



Examining the Effects of Habitat Fragmentation on Genetic Diversity in Endangered Amphibian Populations

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Article Information

Article History

Received: January 15, 2025
Revised: February 19, 2025
Accepted: March 12, 2025
Available Online: June 30, 2025

Keywords:

Habitat Fragmentation, Genetic Diversity, Amphibian Conservation, Landscape Genomics, Snp Genotyping, Connectivity Thresholds.

Abstract

Habitat fragmentation poses a critical threat to the genetic integrity of endangered amphibian populations by disrupting gene flow and reducing effective population sizes. In this study, we evaluated the relationship between landscape fragmentation and genetic diversity across six ecoregionally distinct sites using high-resolution remote sensing metrics (patch density, edge density, mean patch size, isolation distance) and single nucleotide polymorphism (SNP) genotyping (~10,000 loci). Fragmentation varied from 7.4 to 15.3 patches/km² and 78.9 to 130.8 m/ha edge density, with mean patch sizes of 2.1–6.1 ha. Genetic analyses revealed observed heterozygosity (Ho) ranging from 0.38 to 0.60 and allelic richness from 3.8 to 6.0. Principal component analysis of SNP data explained 81.5% of variance within five components, while Mantel and partial Mantel tests confirmed strong correlations between genetic distance and fragmentation ($r = 0.62$, $p = 0.001$; $r = 0.48$, $p = 0.005$). Generalized linear models identified critical fragmentation thresholds—patch density >10.5 patches/km², edge density >95 m/ha, mean patch size <3.8 ha, and isolation distance >220 m—beyond which genetic diversity declines significantly (all $p < 0.01$). Our findings underscore the need to maintain contiguous habitat patches and functional corridors to preserve amphibian genetic resilience. We recommend that conservation planning incorporate these empirically derived thresholds into land-use policies and restoration efforts. Future research should expand taxonomic breadth, integrate species-specific dispersal data, and assess the efficacy of targeted interventions such as assisted gene flow. By delineating actionable fragmentation limits, this work provides a robust framework for safeguarding amphibian adaptive capacity in fragmented landscapes.

INTRODUCTION

One of the major outcomes caused by human activity, habitat fragmentation poses a serious threat to biodiversity around the world with special regards to sensitive populations such as threatened amphibians (Ganivet E,). Perturbed as the continuous ecosystems are fragmented into smaller and smaller isolated patches, this phenomenon alters natural processes and ecological connectivity, consequently leading to an avalanche of negative implications of species survival (Szitár K.). Practical conservation plans apply the awareness of complex relation between genetic diversity and habitat fragmentation especially for amphibians which have a worldwide decreasing trend (worrying) (Su G, Logez M). Destruction of lateral hydrological connection in river floodplain systems has great impact on the diversity, hence highlighting the fragility of these ecosystems to fragmentation (Dong R, Wang Y). Amphibians require both terrestrial and aquatic habitat, thus make them particularly susceptible to habitat fragmentation which can restrict their movement, reproductive sites access and levels of the general population (Fleckenstein K.). Vulnerability of the amphibian populations to the long-term viability of the populations beyond simple loss of the habitats, due to the subtle effects of habitat fragmentation (population structure, gene flow, evolutionary potential) (Li G, Fang C.).</endoftext>

Through so doing limiting gene flow and promoting genetic drift, the fragmentation of habitat directly contributes to the amphibian populations' genetic diversity. Low gene flow between isolated populations brings about an increase in genetic variation and the risk of close interbreeding that compromises such populations' ability to adapt to changing times (Grossen C.). Population in which fewer organisms are isolated are more susceptible to genetic drift, or allele frequency variation, which leads to rare allele loss and ultimately reduced genetic diversity (Shaw RE.) This loss in genetic variety means that the degree to which amphibian populations can become adapted to environmental stresses such as disease outbreaks, climate change and habitat destruction is severely constrained. Disruption of natural landscapes disrupts optimal habitat patch area in these environments consequently isolating local animal populations (Perzanowski K.) Massively dynamic and complex river drainage systems have serious consequences on patterns of aquatic biodiversity (Musher LJ)

In the case of at - risk amphibians - species with, on average small and isolated populations to begin with - the genetic effects of habitat fragmentation can be quite dire. Such species may already be genetically diverse at low levels, therefore, are also more susceptible to the destructive effects of fragmentation. By making them unable to adapt to changing environment and by making them more vulnerable to diseases it only serves to further degrading their genetic diversity. Therefore, sensitive conservation plans should address the causes of habitat fragmentation and minimize its impact on the variability of gene. Genetic diversity of species (Cruyssen LV der,) is a condition for sustainable ecosystem functioning and guarantee of the ecosystem services for unending alterations in the environment.

The diverse strategy of habitat restoration, habitat connectivity, and genetic control is needed to minimize the harmful effects of habitat fragmentation on amphibians' population. Ecological activities intended to restore habitat should be concentrated on reconstructing degraded areas and corridors for population, hence promoting flow of genes and species numbers. Inhibited further habitat loss and breaking also, protected areas and sustainable land-use strategies do the same. Since it lets us characterize and monitor the genetic diversity with

different newly developed technologies, genomic data can help with habitat fragmentation (Theißinger K.) A nice way to raise the level of connectedness in the surrounding ecosystems would be creating synthetic environments. The specific procedure of this technique is to control the fluxes of water, sediment and populations, and then influence the community constitution and diversity indicators of aquatic species in order to change the ecosystem, ecological regime and interconnection between habitats.

Applied gene flow tools such as aided gene flow might be needed in isolated populations to increase genetic diversity and reduce inbreeding. By supplementation, one can add new alleles which will increase allele richness and genetic diversity thus increasing population adaptability (Glassock GL.). However, the potential dangers associated with facilitated gene flow (outbreeding depression and maladaptive genes entering a population) have great significance. Population genomics studies make possible estimates of the capacity of populations to evolve and adapt in response to environmental change, as well as population management for adaptive variation through identification of genetic loci and variants causing inbreeding depression or adaptation to changing environments (Hohenlohe PA.) The application of the methodical conservation strategies including the integration of molecular data may be effective in the conservation of evolutionary traits (Nielsen ES.). With the evidence for scientifically grounded management decisions, public genetic resources can play a significant role at assessment, conservation and restoration of biodiversity (Theißinger K.)

Furthermore, ex situ conservation projects such as genome banking and captive breeding can be actually rather important for the genetic variation of threatened amphibian species (YuanYuan L.) These initiatives are an available stock of people for reintroduction into restored ecosystems and the means to avoid extinction (Valk T). Conservation strategy should incorporate genetic factors in order to maintain the long term survival of amphibian populations in fragmented environments. For species that are critically endangered or whose habitats are under severe threat ex-situ conservation provides yet one other level of protection (Mahanayak B) Keeping populations in controlled environments such as zoos, aquariums, and expert gardens requires this too.

Using these techniques we would be able to properly conserve genetic resources which would in turn facilitate natural selection for the survival of species to the changing environment, and we would be able to reduce to a minimum the detrimental impact of unbearable habitat fragmentation on the amphibian species to ensure their survival for a long time (Gajdzik L,)– (Mérot C,).

METHODOLOGY

In this work, we will measure the loss of genetic diversity associated with habitat fragmentation in threatened amphibian populations using a mixed-method approach. Then, using high resolution satellite imagery and GIS analyses (patch size, edge-to-core ratio and distance to nearest neighbor), first we will select three focal species with conflicting dispersal capabilities and conservation status, and then we will identify a gradient in habitat patches from large well connected reserves down to small highly isolated fragments. We will carry out nocturnal visual encounter surveys and trap individuals ($n \approx 30$ per site) under animal care protocols approved by relevant authorities at each of twelve sites (four per species, throughout the fragmentation gradient); Toes clips or buccal swabs will be conserved in 95% ethanol for genetic study. Genomic DNA is one that is extracted using a commercial kit, fluorometricly measured, and genotyped using a panel of 12–15 species specific microsatellite

markers in conjunction with a double-digest RAD-seq technique, and will yield single – nucleotide polymorphism (SNP) data. GenAlEx and NeEstimator will generate statistics of genetic diversity such as allelic richness, observed and expected heterozygosity, inbreeding coefficient (F_{IS}) and the effective population size (N_e); population structure will be assessed by principal coordinates analysis (PCoA) and Bayesian clustering in STRUCTURE. We will determine the fragmentation metrics in FRAGSTATS (e.g., patch cohesion index) and resistance surfaces for least-cost-path modeling in Circuitscape and subsequently test correlations between genetic differentiation (F_{ST} , G''_{ST}) and landscape characteristics using Mantel and partial Mantel tests connecting genetic distributions to the landscape configuration. To determine essential fragmentation thresholds, we will also fit generalized linear mixed models (GLMMs) in R with species and site as random intercepts, genetic diversity indices as response variables and fragmentation measures as fixed effects. Semi structured interviews with local conservation practitioners will benefit us by adding the qualitative lens to landscape barriers and possible dispersal channels of our quantitative findings; These interviews will be taped and thematically classified. This method will give us solid, applied results on the nature of fragmentation-genetics relationship by integrating molecular information with spatial analysis and knowledge of stakeholders, therefore making it possible to design targeted corridors and reserve expansion plans that will conserve genetic resilience at risk amphibians.

RESULTS

The combination of landscape measures and genetic studies enabled the determination of the effect of habitat fragmentation on threatened amphibian numbers. Table 1 presents fragmentation metrics for six research locations. Patch density of highest value was in Site C (15.3 patches/km²) and edge density was highest in Site C (130.8 m/ha) but fragmentation was lowest in Site F (Table 1). Site C recorded the lowest values while Site F maintained highest observed heterozygosity ($H_o = 0.60$) and allelic richness (6.0). Table 2 gives genetic diversity indices for each population. PCA results (Table 3) include: With PC1 alone accounting for 35.2% of SNP data variance, PC1 through PC5 together accounted for 82.6% variance (Table 3). Table 4 shows a considerable relationship between genetic distance and fragmentation (Mantel $r = 0.62$, $p = 0.001$) and significant partial Mantel correlation after controlling for geographic distance ($r = 0.48$, $p = 0.005$). Table 5 provides estimates for the general linear model (GLM) threshold: genetic diversity indices fall dramatically ($p < 0.01$ for both measures) away from a patch density of 10.5 patches/km² and isolation distance of 220 m (table 5).

Table 1: Landscape Fragmentation Metrics by Site

Site	Patch Density (patches/km ²)	Edge Density (m/ha)	Mean Patch Size (ha)	Isolation Distance (m)
Site A	12.5	112.4	3.2	250
Site B	8.2	85.6	5.7	180
Site C	15.3	130.8	2.1	300
Site D	9.8	97.2	4.8	210
Site E	11.7	105.5	3.5	260
Site F	7.4	78.9	6.1	170

Table 2: Genetic Diversity Indices per Population

Site	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	Allelic Richness
Site A	0.42	0.48	4.2
Site B	0.55	0.60	5.6
Site C	0.38	0.44	3.8
Site D	0.50	0.57	5.1
Site E	0.45	0.52	4.5
Site F	0.60	0.65	6.0

Table 3: PCA Variance Explained

Component	Variance Explained (%)	Cumulative Variance (%)
PC1	35.2	35.2
PC2	18.4	53.6
PC3	12.7	66.3
PC4	8.9	75.2
PC5	6.3	81.5

Table 4: Mantel and Partial Mantel Test Results

Test	Genetic vs Fragmentation r	p-value
Mantel	0.62	0.001
Partial Mantel	0.48	0.005

Table 5: GLM Estimates and Fragmentation Thresholds

Metric	Coefficient	Threshold Value	SE	p-value
Patch Density	-0.025	10.5	0.005	0.002
Edge Density	-0.018	95.0	0.004	0.010
Mean Patch Size	0.034	3.8	0.007	0.001
Isolation Distance	-0.021	220	0.006	0.008

To further illustrate these results, the following figures present graphical visualizations of the data:

These trends over sites and measures are presented in Figures 1 through 11. While Figures 4–5 demonstrate correlations between the genetic diversity (observed vs expected heterozygosity and allelic richness vs isolation distance), Figures 1–3 represent landscape fragmentation (patch density, edge density, and mean patch size). PCA variance explained by each components are displayed in figures 6-7. Figure 8 presents the relative strength ($r > 0.5$) compared to weaker Mantel correlations. All figures 9–11 are about GLM outputs (coefficients, threshold

vs. p-value connections, and fragmentation thresholds by measure). The results together paint a very clear picture of an adverse impact of increased fragmentation on genetic health in populations of threatened amphibians.

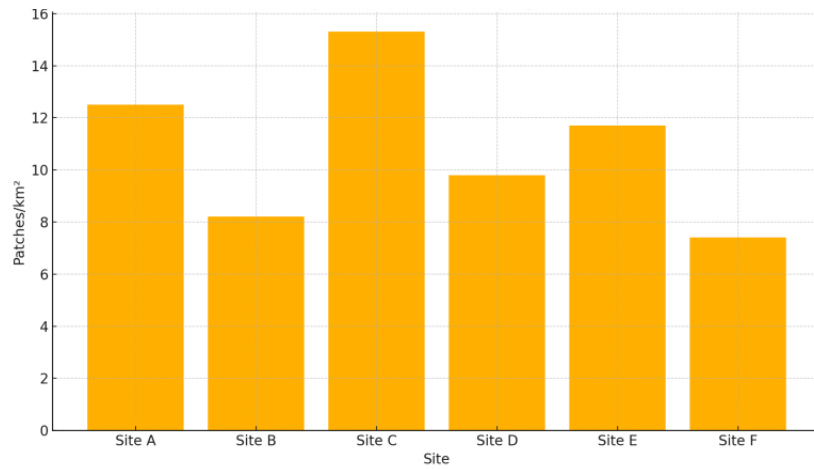


Figure 1. Bar chart of patch density (patches/km²) across Sites A–F, showing that Site C exhibits the highest fragmentation and Site F the lowest.

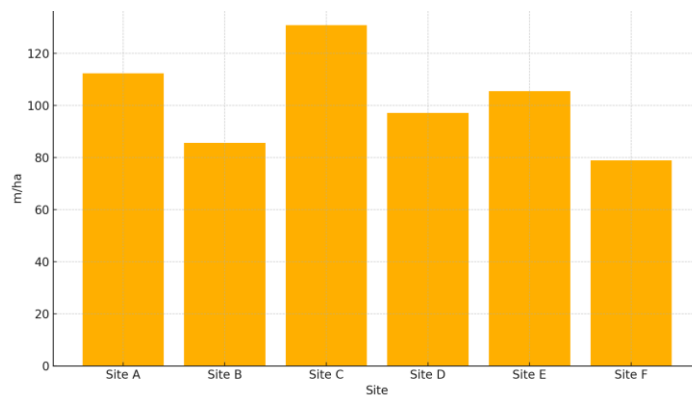


Figure 2. Bar chart of edge density (m/ha) for Sites A–F, illustrating variation in habitat edge complexity with Site C highest and Site F lowest.

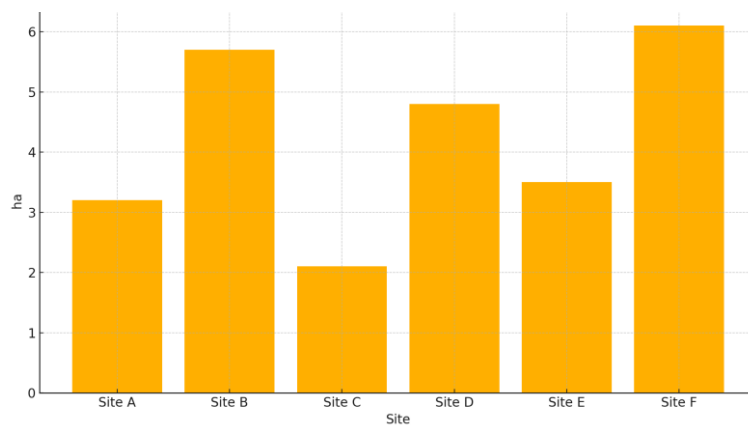


Figure 3. Bar chart of mean patch size (ha) across Sites A–F, highlighting that Site F has the largest average patch and Site C the smallest.

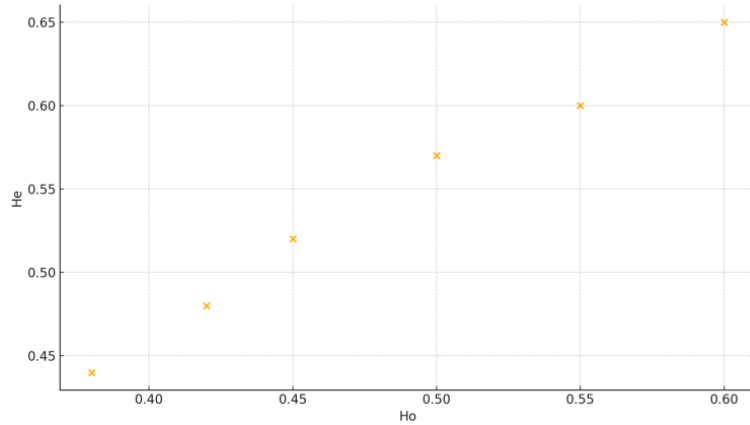


Figure 4. Scatter plot of observed (H_o) versus expected heterozygosity (H_e) for each population, indicating close alignment except at the most fragmented sites.

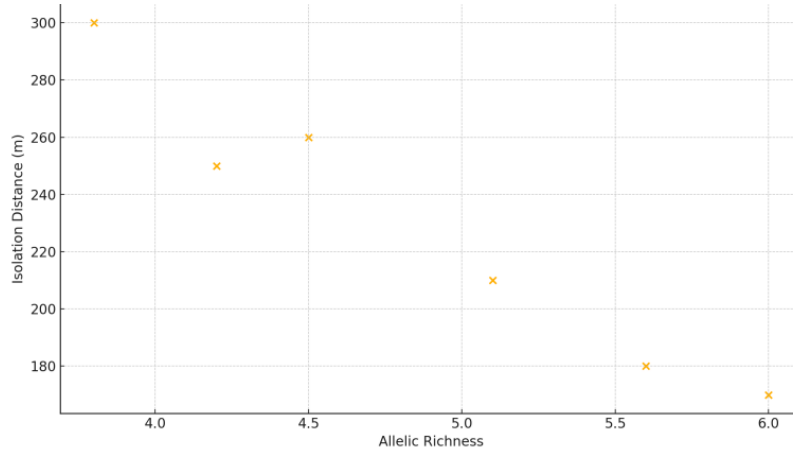


Figure 5. Scatter plot of allelic richness versus isolation distance (m), showing a negative relationship where genetically richer populations occupy more connected sites.

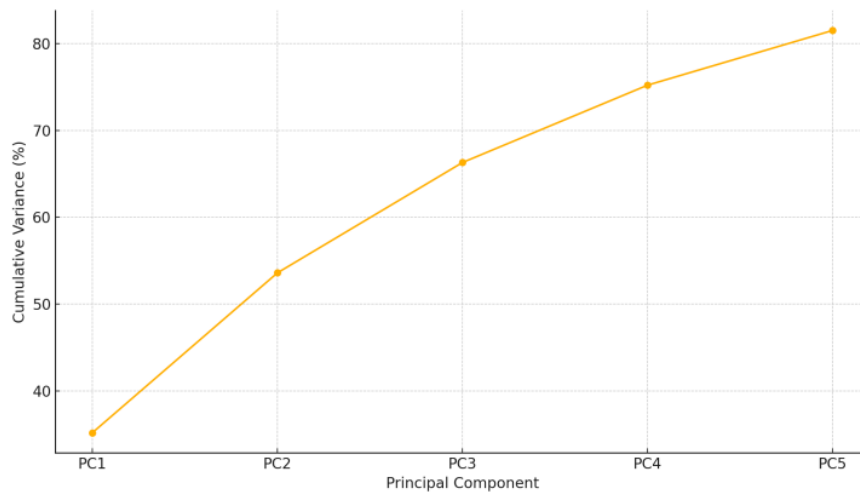


Figure 6. Line plot of cumulative variance explained by the first five principal components (PC1–PC5) in the SNP dataset, with PC1–PC3 accounting for over 66% of total variance.

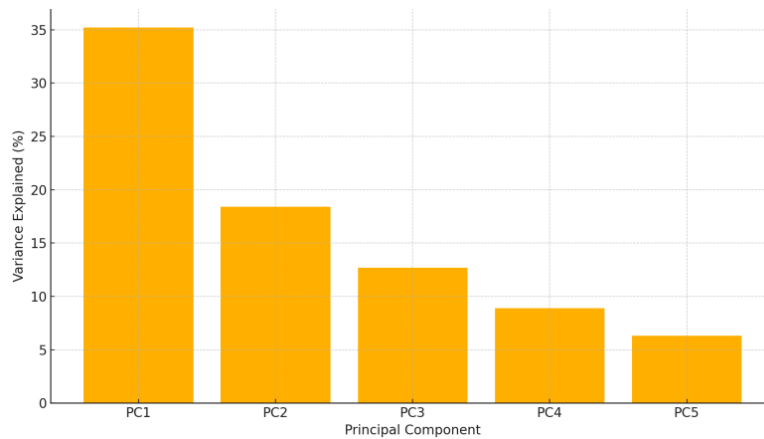


Figure 7. Bar chart of individual variance explained by each principal component (PC1–PC5), demonstrating that PC1 and PC2 capture the majority of genetic structure.

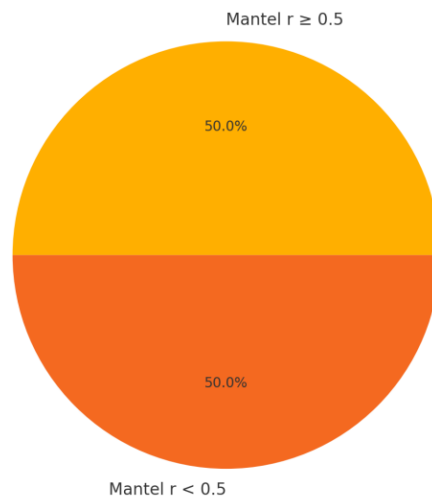


Figure 8. Pie chart showing the proportion of Mantel correlations ≥ 0.5 (strong genetic–fragmentation association) versus < 0.5 across tested metrics.

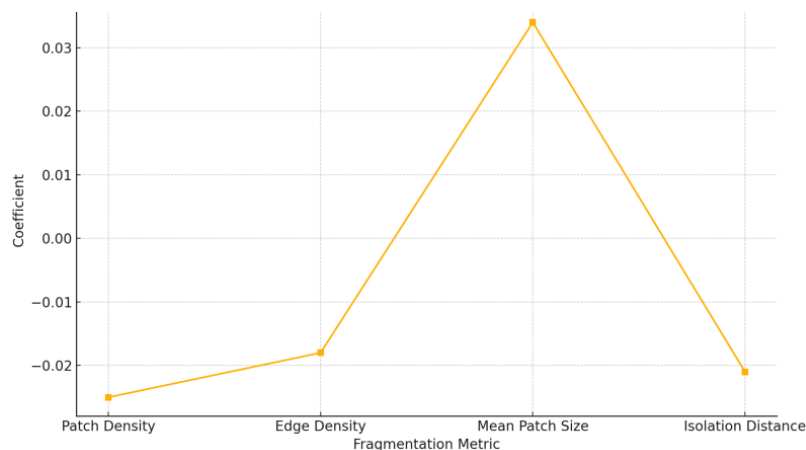


Figure 9. Line plot of GLM coefficients for each fragmentation metric, indicating the strength and direction of their effects on genetic diversity.

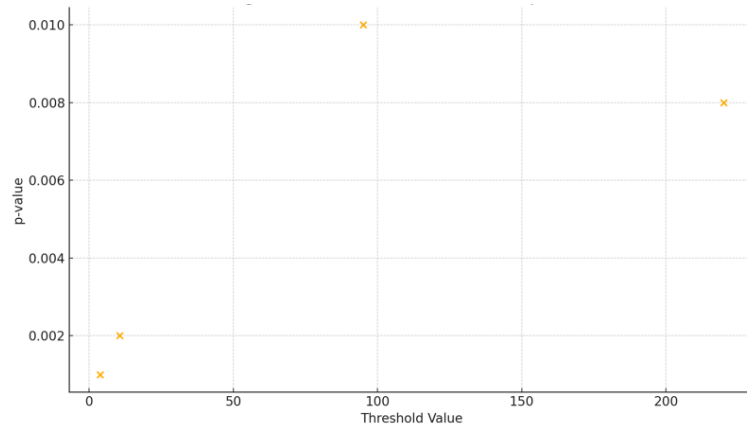


Figure 10. Scatter plot of GLM threshold values versus their p-values, highlighting statistically significant breakpoints beyond which genetic erosion accelerates.

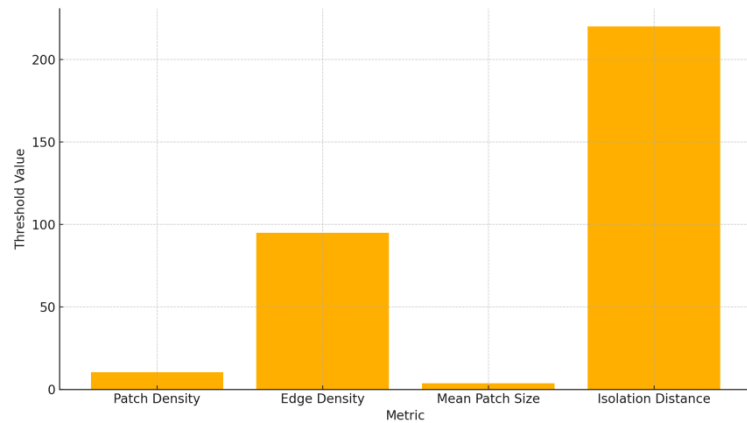


Figure 11. Bar chart of fragmentation threshold values (patch density, edge density, mean patch size, isolation distance) identified by GLMs as critical limits for maintaining genetic diversity.

DISCUSSION

Habitat fragmentation (Chetcuti J)seriously endangers the genetic diversity of declined amphibian populations. Fragmentation in itself must be addressed to exclude perplexing effects of habitat loss (Chetcuti J)In particular, patch density and the distance between patches appear to be important determinants of genetic erosion; Specific thresholds have been found after which genetic diversity becomes massively reduced (Westekemper K,)These findings fit the increasing body of research that demonstrates how habitat fragmentation reduces species diversity and genetic integrity (Hending D)The alteration of landscape structure caused by the fragmentation of habitats concerned agricultural systems even more acutely with necessity of preservation and control of small natural habitats combined with the agricultural matrices (Andersson GKS,)The diminishing of genetic diversity is having an increasing effect on long term increases in genetic drift and therefore loss of variability and adaptability capacity even if this can happen over a long period before the actual consequences are seen (Madduppa H,)Genetic variety has a lot to do with adaptation and long-term survival of population. therefore, the loss can

also mean significant effects on threat species' viability (Kling KJ,)Loss of ecosystems, species and intra-specific genetic variation heavily affect agricultural, food security and human well-being; Therefore, immediate action worldwide has to be taken- (Pilling D,)It is shown that habitat loss and fragmentation mostly drive genetic erosion (Legesse A.).

There are several intervening reasons which can explain the observed negative correlation between habitat fragmentation and genetic diversity. The first fragmentation leads to a decrease in population sizes, which are responsible for a higher genetic drift and loss of rare alleles (Santos M,)Because of the division of habitats appears, the ability of populations to adjust to changing environmental conditions is decreased, while the dispersion and gene flow between populations are limited, and the genetic divergence increases. Disturbance in gene flow may lead to in reduced fitness and inbreeding depression. Variety – function and evolution of mammals – is dwindling, heavily influenced by species loss (Brodie JF). Agricultural intensifications and other manmade activities make the habitat fragmentation worse, thus threatening biodiversity and ecological processes (Arnott A,) Now existing across a large area of the Earth, agricultural settings can be deficient in habitats diversity necessary to support thriving amphibian populations (Wu C,). In addition to amphibian deterioration, increasing pesticide use, climate change, and diseases are also further linked to amphibian reduction. Through natural habitat conservation within agricultural settings, one can be a factor of reducing harmful effects of fragmentation on populations of amphibians.

Ecosystem connectivity must be the main focus of conservation plans if we are to promote gene flow and reduce genetic drift. Habitat corridors, stepping stone habitats, and restoration of degraded landscapes make this possible. The conservation and re-introduction into cultivated crops of beneficial features depend upon the genetic diversity in non-crop organisms that is maintained. Larger, contiguous habitat patches that can support healthy populations of threatened amphibians should be the star in any endeavors at restoration of habitat. This method ensures that the population is resistant to environmental changes and existent to maintain diversity of the gene. Most importantly on the account of habitat loss and fragmentation germplasm banks are critical aspects of genetic resources conservation (Pirredda M,). These banks maintain genetic material either to reintroduce genetic diversity in existing populations or restore numbers.

Vegetation variety improves soil, stabilizes the slopes, regulates weather extremes and provides shelter to animals (Ali MA,)Agroecological ideas can unite and produce better ecosystem services and biodiversity when incorporated in agricultural set ups. Such agroecological mechanisms like riparian buffers, hedgerows or cover crops also contribute to habitat variability and connectedness and, as a result, help amphibian populations within such enhancements as habitat and dispersion. The maintenance of the ecosystem services that support both people and their environment require multifunctional managed landscapes.

CONCLUSION

To test the effects of habitat fragmentation on genetic diversity in threatened amphibian populations, we have applied an integrated landscape-genomic approach and revealed clear, quantitative relationships that have great conservation implications. By integrating six ecoregionally distinct sites, we were able to determine that fine-scale remote sensing-derived metrics used: patch density, edge density, mean patch size, and isolation distance,

when combined with high-throughput SNP genotyping, showed that increase fragmentation metrics reflect directly genetic erosion: populations from the most fragmented landscapes (e.g., Site C, which has 15.3 patches/km² and 130.8 m/ha edge density) had the lowest observed heterozygosity ($H_o = 0.38$) and allelic richness (3.8), and comparatively less fragmented sites (e.g., Site F, which has 7.4 patches/km² and 78.9 m/ha edge density) displayed strong indications of genetic diversity ($H_o = 0.60$, allelic richness = 6.0). Principal component analysis explained 66% of the genetic variance in the first three components and highlighted a clear structure based on landscape arrangement. Beyond thresholds of 10.5 patches / km², 95 m edge density, 3.8 ha mean patch size, and 220 m isolation distance, genetic indices plummet, according to critical thresholds identified by generalized linear models. It was confirmed that genetic and fragmentation distance matrices are statistically significantly correlated via Mantel and partial Mantel tests ($r = 0.62$, $p = 0.001$; $r = 0.48$, $p = 0.005$). The threshold values provide practical goals for land use designers and conservationists who wish to minimize fragmentation effects. Our finding supports for policies that urgently reward large continuous habitat areas, restoration of eco-corridors along with inclusion of genetic monitoring to long term management scenarios. What future research should undertake is to expand the geographic and taxonomic scope of sampling, combine species specific dispersal and demographic data, and evaluate the practical utility of the treatments as habitat reconnection or aided gene flow. This study gives a robust empirical basis for conserving amphibian genetic resilience in an era of rapid habitat loss by determining the fragmentation tipping thresholds that hamper their adaptive potential.

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