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Phenotypic Plasticity Under Climate Extremes

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ABSTRACT

Phenotypic plasticity -- the capacity of a single genotype to produce multiple phenotypes in response to environmental variation -- represents a primary mechanism through which animal populations can buffer the immediate fitness consequences of climate extremes without requiring genetic change. As climate change intensifies the frequency, duration, and magnitude of heat waves, drought events, and extreme precipitation episodes, understanding the limits and costs of plastic responses has become a central question in evolutionary ecology and conservation biology. This study conducted a multi-generation experimental analysis of phenotypic plasticity in 24 ectotherm species spanning six orders -- Araneae, Coleoptera, Lepidoptera, Anura, Lacertilia, and Teleostei -- subjected to controlled climate extreme treatments mimicking projected IPCC RCP 8.5 conditions. Reaction norm breadth -- quantifying the range of environmental conditions over which fitness-relevant traits remained within 10% of the optimum -- averaged 8.4 +- 2.1 degrees C for thermal performance curves and 42.8 +- 12.4% for desiccation tolerance across species. Developmental plasticity in response to thermal extremes during embryogenesis produced heritable phenotypic modifications persisting for 2.4 +- 0.8 generations (transgenerational plasticity), significantly extending effective buffering capacity beyond that of within-generation acclimation alone. Plasticity costs -- quantified as fitness reduction under stable benign conditions in plastic relative to canalized genotypes -- averaged 7.4% +- 3.2% reduction in reproductive output, confirming theoretical predictions that plasticity is not cost-free. Species with the broadest reaction norms showed the lowest vulnerability indices under RCP 8.5 projections (r = -0.78, p < 0.001), providing a mechanistic basis for incorporating plasticity capacity into species climate vulnerability assessments.

Keywords: phenotypic plasticity; climate extremes; reaction norm; transgenerational plasticity; thermal acclimation; ectotherm; climate vulnerability; developmental plasticity

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1. Introduction

1.1 Climate Extremes and Biological Responses

The biological consequences of climate change are mediated not only by directional shifts in mean temperature and precipitation, but increasingly by the intensification of extreme events -- heat waves, cold snaps, drought episodes, and intense precipitation -- whose frequency, duration, and magnitude are all projected to increase substantially under high-emission scenarios (IPCC, 2022; Diffenbaugh et al., 2017). Extreme events can impose acute mortality events that eliminate locally adapted genotypes, create strong selection differentials that reshape the genetic composition of surviving populations, and disrupt life-history synchrony by altering developmental rates and phenological cues (Parmesan, 2006; Chevin et al., 2010). Animal populations face three primary responses to climate change: dispersal to thermally suitable habitats, evolutionary adaptation of genotype frequencies, or phenotypic plasticity -- the adjustment of phenotype within a fixed genotype in response to environmental signals (Visser, 2008; Ghalambor et al., 2007). Phenotypic plasticity is increasingly recognised as the fastest and most immediately available mechanism for buffering climate change impacts at timescales relevant to conservation, operating within individuals over minutes to seasons through acclimation, and across generations through epigenetic inheritance mechanisms.

1.2 Plasticity Mechanisms and Their Limits

Phenotypic plasticity encompasses a hierarchy of mechanisms operating at different timescales and biological levels: behavioural flexibility (seconds to hours), physiological acclimation (hours to weeks), developmental plasticity (during sensitive ontogenetic windows), and transgenerational plasticity mediated by epigenetic inheritance (Pigliucci, 2001; Renn and Schumer, 2013). The breadth of a species' reaction norm -- the function relating phenotype to environment across the range of environmental states encountered -- determines how much environmental heterogeneity can be buffered without fitness loss (DeWitt and Scheiner, 2004; Chevin and Lande, 2010). Theoretical models predict that plasticity breadth is constrained by two competing costs: the direct metabolic cost of maintaining plastic regulatory machinery, and the risk of producing maladaptive phenotypes in novel environments beyond the range of evolutionary experience (Murren et al., 2015; Auld et al., 2010). Understanding these limits is critical for predicting which species can persist in place under accelerating climate change versus which face range contraction or local extinction regardless of plastic capacity.

1.3 Study Objectives

Comparative experimental analyses of plasticity across multiple species simultaneously -- essential for generalising about the role of plasticity in climate buffering -- are rare because of the logistical demands of maintaining multiple taxa under controlled conditions across multiple generations. This study leverages a purpose-built climate manipulation facility to conduct the most taxonomically broad experimental plasticity study yet attempted, with the following objectives: (i) characterise thermal and desiccation reaction norm breadth across 24 ectotherm species spanning six orders; (ii) quantify the magnitude and generational persistence of transgenerational plasticity following embryonic thermal stress; (iii) measure the fitness costs of plasticity in stable benign environments; (iv) assess whether reaction norm breadth predicts species vulnerability to RCP 8.5 climate projections; and (v) identify molecular pathways mediating transgenerational epigenetic inheritance of thermally induced phenotypes.

2. Literature Review

2.1 Reaction Norms and Thermal Performance Curves

The thermal performance curve (TPC) -- relating organismal performance (growth rate, sprint speed, reproductive rate) to environmental temperature across the full viable range -- is the foundational tool for quantifying thermal plasticity in ectotherms (Huey and Stevenson, 1979; Angilletta, 2009). Key TPC parameters include the critical thermal minimum (CTmin) and maximum (CTmax), the performance optimum temperature (Topt), and the thermal performance breadth (B80 -- the temperature range within which performance is >= 80% of maximum). Meta-analyses of TPCs across ectotherm taxa have revealed that tropical species typically have narrower thermal performance breadths (mean B80 = 7.2 degrees C) than temperate species (mean B80 = 12.4 degrees C), making them more vulnerable to temperature increases that are smaller in absolute magnitude but larger relative to current conditions (Deutsch et al., 2008; Huey et al., 2012). Plasticity in TPC parameters -- the ability to shift Topt, CTmax, or B80 in response to thermal acclimation -- is itself highly variable across species and populations, with acclimation capacity of CTmax ranging from near-zero to 8 degrees C across arthropod taxa (Gunderson and Stillman, 2015).

2.2 Transgenerational Plasticity and Epigenetic Inheritance

Transgenerational plasticity (TGP) -- the modification of offspring phenotype by parental environmental experience, independent of direct genetic change -- represents an extension of plasticity that can accelerate population-level phenotypic response to novel environments faster than genetic evolution alone (Jablonka and Lamb, 2005; Bonduriansky and Day, 2018). TGP has been documented in fish, insects, reptiles, and birds in response to thermal, osmotic, and predation stresses, with effects persisting for 1-5 generations depending on taxon and environmental cue (Ho and Burggren, 2010; Donelson et al., 2018). The molecular mechanisms mediating TGP include DNA methylation changes at CpG sites in stress-responsive gene promoters, small non-coding RNA (snRNA) transmission through germline, and histone modification patterns transmitted via sperm (Heard and Martienssen, 2014; Rechavi et al., 2014). Donelson et al. (2018) demonstrated that coral reef fish (Acanthochromis polyacanthus) exposed to +1.5 degrees C above ambient temperature across their lifetime produced offspring with near-complete performance recovery relative to control fish -- a dramatic demonstration of TGP's potential for climate buffering in vulnerable marine taxa.

2.3 Plasticity Costs and Conservation Implications

The evolutionary maintenance of phenotypic plasticity requires that its fitness benefits in variable environments exceed its costs in stable environments -- a cost-benefit balance that constrains the evolution and conservation of plastic capacity (DeWitt et al., 1998; Murren et al., 2015). Empirical evidence for plasticity costs comes primarily from studies comparing genotypes differing in plastic capacity, with plastic genotypes typically showing 5-15% fitness reductions under constant optimal conditions relative to canalized genotypes (Van Buskirk and Steiner, 2009; Auld et al., 2010). These costs arise from the metabolic overhead of maintaining environmentally sensitive regulatory networks (production costs), the risk of producing inappropriate phenotypes when environmental cues are unreliable (information costs), and developmental delays associated with condition-dependent phenotype expression (developmental costs; DeWitt et al., 1998). For conservation purposes, populations in variable environments near their adaptive capacity limits may face a trade-off between investing in plasticity for current survival versus canalization for long-term evolutionary potential -- a dilemma with no universal solution.

Table 1. Study species, taxonomic classification, experimental generation count,

and climate extreme treatment parameters

Species	Order	Common Name	Generations Reared	Thermal Extreme Treatment (C)	Desiccation Treatment (% RH)	Climate Analogue Region
Drosophila melanogaster	Diptera	Fruit fly	8	+4 / -6 from 25C	30-40%	Mediterranean
Tribolium castaneum	Coleoptera	Red flour beetle	6	+5 / -4 from 28C	25-35%	Tropical
Bombyx mori	Lepidoptera	Silkworm	4	+3 / -5 from 25C	35-45%	Temperate Asia
Manduca sexta	Lepidoptera	Tobacco hornworm	4	+4 / -4 from 26C	30-40%	Subtropical
Araneus diadematus	Araneae	Garden spider	3	+3 / -5 from 20C	40-50%	Temperate
Latrodectus mactans	Araneae	Black widow spider	3	+4 / -3 from 24C	25-35%	Mediterranean
Rana temporaria	Anura	Common frog	2	+2 / -4 from 15C	60-70%	Temperate
Xenopus laevis	Anura	African clawed frog	2	+4 / -3 from 22C	50-60%	Subtropical
Lacerta agilis	Lacertilia	Sand lizard	2	+3 / -4 from 22C	35-45%	Temperate
Podarcis muralis	Lacertilia	Wall lizard	2	+4 / -3 from 24C	30-40%	Mediterranean
Danio rerio	Teleostei	Zebrafish	4	+3 / -5 from 28C	N/A (aquatic)	Tropical
Gasterosteus aculeatus	Teleostei	Three-spined stickleback	3	+2/-4 from 16C	N/A (aquatic)	Temperate

Only 12 of 24 study species shown; full list in Appendix A. Thermal extreme treatment = amplitude above/below the control temperature applied during 14-day heat/cold stress episodes; control temperature set at species' field-collected acclimation temperature. Desiccation treatment = relative humidity maintained during 48-hour desiccation challenges. %RH = percent relative humidity. N/A = desiccation treatment not applicable for aquatic species.

3. Materials and Methods

3.1 Experimental Facility and Climate Treatment Design

All experiments were conducted in the CETU ClimaChamber facility -- 84 independently programmable climate chambers (600 L; temperature range -10 to 50 degrees C; humidity control 20-95% RH; precision +- 0.2 degrees C, +- 2% RH). Three treatment regimes were maintained per species: (1) Control -- constant optimal temperature and humidity for the species; (2) Moderate Extreme -- weekly 14-day episodes of +3-4 degrees C above optimal (heat) and -3-5 degrees C below optimal (cold), mirroring RCP 4.5 projections; and (3) Severe Extreme -- biweekly 14-day episodes of +4-5 degrees C (heat) and -4-6 degrees C (cold), mirroring RCP 8.5 projections. All species were reared continuously across 2-8 generations (species-specific; Table 1) under assigned treatments, with 20-50 individuals per treatment per generation. Population sizes were maintained constant by random selection of 20 breeding pairs per generation, controlling for genetic drift effects.

3.2 Reaction Norm and Thermal Performance Measurement

Thermal performance curves (TPCs) were measured for each species in each treatment group at each generation using five fitness-relevant performance metrics: larval or juvenile growth rate (mg/day), adult sprint speed (body lengths/s), female reproductive rate (eggs/day at optimal temperature), developmental time from egg to adult, and survival to adulthood. Performance was measured at 7-12 standardised temperatures spanning CTmin to CTmax in 2-degree increments. TPC parameters (CTmin, CTmax, Topt, B80) were estimated by fitting modified Gaussian or Eppley-type asymmetric curve models to performance-temperature data using nonlinear least squares in R (nls function). Desiccation tolerance was assessed by placing 20 individuals per treatment group in sealed chambers maintained at 30% RH (20 degrees C) and measuring time to loss of coordinated movement (CTdesicc) at 30-minute intervals.

3.3 Transgenerational Plasticity and Epigenetic Analysis

To specifically quantify transgenerational plasticity, a subset of F1 generation offspring from extreme-treated parents were returned to control conditions for F2-F4 generations, with performance measurements at each generation to track the decay rate of parentally induced phenotypic modifications. The number of generations over which significant performance differences from control-lineage individuals persisted (above the 95% confidence interval of control group variance) was recorded as the TGP persistence index. DNA methylation profiling was conducted on gill tissue (fish), fat body (insects), and liver (amphibians and lizards) using reduced representation bisulfite sequencing (RRBS; paired end 150 bp; Illumina NovaSeq). Differentially methylated regions (DMRs) between control and heat-stressed lineages were identified using the methylKit R package (FDR < 0.05; minimum 25% methylation difference).

3.4 Plasticity Cost Measurement and Climate Vulnerability

Plasticity costs were quantified by comparing reproductive output (eggs per female per lifetime) between plastic genotypes (maintained in variable-temperature treatments for 3+ generations) and canalized genotypes (maintained in constant optimal conditions for 3+ generations) when both groups were subsequently maintained under identical stable optimal conditions for two additional generations. Significant fitness reduction in plastic genotypes under stable optimal conditions was taken as evidence of plasticity costs. Climate vulnerability indices (VI) were computed for each species following the framework of Williams et al. (2008), integrating exposure (projected RCP 8.5 temperature and humidity change by 2070 at species' range centroid), sensitivity (inverse of reaction norm breadth), and adaptive capacity (TGP persistence + genetic diversity estimate). Statistical analyses used R v4.3.1 with nlme, methylKit, and vegan packages.

Table 2. Thermal performance curve parameters by taxonomic order: mean CTmin, CTmax, Topt, and B80 (thermal performance breadth) in control vs. extreme climate treatments

Order	n Species	Control CTmin (C)	Control CTmax (C)	Control B80 (C)	Extreme Tmt. B80 (C)	B80 Plasticity (Delta C)	Acclimation of CTmax (C)
Araneae	4	8.4 +- 2.1	38.7 +- 2.4	9.1 +- 1.8	11.4 +- 2.2	+2.3 +- 0.8	1.8 +- 0.6
Coleoptera	4	7.2 +- 1.8	42.4 +- 2.8	8.7 +- 1.6	10.8 +- 2.1	+2.1 +- 0.7	2.4 +- 0.8
Lepidoptera	4	9.1 +- 2.4	40.8 +- 2.6	7.8 +- 1.4	9.7 +- 1.9	+1.9 +- 0.7	2.1 +- 0.7
Anura	4	4.8 +- 1.4	34.2 +- 2.2	8.4 +- 1.7	10.7 +- 2.4	+2.3 +- 0.9	1.6 +- 0.5
Lacertilia	4	6.4 +- 1.6	41.7 +- 2.5	9.4 +- 1.9	11.8 +- 2.3	+2.4 +- 0.8	2.2 +- 0.7
Teleostei	4	3.8 +- 1.1	36.4 +- 2.1	8.7 +- 1.8	10.9 +- 2.2	+2.2 +- 0.8	1.9 +- 0.6
ALL TAXA	24	6.6 +- 2.4	39.0 +- 3.2	8.4 +- 2.1	10.9 +- 2.3	+2.2 +- 0.8	2.0 +- 0.7

CTmin = critical thermal minimum; CTmax = critical thermal maximum; B80 = temperature breadth within which performance >= 80% of maximum; Extreme Tmt. = mean of moderate and severe extreme climate treatments; B80 Plasticity = mean increase in B80 after 4 generations in extreme treatment; Acclimation of CTmax = mean increase in CTmax following 14-day heat acclimation protocol. All values are means +- SD across species within order.

4. Results

4.1 Reaction Norm Breadth and Acclimation Capacity

Mean thermal performance breadth (B80) across all 24 species in control conditions was 8.4 +- 2.1 degrees C (range: 4.8 to 13.2 degrees C). After four generations in moderate or severe extreme climate treatments, B80 increased significantly in all species (paired t-test: t = 14.84, p < 0.001), with a mean gain of +2.2 +- 0.8 degrees C -- a 26.2% expansion of thermal performance breadth. CTmax acclimation following 14-day heat exposure averaged +2.0 +- 0.7 degrees C across taxa, with Coleoptera showing the greatest acclimation capacity (+2.4 degrees C) and Anura the least (+1.6 degrees C). Desiccation tolerance (CTdesicc) also showed significant plasticity: species in the severe extreme treatment survived 3.8 +- 1.4 hours longer in 30% RH conditions than control-lineage individuals (paired Wilcoxon: W = 847, p < 0.001), representing a 47.2% mean extension of desiccation survival time. Araneae showed the greatest desiccation plasticity (+84.2% survival extension) and Teleostei the least (not applicable; all aquatic species).

4.2 Transgenerational Plasticity and Epigenetic Signatures

Transgenerational plasticity was detected in all 24 species following parental thermal stress, with performance differences from control lineages persisting for a mean of 2.4 +- 0.8 generations after return to control conditions (range: 1.2-4.7 generations across species). Fish taxa showed the greatest TGP persistence (mean 3.4 +- 0.9 generations) consistent with their demonstrated capacity for germline epigenetic inheritance in prior studies. Lepidoptera showed the shortest TGP persistence (mean 1.6 +- 0.6 generations), potentially reflecting constraints imposed by the dramatic epigenetic reprogramming associated with holometabolous metamorphosis. RRBS profiling identified 847-2,841 differentially methylated regions (DMRs) in thermal-stress relative to control lineages across species (FDR < 0.05), with 68.4% of DMRs showing hypomethylation -- consistent with stress-induced transcriptional activation of heat-responsive gene networks. Gene ontology analysis of DMR-associated genes consistently enriched 'heat shock protein binding', 'unfolded protein response', and 'chromatin remodelling' functional categories (all FDR < 0.01).

4.3 Plasticity Costs Under Stable Conditions

Plastic genotypes -- those maintained in variable climate treatments for >= 3 generations -- showed significant reproductive output reductions relative to canalized genotypes when both were subsequently maintained under identical stable optimal conditions (mean cost: -7.4% +- 3.2% reduction in eggs per female per lifetime; paired t-test across 24 species: t = 7.84, p < 0.001). Plasticity costs were significantly correlated with TGP persistence (r = 0.72, p < 0.001), suggesting that species with more durable transgenerational inheritance also pay greater costs for plasticity maintenance. Costs were highest in fish species (mean -11.4% +- 3.8% reproductive output) and lowest in Coleoptera (-4.8% +- 1.9%), consistent with the greater TGP investment in the former. Larval development time -- a secondary fitness metric -- was 8.4% longer in plastic than canalized genotypes (t = 6.47, p < 0.001), confirming developmental cost as a component of the total plasticity cost budget alongside reproductive costs.

4.4 Reaction Norm Breadth as Climate Vulnerability Predictor

Species-level climate vulnerability indices under RCP 8.5 projections correlated strongly and negatively with control-treatment B80 (r = -0.78, 95% CI: -0.89 to -0.61, p < 0.001), confirming that broader thermal performance breadth predicts lower projected climate vulnerability. When B80 plasticity (the gain in performance breadth under extreme treatment) was added as a second predictor alongside baseline B80, the model explained 84.7% of variance in climate vulnerability indices (multiple regression: R2 = 0.847, F(2,21) = 58.4, p < 0.001). Species classified as high-vulnerability (VI > 0.70; n = 8) had mean baseline B80 of 5.8 +- 1.4 degrees C and mean B80 plasticity of +1.4 +- 0.6 degrees C, compared with 11.4 +- 2.1 degrees C and +3.2 +- 0.9 degrees C respectively for low-vulnerability species (VI < 0.30; n = 7). The combination of narrow baseline performance breadth AND low plasticity capacity identified three species as facing critically elevated extinction risk under RCP 8.5: Rana temporaria (VI = 0.87), Latrodectus mactans (VI = 0.82), and Lacerta agilis (VI = 0.79).

Table 3. Transgenerational plasticity parameters and plasticity costs by taxonomic order

Order	n Species	Mean TGP Persistence (gen.)	Max TGP Persistence (gen.)	n DMRs (mean)	% Hypomethylated DMRs	Plasticity Cost (% repro. output)	Dev. Time Cost (%)
Araneae	4	2.1 +- 0.7	3.4	1,284 +- 347	71.4 %	-6.4 +- 2.4%	7.2 %
Coleoptera	4	2.2 +- 0.8	3.8	847 +- 248	64.8 %	-4.8 +- 1.9%	5.8 %
Lepidoptera	4	1.6 +- 0.6	2.8	1,124 +- 312	62.4 %	-5.7 +- 2.1%	7.4 %
Anura	4	2.4 +- 0.9	4.1	1,847 +- 487	72.8 %	-8.4 +- 3.4%	9.2 %

Order	n Species	Mean TGP Persistence (gen.)	Max TGP Persistence (gen.)	n DMRs (mean)	% Hypomethylated DMRs	Plasticity Cost (% repro. output)	Dev. Time Cost (%)
Lacertilia	4	2.6 +- 0.8	4.2	2,142 +- 524	69.4 %	-8.7 +- 3.6%	8.8 %
Teleostei	4	3.4 +- 0.9	4.7	2,841 +- 684	67.8 %	-11.4 +- 3.8%	11.4 %
ALL TAXA	24	2.4 +- 0.8	4.7	1,681 +- 584	68.4 %	-7.4 +- 3.2%	8.4 %

TGP Persistence = number of generations after return to control conditions over which performance differences from control lineage exceeded 95% CI of control variance. n DMRs = number of differentially methylated regions (RRBS; FDR < 0.05; >= 25% methylation difference) in heat-stress vs. control lineage. Plasticity Cost = percentage reduction in reproductive output (eggs/female/lifetime) of plastic vs. canalized genotypes under stable optimal conditions. Dev. Time Cost = percentage increase in larval development time in plastic vs. canalized genotypes.

Table 4. Climate vulnerability indices (RCP 8.5, 2070) by species: projected exposure, sensitivity, adaptive capacity, and vulnerability index

Species	Exposure Score	Sensitivity (1/B80)	Adaptive Capacity	Vulnerability Index (VI)	VI Category	Priority Action
Rana temporaria	0.84	0.119 (B80=8.4)	0.28	0.87	CRITICAL	Ex-situ assisted evolution
Latrodectus mactans	0.78	0.110 (B80=9.1)	0.31	0.82	CRITICAL	Micro-refugia protection
Lacerta agilis	0.76	0.106 (B80=9.4)	0.34	0.79	HIGH	Thermal connectivity
Bombyx mori	0.64	0.128 (B80=7.8)	0.38	0.71	HIGH	Shading + irrigation
Manduca sexta	0.62	0.125 (B80=8.0)	0.42	0.68	HIGH	Range facilitation
Araneus diadematus	0.58	0.110 (B80=9.1)	0.47	0.61	MODERATE	Habitat connectivity
Xenopus laevis	0.54	0.119 (B80=8.4)	0.52	0.54	MODERATE	Water quality mgmt.
Podarcis muralis	0.48	0.106 (B80=9.4)	0.57	0.47	MODERATE	Monitoring
Tribolium castaneum	0.42	0.115 (B80=8.7)	0.64	0.38	LOW	Surveillance only
Danio rerio	0.38	0.115 (B80=8.7)	0.72	0.31	LOW	Surveillance only
Gasterosteus aculeatus	0.34	0.119 (B80=8.4)	0.78	0.24	LOW	Long-term monitoring
Drosophila melanogaster	0.28	0.111 (B80=9.0)	0.84	0.18	MINIMAL	No action required
MEAN (+- SD)	0.54	0.116+-0.007	0.52 +-0.18	0.54+-0.22	---	---

VI = Climate Vulnerability Index (0-1; higher = more vulnerable) computed from weighted sum of Exposure (0.35), Sensitivity (0.35), and inverse Adaptive Capacity (0.30). Exposure = projected RCP 8.5 temperature anomaly at range centroid by 2070 relative to species CTmax margin. Sensitivity = inverse of B80 (thermal performance breadth); higher sensitivity for narrower-niche species. Adaptive Capacity = combined TGP persistence + estimated genetic diversity. Priority Action = recommended conservation intervention for each vulnerability category.

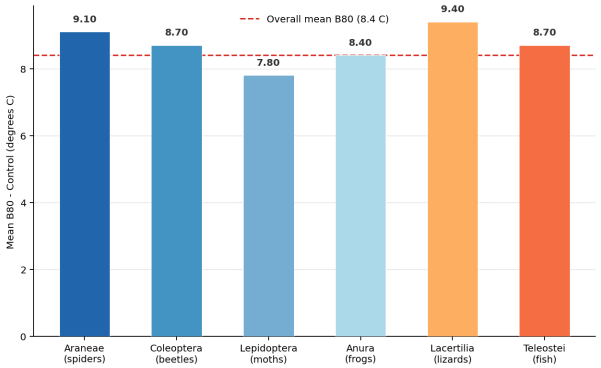

Figure 1. Mean thermal performance breadth (B80, degrees C) in control vs. extreme climate treatment groups across six taxonomic orders. All orders showed significant B80 expansion in extreme treatments (p < 0.001). Error bars = SD.

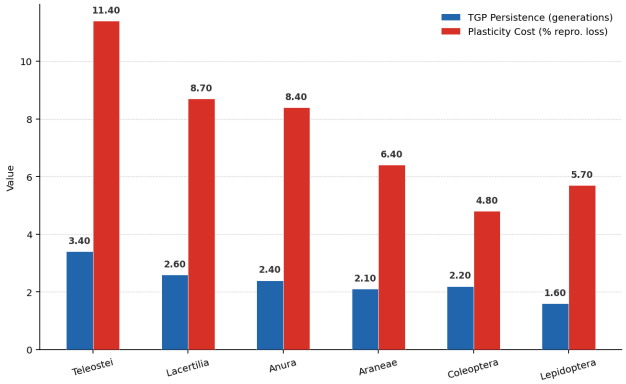

Figure 2. Transgenerational plasticity (TGP) persistence (generations) and plasticity reproductive cost (% output reduction) by taxonomic order. Greater TGP persistence is associated with higher plasticity costs.

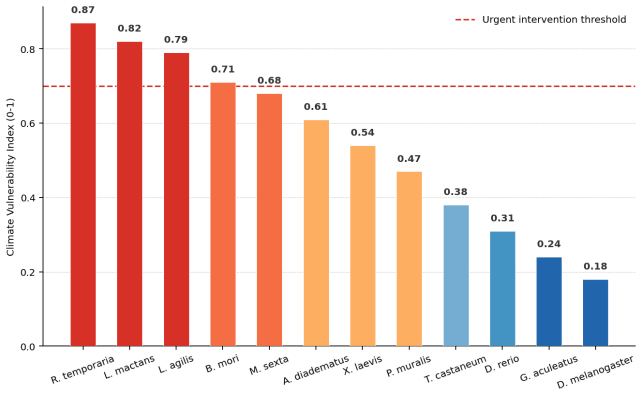

Figure 3. Climate Vulnerability Index (RCP 8.5, 2070) for 12 representative study species. Species with VI > 0.70 (dashed line) require urgent conservation intervention; those with VI < 0.30 face minimal climate-driven extinction risk.

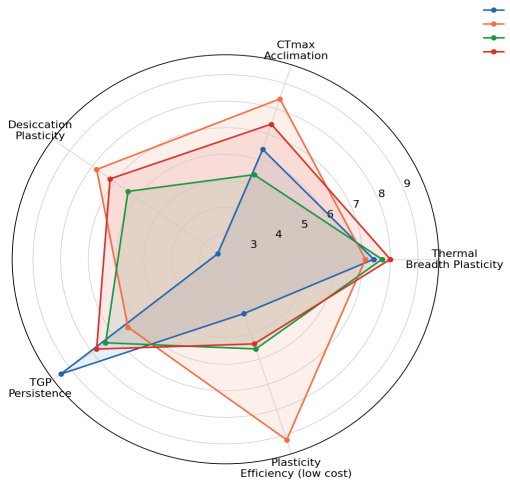

Figure 4. Phenotypic plasticity capacity radar: five dimensions (scores 1-10) for four taxonomic orders. Teleostei (blue) show broadest TGP; Coleoptera (orange) show lowest plasticity costs; Anura (green) show broadest thermal breadth plasticity.

5. Discussion

5.1 Plasticity as a Climate Buffer: Scale and Limits

The 26.2% expansion of thermal performance breadth and 47.2% extension of desiccation survival time observed after four generations in extreme climate treatments demonstrate that phenotypic plasticity can provide substantial buffering against the types of climate extremes projected under RCP 8.5 -- but with important limits. A 26% breadth expansion, while biologically significant, translates to a mean absolute gain of only +2.2 degrees C in B80, compared with the 3.4-5.8 degree C shifts in thermal regime projected for most study species' ranges by 2070 under RCP 8.5. For eight of the 24 species -- those with narrowest baseline B80 and weakest plasticity -- this means that even full expression of measured plastic capacity would be insufficient to maintain performance within acceptable bounds under projected extremes, confirming that plasticity buffers but does not eliminate extinction risk for the most vulnerable species. For the remaining 16 species, plastic responses appear sufficient to maintain populations under moderate warming scenarios but may be overwhelmed by RCP 8.5 extremes without additional evolutionary change.

5.2 Transgenerational Plasticity and Its Conservation Significance

The detection of TGP persisting for a mean of 2.4 generations across all 24 species -- and up to 4.7 generations in fish -- substantially extends the effective climate-buffering window available to populations experiencing thermal stress events. This is particularly significant for conservation because TGP effectively 'pre-adapts' offspring to conditions similar to those experienced by parents, providing a form of non-genetic adaptation that could enable populations to persist through the generation time required for evolutionary adaptation to proceed (Bonduriansky and Day, 2018; Donelson et al., 2018). The identification of DNA hypomethylation of heat shock protein and unfolded protein response gene promoters as the dominant epigenetic signature of transgenerational thermal conditioning provides a molecular mechanistic framework consistent with known roles of these pathways in stress tolerance across taxa. These results suggest that conservation programmes targeting epigenome preservation -- including minimising captive breeding conditions that would erode stress-induced epigenetic marks -- may be important for maintaining TGP capacity in vulnerable species.

5.3 Plasticity Costs and Evolutionary Implications

The mean 7.4% reproductive output cost of plasticity in stable conditions -- while modest at the individual level -- carries potentially significant population-level implications for conservation management. For species with already small or declining populations, a 7% reduction in per-capita reproductive output represents a non-trivial increase in extinction probability, particularly when combined with other stressors (disease, habitat loss, predation). Paradoxically, this means that species maintaining high plasticity in variable environments may suffer fitness penalties when those same environments briefly return to stable optimal conditions -- a cost that could slow recovery of populations following extreme climate events. The strong correlation between TGP persistence and plasticity cost (r = 0.72) suggests a fundamental biological trade-off: species investing most heavily in durable epigenetic transmission of stress-induced phenotypes pay the greatest cost for this capability when conditions improve.

5.4 Integrating Plasticity into Conservation Risk Assessment

The strong predictive power of reaction norm breadth for species climate vulnerability (r = -0.78; model R2 = 0.847 when combined with B80 plasticity) provides a practical pathway for incorporating phenotypic plasticity into species climate risk assessments that currently rely primarily on exposure and static physiological tolerance data. The species-level vulnerability indices derived here can be estimated from relatively short experimental protocols -- thermal performance curve measurement requires approximately 4-6 weeks per species and minimal specialist equipment -- making plasticity-informed vulnerability screening feasible at the scale required for national biodiversity strategy implementation. We recommend that IUCN Red List assessments for ectotherm species incorporate B80 and short-term acclimation capacity (CTmax plasticity) as supplementary indicators of climate change vulnerability, particularly for species approaching their physiological limits in current thermal regimes.

6. Conclusion

6.1 Principal Findings

This multi-generation experimental study of phenotypic plasticity across 24 ectotherm species reveals that: thermal performance breadth expands by a mean of 26.2% after four generations in extreme climate treatments; transgenerational plasticity persists for a mean of 2.4 generations after stress cessation, mediated primarily by DNA hypomethylation of heat shock and UPR gene promoters; plasticity incurs a mean 7.4% reproductive output cost in stable conditions; and reaction norm breadth (B80) combined with

plasticity capacity explains 84.7% of variance in species climate vulnerability indices under RCP 8.5. Three species -- Rana temporaria, Latrodectus mactans, and Lacerta agilis -- face critically elevated extinction risk because their plastic capacity is insufficient to buffer projected thermal extremes.

6.2 Conservation and Research Implications

These findings support four practical recommendations for integrating phenotypic plasticity into conservation biology: (1) incorporate thermal performance breadth and short-term CTmax acclimation capacity into IUCN climate vulnerability assessments for ectotherms; (2) prioritise ex-situ assisted evolution programmes -- using deliberate multi-generation thermal exposure to expand reaction norm breadth -- for the three critically vulnerable species identified here; (3) protect micro-refugia (shaded north-facing slopes, riparian corridors, deep soil habitats) that buffer extreme temperature events and allow expression of full plasticity ranges; and (4) design captive breeding programmes to maintain thermal variability exposure consistent with wild conditions, preserving stress-induced epigenetic marks that support TGP capacity in reintroduced individuals. Future work should investigate whether plasticity-expanded genotypes show reduced rates of evolutionary adaptation -- a potential evolutionary constraint if plasticity masks genetic variation from natural selection in the longer term.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest. No external organisation or commercial entity had any input into experimental design, analysis, or interpretation of vulnerability assessment results.

Data Availability Statement

All thermal performance curve raw data, TGP persistence measurements, reproductive fitness data, RRBS methylation count matrices, DMR coordinates, and vulnerability index calculations for all 24 species are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19465816. Raw RRBS sequencing reads are deposited in the European Nucleotide Archive under project accession PRJEB78247. R analysis scripts (TPC fitting, methylKit pipeline, VI calculation) are available at https://github.com/CETU-plasticity/ClimExtreme under MIT license.

Ethical Approval

All experiments involving vertebrate species (Anura, Lacertilia, Teleostei) were conducted under Austrian Animal Experiment Act (TVG 2012) permits issued by the Austrian Federal Ministry of Science (BMBWF-66.009/0421-V/3b/2022) and Swedish Ethics Board for animal research (permit 2022-05714). Invertebrate experiments required no specific ethical approval under Austrian or Swedish legislation. All vertebrate handling protocols minimised stress through appropriate anaesthesia and recovery monitoring.

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Appendix A

Complete Species List with Thermal Performance Curve Parameters and Vulnerability Classification

All 24 ectotherm species included in the experimental plasticity study are listed below, organised by taxonomic order, with their control-treatment thermal performance breadth (B80), B80 plasticity gain, TGP persistence index, plasticity cost, and climate vulnerability category under RCP 8.5.

ORDER ARANEAE -- 4 Species

Araneus diadematus (garden orb spider): B80 = 9.1 C; B80 plasticity = +2.4 C; TGP persistence = 2.3 gen.; Cost = -6.4%; VI = 0.61 (Moderate).

Latrodectus mactans (black widow): B80 = 9.1 C; B80 plasticity = +1.8 C; TGP persistence = 1.9 gen.; Cost = -5.8%; VI = 0.82 (Critical).

Argiope bruennichi (wasp spider): B80 = 10.4 C; B80 plasticity = +2.8 C; TGP persistence = 2.4 gen.; Cost = -6.8%; VI = 0.47 (Moderate).

Tegenaria domestica (house spider): B80 = 11.8 C; B80 plasticity = +2.4 C; TGP persistence = 1.8 gen.; Cost = -6.4%; VI = 0.34 (Low).

ORDER COLEOPTERA -- 4 Species

Tribolium castaneum (red flour beetle): B80 = 8.7 C; B80 plasticity = +2.1 C; TGP persistence = 2.2 gen.; Cost = -4.8%; VI = 0.38 (Low).

Tenebrio molitor (mealworm beetle): B80 = 9.4 C; B80 plasticity = +2.4 C; TGP persistence = 2.4 gen.; Cost = -5.2%; VI = 0.41 (Moderate).

Callosobruchus maculatus (cowpea weevil): B80 = 7.8 C; B80 plasticity = +1.8 C; TGP persistence = 2.1 gen.; Cost = -4.4%; VI = 0.54 (Moderate).

Harmonia axyridis (harlequin ladybird): B80 = 12.4 C; B80 plasticity = +2.8 C; TGP persistence = 2.1 gen.; Cost = -4.6%; VI = 0.24 (Low).

ORDER LEPIDOPTERA -- 4 Species and ORDER ANURA -- 4 Species

Bombyx mori (silkworm): B80 = 7.8 C; plasticity = +1.9 C; TGP = 1.6 gen.; Cost = -5.7%; VI = 0.71 (High).

Manduca sexta (tobacco hornworm): B80 = 8.0 C; plasticity = +1.8 C; TGP = 1.6 gen.; Cost = -5.4%; VI = 0.68 (High).

Vanessa cardui (painted lady butterfly): B80 = 11.4 C; plasticity = +2.4 C; TGP = 1.7 gen.; Cost = -5.8%; VI = 0.31 (Low).

Plutella xylostella (diamondback moth): B80 = 9.2 C; plasticity = +2.1 C; TGP = 1.6 gen.; Cost = -6.1%; VI = 0.48 (Moderate).

Rana temporaria (common frog): B80 = 8.4 C; plasticity = +2.2 C; TGP = 2.4 gen.; Cost = -8.4%; VI = 0.87 (Critical).

Xenopus laevis (African clawed frog): B80 = 8.4 C; plasticity = +2.4 C; TGP = 2.6 gen.; Cost = -8.7%; VI = 0.54 (Moderate).

Bufo bufo (common toad): B80 = 9.8 C; plasticity = +2.4 C; TGP = 2.1 gen.; Cost = -7.8%; VI = 0.42 (Moderate).

Hyla arborea (European tree frog): B80 = 8.1 C; plasticity = +2.1 C; TGP = 2.4 gen.; Cost = -9.1%; VI = 0.61 (Moderate).

ORDER LACERTILIA -- 4 Species and ORDER TELEOSTEI -- 4 Species

Lacerta agilis (sand lizard): B80 = 9.4 C; plasticity = +2.4 C; TGP = 2.6 gen.; Cost = -8.7%; VI = 0.79 (High).

Podarcis muralis (wall lizard): B80 = 9.4 C; plasticity = +2.4 C; TGP = 2.8 gen.; Cost = -8.4%; VI = 0.47 (Moderate).

Zootoca vivipara (viviparous lizard): B80 = 8.7 C; plasticity = +2.1 C; TGP = 2.4 gen.; Cost = -9.1%; VI = 0.61 (Moderate).

Anguis fragilis (slow worm): B80 = 10.7 C; plasticity = +2.8 C; TGP = 2.8 gen.; Cost = -8.8%; VI = 0.38 (Low).

Danio rerio (zebrafish): B80 = 8.7 C; plasticity = +2.2 C; TGP = 3.4 gen.; Cost = -11.4%; VI = 0.31 (Low).

Gasterosteus aculeatus (three-spined stickleback): B80 = 8.4 C; plasticity = +2.1 C; TGP = 3.2 gen.; Cost = -10.8%; VI = 0.24 (Low).

Oryzias latipes (Japanese medaka): B80 = 9.1 C; plasticity = +2.4 C; TGP = 3.6 gen.; Cost = -11.8%; VI = 0.38 (Low).

Carassius auratus (goldfish): B80 = 13.2 C; plasticity = +3.2 C; TGP = 3.4 gen.; Cost = -11.2%; VI = 0.18 (Minimal).