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ORIGINAL RESEARCH

Intraspecific Genome Size Variation in *Rorippa indica* Reveals a Tropical Adaptation by Genomic Enlargement

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ABSTRACT

Genome size exhibits substantial variation across organisms, but its causes and ecological consequences remain incompletely understood. While interspecific comparisons have suggested selective pressures against large genomes, intraspecific variation has been less explored. Here, we investigate genome size diversity within the hexaploid yellowcress *Rorippa indica* by integrating flow cytometry, plastome phylogeography, genomic repeat profiling, and reciprocal common garden experiments. Across 192 accessions from 83 natural populations, genome size ranged from 764 to 892 Mb, a 15.8% difference relative to the mean (812 Mb), representing the widest range yet reported within *Rorippa*. Plastome haplotype analysis revealed that lineages colonizing tropical habitats tended to retain or enlarge genome size, whereas northern lineages exhibited reductions. Genome size was significantly correlated with tropical environments characterized by higher winter temperatures and reduced seasonality. Variation was largely attributable to repetitive DNA, with 45S rDNA and *Ty1-copia* retrotransposons (*Bianca*) explaining up to 15.5% and 26.1% of the differences, respectively. Reciprocal transplantation experiments demonstrated that plants with larger genomes had higher fitness in tropical conditions, producing 32% more fruits. These findings indicate that genome size in *R. indica* is not a neutral trait but is selectively expressed, with both shrinkage and enlargement representing adaptive strategies under contrasting environments. We propose that genome enlargement, driven primarily by specific repetitive elements, constitutes an adaptive response to stable tropical climates. As global warming progresses, species with larger genomes may exhibit slower growth but increased reproductive output, with broad implications for ecosystem dynamics and agricultural productivity.

1 | Introduction

A well-known phenomenon about biodiversity is that genomes come in strikingly diverse packages across the tree of life. From the bacterium *Nasuia deltocephalinicola* with only 112,000 base pairs to the fork fern *Tmesipteris oblanceolata* boasting 160 billion base pairs, genome size spans six orders of magnitude (Hidalgo et al. 2017; Fernández et al. 2024). Such variation plays crucial roles in biological processes, influencing genomic plasticity (Lynch and Conery 2003; Leitch and Leitch 2008), cellular dynamics (Beaulieu et al. 2008; Francis et al. 2008), environmental adaptation (Knight and Ackerly 2002; Faizullah et al. 2021),

and species diversification (Simonin and Roddy 2018; Gomez et al. 2024). Although evidence remains limited, adjustable genome size has been proposed as a way of balancing a relatively low mutation rate with the need for rapid responses to environmental change (Mei et al. 2018). Yet the dynamics of genome size variation, particularly its evolutionary trajectory and fitness consequences in natural contexts, remain incompletely understood.

A central question is whether genome size variation is driven primarily by neutral processes or by natural selection. Neutral hypotheses, such as the mutational hazard and mutational equilibrium models, suggest that genome size reflects random drift rather than adaptive forces (Petrov 2002; Lynch and Conery 2003). In contrast, adaptive hypotheses posit that genome size directly affects phenotypic traits such as cell size and metabolism, thereby influencing fitness under particular environmental conditions (e.g., CO_2, water, or nutrient stresses) (Mei et al. 2018; Faizullah et al. 2021). Increasing evidence supports adaptive roles for genome size variation, which can arise from a range of evolutionary and ecological factors. Beyond whole-genome duplication or polyploidy, intrinsic processes such as the accumulation of repetitive and transposable elements (Bourque et al. 2018), as well as extrinsic influences including climate (Bureš et al. 2024), mating system (Roessler et al. 2019), or demographic history (Lefebure et al. 2017; Bilinski et al. 2018), have all been implicated. Collectively, these findings suggest that genome size can function as an ecologically important trait subject to selection.

Most insights into genome size evolution have come from interspecific comparisons, which led to the development of several key hypotheses (Bureš et al. 2024). For example, the "large genome constraint" hypothesis suggests evolutionary disadvantages to genome enlargement, with selection favoring a reduction in genome size (Knight 2005; Hidalgo et al. 2017; Gomez et al. 2024). In addition, the "environmentally selected" hypothesis emphasizes the role of genome streamlining in coping with stresses such as drought, tidal fluctuations, or nutrient limitation (Lyu et al. 2018; Greenhalgh et al. 2020; Faizullah et al. 2021). Still, genomic enlargement has also been associated with demographic fluctuations (Lefebure et al. 2017) and parallel adaptation along altitudinal gradients (Díez et al. 2013). For example, larger genomes tend to occur in stable environments with lower seasonality and longer growing seasons (Knight and Ackerly 2002; Díez et al. 2013; Carta and Peruzzi 2016; Qiu et al. 2019; Záveská et al. 2024), where relaxed constraints on growth rate and temperature-activated transposable element activity may facilitate their persistence. Thus, both shrinkage and enlargement of genomes can represent adaptive strategies, yet their ecological and evolutionary significance remains incompletely resolved (Mei et al. 2018; Blommaert 2020), particularly by attaining more insights from intraspecific investigations on larger genomes. Such studies at the population level can offer critical opportunities to disentangle the forces shaping genome size variation.

Here, we focus on intraspecific genome size variation in *Rorippa indica*, an allohexaploid herb with broad ecological amplitude. This species is native to temperate East Asia but has recently colonized tropical regions, including Southeast Asia and Central America (Setyawati et al. 2015). With its hybrid origin from tetraploid *Rorippa dubia* ($2n = 4x = 32$, AACC) and diploid *Rorippa globosa* ($2n = 2x = 16$, BB) (Han et al. 2024), *R. indica* ($2n = 6x = 48$, AACCBB) retains genomic redundancy that may facilitate genome size shifts. Its wide distribution and ecological breadth make it an excellent system for investigating the evolutionary and ecological consequences of intraspecific genome size variation. Specifically, we ask: (1) what is the extent and pattern of genome size variation among natural populations of *R. indica*; (2) does genome size correlate with fitness-related traits; and (3) what is the evolutionary trajectory of genome size within this species? Addressing these questions will provide new insights into the drivers and consequences of genome size variation and illuminate how organisms adapt their genomic architecture in response to environmental change.

2 | Materials and Methods

2.1 | Population Sampling

A total of 192 accessions from 83 natural populations of *Rorippa indica* were sampled during 2017–2019 (Figure 1A), excluding the closely related species *R. hengduanshanensis* (Zheng et al. 2021). In 2020, 8–10 seeds for each accession were stratified on the half-strength Murashige and Skoog medium under 4°C and dark for 2 weeks, and then germinated under 22°C and long day conditions (16 h light + 8 h dark) for 10 days. Seedlings with at least two true leaves were transplanted into mediums mixed with nutrient soil and vermiculite (1:3) and grown under climate-controlled chambers (16 h light/8 h dark, 22°C, 60% relative humidity). To reduce systematic and artificial influences on the following analysis, all plants were randomized into trays of 4 × 8 grids using the default method of completely randomized design function design.crd() in the R package agricolae v.1.3–3.

2.2 | Genome Size Estimation

Flow cytometry (FCM) was used to measure genome size variation in *R. indica* (Doležel et al. 2007). Fully developed fresh leaves around 20 mg were chopped under 1 mL fresh ice-cold Otto I solution (0.1 M citric acid, 0.5% Tween-20), with plants of *Capsella rubella* MTE (219 Mb) as the internal standard (Figure S1). The mixed homogenate was filtered through a 42 μm nylon mesh. Propidium iodide (PI, 50 μg/mL) was used as a fluorochrome, together with 0.1 mg/mL RNase to digest potential RNA and Otto II solution with 2 μL β-mercaptoethanol. The FCM analysis was performed using a BD FACSVerse Flow Cytometer. To accurately distinguish the G1 and G2 peaks of *R. indica*, FCM tests were performed with or without *C. rubella* leaves for each sample, separately. The DNA peaks exhibited high precision, with coefficients of variation (CV) consistently below 5%. Specifically, the G1 peaks of the internal standards *C. rubella* MTE were often undetectable, while the G2 peaks were more prominent and generated low average CV values (mean ± SD = 3.75 ± 0.53%; Figure S1). Tested samples had single prominent G1 peaks with lower CV values (1.25% ± 0.13%). Genome size was estimated for mixed samples as the total nuclear DNA content (i.e., 2C value) according to the formula: sample genome size = 2 × (sample mean value of G1 peak/internal standard mean value of G2 peak) × 219 Mb. A total of 3–4 replicates per accession were

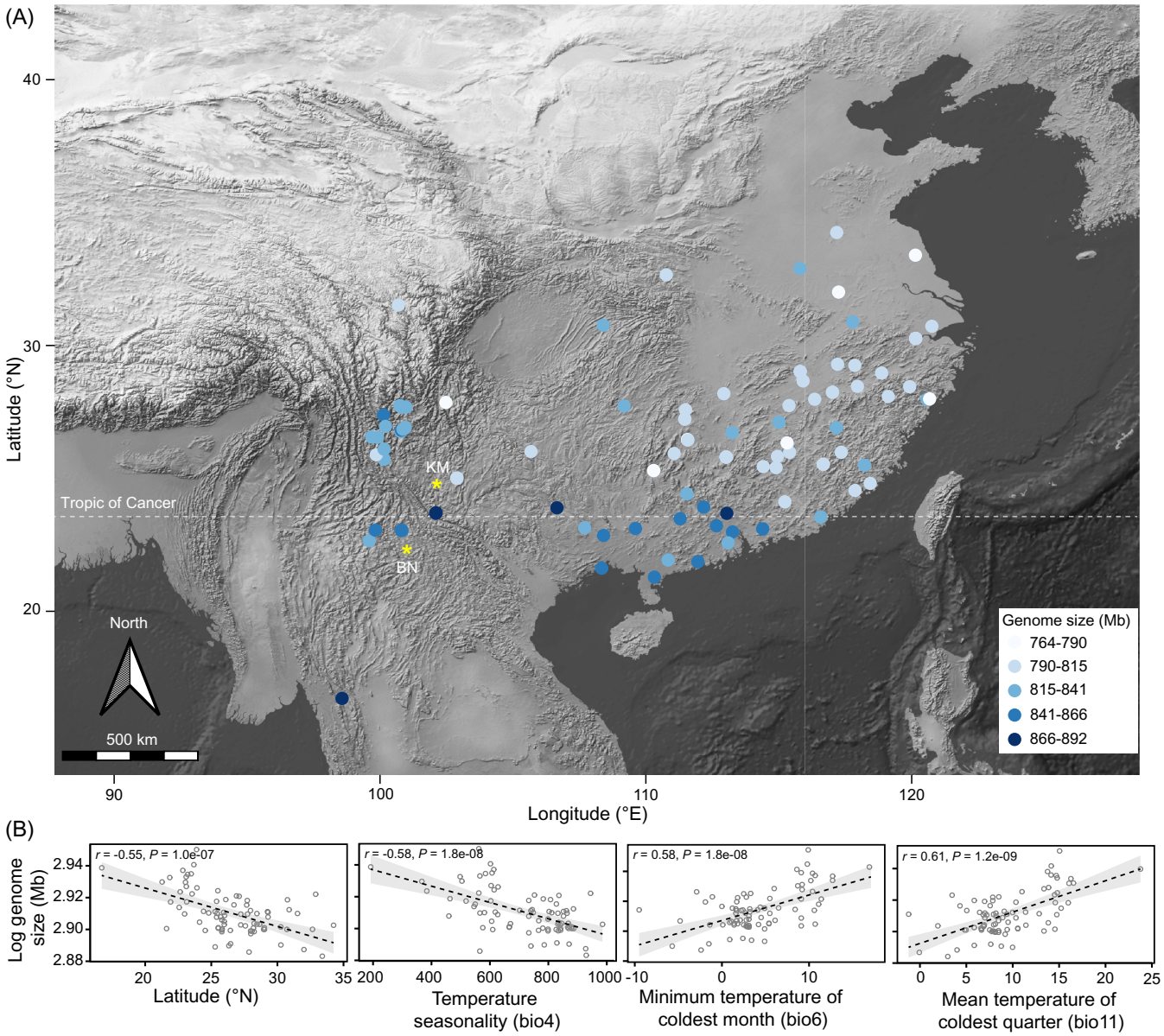

FIGURE 1 | Natural variation in genome size of *Rorippa indica*. (A) Geographic distribution of genome sizes in *R. indica*. The genome size is represented by the color of each accession dot, ranging from light (smaller genome) to dark blue (larger genome). Yellow asteroids indicate the locations of two common gardens: Kunming (KM) and Xishuangbanna (BN). (B) The relationship between log-transformed genome size and several environmental factors: Latitude, temperature seasonality (bio4), minimum temperature of coldest month (bio6), and mean temperature of coldest quarter (bio11). In each panel, the dashed line represents the best-fitting linear regression model; the grey zone indicates the 95% confidence intervals for the regression line; and the Pearson's correlation coefficient (*r*) and *P*-values are shown above.

sampled and measured at two or three different developmental stages. Each replicate involved FCM analysis of over 3000 nuclei (mean ± SD = 8749 ± 5540). Estimates from multiple outputs of FCM analyses were extracted and combined using a custom R script. To check the chromosome numbers of *R. indica* plants, slides were prepared for selected root samples representing different genome sizes.

2.3 | Correlation With Climatic Variables

Bioclimatic variables (bio1–19) for the 83 natural populations were extracted from WorldClim (https://www.worldclim.org/) using the R package Raster v.2.6-6. Log-transformed median values of intrapopulation genome size were used to estimate Pearson's correlation (*r*) with each bioclimatic or geographic variable (i.e., latitude, longitude, and elevation) in R v.4.0.2.

2.4 | Genomic Sequencing and Chloroplast Genome Assembly

High-quality DNA was extracted for 64 randomly selected accessions spanning the native range of *R. indica* with Qiagen DNeasy Plant Kits. Whole-genome sequencing was performed on the Illumina Novaseq platform, generating a minimum of 4 Gb clean data and approximately 5× coverage for each sample, with 150 bp

paired-end reads. Chloroplast genome assembly and annotation were performed by the default parameters of GetOrganelle v.1.4.0 (Jin et al. 2020) and CPGAVAS v.2.0 (Shi et al. 2019) (http://47.96.249.172:16019/analyzer/home), respectively. Protein coding genes (PCGs) were extracted and concatenated using a custom R script (Han et al. 2024). Sequences were aligned using the MAFFT method in Geneious Prime v.2019.0.3 (Kearse et al. 2012).

2.5 | Phylogeny and Network Analysis

Chloroplast haplotypes were inferred from the aligned PCGs sequences using DnaSP v.6.10.04 (Rozas et al. 2017). Haplotype networks were built under the TCS method using PopART v.1.7 (Leigh and Bryant 2015), with gap as the fifth variant, limit of parsimony connection as 95%, and long gaps as additional characters. Phylogeny was constructed using BEAST v.2.6.0, under a relaxed molecular clock and uniform prior distributions (Han et al. 2024). A total of four independent runs were carried out, each with 200 million Markov Chain Monte Carlo simulations. Convergence was ensured by the effective sample size of parameters larger than 200 using TRACER v.1.7. Tree files were combined in LogCombiner v.2.6.0 (BEAST Developers), with 10% of runs as burn-in. Based on a set of 1000 resampled trees, a final maximum clade credibility tree was annotated under mean node heights in TreeAnnotator v.1.8.4 (BEAST Developers). The resampled trees were dated using penalized likelihood and maximum likelihood methods by the chronos() function in the R package ape v.5.5 (Paradis and Schliep 2019), under the selected relaxed clock model and lambda = 1.0. The maximum and minimum crown ages of *R. indica* were assigned as 1.47 and 0.71 Myr using the makeChronosCalib() function in ape v.5.5, respectively, according to the published dating results using a plastome tree (Han et al. 2024). The mean node ages were calculated using the branching.times() function in ape v.5.5, as well as their corresponding values of 95% highest posterior density (HPD). Integrated evidence for closely related haplotypes was clarified as haplogroup (HG) for subsequent analysis. Specifically, the assigned haplogroups may have been separated from each other by accumulated plastid variation, which was confirmed by both robust phylogenetic supports (i.e., posterior probabilities = 1.0) and more convergent network clusters among haplogroups than within haplogroups.

2.6 | Phylogeny-Corrected Correlation With Climatic Variables or Repeat Elements

To test the effect of climatic variables or repeat elements on genome size variation without phylogenetic inference, multiple phylogenetic generalized least squares (PGLS) tests were implemented using the R package caper v.1.0.1 (Orme et al. 2013). Climatic variables (bio1–19) were obtained as described above.

To characterize the repetitive DNA from unassembled sequences, paired-end raw reads under genomic coverage of 0.1× were sampled and merged from Illumina sequencing data for each sample using seqtk (https://github.com/lh3/seqtk). Genomic repeats were captured using the protein domain database of Viridiplantae v.3.0 in RepeatExplorer2 (Novak et al. 2020) (Table S1). After filtering the sequencing depth (> 1 million reads) and read quality by Trimmomatic (Bolger et al. 2014), a total of 56 accessions were used. For PGLS analyses, we identified the transformation structure for the covariance matrix (λ, κ, and δ) through maximum likelihood (ML) model. They adjusted phylogenetic signal (λ), branch lengths (κ), and divergence (δ), fitting correlation structure to match the data (Table S2). Two of the parameters were held constant while the remaining one was optimized. Final PGLS analysis was conducted using the transformation structure according to the ML model.

2.7 | Common Garden Experiment

To evaluate the fitness effect of genome size (GS) variation, a reciprocal transplantation experiment was performed for accessions with small (< 840 Mb) or large (> 840 Mb) genome sizes. Types of genome size were divided according to comparison with that of the constructed ancestral haplotype (H01 or HG-I, see the result section for details). Two common gardens were established: one in temperate Kunming (KM; mean temperature of winter = 5°C–10°C) and one in tropical Xishuangbanna (BN; 15°C–20°C) (Figure 1A). To reduce the influence of phylogenetic interference, accessions from one specific haplogroup (HG-III) with varying genome sizes were used, including 11 accessions with small genome sizes and nine with large ones. For these accessions, no significant correlation was detected between genome sizes and source latitudes (the Pearson's correlation coefficient $r = -0.26$, $p = 0.30$). This allowed us to isolate the fitness effect of genome size variation from the influence of latitude or the environment of origin.

The seeds were collected from plants used for FCM and growing in the climate-controlled chamber for one generation (16 h light/8 h dark, 22°C, 60% relative humidity). They were randomized into trays of 4 × 8 grids using the default method and design.crd() function in the R package agricolae v.1.3–3, with 15 biological replicates per accession per site. About 10 seeds per replicate were sown directly into each grid with a medium made of nutrient soil to vermiculite = 1:3. Only one seedling was randomly kept after germination per grid. The experiments were performed from October 2021 to August 2022 to cover the natural growing periods of *R. indica* during the autumn-spring seasons in temperate areas and the dry season in tropical areas.

A total of 22 traits were collected across the whole life history of *R. indica* plants. The original traits included the germination rate recorded at 7 days after sowing (GR), survival rates at 10/14/18 weeks (SR_10W/14W/18W), mean or maximum rosette diameters at 10/14/18 weeks (RD_10W/14W/18W_mean/max), flowering time (FT), total number and total/mean length of primary inflorescence (TN/TL/ML_PI), the tallest length of primary inflorescence as plant height (PH), total number and total/mean/maximum length of secondary inflorescence (TN/TL/ML/MaxL_SI), and total number of fruits (TN_F). The calculated traits included the mean or maximum relative growth rates (RGR_mean/max) evaluated as the average increase of rosette diameters per 4 weeks. The effects of site (KM vs. BN), genome size/GS type (small vs. large), or their interaction (site × GS) on trait variation were measured

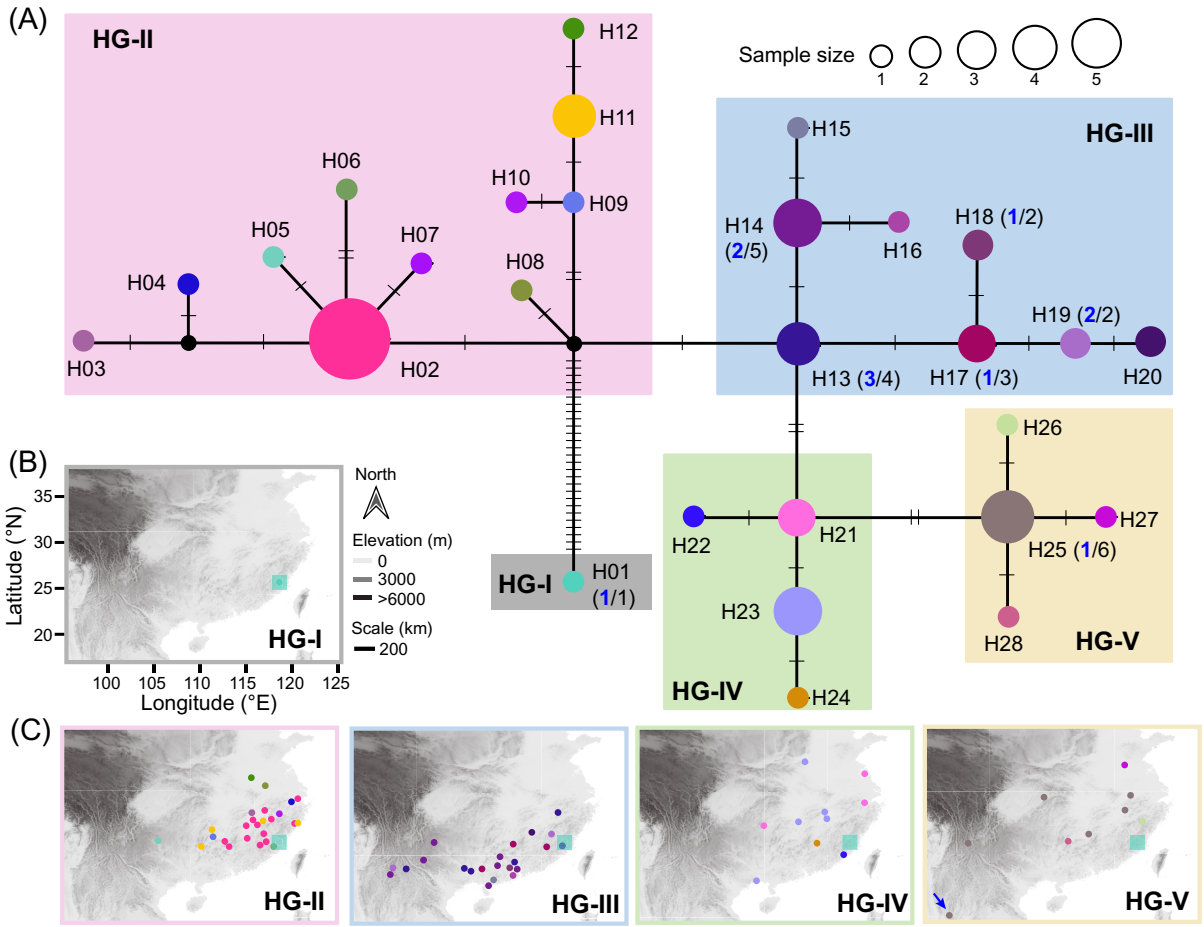

FIGURE 2 | Phylogeography of plastome haplotypes in *Rorippa indica*. (A) Network of plastid haplotypes in *R. indicia*. Haplotypes are represented as colored pie charts, with names (H01–28) and relationships indicated by connecting lines. The number of mutations between haplotypes is shown by bars on connecting lines. The size of each pie chart corresponds to the number of individuals with that haplotype. Haplotypes with larger genomes are labeled with blue numbers out of the total haplotype size indicated in parentheses. Haplogroups are labeled as HG I-V under shades with different colors. (B) Map of haplotype from the ancestral HG-I. (C) Maps of haplotypes from the derived HG-II, HG-III, HG-IV and HG-V, respectively. In (B–C), haplotypes are illustrated as colored dots similar to (A), with the position of HG-I marked as a square in each panel. The location of the Southeast Asia accession with a larger genome from HG-V/H25 is indicated by a blue arrow line on the map.

using a generalized linear mixed model (GLMM) in the R package glmmTMB v.1.9.4 (Brooks et al. 2017). The influence of random effects (population and tray) was determined by our published method (Du et al. 2024).

3 | Results

3.1 | Genome Size Variation in *Rorippa indica*

Flow cytometry (FCM) of 192 accessions from 83 natural populations revealed extensive intraspecific genome size variation in *R. indica* (Figure 1A). The mean (± standard deviation/SD) genome size was 811.71 ± 36.74 Mb, ranging from 764 to 892 Mb, representing a 15.8% difference relative to the mean. This is the largest range observed among *Rorippa* species (Figure S2), exceeding reported variation in *R. elata* (SD = 11.22 Mb, 186 accessions) and *R. palustris* (SD = 10.78 Mb, 62 accessions) (Han et al. 2022). Chromosome counts confirmed $2n = 6x = 48$ across sampled individuals with different genome sizes. Correlation analyses identified significant associations between genome size and geographic or bioclimatic variables (Figures S3 and S4). The strongest correlations were with latitude ($r = -0.55$, $p < 0.0001$), temperature seasonality (bio4, $r = -0.58$, $p < 0.0001$), minimum temperature of the coldest month (bio6, $r = 0.58$, $p < 0.0001$), and mean temperature of the coldest quarter (bio11, $r = 0.61$, $p < 0.0001$) (Figure 1B).

3.2 | Phylogeography of Genome Size Variation

Chloroplast genome sequencing of 64 accessions yielded 28 haplotypes, which clustered into five haplogroups (HG I–V) (Figure 2A and Figure S5). HG-I, sampled from southeast China (25.50°N, 118.23°E) (Figure 2B), was basal in the haplotype tree and originated approximately 1.40 Ma (95% HPD: 1.47–1.14 Ma), representing the ancestral lineage. Haplogroups HG II–V diverged subsequently. Clear phylogeographic patterns were observed across latitudes among haplogroups (Figures 2C and 3A, and Figure S5) (ANOVA, df = 4, $F = 5.21$, $p = 1.20e\text{-}03$). HG-III was distributed significantly southward into lower latitudes (mean ± SD = 24.05 ± 2.07°N; one-sample Wilcoxon test, $V = 29$, $p = 0.01$) from *c.* 1.07 Ma (95% HPD: 1.16–0.90 Ma), while HG-II, -IV, and -V were

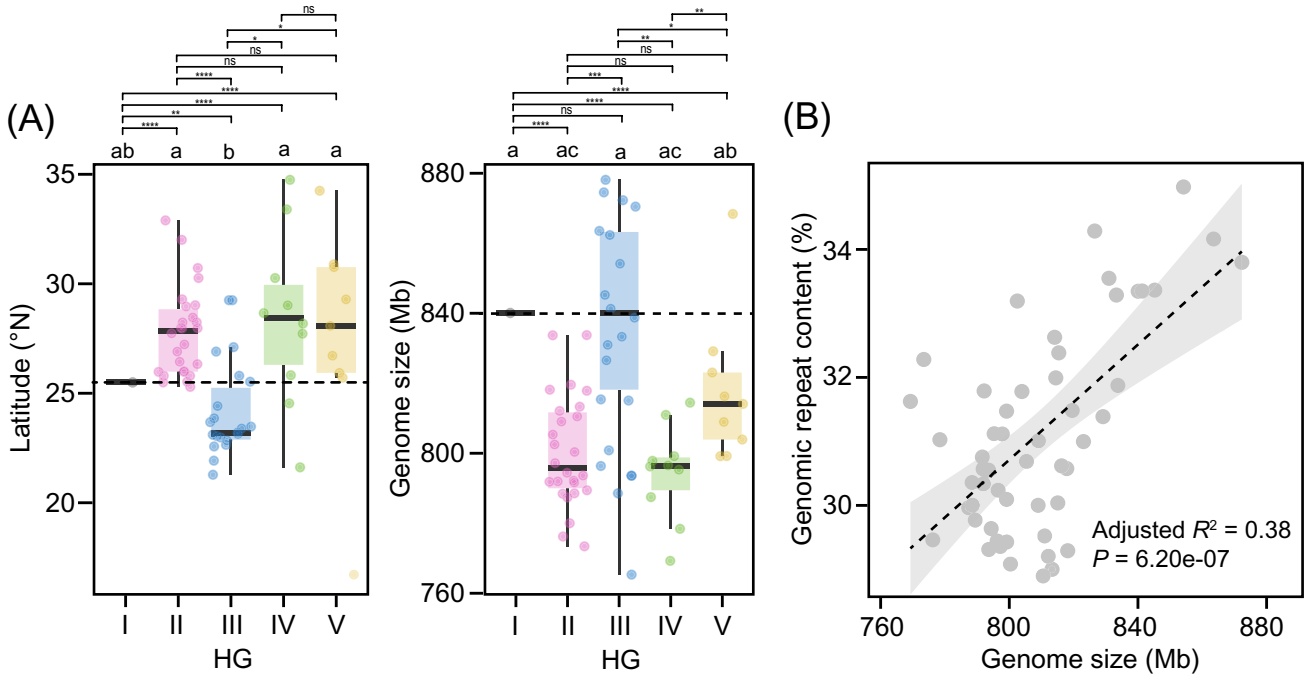

FIGURE 3 | Geographic and genomic components of genome size variation in *Rorippa indica*. (A) Box-and-whisker plots illustrate the distribution of latitude (left) and genome size (right) across haplogroups (HG I-V). The median value is represented by the horizontal line within each box. The 25th and 75th percentiles mark the bottom and top of the box, respectively. Whiskers extend to the limits of the 95% confidence intervals. Scattered dots represent individual data points. Horizontal dashed lines indicate the values of the ancestral HG-I. Letters above boxes denote results of LSD tests. Asterisks between HG-I and the others indicate significance of one-sample Wilcoxon tests comparing the deviation from HG-I. Asterisks between haplogroups except HG-I reveal pairwise differences between haplogroups using Bonferroni-corrected Wilcoxon rank-sum tests. Levels of statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant. (B) Linear relationship between genome size and genomic repeat content. The dashed line represents the best-fitting linear regression model. The grey zone indicates the 95% confidence intervals for the regression line. The adjusted R^2 and p-value quantify the strength and significance of the relationship, respectively.

distributed northward (mean ± SD = 27.89 ± 2.07°N; $V = 899$, $p < 0.0001$), diverging between 1.25 and 0.75 Ma (Figure 2C, and Figure S5).

Genome size patterns differed among haplogroups (ANOVA, df = 4, $F = 10.02$, $p < 0.0001$). HG-I had an intermediate size (~840.08 Mb). Northern haplogroups (HG-II, -IV, -V) were significantly smaller (mean ± SD = 802.67 ± 18.25 Mb; $V = 13$, $p < 0.0001$). In contrast, HG-III showed the highest variability (mean ± SD = 837.62 ± 31.59 Mb), comparable to HG-I ($V = 82$, $p = 0.90$) but significantly larger than northern haplogroups (pairwise Wilcoxon test, Bonferroni-adjusted $p < 0.05$) (Figure 3A). An exception was an accession from Mae Sot, Thailand (16.72°N; HG-V), which had a genome size of 868.38 Mb, exceeding its HG-V relatives (mean ± SD = 811.72 ± 10.96 Mb). Overall, populations at lower latitudes, including 55% of HG-III accessions south of the Tropic of Cancer (23.44°N), tended to have larger genomes than both the ancestral and northern haplogroups (Figures 1A, 2, and 3).

3.3 | Genomic Repeat

Repetitive DNA content, estimated from unassembled sequence reads, ranged from 28.90% to 34.98% (mean ± SD = 31.10 ± 1.55%) (Table S1). *Athila*, a *Ty3-gypsy* retrotransposon, was the most abundant repetitive element, comprising ~30% of the total repeats (mean ± SD = 30.82 ± 2.21%). Genome size was positively correlated with repeat content, with a significant linear regression (adjusted $R^2 = 0.38$, $p < 0.0001$) (Figure 3B).

3.4 | Ecological and Genomic Drivers of Genome Size Variation

Phylogenetically corrected analyses (PGLS) revealed that genome size was strongly associated with tropical environments (Tables 1 and S2). Larger genomes occurred at lower latitudes, in regions with higher winter temperatures (bio11) and lower temperature seasonality (bio4) (adjusted $R^2 = 0.34$, 0.26, 0.28, $p = 0.02$, 0.04, 0.03, respectively). Genome size was also positively associated with the abundance of specific repetitive elements, particularly the 45S rDNA and *Ty1-copia* repeats (*Bianca*) (adjusted $R^2 = 0.16$ and 0.26, $p = 0.04$ and 0.04, respectively) (Table 2).

3.5 | Fitness Effect of Genome Size Variation

Common garden experiments demonstrated local adaptation associated with genome size. Significant site effects were observed for traits including germination, seedling survival, growth, and architecture (Table 3; Figures S5–S6). In Kunming (KM, temperate), germination and seedling survival were higher than in Xishuangbanna (BN, tropical)

TABLE 1 | Statistics for PGLS models detecting the significant effects of bioclimatic or geographic variables on genome size variation across populations of *Rorippa indica*.

Variable	df	AIC	*F*-Statistic	Adjusted R^2	*p*-Value
Annual mean temperature/bio1	2	−72.68	5.33	0.25	**0.04**
Mean diurnal range of temperature/bio2	2	−67.98	0.39	0.05	0.55
Isothermality/bio3	2	−72.80	5.48	0.26	**0.04**
Temperature seasonality/bio4	2	−73.18	5.95	0.28	**0.03**
Max temperature of warmest month/bio5	2	−68.14	0.53	0.04	0.48
Minimum temperature of coldest month/bio6	2	−72.80	5.47	0.26	**0.04**
Temperature annual range/bio7	2	−71.80	3.66	0.17	0.08
Mean temperature of wettest quarter/bio8	2	−68.28	0.17	0.07	0.69
Mean temperature of driest quarter/bio9	2	−69.77	1.54	0.04	0.24
Mean temperature of warmest quarter/bio10	2	−68.34	0.71	0.02	0.42
Mean temperature of coldest quarter/bio11	2	−72.94	5.65	0.26	**0.04**
Annual precipitation/bio12	2	−71.95	4.45	0.21	0.06
Precipitation of wettest month/bio13	2	−68.94	1.26	0.02	0.28
Precipitation of driest month/bio14	2	−69.59	1.89	0.06	0.19
Precipitation seasonality/bio15	2	−67.53	0.00	0.08	1.00
Precipitation of wettest quarter/bio16	2	−69.77	2.07	0.08	0.18
Precipitation of driest quarter/bio17	2	−67.56	0.02	0.08	0.88
Precipitation of warmest quarter/bio18	2	−70.64	2.98	0.13	0.11
Precipitation of coldest quarter/bio19	2	−67.54	0.00	0.08	0.97
Latitude	2	−74.45	7.68	0.34	**0.02**
Longitude	2	−72.85	4.16	0.20	0.06
Altitude	2	−70.64	2.41	0.10	0.15

Note: Values of significant *p*-values by *F*-tests are shown in bold.
Abbreviations: AIC, Akaike information criterion; df, degree of freedom.

($p \leq 0.0001$). Genome size effects were detected for flowering time (Chisq = 11.81, $p = 5.90e\text{-}04$), with larger-genome plants flowering earlier in both BN (large vs. small: 90.57 ± 25.77 vs. 109.94 ± 28.76 days; $p = 0.002$) and KM (212.83 ± 43.69 vs. 234.92 ± 28.39 days; $p = 0.001$) (Figure S7). Site × genome size interactions were significant for relative growth rate and fruit number in BN (Chisq = 11.59 and 9.40; $p = 1.14e\text{-}03$ and $2.17e\text{-}03$). Larger-genome plants produced 32% more fruits in the tropics (Figure 4), indicating context-dependent rather than universal fitness benefits.

4 | Discussion

This study investigates intraspecific genome size variation in the widespread hexaploid herb *Rorippa indica*. Our findings indicate that genome size differences are largely driven by the activity of specific repetitive elements, particularly 45S rDNA and *Ty1-copia* type long tandem repeats. Such variation can be interpreted in two complementary ways: as genome enlargement in southern tropical lineages or as genome shrinkage in northern temperate lineages. Both perspectives highlight the role of repetitive elements in shaping genome architecture under contrasting climatic regimes. Evidence from reciprocal transplantation supports the adaptive significance of these changes. Together, our results provide new insights into the drivers and consequences of genome size variation in *R. indica*, contributing to a broader understanding of genome evolution and plant adaptation.

4.1 | Natural Variation in Genome Size of *Rorippa indica*

We detected substantial intraspecific variation in genome size, ranging from 764 to 892 Mb (128 Mb difference, representing 15.8% relative to the mean), among natural populations of *R. indica* (Figure 1). This variation exceeds that reported for *Arabidopsis thaliana* (18–23 Mb) (Long et al. 2013; Lian et al. 2024; Igolkina et al. 2025) and other relative *Rorippa* species (Han et al. 2022). Such breadth suggests high cytological diversity within *R. indica*, attributable to several evolutionary

TABLE 2 | Statistics for PGLS models detecting the significant effects of genomic repeat variables on genome size variation across populations of *Rorippa indica*.

Hierarchical group	df	AIC	*F*-Statistic	Adjusted R^2	*p*
rDNA					
45S	2	−71.02	3.38	0.16	**0.04**
5S	2	−68.05	0.40	0.05	0.54
Class I/*Ty1-copia*					
Bianca	2	−72.89	5.59	0.26	**0.04**
SIRE	2	−69.81	1.18	0.01	0.30
TAR	2	−67.92	0.33	0.05	0.58
Ivana	2	−67.74	0.18	0.07	0.68
Tork	2	−67.72	0.16	0.07	0.70
Ale	2	−67.65	5.13	0.07	0.73
Class I/*Ty3-gypsy*					
Tekay	2	−70.49	2.82	0.12	0.12
Retand	2	−68.38	0.78	0.02	0.40
CRM	2	−67.58	0.03	0.08	0.86
Athila	2	−67.58	0.03	0.08	0.86
Galadriel	2	−67.55	0.01	0.08	0.93
Class I/*LINE*	2	−67.61	0.06	0.08	0.81
Class I/Total	2	−67.60	0.05	0.08	0.83
Class II/*TIR*					
hAT	2	−68.92	1.24	0.02	0.29
MuDR Mutator	2	−68.50	0.86	0.01	0.37
PIF Harbinger	2	−67.68	0.13	0.07	0.73
EnSpm CACTA	2	−67.54	0.00	0.08	0.99
Others/*Satellite*	2	−68.97	1.29	0.02	0.28

Note: Values of significant *p*-values by *F*-tests are shown in bold.
Abbreviations: AIC, Akaike information criterion; df, degree of freedom.

processes associated with its polyploid origin and subsequent establishment.

First, the allohexaploid origin of *R. indica* from parents with differing genome sizes provided opportunities for extensive genomic reshuffling. The likely maternal progenitor, *R. dubia*, is itself an allotetraploid that exhibits wide intraspecific genome size variation (Tu et al. 2019; Zheng et al. 2021; Han et al. 2024). Thus, *R. indica* inherited considerable potential for genome size diversity, as exemplified by the relatively large genome of HG-I plants (Figure 2).

Second, polyploidization can trigger "genome shock" (McClintock 1984), whereby relaxed selection on duplicated elements leads to dynamic genome restructuring (Cheng et al. 2018). Consistent with findings in *A. thaliana*, maize, and *Amomum* gingers (Long et al. 2013; Bilinski et al. 2018; Hlavatá et al. 2024; Igolkina et al. 2025), our results indicate that genome size changes in *R. indica* are driven largely by the activity of specific repeats, notably 45S rDNA and *Ty1-copia* elements (Table 2), although coding gene duplications may also contribute. This highlights that particular classes of repetitive elements, rather than overall TE abundance, can disproportionately shape intraspecific genome size diversity (Igolkina et al. 2025).

Third, genome size variation in *R. indica* is unlikely to be simply neutral but instead reflects selective pressures under contrasting environments. Phylogeographic analyses show that HG-I, with ~840 Mb, represents the ancestral haplogroup, from which both northern and southern lineages derived (Figures 2–3). Southern HG-III maintained or expanded genome size, while northern haplogroups (HG-II, -IV, -V) exhibited reductions. This dual pattern supports the hypothesis that both shrinkage and enlargement may represent adaptive strategies under different ecological conditions (Faizullah et al. 2021).

TABLE 3 | Summary of the GLMM analysis for detecting local adaptation of genome size variation in *Rorippa indica*.

		Variables						Random factors			
		Site		GS		Site × GS		Population		Tray	
Life cycles	Traits	Chisq	*p*	Chisq	*p*	Chisq	*p*	Chisq	*p*	Chisq	*p*
Germination	GR	**18.79**	**1.46e-05**	0.70	0.40	2.21	0.14	0	1	0	1
Seedling	SR_10W	**14.25**	**1.60e-04**	0.49	0.49	2.03	0.16	0	1	0	1
	SR_14W	**16.48**	**4.91e-05**	0.01	0.93	0.99	0.32	0	1	0	1
	SR_18W	4.42	0.04	3.32	0.07	0.15	0.70	0	1	0	1
	RD_10W_mean	6.10	0.01	0.17	0.68	0.57	0.45	3.30	0.07	0	1
	RD_14W_mean	**19.71**	**9.00e-06**	1.01	0.31	3.24	0.07	0.66	0.42	0	1
	RD_18W_mean	1.38	0.24	1.03	0.31	3.11	0.08	0	1	0	1
	RD_10W_max	0.75	0.39	0.11	0.74	0.32	0.57	1.66	0.20	0	1
	RD_14W_max	6.15	0.01	1.30	0.25	1.37	0.24	0.16	0.69	0	1
	RD_18W_max	2.76	0.10	0.37	0.54	2.00	0.16	0	1	0	1
	RGR_mean	**16.55**	**4.75e-05**	0.26	0.61	**10.59**	**1.14e-03**	0	1	0	1
	RGR_max	**12.53**	**4.01e-04**	0.06	0.81	8.30	3.97e-03	0	1	0	1
Flowering	FT	**654.14**	**2.00e-16**	**11.81**	**5.90e-04**	0.26	0.61	**10.31**	**1.33e-03**	2.19	0.14
	TN_PS	0.19	0.66	0.00	0.96	0.19	0.66	0	1	0	1
	TL_PS	4.84	0.03	0.00	0.98	7.77	5.30e-03	0.89	0.35	1.67	0.20
	ML_PS	0.04	0.84	0.07	0.79	0.94	0.33	2.99	0.08	3.03	0.08
	PH	0.07	0.79	0.06	0.82	1.67	0.20	2.65	0.10	3.03	0.08
	TN_SI	**12.22**	**4.72e-04**	0.70	0.41	0.06	0.81	0	1	0	1
	TL_SI	**38.53**	**5.39e-10**	3.80	0.05	1.28	0.26	1.67	0.20	0	1
	ML_SI	**18.95**	**1.34e-05**	3.40	0.07	0.07	0.79	0.00	0.97	0	1
	MaxL_SI	**25.52**	**4.38e-07**	3.09	0.08	0.27	0.60	0.25	0.62	0	1
Fruiting	TN_F	**91.18**	**2.00e-16**	0.92	0.34	**9.40**	**2.17e-03**	**50.31**	**1.31e-12**	2.64	0.10

Note: Abbreviation for traits across the life cycles of *Rorippa indica*: (1) germination (GR, germination rate recorded at 7 days after sowing); (2) seedling (SR_10W/14 W/18 W, survival rate recorded at 10/14/18 weeks after sowing; RD_10W/14 W/18 W/_mean/max, mean or maximum rosette size for 10/14/18 weeks-old seedlings; RGR_mean/max, mean or maximum relative growth rate calculated based on RD values); (3) flowering (FT, flowering time; TN/TL/ML_PI, total number/total length/mean length of primary inflorescence; PH, plant height measured as the tallest length of primary inflorescence; TN/TL/ML/MaxL_SI, total number and total/mean/maximum length of secondary inflorescence); and (4) fruiting (TN_F, total number of fruits). Degree of freedom = 1. Values of significant Wald chi-squared statistics (Chisq) and Bonferroni corrected *p*-values ($\alpha = 0.05/22 = 2.27e\text{-}03$) are shown in bold.

Finally, *R. indica* established distinct phylogeographic lineages within the last one million years (Han et al. 2024) (Figures 2–3). Such rapid divergence may have promoted differential resolution of repetitive elements through independent gain or loss, then generating substantial genome size variation despite limited overall polymorphism (Lefebure et al. 2017). The strong associations we observed between genome size and both geography and temperature further point to a potential adaptive role of natural variation in genome size (Figure 1B; Table 1).

4.2 | Temperature as a Significant Driver of Genome Size Variation

Intraspecific genome size variation is widespread across taxa (Šmarda and Bureš 2010; Díez et al. 2013; Bilinski et al. 2018), but its ecological drivers remain debated. In *R. indica*, our analyses revealed that temperature-related variables are major determinants of genome size (Figure 1b; Table 1), highlighting the influence of climate on genomic architecture.

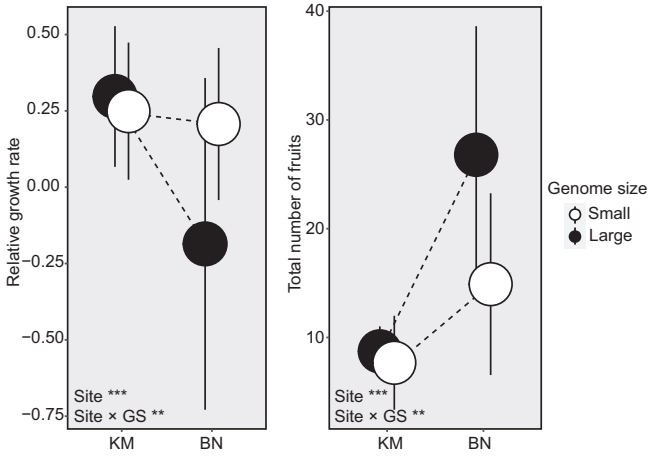

FIGURE 4 | Local adaptation of larger genomes to the tropical environment in *Rorippa indica*. Reaction norms of phenotypic variation in relative growth rate (left) and total number of fruits (right) for small (open dots) and large genomes (closed dots) in two contrasting common gardens: Kunming (KM) and Xishuangbanna (BN). The dashed lines connect the mean values for each trait and genome size type across the two common gardens. Error bars represent the standard deviations. Asterisks indicate statistically significant differences between sites or interactions between site and genome size (Site × GS) (**$p < 0.01$, ***$p < 0.001$), as determined by Bonferroni-corrected Wald chi-squared tests (Table 3).

Links between genome size and temperature are complex. In some organisms, larger genomes are associated with cold environments, such as the Antarctic krill (Shao et al. 2023), certain microbes (Ngugi et al. 2023), and perennial grasses (Simpson et al. 2024), possibly due to slower metabolism and cell cycling. However, this trend is not universal, as life-history strategies and ecological contexts mediate outcomes (Simonin and Roddy 2018; Bureš et al. 2024). Conversely, in several plant systems, larger genomes are favored in warm, stable climates, as observed in *Helianthus* sunflowers (Qiu et al. 2019), wild maize (Díez et al. 2013), tropical gingers (Hlavatá et al. 2023; Xavier et al. 2024; Záveská et al. 2024), and lilies (Carta and Peruzzi 2016).

Several mechanisms may explain the adaptive advantage of larger genomes in such tropical environments. Enlarged genomes can increase genetic diversity, enhance stress tolerance, or alter developmental trajectories, thereby providing raw material for selection (Bennett 1987; Lefebure et al. 2017). For example, relaxed selection on traits such as stomatal size or growth rate may permit greater biomass accumulation and fecundity (Schley et al. 2022). Furthermore, stable warm climates may promote transposable element activity and limit DNA elimination, relaxing the "large genome constraint" and facilitating repeat accumulation (Kelly et al. 2015; Lefebure et al. 2017; Hlavatá et al. 2024). Notably, specific repeats, rather than total TE content, appear to be critical drivers of genome size evolution (Igolkina et al. 2025).

Still, the relationship between genome size and temperature is not strictly linear. Mixed or contrasting patterns have been observed across scales (e.g., Díez et al. 2013; Cacho et al. 2021). These discrepancies highlight the importance of accounting for phylogenetic autocorrelation, demographic history (Table 1; Figures 2–3), and environmental heterogeneity. Importantly, the distribution of *R. indica* haplogroups suggests that genome size variation may reflect both retention of ancestral states in tropical lineages and reduction in temperate ones. Controlled experiments, such as reciprocal transplantations (Table 3; Figure 4), remain essential to directly test the causal fitness effects of genome size under different climatic regimes.

4.3 | Fitness Effects of Genome Size Variation

Our reciprocal transplant experiments demonstrated that larger genomes in *R. indica* confer conditional fitness advantages under tropical conditions. Accessions with larger genomes produced significantly more fruits (Figure 4), suggesting that genome enlargement can enhance reproductive success in newly colonized tropical environments (Setyawati et al. 2015).

Several mechanisms may underlie this advantage. First, larger genomes typically harbor more repetitive elements, providing a reservoir of genetic material that can fuel novel variation via recombination or mutation (Blommaert 2020). Second, genome enlargement may support more complex regulatory networks, enabling fine-tuned gene expression and epigenetic responsiveness to environmental cues (Leitch and Leitch 2008; Satake et al. 2022). Third, in thermally stable tropical environments, energetic and developmental constraints on genome size may be relaxed, allowing the benefits of enlargement to outweigh its costs (Mei et al. 2018; Schley et al. 2022).

At the same time, larger genomes impose disadvantages, including greater replication costs and higher susceptibility to deleterious mutations (Petrov 2002; Lynch and Conery 2003; Francis et al. 2008). Thus, the optimal genome size likely reflects a balance between benefits and costs that shifts depending on ecological context. While our experiments clearly demonstrate fitness advantages of larger genomes under tropical conditions, they capture only part of the adaptive landscape of genome size variation in *R. indica*. Comparative studies across species and environments will be essential to assess the generality of these findings.

5 | Conclusion

By investigating the natural variation of genome size in *R. indica*, we demonstrate that the expansion or contraction of repetitive elements has generated substantial intraspecific diversity. Our findings highlight genome size as a conditionally adaptive trait, shaped by both intrinsic genomic processes and extrinsic climatic factors. This work provides a foundation for exploring how genome architecture contributes to ecological adaptation and underscores the importance of intraspecific perspectives in understanding genome evolution.

Author Contributions

T.-S.H. and Y.-W.X. conceived the project. T.-S.H. collected the data, performed analyses, and drafted the manuscript. Q.-J.Z. performed the

flow cytometry experiment and phylogenetic network analysis. J.-X.L. and S.L. participated in the common garden experiments. Z.-Q.D. analyzed the phenotypic data. All authors reviewed the final manuscript.

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No AI tools were used to generate data, perform analyses, create, or modify figures.

Data Availability Statement

The sequences can be accessed by GenBank IDs: PQ433226–PQ433289. Custom codes can be found on GitHub (https://github.com/Ting-Shen/riGS).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** FCM histograms of genome size variation in *Rorippa indica*. **Figure S2:** FCM-estimated genome size variation in *Rorippa indica* and its relatives. **Figure S3:** Correlation between genome size and bioclimatic or geographic variables. **Figure S4:** Correlation matrix of geographic and bioclimatic variables. **Figure S5:** Bayesian tree of chloroplast haplotypes in *Rorippa indica*. **Figure S6:** Experimental test of the fitness effect of larger genomes. **Figure S7:** Traits collected for plants with different genome sizes. **Figure S1:** FCM histograms of genome size variation in *Rorippa indica*. **Figure S2:** FCM-estimated genome size variation in *Rorippa indica* and its relatives. **Figure S3:** Correlation between genome size and bioclimatic or geographic variables. **Figure S4:** Correlation matrix of geographic and bioclimatic variables. Pairwise Pearson correlation coefficients among geographic factors (latitude, longitude, altitude) and 19 bioclimatic variables (bio01–bio19) are shown. Positive correlations are represented in red and negative correlations in blue, with color intensity proportional to the correlation coefficient (see scale bar). Variables are clustered by hierarchical clustering to group those with similar correlation patterns. **Figure S5:** Bayesian tree of chloroplast haplotypes in *Rorippa indica*. **Figure S6:** Experimental test of the fitness effect of larger genomes. **Figure S7:** Traits collected for plants with different genome sizes. **Table S1:** Repetitive DNA content. **Table S2:** PGLS transformation structure of the covariance matrix.