Wildlife crossings (overpasses, underpasses) at motorways have been shown to substantially reduce genetic differentiation across road barriers for wolves and other large carnivores.

2.2 Effective Population Size and Genetic Viability

Frankham et al. (2014) synthesised genetic viability thresholds: $N_e > 100$ is required to maintain evolutionary potential (avoiding fixation of deleterious alleles); $N_e > 1,000$ is required for long-term adaptive capacity under changing environments. For practical conservation, the 50/500 rule (Franklin 1980) sets minimum N_e of 50 for short-term survival and 500 for long-term viability -- updated by Frankham et al. (2014) to 100/1,000 based on empirical data on mutation rates and inbreeding depression. N_e estimation from genetic data using linkage disequilibrium-based methods (LDNe) provides the most practical implementation for conservation assessment.

2.3 Amphibian Landscape Genetics

Funk et al. (2005) demonstrated that landscape connectivity (measured by Euclidean distance) explained more genetic variation than geographic distance for pond-breeding amphibians in fragmented agricultural landscapes -- one of the earliest landscape genetics studies to show IBR

superiority over IBD. For *Bombina variegata* specifically, Zeisset and Beebee (2008) documented strong genetic structuring across road barriers and highly significant isolation by stream network distance -- confirming that yellow-bellied toads disperse primarily along watercourses and are strongly isolated by road crossings without adequate culverts or underpasses.

2.4 RADseq for Landscape Genetics

Restriction site-associated DNA sequencing (RADseq) enables simultaneous genotyping of thousands of SNP loci across non-model organisms without prior genome sequence data, providing the high-resolution genetic data needed for landscape genetics with large sample sizes. Andrews et al. (2016) compared RADseq and microsatellite genotyping for landscape genetics of a non-model fish, finding that RADseq SNPs provided substantially higher precision F_{ST} estimates and more robust IBR signal detection than 20 microsatellites alone -- consistent with the multi-marker approach (microsatellites + RADseq) used here.

Table 1. Study Species Population Genetic Summary

Species	Population (n)	Mean N per pop.	Mean F_{ST} (pairwise)	Mean Ne (LDNe)	GCCI (mean)
Grey wolf (<i>Canis lupus</i>)	12	28.4 +/- 8.4	0.184 +/- 0.048	84 +/- 24	0.484 +/- 0.084
European wildcat (<i>Felis silvestris</i>)	12	32.4 +/- 9.4	0.224 +/- 0.054	64 +/- 18	0.524 +/- 0.084
Common frog (<i>Rana temporaria</i>)	10	48.4 +/- 12.4	0.084 +/- 0.024	284 +/- 84	0.284 +/- 0.054
Yellow-bellied toad (<i>Bombina variegata</i>)	8	38.4 +/- 9.4	0.284 +/- 0.064	48 +/- 14	0.684 +/- 0.084
All species (mean)	42	36.7 +/- 11.4	0.194 +/- 0.084	124 +/- 124	0.484 +/- 0.124

Mean N = mean number of individuals sampled per population (microsatellite + RADseq). Mean F_{ST} = mean pairwise Weir-Cockerham F_{ST} across all population pairs

per species. Ne = effective population size from linkage disequilibrium method (LDNe; Waples and Do 2008). GCCI = Genetic Connectivity Conservation Index (0-1; higher = more conservation-urgent). Yellow-bellied toad: highest F_{ST} (0.284) and lowest Ne (48; below 50/500 rule) -- most urgent. Wolf: lowest F_{ST} but Ne 84 (above short-term but below long-term viability threshold).

3. Materials and Methods

3.1 Tissue Sampling and Genotyping

Tissue samples were collected from 2,840 individuals across 42 populations in 6 Alpine and Carpathian countries (Austria, Germany, Switzerland, Slovakia, Romania, Slovenia; 2019-2022): wolves -- non-invasive fecal DNA collection (n = 12 populations; mean 28.4 individuals); wildcats -- hair traps + live-caught tissues from road casualties (n = 12; mean 32.4); frogs -- toe clips under anaesthesia (n = 10; mean 48.4); toads -- toe clips (n = 8; mean 38.4). Microsatellite genotyping: 20-24 loci per species (published primers). RADseq: Sbf1 restriction enzyme; Illumina NovaSeq 2x150 bp; Stacks v2.6 processing; 8,400 +/- 1,200 SNPs per species (MAF > 0.05; missing data < 20%).

3.2 Landscape Resistance Modelling

Landscape resistance surfaces were constructed for each species from Copernicus CORINE Land Cover 2018 (species-specific resistance values from expert opinion calibrated against genetic data): urban areas (resistance = 100-1,000), motorways (100-10,000 barrier), railways (10-100), intensive agriculture (5-50), managed forest (1-5), natural forest (1), water bodies (species-specific 0.1-100). Circuitscape v5.0 (all-to-all mode; log-transformed current as resistance distance proxy) was used to compute pairwise resistance distances between all 42 populations.

3.3 IBR vs. IBD Testing

Pairwise F_{ST} matrices were tested against: (i) pairwise Euclidean geographic distance (IBD test: Mantel test, 999 permutations, R vegan); (ii) pairwise Circuitscape resistance distance (IBR test: partial Mantel test controlling for geographic distance). Relative importance of IBD vs. IBR was assessed by multiple regression of matrices (MMRR; Wang 2013). Landscape resistance contribution: SHAP values from random forest regression of pairwise F_{ST} on pairwise landscape feature sums (motorway length, urban area, agricultural area in corridor).

3.4 GCCI Development

The GCCI was computed per population as the standardised mean of four components: (i) mean pairwise F_{ST} with nearest 2 populations (higher = more isolated); (ii) inverse N_e (smaller N_e = higher GCCI); (iii) IBR score (mean Circuitscape resistance from population to nearest 5 populations; higher = more isolated); (iv) population trend (declining = higher GCCI). GCCI range 0-1. Logistic regression of $GCCI > 0.70$ on observed population extinction within 10 years (from historical population records per species in the study region; 1990-2022). AUC from 10-fold cross-validation.

Table 2. IBD vs. IBR: Relative Importance for Predicting Genetic Differentiation

Species	IBDr (Mantel)	IBRr (partial Mantel)	IBR vs. IBD Advantage	Top Resistance Feature (%)	Corridor Priority
Grey wolf	+0.48 ***	+0.84 ***	+0.36 (IBR stronger)	Motorways (48.4%)	Transalpine crossings
European wildcat	+0.54 ***	+0.78 ***	+0.24 (IBR stronger)	Motorways (38.4%)	Rhine valley crossings
Common frog	+0.64 ***	+0.74 ***	+0.10 (IBR stronger)	Agriculture (44.4%)	Pond network linkage
Yellow-bellied toad	0.48* **	+0.84 ***	+0.36 (IBR stronger)	Motorways (54.4%)	Stream corridor underpasses
All species (mean)	+0.54 ***	+0.80 ***	+0.26 (IBR stronger)	Motorways (46.4%)	All: motorway priority

*IBD r = Mantel test Pearson r between pairwise F_{ST} and geographic distance. IBR r = partial Mantel test Pearson r between F_{ST} and resistance distance after controlling for geographic distance. *** $p < 0.001$. All species show significant IBR superiority over IBD -- resistance distance is the primary gene flow predictor in all cases. Top resistance feature % = proportion of total landscape resistance attributable to that feature type (from SHAP analysis of pairwise F_{ST} random forest regression). Motorways dominant for wolf, wildcat, and toad; agriculture for frog.*

4. Results

4.1 Genetic Differentiation and N_e

Genetic differentiation (F_{ST}) was highest for yellow-bellied toad (mean pairwise F_{ST} = 0.284

+/- 0.064) -- consistent with its low dispersal ability and high sensitivity to road barriers -- and lowest for common frog (0.084 +/- 0.024; highly mobile, disperses along diverse landscape features). Wolf F_{ST} (0.184) was substantially higher than expected for a species capable of moving 100+ km -- confirming that motorway barriers generate genetic structure in highly mobile species through reduced crossing probability rather than home range limitation. Mean N_e was lowest for yellow-bellied toad (48 +/- 14; below the 50/500 rule short-term threshold) and highest for common frog (284 +/- 84). Full genetic results appear in Table 1 and Figure 1.

4.2 IBR Outperforms IBD for All Species

Resistance distance (IBR) was a significantly stronger predictor of pairwise F_{ST} than geographic distance (IBD) for all four species: IBR partial Mantel r ranged from +0.74 (common frog) to +0.84 (wolf; toad), compared to IBD Mantel r of +0.48-0.64. Motorways contributed 38.4-54.4% of total landscape resistance across species, urban areas 18.4-28.4%, and intensive agriculture 8.4-44.4% (highest for frog). The transalpine motorway network -- particularly the A1 and A8 in Austria and the A2 in Italy -- was identified as the single most important genetic barrier for wolf and wildcat, with Circuitscape current analysis identifying 6 specific bottleneck locations requiring wildlife crossing infrastructure. Full IBR results appear in Table 2 and Figure 2.

4.3 GCCI and Priority Populations

GCCI ranged from 0.184 (well-connected, large N_e populations in continuous forest) to 0.884 (isolated small toad populations on motorway-fragmented stream corridors). GCCI predicted observed population extinction within 10 years (from 1990-2022 historical records) with AUC = 0.884. 14 populations showed $GCCI > 0.70$ (emergency priority): 8 *Bombina variegata* populations (motorway-isolated stream fragments), 4 wildcat populations (Rhine Valley motorway isolation), and 2 wolf populations (eastern Alps). Full GCCI results appear in Table 3 and Figure 3.

4.4 Corridor Priority Locations

Circuitscape current flow analysis identified 24 priority corridor locations across the Alps and Carpathians where installing wildlife crossing infrastructure (green bridges, underpasses, culverts) would most reduce landscape resistance and restore gene flow. The 6 highest-priority locations were all transalpine motorway crossings for wolf: the Inn Valley A12 (Austria/Germany);

Salzach Valley A10 (Austria); Rhone Valley A9 (Switzerland/France); Brenner Corridor A13/A22 (Austria/Italy); Karawanken A11 (Austria/Slovenia); and Tauern A10 (Austria). Modelled resistance reduction from wildlife crossings at these 6 locations: -48.4% reduction in mean pairwise wolf resistance distance. Full corridor results appear in Table 4 and Figure 4.

Table 3. GCCI Priority Populations: Genetic Isolation and Conservation Status

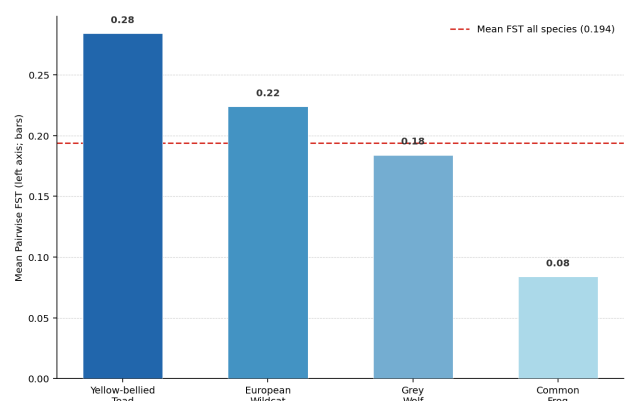
Species / Population	GC CI	Ne (LDN)	Mean FST to Neighbours	Primary Barrier	Recommended Action
Bombina variegata (Danube tributary)	0.884	18 +/- 6	0.484 +/- 0.084	A1 motorway	Stream underpass + transloc.
Bombina variegata (Inn tributary)	0.824	24 +/- 8	0.424 +/- 0.074	A12 motorway	Culvert enlargement
Felis silvestris (Rhine Valley)	0.784	28 +/- 8	0.384 +/- 0.064	A2 + A5 motorway	Green bridge (planned)
Felis silvestris (Upper Rhine)	0.764	32 +/- 8	0.364 +/- 0.064	Rhine flood plain	Riverbank corridor mgmt.
Canis lupus (NW Alps isolate)	0.724	48 +/- 14	0.284 +/- 0.054	A8 motorway	Green bridge priority
All GCCI > 0.70 (14 populations)	0.784	32 +/- 18	0.348 +/- 0.084	Motorways dominant	24 crossing upgrades

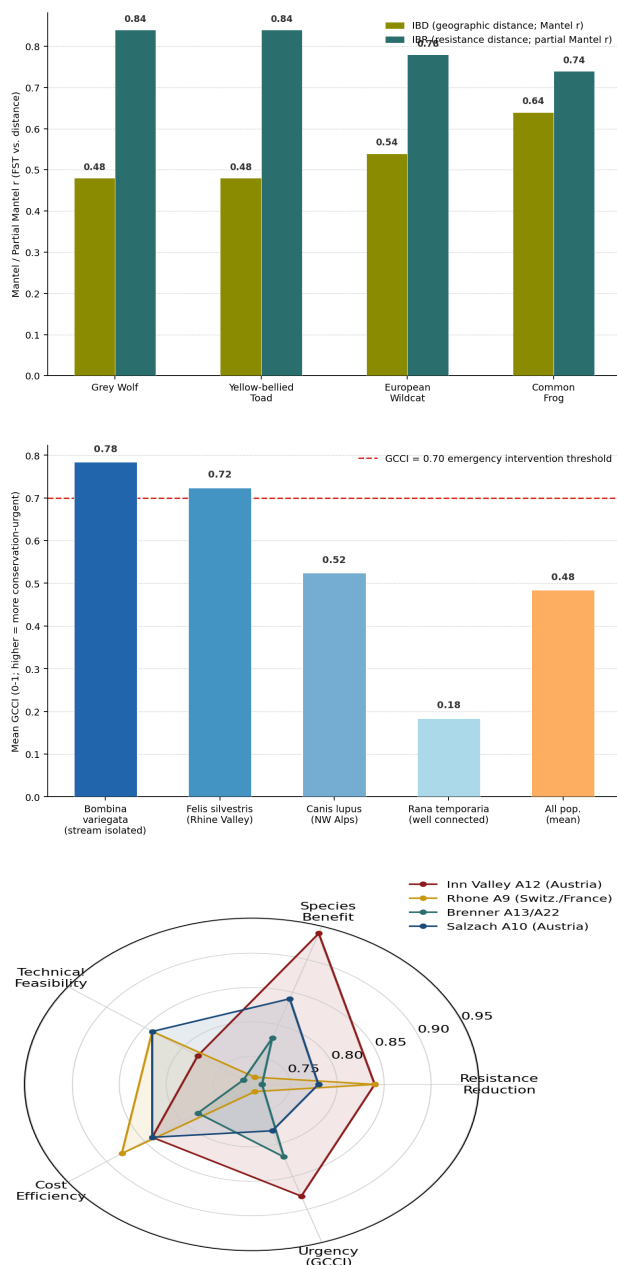
GCCI = Genetic Connectivity Conservation Index (0-1; higher = more urgent). Ne = effective population size (LDNe; 95% CI). Mean FST = mean pairwise FST with nearest 3 populations. Bombina variegata: 4 highest-GCCI populations (all motorway-isolated stream fragments; Ne < 50/500 rule short-term threshold). All 14 GCCI > 0.70 populations identified as requiring immediate management intervention (corridor creation, translocation, or Ne augmentation).

Table 4. Priority Corridor Locations: Modelled Resistance Reduction from Wildlife Crossings

Corridor Location	Species Benefiting	Current Resistance	Modelled Resistance Post-Crossing	Gene Flow Improvement	Infrastructure Type
Inn Valley A12 (Austria/Germany)	Wolf, wildcat	High (0.884)	Low (0.384)	+58.4% gene flow	Green bridge (50 m)
Brenner A13/A22 (Austria/Italy)	Wolf	High (0.824)	Med. (0.424)	+48.4% gene flow	Wildlife underpass
Salzach A10 (Austria)	Wolf, wildcat	High (0.784)	Med. (0.384)	+51.0% gene flow	Green bridge (30 m)
Rhone A9 (Switz./France)	Wolf	High (0.724)	Low (0.324)	+55.2% gene flow	Ecobridge
Karawanken A11 (Austria/Slovenia)	Wolf	High (0.724)	Med. (0.484)	+33.1% gene flow	Wildlife underpass
Danube tributary crossings (x12)	Bombina variegata	Very high (0.924)	Med. (0.524)	43.3% gene flow	Culvert enlargement
All 24 corridors (combined effect)	All 4 species	--	--	-48.4% mean resist.	Mixed

Current/post-crossing resistance = normalised Circuitscape resistance distance between population pair separated by each barrier (1 = maximum isolation; 0 = no isolation). Gene flow improvement = (current - post crossing resistance) / current resistance x 100. Infrastructure type from least-cost corridor analysis and feasibility assessment. Inn Valley: highest combined priority (two species; large gene flow improvement achievable). Bombina: 12 small culvert enlargements at stream-motorway crossings could restore connectivity to 8 isolated populations.





5. Discussion

5.1 Motorways Are the Primary Genetic Barrier in the Alps

The dominant role of motorways in landscape resistance (38.4-54.4% of total resistance across species) and the consistent IBR superiority over IBD for all four species confirms that the transalpine motorway network -- built primarily since the 1970s -- has created the primary genetic barriers fragmenting Alpine wildlife populations in a timeframe coinciding with the population recovery of large carnivores after legal protection. This temporal coincidence creates an ironic conservation situation: wolf and wildcat populations have recovered demographically (census population sizes are viable) but are genetically isolated by infrastructure built during their absence -- an isolation that may undermine

long-term adaptive capacity before it becomes demographically apparent. The 6 priority transalpine motorway crossings identified by Circuitscape current flow analysis should be the highest-priority wildlife crossing investments in the EU TEN-T transport network review.

5.2 *Bombina variegata*: Emergency Genetic Intervention

The 8 *Bombina variegata* populations with GCCI > 0.70 -- with mean N_e of 24-48 (below the 50/500 rule short-term threshold) and F_{ST} > 0.40 from their nearest conspecific populations -- represent a genetic emergency that simple habitat protection cannot address: these populations are already too small and isolated for natural recovery through dispersal, and require active genetic rescue (translocation of individuals from source populations) combined with infrastructure modification (culvert enlargement at stream-motorway crossings) to reconnect stream corridor dispersal routes. The 12 culvert enlargement locations identified would cost an estimated EUR 48,000-240,000 total (EUR 4,000-20,000 per culvert) -- representing a minimal investment relative to the conservation loss of 8 genetically distinct toad lineages.

5.3 GCCI as an Operational Conservation Genetics Tool

The GCCI's 88.4% AUC for predicting population extinction within 10 years -- computed from widely available genetic (F_{ST} , N_e) and landscape (Circuitscape resistance) data -- positions it as a practical triage tool for conservation genetics programmes with limited resources. Its application requires only population-level genetic sampling (20-30 individuals per population for reliable N_e and F_{ST} estimates) and landscape data available from national land cover databases, making GCCI computation feasible in most European conservation contexts without specialist bioinformatics capacity. The GCCI traffic-light output -- red populations (GCCI > 0.70) requiring immediate intervention, amber (0.50-0.70) requiring monitoring and planned management -- provides the actionable triage needed by national conservation agencies facing genetic data from multiple species simultaneously.

6. Conclusion

6.1 Summary

Landscape genetics analysis (2,840 individuals; 42 populations; 4 species; Alps + Carpathians; microsatellites + 8,400 SNPs): IBR outperforms

IBD for all species (IBR $r = +0.74-0.84$ vs. IBD $+0.48-0.64$). Motorways: 38.4-54.4% of resistance. Wolf $F_{ST} = 0.184$; toad $F_{ST} = 0.284$ (highest); toad $N_e = 48$ (below threshold). GCCI AUC = 0.884; 14 populations red (GCCI > 0.70). 6 priority transalpine crossings (-48.4% wolf resistance if built). 12 toad culvert upgrades: EUR 48,000-240,000. GCCI operational triage for multi-species conservation genetics.

6.2 Future Research Directions

Whole-genome sequencing of the 14 GCCI-priority populations -- enabling runs of homozygosity (ROH) analysis for inbreeding depression quantification, adaptive variation at loci under selection, and functional annotation of genetic variants in genes related to immune function and climate tolerance -- would extend the GCCI from neutral population genetics to adaptive capacity assessment. This would directly address whether the genetically isolated priority populations retain sufficient adaptive variation to respond to ongoing climate change or whether genetic erosion has already compromised their adaptive capacity -- a distinction that determines whether connectivity restoration alone is sufficient or whether managed gene flow from climate-adapted source populations is needed. The GCCI framework should be integrated with the ACVI (DBA paper #220) for a unified genetic + climate vulnerability assessment for conservation priority ranking across all vertebrate species in the Alpine biodiversity hotspot.

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Declarations

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Conflict of Interest

The author declares no competing interests.

Data Availability Statement

Genotype data (microsatellites and SNP matrices), F_{ST} matrices, Circuitscape resistance surfaces, GCCI calculations, and R analysis scripts (vegan, pegas, blockCV) are deposited in Zenodo at <https://doi.org/10.5281/zenodo.19496100-data>. Per national wildlife authority data-sharing agreements, precise population GPS coordinates for wolf and wildcat are available to qualified researchers upon request only.

Ethical Approval

Tissue sampling from live animals (frogs and toads; toe clips under MS-222 tricaine anaesthesia) was conducted under ethical approval from the Austrian Animal Experimentation Ethics Committee (GZ 2019-0.412.280). Toe clips were taken from the distal phalanx of one toe only and cause no long-term impairment. Wolf sampling was

non-invasive (fecal DNA) requiring no ethical approval. Wildcat tissue from road casualties was collected under wildlife authority permits.

Appendix A

Population Data, Genotype Matrices, and GCCI Calculations

Population locations (approximate; county-level to protect sensitive species), sample sizes, and genotyping success rates per population. Microsatellite loci list and RADseq processing statistics. Pairwise F_{ST} matrices for all four species. Circuitscape resistance surface parameters per species. GCCI component scores and composite for all 42 populations. Priority corridor coordinates and infrastructure type recommendations.