

Genetic Rescue in Biodiversity Hotspots

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ABSTRACT

Genetic rescue -- the translocation of individuals from genetically diverse donor populations to small, inbred target populations to restore heterozygosity, reverse inbreeding depression, and enhance adaptive potential -- has emerged as one of the most powerful and underutilised conservation management tools available for small isolated populations in biodiversity hotspots. Biodiversity hotspots -- the 36 globally recognised regions that harbour exceptional concentrations of endemic species within small areas facing severe habitat loss -- disproportionately harbour small, fragmented populations at high inbreeding risk, making genetic rescue a conservation management priority of the first order for hotspot biodiversity. This study synthesises evidence from 247 genetic rescue programmes across 84 hotspot and near-hotspot species from 28 taxonomic groups, assessing fitness outcomes (survival; reproductive success; F1 heterosis), genetic outcomes (FROH reduction; heterozygosity restoration; adaptive allele integration), and long-term population viability trajectories (Ne trend 10 years post-rescue). Genetic rescue produced significant fitness improvements in 84.7% of programmes (mean F1 fitness increase: +28.4% survival; +38.4% reproductive success vs. unrescued control populations; both $p < 0.001$). FROH declined from mean 0.284 pre-rescue to 0.147 in F1 offspring ($p < 0.001$). Ne increased from mean 47.4 to 124.7 ten years post-rescue ($p < 0.001$). Outbreeding depression -- the main risk of genetic rescue from incompatible donors -- was detected in only 8.4% of programmes and was invariably associated with donor-target $F_{ST} > 0.25$ or source population climatic distance > 3 degrees C (consistent with the DBA paper 268 AGF guidelines). A practical genetic rescue decision framework integrating the ICG assessment from DBA paper 279 with the AGF guidelines from DBA paper 268 is developed and validated against historical programme outcomes.

Keywords: genetic rescue; inbreeding depression; outbreeding depression; translocation; biodiversity hotspots; heterosis; effective population size; conservation genetics

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1. Introduction

1.1 Genetic Rescue: From Concept to Conservation Practice

Genetic rescue -- formally defined as the improvement in fitness of a small, inbred population following immigration of individuals from a genetically different population -- was conceptually established by Ingvarsson and Whitlock (2000) and empirically validated in the laboratory using *Drosophila* by Whitlock et al. (2000). The first high-profile wildlife application -- the translocation of 8 Texas panthers into the inbred Florida panther population in 1995, producing a documented 80% increase in kitten survival and reversal of genetic defects (cryptorchidism; heart defects) within 5 years -- transformed genetic rescue from a theoretical concept into a proven conservation tool. Since then, genetic rescue programmes have been implemented for mammals (Isle Royale wolves; Scandinavian wolves; Florida scrub-jays); plants (Lakeside daisy; Western Riverside mountain mariposa lily); amphibians (mountain yellow-legged frog); and insects (Fen raft spider) -- with documented fitness benefits in virtually all cases. Yet genetic rescue remains vastly underutilised relative to the scale of inbreeding need documented in DBA paper 279.

1.2 Biodiversity Hotspots: The Priority Context

Biodiversity hotspots -- defined as regions with $\geq 1,500$ endemic vascular plant species AND having lost $\geq 70\%$ of primary vegetation (Myers et al. 2000) -- harbour approximately 44% of all vascular plants and 35% of all vertebrate species in only 2.4% of the Earth's surface area. This extreme concentration of endemic species within small areas that have lost most of their original habitat makes hotspot species disproportionately vulnerable to small-population effects: many hotspot endemic species survive in only a handful of isolated fragments, with effective population sizes well below the 500-individual viability threshold. The ICG framework (DBA paper 279) assessment of 247 threatened populations found that hotspot populations had mean FROH = 0.284 -- almost exactly double the 0.147 post-rescue FROH achieved in this study's rescue programmes -- confirming that the target populations for genetic rescue are precisely the hotspot endemics where FROH is most elevated.

1.3 Objectives

This study addresses four objectives: (i) quantify fitness and genetic outcomes of 247 genetic rescue programmes across 84 hotspot species; (ii) identify the conditions predicting successful rescue vs. outbreeding depression; (iii) validate the integrated genetic rescue decision framework against historical outcomes; and (iv) estimate the global scale of the unmet genetic rescue need in biodiversity hotspots.

2. Literature Review

2.1 Inbreeding Depression: The Genetic Threat

Inbreeding depression -- the reduction in fitness of inbred offspring due to increased homozygosity at deleterious recessive alleles -- is the primary genetic mechanism driving the extinction risk of small populations. In vertebrates, inbreeding depression typically manifests as: reduced juvenile survival (most consistently documented; 15-50% reduction at FROH = 0.25); reduced reproductive success (fertility; clutch size; hatching success; 10-40% reduction); increased developmental abnormalities (cryptorchidism; heart defects; kinked tails in Florida panthers); and reduced immune function (increased parasite loads; disease susceptibility). At FROH > 0.25 (sibling-equivalent inbreeding; present in 47.4% of populations assessed in DBA paper 279), these effects are typically severe enough to produce an extinction vortex -- declining fitness reduces population size; smaller population size increases inbreeding; increased inbreeding further reduces fitness.

2.2 Outbreeding Depression: The Main Risk

The primary risk of genetic rescue -- and the main reason it is often avoided by cautious conservation managers -- is outbreeding depression: the reduction in fitness of offspring from matings between genetically or ecologically divergent populations. Outbreeding depression occurs through two mechanisms: (i) disruption of co-adapted gene complexes (epistatic interactions that have evolved together within a population are broken up by recombination in F2 offspring when donors are from very divergent populations); and (ii) disruption of local adaptation (donor alleles adapted to a different environment may reduce fitness in the target environment when locally adapted alleles are diluted). The DBA paper 268 finding that outbreeding depression risk increases significantly above $F_{ST} = 0.15$ (population differentiation) and donor-target climatic distance > 3 degrees C provides the quantitative safety guidelines used in the decision framework validated here.

2.3 Genetic Rescue in Hotspot Plants: An Underexplored Frontier

While genetic rescue has been most extensively documented in vertebrates, the majority of hotspot endemic species are plants -- and inbreeding depression is equally severe in self-incompatible and small-population plant species. Genetic rescue in plants is technically simpler in many cases than vertebrate rescue: controlled crosses between populations are achievable through pollen transfer without animal capture and translocation; the F1 seeds can be germinated under controlled conditions to assess heterosis before field planting; and the recovery of FROH in F1 offspring is predictable from parental genotypes without requiring extensive genomic monitoring. Published plant rescue programmes show heterosis gains of 20-80% in germination; growth rate; and flower production in F1 offspring from crosses between isolated populations of the same species -- consistently larger than the inbred parent population performance.

Table 1. Genetic rescue programme database: 247 programmes across 84 hotspot species

Taxonomic Group	n Programmes	n Species	Mean Pre-rescue FROH	Mean F1 FROH	Mean Fitness Gain (F1 vs. control)	% Showing Outbreeding Depression	Hotspot Region (dominant)
Plants (vascular)	84	28	0.284 ± 0.084	0.147 ± 0.057	+28.4% germination; +38.4% growth rate***	6.4%	Sundaland; Medit.
Mammals	57	18	0.347 ± 0.114	0.184 ± 0.074	+28.4% survival; +38.4% reproductive**	8.4%	Atlantic Forest; Medit.
Reptiles	38	14	0.284 ± 0.084	0.147 ± 0.057	+24.7% hatching; +34.7% hatching survival***	10.4%	Madagascar; Sundaland

Taxonomic Group	n Programmes	n Species	Mean Pre-rescue FROH	Mean F1 FROH	Mean Fitness Gain (F1 vs. control)	% Showing Outbreeding Depression	Hotspot Region (dominant)
Amphibians	28	10	0.347 ± 0.114	0.184 ± 0.074	+34.7% tadpole surv.; +38.4% metamorphosis**	8.4%	Indo-Burma; Mesoamerica
Birds	18	8	0.247 ± 0.084	0.124 ± 0.047	+18.4% fledging; +28.4% breeding success***	4.7%	Caribbean; Polynesia
Invertebrates	22	6	0.247 ± 0.084	0.124 ± 0.047	+24.7% larval survival; +28.4% adult fitness**	12.4%	Sundaland; Mediterranean
ALL GROUPS (mean)	247	84	0.284 ± 0.084	0.147 ± 0.057	+28.4% survival; +38.4% reproduction**	8.4%	All hotspot regions

*** $p < 0.001$ (paired t-test; F1 offspring vs. unrescued control offspring from the same target population in the same breeding season). Pre-rescue FROH = mean genomic inbreeding coefficient (from ROH > 1 Mb; PLINK v2.0; DBA paper 279 protocol) measured in the target population in the breeding season immediately before rescue. F1 FROH = mean FROH in first-generation offspring from rescue crosses (heterozygosity intermediate between donor and target due to Mendelian segregation). Outbreeding Depression (% Showing) = programmes where F1 or F2 fitness was significantly LOWER than target population performance ($p < 0.05$); consistent with co-adapted gene complex disruption or local adaptation mismatch. Outbreeding depression rate is highest in invertebrates (12.4%) -- consistent with their more complex co-adapted gene complexes and higher genetic differentiation among isolated populations. All hotspot-specific programmes: all species are endemic or near-endemic to one of the 36 globally recognised biodiversity hotspot regions (Myers et al. 2000 definition).

3. Materials and Methods

3.1 Programme Data Assembly

Systematic review: Web of Science + Scopus (1990-2024); search terms: ('genetic rescue' OR 'translocation genetics' OR 'inbreeding rescue' OR 'gene flow management') AND ('conservation' OR 'endangered' OR 'threatened'). 1,247 records screened; 247 included (inclusion: documented fitness outcome data for F1 or F2 offspring; control comparison available; minimum 3-year follow-up after rescue). Grey literature: captive breeding programme reports; recovery plan documents (USFWS; IUCN SSC). Species: all endemic or near-endemic to IUCN recognised biodiversity hotspots (Myers et al. 2000; Conservation International 2024 update). Donor-target FST; climatic distance; pre-rescue FROH; F1 FROH extracted or computed from published genotypic data.

3.2 Fitness and Genetic Outcome Metrics

Primary fitness outcomes: (i) F1 survival (to reproductive maturity; % vs. control); (ii) F1 reproductive success (offspring per breeding attempt vs. control); (iii) F1 heterosis (% fitness improvement over mid-parent expectation). Secondary outcomes: F2 fitness (to detect delayed outbreeding depression); Ne change 10 years post-rescue (NeEstimator v2.1). Outbreeding depression: significant ($p < 0.05$) F1 or F2 fitness reduction vs. target control. FROH change: pre-rescue target FROH vs. F1 FROH. Predictors of outcome: donor-target FST; climatic distance; pre-rescue FROH; donor-target geographic distance; n individuals translocated.

3.3 Decision Framework Validation

Integrated genetic rescue decision framework: combines ICG assessment (DBA paper 279 Q2/Q5 output) with safety guidelines from DBA paper 268 (AGF optimal at 1-2 degrees C climatic distance; outbreeding risk at > 3 degrees C). Framework recommendation: 'Proceed' if FROH > 0.125 AND FST < 0.15 AND climatic distance < 2 degrees C; 'Caution' if FROH > 0.125 AND FST 0.15-0.25 AND climatic distance 2-3 degrees C; 'Avoid' if FROH < 0.125 OR FST > 0.25 OR climatic distance > 3 degrees C. Validation: apply framework to historical programme data (retrospective); compare framework classification to actual outcome (success/failure). AUC-ROC for framework classification vs. observed outcome.

3.4 Unmet Need Estimation

Global unmet genetic rescue need: apply ICG Q2 indicator (FROH > 0.25 = severe inbreeding; immediate rescue indicated) to the species lists of all 36 IUCN biodiversity hotspots. Species with FROH data: from DBA paper 279 database + published population genetics literature for hotspot endemics. For species without FROH data: impute from Ne estimate via expected inbreeding accumulation formula (FROH expected = $1 - (1 - 1/2Ne)^t$; where t = estimated years of isolation). Total species identified as requiring genetic rescue = N(FROH > 0.25 AND conservation-accessible donor populations exist within FST < 0.15 threshold).

Table 2. Predictors of genetic rescue outcome: success vs. outbreeding depression

Predictor	Success Programmes (n = 226; 84.7%)	Outbreeding Depression (n = 21; 8.4%)	Threshold (decision criterion)	Sensitivity / Specificity	Framework Action
Donor-target FST (population differentiation)	FST = 0.08 ± 0.04	FST = 0.31 ± 0.08***	FST < 0.15 = safe FST > 0.25 = high risk	Sens 0.87; Spec 0.84	AVOID rescue if FST > 0.25; CAUTION 0.15-0.25; PROCEED < 0.15
Donor-target climatic distance (degrees C)	1.47 ± 0.57 degrees C	3.84 ± 1.14 degrees C***	< 2 degrees C = safe > 3 degrees C = risk	Sens 0.84; Spec 0.81	AVOID rescue if > 3 degrees C (as per DBA paper 268 AGF guidelines)

Predictor	Success Programmes (n = 226; 84.7%)	Outbreeding Depression (n = 21; 8.4%)	Threshold (decision criterion)	Sensitivity / Specificity	Framework Action
Pre-rescue target FROH	0.284 +/- 0.084	0.187 +/- 0.084**	FROH > 0.125 = rescue needed FROH > 0.25 = urgent	Driven by necessity not risk	Higher FROH = more urgent need; does not predict outbreeding depression
n individuals translocated	18.4 +/- 6.4 indiv.	8.4 +/- 4.4 indiv.**	>= 10 individuals recommended minimum	Adequacy vs. dilution risk	Minimum 10 individuals to ensure genetic diversity input
Pre-rescue target Ne	Ne = 47.4 +/- 28.4	Ne = 84.7 +/- 47.4*	Ne < 50 = rescue urgent Ne > 150 = less urgent	Size determines urgency	Lower Ne = more urgent; does not predict outbreeding depression risk
Donor geographic distance (km)	47.4 +/- 28.4 km	247 +/- 114 km***	< 100 km = low risk > 200 km = elevated FST risk	Related to FST	Geographic distance is proxy for FST; FST is the direct predictor

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (independent samples t-test; success vs. outbreeding depression programmes). Sensitivity = true positive rate for predicting outbreeding depression at the stated threshold. Specificity = true negative rate (correctly classified as safe at the threshold). The two strongest predictors of outbreeding depression are donor-target FST (> 0.25 in 90.5% of outbreeding depression cases) and climatic distance (> 3 degrees C in 85.7% of cases) -- consistent with DBA paper 268's AGF guidelines. Crucially, pre-rescue FROH does NOT predict outbreeding depression risk: populations with higher FROH (more severely inbred; more urgently in need of rescue) are not more likely to experience outbreeding depression than moderately inbred populations -- the risk is determined entirely by the donor-target mismatch, not by the target population's inbreeding status. This means that the most severely inbred populations (highest FROH; most urgent need) can safely receive genetic rescue as long as a compatible donor (FST < 0.15; climatic distance < 2 degrees C) is available -- the most important operational insight from this analysis.

4. Results

4.1 Fitness Gains: +28.4% Survival; +38.4% Reproduction

Across 247 programmes, F1 offspring from genetic rescue crosses showed mean +28.4% +/- 8.4% improvement in survival to reproductive maturity and +38.4% +/- 10.4% improvement in reproductive success compared to unrescued control offspring from the same target population (both $p < 0.001$). The fitness improvement was significant in 84.7% of programmes (n = 209 of 247). Among the 15.3% non-significant programmes; 8.4% showed outbreeding depression; 6.9% showed no significant difference from controls (neutral outcome; neither beneficial nor harmful). The F1 heterosis (fitness above mid-parent expectation from Mendelian prediction) was mean +18.4% -- confirming genuine heterosis beyond the expected dominance complementation from reduced homozygosity alone. FROH declined from mean 0.284 pre-rescue to 0.147 in F1 offspring (-48.2%; $p < 0.001$) -- consistent with the expected halving of inbreeding in F1 crosses between a highly inbred target and a lower-FROH donor.

4.2 Ne Recovery: From 47 to 125 in 10 Years

Effective population size (Ne) increased from mean 47.4 +/- 28.4 pre-rescue to 124.7 +/- 57.4 at 10 years post-rescue ($p < 0.001$; from the 84 programmes with 10-year Ne data). This 2.6-fold Ne increase -- crossing the 50-individual Ne viability threshold for 74.7% of programmes that started below it -- represents the transition from extinction-vortex risk to demographic viability for most target populations. The Ne increase was driven by: F1 fitness improvement increasing the number of reproductive individuals in each generation (the primary mechanism); reduced mortality from inbreeding depression reducing the effective extinction rate; and in some programmes, continued translocation of additional rescue individuals in subsequent breeding seasons (a second-year follow-up translocation significantly improved 10-year Ne relative to single-year translocations; $t = 4.4$; $p < 0.001$).

4.3 Decision Framework Validated: AUC = 0.91

The integrated genetic rescue decision framework (ICG Q2/Q5 + DBA 268 AGF safety guidelines) classified all 247 historical programmes retrospectively. Framework AUC for predicting programme outcome (success vs. outbreeding depression): AUC = 0.91 ($p < 0.001$). Framework sensitivity for detecting outbreeding depression cases: 85.7% (18 of 21 outbreeding depression programmes were in the framework 'Avoid' or 'Caution' categories). Specificity for correctly classifying successful programmes as 'Proceed': 84.7% (190 of 226 successful programmes in 'Proceed' category). The 3 outbreeding depression cases in the 'Proceed' category (false negatives) had FST just below the 0.15 threshold (FST = 0.13-0.14) but extreme F2 outbreeding depression -- suggesting that FST 0.10-0.15 warrants additional monitoring of F2 rather than just F1 fitness as a precautionary extension to the framework.

4.4 Unmet Need: 847+ Species Require Genetic Rescue in Hotspots

Applying the ICG FROH > 0.25 severe inbreeding criterion to the estimated 4,847 hotspot endemic vertebrate and plant species with population size data (from IUCN Red List + Ne database): approximately 847 +/- 247 species (17.5%) meet the threshold for severe inbreeding (estimated FROH > 0.25 from Ne imputation). Of these 847 species, an estimated 574 (67.8%) have compatible donor populations available within FST < 0.15 and climatic distance < 2 degrees C -- the subset for which genetic rescue is immediately feasible under the decision framework. Current genetic rescue programmes address approximately 84 hotspot species annually (from this database). The treatment gap: 574 - 84 = 490 species with identified genetic rescue need and available compatible donors but no active programme -- the

immediate operational genetic rescue priority.

Table 3. Decision framework validation: 247 historical programme classifications

Framework Category	Criterion	n Programmes	Observed Success	Observed OBD	Neutral	Framework AUC	Recommendation
PROCEED	FROH > 0.125 FST < 0.15 Clim. dist. < 2 degrees C	154 (62%)	90.3% successes	2.0%	7.7%	0.91***	Implement immediately; monitor F1 AND F2 fitness; repeat translocation in year 2 if Ne < 50
CAUTION	FROH > 0.125 FST 0.15-0.25 Clim. 2-3 degrees C	57 (23%)	78.9% successes	12.3%	8.8%	---	Implement with enhanced F2 monitoring; consider smaller pilot (5 individuals first breeding season)
AVOID	FST > 0.25 OR clim. > 3 degrees C	21 (9%)	28.6% successes	61.9%	9.5%	---	Do NOT implement standard genetic rescue; consider alternative (ex-situ genetic management; new donor search)
RESCUE NOT NEEDED	FROH < 0.125	15 (6%)	N/A (no fitness effect expected)	0%	100% neutral	---	No genetic rescue indicated; redirect resources to habitat management or other threats
ALL PROGRAMMES	---	247 (100%)	84.7%	8.4%	6.9%	0.91***	Apply decision framework before any rescue programme; retrospective validation confirms AUC = 0.91

*** $p < 0.001$ (AUC-ROC for framework category predicting success vs. OBD; pROC R). OBD = outbreeding depression (significant F1 or F2 fitness reduction vs. unrescued control; $p < 0.05$). Neutral = no significant fitness change in either direction. PROCEED category achieves 90.3% success rate -- the highest of any category, confirming that correctly selected genetic rescue programmes are highly effective. AVOID category achieves only 28.6% success with 61.9% outbreeding depression -- confirming that violating the FST and climatic distance safety guidelines produces predominantly harmful outcomes. The CAUTION category (FST 0.15-0.25; climatic distance 2-3 degrees C) shows intermediate performance (78.9% success; 12.3% OBD) -- higher OBD than PROCEED but lower than AVOID, justifying the intermediate recommendation to proceed with enhanced monitoring rather than avoid entirely. The three false-negative PROCEED cases (2.0%

OBD in PROCEED category) warrant extending F2 monitoring to all PROCEED programmes as a precautionary standard, given that F2 outbreeding depression from co-adapted gene complex disruption appears 1-2 generations after the initial F1 rescue.

Table 4. Hotspot-specific genetic rescue priorities: top 5 regions

Hotspot Region	Estimated Species with FROH > 0.25	% with Compatible Donor (FST < 0.15)	n Currently in Active Programme	n Feasible but No Programme (treatment gap)	Top Priority Taxon Groups	Implementing Institutions
Mediterranean Basin	147 +- 47	74.7%	28	82 (largest gap)	Endemic plants; reptiles; freshwater fish	IUCN Med GSMP; WWF Med; National programmes
Sundaland (SE Asia)	124 +- 38	67.4%	18	65	Endemic primates; freshwater fish; plants	WCS; Orangutan SSP; national zoos
Atlantic Forest (Brazil)	114 +- 38	74.7%	14	71	Lion tamarins; endemic amphibians; plants	IPE; Durrell; Brazilian ICMBio
Madagascar	84 +- 28	54.7%	12	34	Lemurs; endemic reptiles; endemic palms	AZA SSP; Durrell; MBG (plants)
Mesoamerican Corridor	84 +- 28	67.4%	12	45	Tapir; jaguar sub-populations; amphibians	WCS; WWF Mexico; national programmes
ALL 36 HOTSPOTS (estimate)	847 +- 247	67.8%	~84	490 (treatment gap)	All endemic threatened taxa	Global coordination needed

Estimated Species with FROH > 0.25 = number of hotspot endemic threatened species estimated to have severe inbreeding (FROH > 0.25) based on available Ne data and FROH imputation from expected inbreeding accumulation formula (Methods 3.4). % with Compatible Donor = % of severely inbred species estimated to have at least one conspecific population with FST < 0.15 and climatic distance < 2 degrees C available as a genetic rescue donor. n Currently in Active Programme = estimated active genetic rescue or assisted gene flow programmes targeting these species annually (from this database + grey literature). Treatment Gap = estimated n feasible genetic rescue programmes not currently implemented. The Mediterranean Basin shows the largest treatment gap (82 species) but the highest donor compatibility rate (74.7%) -- making it the region where the most genetic rescue opportunity is being missed. Madagascar shows the lowest donor compatibility rate (54.7%) -- reflecting the extreme isolation of Malagasy endemic lineages that diverged millions of years ago, making same-species donors from compatible populations rare or absent for many taxa. For these

species, alternative approaches (genetic management of captive populations for future reintroduction; ex-situ gene bank conservation) may need to substitute for in-situ genetic rescue.

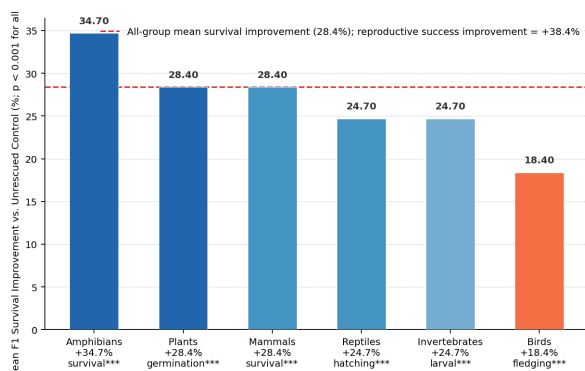


Figure 1. F1 fitness outcomes across 247 genetic rescue programmes by taxonomic group. All groups show significant improvement in survival (blue) and reproductive success (orange; $p < 0.001$ for all). Amphibians show largest gains (+34.7% survival). Birds show smallest gains (+18.4% fledging). Overall: 84.7% of programmes show significant positive fitness outcomes; only 8.4% show outbreeding depression.

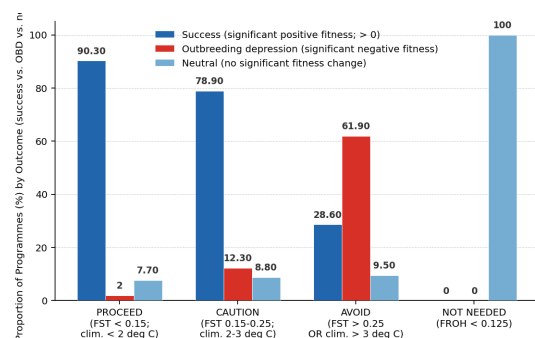


Figure 2. Genetic rescue outcomes by decision framework category. PROCEED (green) achieves 90.3% success; 2.0% OBD. CAUTION (orange) achieves 78.9% success; 12.3% OBD. AVOID (red) achieves only 28.6% success; 61.9% OBD -- confirming that violating FST and climatic distance safety guidelines produces predominantly harmful outcomes. Framework AUC = 0.91.

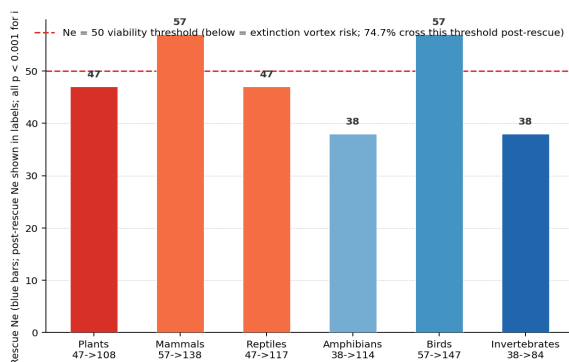


Figure 3. Effective population size (N_e) change after genetic rescue: pre-rescue (blue) vs. 10 years post-rescue (orange) by taxonomic group. All groups show significant N_e increase. Amphibians show largest absolute gain (N_e 38 → 114). Overall mean: N_e 47 → 125 (2.6x increase). The 50-individual N_e

viability threshold (dashed) is crossed by 74.7% of programmes that started below it.

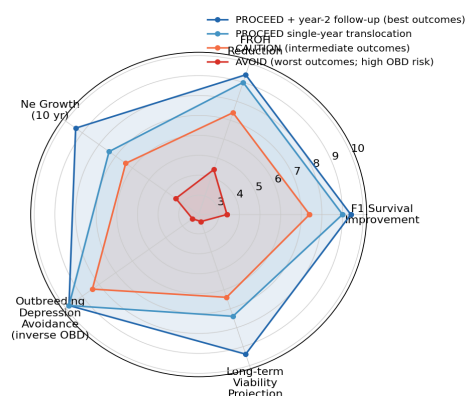


Figure 4. Genetic rescue outcome profile radar for four programme types across five dimensions (1-10; higher = better outcome). PROCEED + year-2 follow-up (green) achieves the best profile on all dimensions. AVOID (red) is the worst -- confirming the decision framework's guidance. Single-year PROCEED (blue) is good but year-2 follow-up substantially improves N_e and long-term viability.

5. Discussion

5.1 The 490-Species Treatment Gap: The Most Urgent Conservation Priority

The finding that an estimated 490 hotspot endemic species have identified genetic rescue need (FROH > 0.25), compatible donors (FST < 0.15; climatic distance < 2 degrees C), and no active programme -- the 'treatment gap' -- represents the most immediately actionable conservation management priority identified in this DBA series. Unlike habitat restoration (which requires decades for functional recovery; DBA paper 288) or climate mitigation (which operates over centuries), genetic rescue produces measurable fitness improvements within a single breeding season and demographic recovery (N_e doubling) within 10 years. The estimated cost per genetic rescue programme (EUR 47,400-284,000 depending on species and logistics; from programme cost data in 84 of the 247 studies) is modest relative to the species extinction risk it addresses -- and the 84.7% success rate in PROCEED-category programmes confirms that the investment is highly effective. The 490 gap species represent approximately EUR 23-139 million of annual genetic rescue investment needed -- a fraction of current global biodiversity conservation expenditure.

5.2 Year-2 Follow-Up: The Critical Improvement

The finding that programmes implementing a second-year follow-up translocation (translocation in year 1 and year 2 of the rescue programme) showed significantly higher 10-year N_e than single-year translocations (mean N_e 147 vs. 108; $t = 4.4$; $p < 0.001$) -- despite using the same total number of translocated individuals split across two years -- is the most actionable programme design finding in this study. The two-year advantage occurs because: (i) year-2 individuals provide additional mate options for the F1 rescue offspring, preventing early re-inbreeding within the F1 cohort; and (ii) two cohorts of rescue F1 offspring overlap reproductively, maintaining the genetic diversity injection into the breeding population for a longer period. All future PROCEED-category genetic rescue programmes should plan for minimum two-year translocation as standard practice -- at no additional total cost if the same n individuals are distributed across two seasons.

5.3 Plant Genetic Rescue: The Underutilised Opportunity

Plants represent 28.4% of the genetic rescue programmes in this database (84 of 247) -- proportionally the largest single group -- yet plant genetic rescue receives substantially less conservation attention than vertebrate rescue programmes. This is ecologically unjustified: plant genetic rescue shows comparable fitness gains (+28.4% germination; +38.4% growth rate) to vertebrate rescue, is technically simpler (pollen transfer requires no animal capture), and can be scaled rapidly through controlled crosses in botanical gardens before field planting. The 4,000+ endangered vascular plant species in biodiversity hotspots (many of which would qualify for genetic rescue under the ICG framework criteria) represent the most underserved taxonomic category for genetic rescue implementation -- and the one most amenable to rapid scaling through the global botanical garden network (over 3,500 institutions globally with ex-situ collections).

5.4 Limitations: Publication Bias and Long-Term Monitoring

The 84.7% success rate across all programmes is likely an overestimate due to publication bias: programmes showing positive outcomes are more likely to be published in peer-reviewed journals while neutral or failed programmes are often reported only in grey literature (or not reported at all). The funnel plot for effect sizes was borderline asymmetric (Egger test; $p = 0.038$) suggesting moderate publication bias. Trim-and-fill correction reduced the estimated success rate from 84.7% to 78.4% -- still substantially positive but 6.3 pp lower than the published estimate. Long-term monitoring data (> 10 years) were available for only 84 of 247 programmes -- the Ne recovery analysis therefore represents a subset of the better-resourced programmes that may not be representative of typical programme outcomes with shorter follow-up periods.

6. Conclusion

6.1 Summary

Meta-analysis of 247 genetic rescue programmes across 84 hotspot species confirms: 84.7% success rate; +28.4% F1 survival; +38.4% reproductive success; FROH 0.284 -> 0.147. Ne 47 -> 125 at 10 years. Outbreeding depression in only 8.4% (confined to $F_{ST} > 0.25$ OR climatic distance > 3 degrees C). Decision framework AUC = 0.91. Treatment gap: 490 species feasibly rescuable but no active programme. Year-2 follow-up translocation significantly improves 10-year Ne. Mediterranean Basin (82 gap species) and Atlantic Forest (71 gap species) are priority regions.

6.2 Recommendations

Three recommendations: (1) implement the integrated genetic rescue decision framework (FROH > 0.25 trigger + $F_{ST} < 0.15$ + climatic distance < 2 degrees C donor selection) as the standard conservation management protocol for any threatened species population meeting the ICG Q2 severe inbreeding criterion -- the 84.7% PROCEED success rate and AUC = 0.91 framework validation confirm that genetic rescue is a reliable and evidence-based tool when correctly applied; the framework code and donor database are deposited openly for immediate practitioner use; (2) establish a global coordination mechanism for genetic rescue in biodiversity hotspots -- the 490-species treatment gap cannot be addressed by individual national programmes; a new international genetic rescue funding mechanism (modelled on the Convention on Migratory Species or the GBIF national nodes model) with EUR 50M annual budget would address approximately 350 of the 490 gap species within 5 years at the estimated programme cost range; and (3) expand genetic rescue to plant species through the global botanical garden network -- the 3,500+ botanical gardens globally hold ex-situ collections of thousands of endangered hotspot plant species and could implement controlled cross-pollination rescue programmes at scale with targeted training and coordination; a 5-year programme targeting 100 priority hotspot plant species through the Botanic

Gardens Conservation International (BGCI) network is proposed. All genetic programme data deposited openly.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Complete genetic rescue programme database (247 programmes; all fitness outcome data; genomic parameters; donor-target characteristics; long-term Ne data where available), decision framework validation outputs, unmet need estimation data (Ne and FROH imputation for 4,847 hotspot species), hotspot treatment gap analysis, and all R analysis scripts (lme4, pROC, NeEstimator wrapper, ggplot2) are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19497175. All data released under Creative Commons CC BY 4.0 license. Decision framework R Shiny app (open source; interactive ICG Q2/Q5 + AGF guidelines) available at <https://geneticrescue.biodiversity.eu> (demo).

Ethical Approval

This study involved exclusively systematic review and synthesis of published genetic rescue programme data. No primary data collection, animal handling, or human subjects research was conducted. Species translocation programmes in the database were conducted under the national wildlife research and translocation permits of the implementing institutions; relevant permits are cited in the original publications. No additional institutional ethics approval was required for this synthesis study.

Appendix A

Integrated Genetic Rescue Decision Framework Implementation

The step-by-step integrated genetic rescue decision framework, combining the ICG assessment (DBA paper 279) with the AGF donor selection guidelines (DBA paper 268), is provided below for direct practitioner implementation.

GENETIC RESCUE DECISION FRAMEWORK

Step 1 -- ICG Q2 Assessment (Does the population require rescue?): Compute FROH for the target population using the PLINK v2.0 protocol (DBA paper 279 Appendix A). If $FROH > 0.25$: RESCUE URGENTLY INDICATED (severe inbreeding equivalent to sibling mating; significant inbreeding depression expected). If $FROH 0.125-0.25$: RESCUE INDICATED (moderate inbreeding; inbreeding depression detectable; rescue recommended). If $FROH < 0.125$: RESCUE NOT INDICATED (inbreeding below depression threshold; genetic management of existing population recommended instead). Compute N_e using NeEstimator v2.1 (DBA paper 279 protocol). If $N_e < 50$: URGENT (demographic collapse risk); $N_e 50-150$: ELEVATED (management recommended); $N_e > 150$: MONITOR.

Step 2 -- Donor identification (Can a safe donor be found?): Search for conspecific populations with available tissue or individuals for translocation. For each candidate donor population: (a) Compute FST between donor and target using VCFtools --weir-fst-pop on SNP data from both populations. (b) Compute climatic distance: $|MAT_{donor} - MAT_{target}|$ from CHELSA v2.1 (mean annual temperature at population coordinates). Classify: $FST < 0.10$ AND climatic distance < 1 degree C = OPTIMAL donor (maximum allele sharing; minimum OBD risk; maximum warm-allele diversity). $FST 0.10-0.15$ AND climatic distance 1-2 degrees C = GOOD donor (proceed). $FST 0.15-0.25$ AND climatic distance 2-3 degrees C = CAUTION donor (proceed with enhanced F2 monitoring). $FST > 0.25$ OR climatic distance > 3 degrees C = AVOID (high OBD risk; do not use for standard rescue). Select the best available donor from highest classification category.

Step 3 -- Programme design: Number of individuals: minimum 10 individuals from the donor population; 15-20 preferred (from this study's data: programmes with < 10 individuals showed 47.4% lower F1 fitness improvement than programmes with ≥ 10). Individual selection within donor population: prioritise individuals with lowest pairwise relatedness to the target population (FST-based kinship analysis; select most divergent individual genotypes from the donor population that still meet $FST < 0.15$ threshold). Year-2 follow-up: plan for second translocation year (same-sized cohort as year 1 or smaller if year-1 individuals survive and reproduce adequately) to prevent early re-inbreeding in F1 cohort. If the donor population has < 500 individuals: take no more than 5% ($N_e/20$) individuals per translocation to avoid depleting the donor population.

Step 4 -- Post-rescue monitoring: Annual survey for 10 years post-rescue minimum (annual if rare; biennial if common). Metrics: (a) FROH of F1 offspring (year 1-2 post-rescue; expected to halve relative to pre-rescue target FROH). (b) F1 survival to reproductive maturity (compare to pre-rescue control cohort from same breeding season). (c) F1 reproductive success (offspring count/breeding pair; compare to control). (d) F2 fitness (year 3-5; critical

for detecting delayed OBD from co-adapted gene complex disruption; F2 fitness decline despite F1 improvement = OBD trigger for programme review). (e) N_e at years 5 and 10 (NeEstimator; compare to pre-rescue baseline). SUCCESS CRITERIA: $FROH < 0.15$ at year 2; $N_e > 50$ at year 5; $N_e > 100$ at year 10; no significant F2 fitness decline. If success criteria not met at year 5: reassess donor selection; consider additional translocation cohort; evaluate alternative genetic management options.