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LIMNOLOGY and OCEANOGRAPHY

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Limnol. Oceanogr. 71, 2026, e70498
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doi: 10.1002/lno.70498

RESEARCH ARTICLE

Genomic adaptation of the water flea *Daphnia* to elevated temperatures: Insights from power plant-heated lakes

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Abstract

Global warming increasingly threatens freshwater biodiversity, highlighting the need to understand how key ecological interactors, such as the zooplankton water flea *Daphnia*, genetically adapt to rising temperatures. Although local thermal adaptation has been documented in *Daphnia*, the underlying genomic changes remain poorly resolved. Here, we examined genomes of *Daphnia cucullata* (a member of the *D. longispina* complex) from five lakes artificially warmed for six decades by cooling water from power plants (increase of about 2–4°C), and from seven adjacent, control (i.e., unheated) lakes. Heated-lake and unheated-lake populations showed pronounced genomic divergence. Using a Hidden Markov Model framework, we identified highly differentiated genomic intervals (HDGIs) between the two groups, which were significantly enriched near 188 thermal-adaptation candidate genes, supporting a polygenic basis of thermal adaptation. Contrary to previous studies on the *D. longispina* complex that identified hybridization as a key driver of local adaptation, we found no evidence for adaptive introgression in the heated lakes, despite the presence of *D. cucullata* × *D. galeata* hybrids. Adaptive potential, measured as cumulative allelic richness across the set of differentiated loci overlapping or located near thermal adaptation candidate genes, was high across all populations. Strikingly, shifts of formerly rare alleles toward intermediate frequencies have led to a higher genetic diversity at adaptive loci in heated-lake than in unheated-lake populations. Our results identify specific loci associated with thermal adaptation in *D. cucullata*, demonstrate that adaptation has occurred independently of hybridization, and underscore the importance of standing genetic variation in local populations for adaptive evolution to warming.

Global warming poses major challenges to natural systems, affecting individuals, populations, communities, and ecosystems (Scheffers et al. 2016). Freshwater biodiversity is particularly vulnerable because freshwater habitats are spatially fragmented, integrate multiple human stressors, and are exposed to interacting pressures such as warming, eutrophication, pollution, hydrological alteration, and biological invasions (Reid et al. 2019). While rapid environmental change can impose intense selection, fragmented landscapes may restrict gene flow and limit access to adaptive genetic variation in local populations. Understanding both the capacity for and the mechanisms underlying thermal adaptation is therefore crucial for persistence and biodiversity maintenance, and for predicting responses to climate warming and informing conservation strategies (De Meester et al. 2011; Stoks et al. 2014). In this context, genomic approaches provide valuable insights into the molecular basis of adaptation and the adaptive potential of natural populations.

Adaptive responses to environmental change can draw on several sources of genetic variation, each leaving distinct

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Associate editor: Michaela M. Salcher

genomic signatures. De novo mutations introduce novel beneficial variants, but their contribution to rapid adaptation is often limited on ecological timescales because it depends on the supply of new mutations—set by the mutation rate and effective population size—and on the strength of selection (Hermisson and Pennings 2005). In contrast, adaptation from standing genetic variation can proceed more rapidly because beneficial alleles are already present before environmental change, and is characterized by allele-frequency shifts at pre-existing polymorphisms, often without strong reductions in genetic diversity (Barrett and Schluter 2008; Pritchard et al. 2010). Introgression can further facilitate rapid adaptive evolution by introducing beneficial alleles from related taxa (Hedrick 2013) and is often inferred from localized genomic regions with excess donor ancestry, reduced genetic distance between recipient and donor populations, and discordant genomic topologies (Martin et al. 2015; Martin and Van Belleghem 2017). In contrast to adaptation driven by a few major-effect loci, adaptation might be polygenic, involving coordinated allele-frequency shifts across many loci of small effect distributed throughout the genome (Pritchard et al. 2010; Barghi et al. 2020). Distinguishing among these mechanisms therefore requires combining genome-wide scans for differentiation, analyses of genetic diversity and allelic richness, and explicit tests for introgression.

The zooplankton water flea *Daphnia* is an excellent model for studying the genomic underpinnings of thermal adaptation. As a dominant phytoplankton grazer and key prey item for planktivorous fish and invertebrates, *Daphnia* plays a crucial role in lake and pond food webs. It is a long-established model for ecological, evolutionary, and ecotoxicological research and has more recently become a prominent system in environmental genomics (Ebert 2022). Both field and laboratory studies show that *Daphnia* can respond to warming and local thermal regimes via phenotypic plasticity and evolution (Brans et al. 2017; Seefeldt and Ebert 2019; Dziuba et al. 2021). Although candidate genes associated with thermal adaptation have been identified in *Daphnia* (Geerts et al. 2015; Jansen et al. 2017; Herrmann and Yampolsky 2021), the spatial distribution of adaptive variation and the underlying genomic architecture—often assumed to be polygenic (Barghi et al. 2020)—remain poorly understood. This gap is critical because the distribution of adaptive variation within the metapopulation, the regional pool of populations connected by gene flow, may strongly influence long-term resilience to warming.

Artificially heated lakes near Konin, Poland (Fig. 1a), provide a unique natural experiment to investigate thermal adaptation in *Daphnia* (Dziuba et al. 2020, 2021). These lakes have experienced a sustained 2–4°C temperature increase for six decades due to thermal discharge from power plants, matching projections for late 21st century warming under high-emission scenarios (IPCC 2021). The heated lakes are interconnected by canals (Zdanowski et al. 2020), allowing potential dispersal among sites. Long-term warming has led to ice-free winters and triggered evolutionary adaptations in *Daphnia galeata*, including increased body size that enhances overwinter survival and early-spring competitiveness (Dziuba et al. 2021). It has also altered *Daphnia* community composition, host–parasite interactions, and plankton and fish assemblages (Kapusta and Bogacka-Kapusta 2015; Dziuba et al. 2023; Beng et al. 2025). These changes might interact with thermal selection and jointly shape evolutionary responses in resident populations. Despite these ecosystem changes, the sustained 2–4°C temperature difference between heated and control (i.e., unheated) lakes provides a unique opportunity to investigate genomic signatures of long-term warming under natural conditions. Throughout this paper, we use "unheated" and "control" interchangeably to refer to reference lakes not receiving thermal discharge.

Three species from the *Daphnia longispina* complex coexist in these lakes: *D. cucullata*, *D. galeata*, and *D. longispina* (Dziuba et al. 2020). Hybridization is common within the complex and often associated with environmental disturbance such as eutrophication or warming (Keller et al. 2008; Brede et al. 2009). Within the complex, *D. galeata* is generally associated with warmer, more eutrophic habitats, whereas *D. cucullata* is often less dominant under such conditions (Keller et al. 2008). In the Konin heated lakes, long-term warming has been accompanied by substantial shifts in the relative abundance of *D. galeata* and *D. cucullata*, with *D. cucullata* becoming dominant in recent years (Dziuba et al. 2020). Given the ecological association of *D. galeata* with warm environments, frequent hybridization in the complex, and documented *D. cucullata* × *D. galeata* hybrids in our studied lakes (Dziuba et al. 2020), adaptive introgression from *D. galeata* into *D. cucullata* is a plausible mechanism facilitating thermal adaptation. While adaptive introgression—the transfer of beneficial alleles between taxa through hybridization—has been shown to facilitate adaptation in diverse organisms, such as hypoxia tolerance in Tibetan dogs (Miao et al. 2016) and pesticide resistance in mosquitoes (Norris et al. 2015), its role in *Daphnia* responses to environmental change remains largely unexplored.

Here, we conducted a landscape genomic analysis of *D. cucullata* populations from artificially heated and neighboring unheated lakes near Konin, Poland. We focused on *D. cucullata* because it is consistently present across all lakes, enabling direct comparisons. We tested three hypotheses regarding genomic responses to long-term warming. First, we hypothesized that sustained warming would generate detectable genomic differentiation consistent with thermal adaptation, that is, involving candidate loci that have been shown to be linked to thermal adaptation in other model systems and studies (H1). Second, we hypothesized that the observed genetic differentiation is primarily fueled by standing genetic variation and reflects a polygenic architecture (H2). Thermal adaptation is expected to involve many loci with small individual effects (Dayan et al. 2019; Herrmann and Yampolsky 2021). Consistent with the presence of standing genetic variation, Chaturvedi et al. (2021) demonstrated that multi-trait adaptation to novel predation pressure in *Daphnia*

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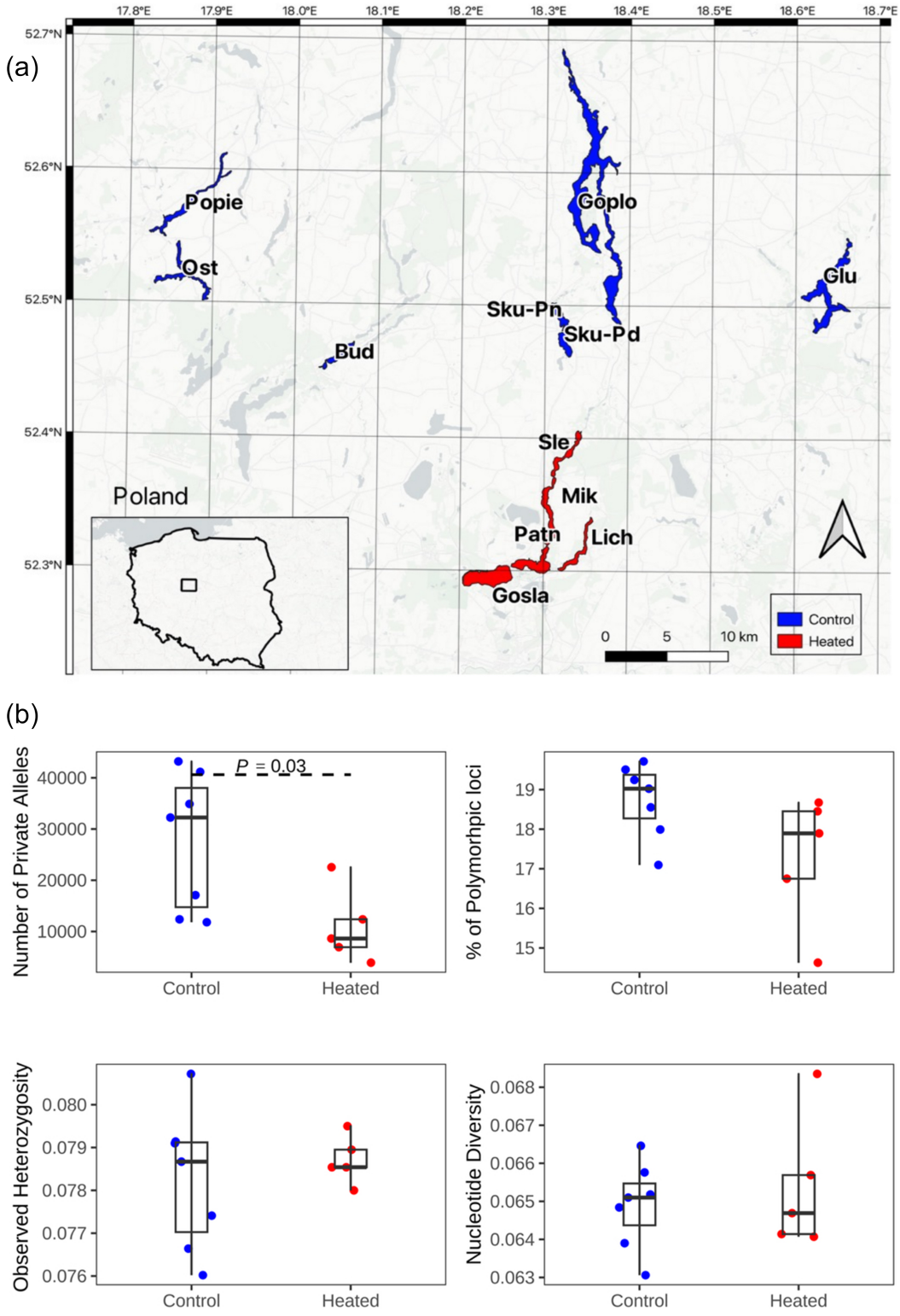

Fig. 1. (**a**) Locations of lakes where *Daphnia cucullata* populations were sampled. For lake labels, *see* Supporting Information Table S1. (**b**) Comparison of population genetic diversity between unheated-lake and heated-lake populations based on the *SNP_whole* dataset. Four metrics were calculated: number of private alleles, percentage of polymorphic loci, observed heterozygosity, and nucleotide diversity. Significant differences between unheated-lake and heated-lake populations are indicated by dashed lines with corresponding *p* values (Mann–Whitney *U* test). Colors are consistent across all plots: red for heated-lake populations and blue for unheated-lake populations.

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was driven entirely by standing local genetic variation without detectable loss of diversity. Under this hypothesis of polygenic standing genetic variation, alleles associated with thermal adaptation in heated-lake populations should also be present in unheated-lake populations or the regional metapopulation. We also expect that the set of thermal adaptation candidate loci would collectively retain substantial allelic richness in heated-lake populations, rather than showing pronounced diversity loss characteristic of adaptation driven by a limited number of de novo mutations or hard selective sweeps (Hermisson and Pennings 2017).

Third, we hypothesized that adaptive introgression contributed to thermal adaptation (H3). Introgression can introduce adaptive alleles at higher initial frequencies than de novo mutations and might complement standing genetic variation (Hedrick 2013). Although introgression is common among *D. galeata*, *D. cucullata*, and *D. longispina* in the *D. longispina* species complex (Nickel et al. 2021), its role in thermal adaptation remains largely unexplored. Given frequent hybridization among *D. galeata*, *D. cucullata*, and *D. longispina* (Brede et al. 2009; Griebel et al. 2015), adaptive introgression might have contributed to responses in the heated lakes. Under this scenario, thermally differentiated genomic regions should show excess *D. galeata* ancestry in *D. cucullata* from heated lakes, reduced genetic distance between *D. cucullata* from heated lakes and *D. galeata* compared with *D. cucullata* from unheated lakes and *D. galeata*, and phylogenetic patterns grouping *D. cucullata* from heated lakes with *D. galeata* rather than with *D. cucullata* from unheated lakes for specific genomic regions (Martin et al. 2015; Martin and Van Belleghem 2017). Conversely, if hybridization occurred without adaptive contribution, hybrid individuals might be present, but introgression signals should not be enriched in thermally divergent genomic intervals.

Methods

Sample collection and DNA extraction

In October 2022, zooplankton was collected from 12 lakes near Konin, Poland (Fig. 1a; Supporting Information Table S1) using a 100 μm plankton net. Samples were preserved in 95% p.a. ethanol and stored at 4°C. Individual *Daphnia* were initially identified morphologically under a stereomicroscope following Benzie (2005), based on diagnostic characters of the *D. longispina* complex, including head shape, helmet development, rostrum morphology, body outline, and tail spine morphology. Individuals with intermediate morphological characters were provisionally classified as putative hybrids. Species assignment was subsequently validated using mitochondrial and nuclear data and published reference genomes representing the parental species *D. cucullata*, *D. galeata*, and *D. longispina* (Nickel et al. 2021).

Daphnia cucullata specimens were picked under a microscope, rinsed with ultrapure water, and DNA was extracted using the GeneJET Genomic DNA Purification kit. DNA was sequenced at BGI HK on the DNB-seq platform targeting ~ 35× coverage. Six to eight individuals were sequenced per lake (*see* Supporting Information Table S1 for the exact number of individuals sequenced and retained per lake), with fewer individuals from Bud, Patn, and Goplo due to library preparation failures. Because different analyses required different subsets of samples, final sample sizes varied among analyses (Supporting Information Table S3). Sequencing initially used PE150 bp reads but was later switched to PE100 bp reads due to short insert sizes caused by endonuclease digestion (Supporting Information Fig. S1). Additionally, we sequenced 14 individuals showing intermediate characters between *D. cucullata* and *D. galeata* (6 from Gosla, 6 from Patn, and 2 from Mik) to characterize hybrids.

Mapping, SNP calling, and filtering

Reads were quality-filtered with Fastp (Chen et al. 2018) using default parameters. All samples were mapped to the *D. galeata* reference genome assembly DGM5 (Dgal_M5_v1; GCA_918697745.1; Nickel et al. 2021), which represented the best available reference for this species complex at the time of this study. We used Bwa-Mem2 (Li 2013) for indexing and mapping, followed by duplicate removal with Samblaster (Faust and Hall 2014) and Samtools (Danecek et al. 2021). We combined our data (92 *D. cucullata* and 14 putative hybrid individuals) with sequences of 17 individuals (Supporting Information Table S2) downloaded from the Sequence Read Archive (SRA) representing reference parental species (*D. longispina*, *D. cucullata*, and *D. galeata*) and *D. cucullata* × *D. galeata* hybrids. Reads with a mapping quality below 20 were excluded from single nucleotide polymorphism (SNP) calling. We used HaplotypeCaller from the Genome Analysis Toolkit (GATK) (McKenna et al. 2010) for SNP calling on individual whole-genome sequencing datasets. The resulting GVCF files were combined with CombineGVCF and subjected to multi-sample SNP calling using GenotypeGVCF. Consistent with the evolutionary divergence between *D. cucullata* and *D. galeata*, the coverages of the DGM5 assembly by *D. cucullata* reads were on average 81.5%, while the average coverage by *D. galeata* sequencing datasets was 93.0% (*see* Data File 1 in the Zenodo repository of this study). We may therefore have missed roughly 11.5% of SNPs but because all *D. cucullata* individuals were equally affected, the relative comparisons between heated- and unheated-lake populations—the core of our study—should not be systematically biased.

After SNP calling, we removed loci with more than two alleles and those failing any of the following quality criteria: Fisher Strand bias > 60.0, Strand Odds Ratio > 3.0, Root Mean Square Mapping Quality < 40.0, Mapping Quality Rank Sum Test < −12.5, QualByDepth < 2.0, Read Position Rank Sum Test < −8.0, and variant confidence/quality score < 30.0, following GATK best-practice recommendations (DePristo et al. 2011). Singletons were excluded, and genotypes with a coverage of less than three reads per sample, or a genotype

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quality score below 20 were set to missing. Additional filtering removed loci in the upper 95% depth quantile, loci with > 20% missing data, samples with > 40% missing genotypes, and loci within repeats or CpG islands. We used RepeatModeler (Flynn et al. 2020) to identify and model repeat families, and subsequently applied RepeatMasker (Tarailo-Graovac and Chen 2009) to annotate the repetitive regions. CpG islands were predicted using CpGIScan (Fan et al. 2017). The variants in the final SNP dataset were categorized into types such as intron variant, missense variant, and synonymous variant using snpEff (Cingolani et al. 2012). The resulting dataset (*SNP_whole*) and subsets were used for all subsequent analyses.

Population genomic structure

Population structure was assessed using discriminant analysis of principal components (DAPC) (Jombart et al. 2010), which maximizes among-group variation through discriminant functions. Putative hybrid individuals were excluded from this analysis because they would strongly reduce variation among pure *D. cucullata* populations, obscuring finer-scale intraspecific structure. Fourfold degenerate sites were extracted from the *SNP_whole* dataset using a custom Python script and pruned with PLINK (Purcell et al. 2007), yielding the *SNP_4dtv_dcucu* dataset used as input for DAPC. The DAPC analysis was performed by grouping the *Daphnia* samples by lake, and the number of principal components was determined through cross-validation (xvalDapc function of the adegenet package in R). As reads of different lengths can differ in mappability, we performed an unsupervised PCA using the *SNP_4dtv_dcucu* dataset with PLINK to assess potential batch effects associated with differences in sequencing read length (PE100 bp vs. PE150 bp).

Genomic diversity

Within-population genomic diversity was calculated using Stacks (Catchen et al. 2013). Using the *SNP_whole* dataset, we calculated observed heterozygosity, the proportion of polymorphic loci, nucleotide diversity (π), and the number of private alleles per population. To evaluate diversity at putatively adaptive loci, we repeated these analyses using the *SNP_thermal_outlier* dataset, which includes only those SNPs from HDGIs that also fall within or near (≤ 10 kb) thermal candidate genes (*see* Identifying signals of thermal adaptation section for details). Differences between heated-lake and unheated-lake populations were assessed using Mann–Whitney *U* tests.

Mitochondrial and nuclear phylogeny

Consensus mitochondrial genomes were generated for 106 newly sequenced individuals and 17 publicly available genomes from the SRA, using the *D. galeata* mitochondrial reference genome (OM397534.1; Choi et al. 2023). SNP calling followed the pipeline described in Mapping, SNP calling, and filtering section, with consensus calling via bcftools commands: mpileup, call, and consensus (Danecek et al. 2021). A phylogeny based on the whole mitochondrial genome was inferred in IQ-TREE2 (Minh et al. 2020), with the best-fitting model (TIM + F + I + R4, as detailed in the IQ-TREE2 manual) selected with ModelFinder (Kalyaanamoorthy et al. 2017). Branch support was assessed with 1000 ultra-fast bootstrap replicates and 1000 SH-like approximate likelihood ratio tests. Trees were visualized with iTOL (Letunic and Bork 2021).

For nuclear phylogeny, fourfold degenerate sites were extracted from the *SNP_whole* dataset (*SNP_4dtv_phylogeny* dataset) using a custom Python script. Unlike *SNP_4dtv_dcucu* dataset described in the Population structure section, all samples from the *SNP_whole* dataset were retained after filtering. The nuclear phylogeny was inferred using the calc_tree function from the snpR package (Hemstrom and Jones 2023). Genetic distances were calculated using Edwards' Angular distance (Edwards 1971), and the resulting distance matrix was used to construct a Neighbor-Joining phylogeny with branch support assessed from 1000 bootstrap replicates.

Identifying signals of thermal adaptation

We retained high-quality SNPs (hereafter referred to as *SNP_HighQuality* dataset) from the *SNP_whole* dataset by selecting variants with a minor allele frequency (MAF) > 0.05 and a missing genotype call rate threshold of < 0.1 (i.e., excluding SNPs with more than 10% missing genotype calls across all individuals). Next, for each population, we used KING (Manichaikul et al. 2010) to infer KING-robust coefficients and excluded individuals with a higher number of missing genotypes from all pairs with kinship coefficients > 0.354—the expected value for genetically identical individuals (e.g., monozygotic twins or members of the same clone), as defined by KING.

To test the thermal adaptation hypothesis (H1), we combined candidate gene and outlier-based approaches. Because long-term heating in the Konin lakes is accompanied by broader ecological changes (Kapusta and Bogacka-Kapusta 2015; Dziuba et al. 2023; Beng et al. 2025), we used this strategy to increase the specificity of our inference toward temperature-related adaptation. In brief, outlier analyses identify genomic regions differentiated between heated and unheated lakes, whereas overlap with independently defined thermal candidate genes provides functional support for an interpretation in terms of thermal adaptation. Thermal adaptation candidate genes were derived from the compilation of previously reported thermal-response genes in Herrmann and Yampolsky (2021). Because this compilation includes genes supported by different evidence types, we applied a conservative filter and retained only genes reported in at least three independent genotyping-based studies summarized in that review, thereby reducing false positives. The retained candidate gene symbols are listed in Data File 2. Gene symbols were converted to FBID_KEYs using the ID Validator tool from FlyBase, and corresponding protein sequences were retrieved from FlyBase (R6.15). Using these sequences, we constructed a BLAST database and performed BLASTp searches (Camacho et al. 2009) with protein sequences

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taken from the annotated reference genome of *D. galeata* we utilized (DGM5). Proteins from DGM5 with hits with e-values lower than 10^{-10} were considered thermal candidate genes of *D. galeata*. Loci within candidate gene regions were extracted from the *SNP_whole* dataset using bedtools to form the *SNP_thermal* dataset, which comprises all SNPs within thermal candidate gene regions regardless of differentiation state.

Highly differentiated genomic regions between the heated and unheated-lake populations were identified following the approach of Chaturvedi et al. (2021) using the *SNP_HighQuality* dataset as input. Briefly, we combined variation partitioning with Hidden Markov Models (HMMs) to identify genomic intervals exhibiting significant differentiation between heated and unheated-lake populations. Hierarchical F-statistics (Yang 1998) partition genomic variation into three levels—individuals, populations, and population types (or groups). We quantified the component of variation attributable to differences between the unheated and heated-lake populations using the *varcomp* function in the HIERFSTAT package (Goudet 2004). This component corresponds to the third hierarchical level and was subsequently modeled with an HMM featuring three hidden states—low, moderate, and high differentiation—each linked to an observed emission value. We applied the HMMs following the approach of Soria-Carrasco et al. (2014), assuming a Gaussian distribution of variation between the two lake types for each state. The transition matrix was designed to prevent direct transitions from the low to high differentiation state. The HMM was constructed and optimized with depmixS4 (Visser and Speekenbrink 2010) using the Expectation–Maximization algorithm, and the hidden states of each SNP locus were reconstructed using the Viterbi algorithm. Genomic regions with at least three consecutive highly differentiated loci (identified by the HMM) were classified as HDGIs. We assessed the significance of differentiation between the heated and unheated groups using a permutation test (with the test.between function from the HIERFSTAT package, where the group identities of *D. cucullata* populations were permuted 1000 times). Genomic intervals showing significant differentiation ($p < 0.01$) were classified as HDGIs.

We calculated the distance between HDGIs and their nearest thermal adaptation candidate genes. HDGIs that overlapped with or were located within 10 kb of a thermal candidate gene were defined as thermal candidate genomic intervals. The associated genes are hereafter referred to as diverging thermal candidate genes. High-quality SNPs (*SNP_HighQuality* dataset) falling within thermal candidate genomic intervals were used to define a subset, *SNP_thermal_outlier* dataset, which represents variants showing substantial divergence between heated and unheated lakes and are potentially linked to thermal adaptation.

We applied a randomization test for the association between thermal adaptation candidate genes and HDGIs. First, we quantified the number of thermal adaptation candidate genes located within a 10 kb distance of HDGIs ($N_{obs}^{thermal_genes}$). Enrichment was calculated as the proportion of thermal adaptation candidate genes among genes located within 10 kb of HDGIs divided by the proportion of thermal adaptation candidate genes in the genomic background. Second, we randomly sampled the same number of loci as HDGIs from the *SNP_HighQuality* dataset, constructed genomic intervals with the same width profiles as HDGIs, and recorded the number of thermal adaptation candidate genes located within 10 kb of those genomic intervals. Repeating this 1000 times generated a null distribution, which we summarize with the 95% randomization interval (2.5^{th}–97.5^{th} percentiles). A one-sided empirical p value was calculated as the proportion of randomizations in which the randomized count equaled or exceeded the observed count; enrichment was considered significant at $p < 0.01$.

The HMM-based approach leverages the spatial clustering of differentiated SNPs along chromosomes and is therefore sensitive for detecting extended genomic regions potentially affected by selection (Soria-Carrasco et al. 2014). However, because this method does not explicitly model population structure, some differentiated regions may also reflect demographic history or background population divergence (Lotterhos and Whitlock 2014). We therefore additionally used BayEnv2 (Günther and Coop 2013), which explicitly incorporates neutral covariance structure among populations and therefore provides a more conservative test for temperature-associated allele frequency shifts. Because the two approaches differ in sensitivity, genomic resolution, and treatment of population covariance, we interpreted BayEnv2 outliers and their overlap with HDGIs as conservative support for temperature-associated divergence rather than expecting extensive one-to-one correspondence. We executed BayEnv2 to identify outlier loci from the *SNP_HighQuality* dataset, with the neutral background explicitly modeled. The covariance matrix was estimated using *SNP_4dtv_dcucu* dataset. We combined both Bayes factors (> 3) and Spearman's ρ (top 95%) to select temperature-adaptation-related outliers. The overlap between HDGIs located within 10 kb of thermal candidate genes and the outlier loci identified by BayEnv2 was quantified, and its significance was evaluated using the same randomization procedure described earlier.

Finally, we conducted functional enrichment analysis of diverging thermal candidate genes to detect enriched KEGG pathways and GO terms across Molecular Function, Cellular Component, and Biological Process categories. We first ran GO and KEGG annotation of the protein sequences from DGM5 assembly with eggNOG (Huerta-Cepas et al. 2019). We built an AnnotationDb based on the annotation result with AnnotationForge (Carlson and Pagès 2019). Lastly, we executed the enrichment analysis with clusterProfiler (Wu et al. 2021) with the p values corrected using BH correction and a q value cutoff set to 0.05.

Quantifying standing genetic variation for outlier loci

To test whether adaptation is fueled by standing genetic variation (H2), we quantified genomic variation within individual populations and population groups (heated vs. unheated) using

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rarefaction analysis following Chaturvedi et al. (2021). Random subsets of individuals were drawn from each population or group, and for each subset we calculated the proportion of alleles in the *SNP_thermal_outlier* dataset (derived from heated-lake populations) that could be recovered. Per population subsamples included 5–8 individuals, while group level analyses used 29 individuals (the post-filtering sample size in the heated group).

To further characterize allele frequency patterns at putatively adaptive loci, we used VCFTOOLS (Danecek et al. 2011) to calculate Tajima's *D* and heterozygosity excess from the *SNP_thermal_outlier* dataset. Tajima's *D* was calculated for each contig within each population. To test for differences in Tajima's *D* between populations from heated and unheated lakes, we fitted a linear mixed-effects model with lake type (heated vs. unheated, i.e., control) as a fixed effect and population and contig as random intercepts, accounting for the non-independence of multiple contig-level estimates within populations. Significance was assessed using Satterthwaite's approximation for degrees of freedom as implemented in the lmerTest package (Kuznetsova et al. 2017). For heterozygosity, we calculated the proportion of loci showing significant heterozygosity excess per population and compared populations from heated and unheated lakes using Mann–Whitney *U* tests.

Introgression analysis

To test the adaptive introgression hypothesis (H3), hybridization was first assessed with admixture analysis. Known ancestry data (Nickel et al. 2021)—comprising three pure *D. cucullata*, six *D. galeata*, and three *D. longispina* individuals—was used to estimate the ancestry profiles of focal samples with ADMIXTURE (Alexander et al. 2009). Closely related individuals were excluded from the *SNP_4dtv_phylogeny* dataset based on KING kinship estimates (*see* Identifying signals of thermal adaptation section), and a filtered *SNP_4dtv_admixture* dataset was generated. The number of ancestral populations was set to $K = 3$, corresponding to the three species. Results were visualized with pophelper (Francis 2016).

To detect introgression from *D. galeata* into *D. cucullata* from heated lakes, we calculated window-based statistics and complementary measures of local phylogenetic relationships and absolute divergence. We estimated *D*, fd, and fdM using Dsuite (Dinvestigate) and the topology (((unheated-lake *D. cucullata*, heated-lake *D. cucullata*), *D. galeata*), *D. longispina*), with *D. longispina* as outgroup (Durand et al. 2011; Malinsky et al. 2021). We favored fd and fdM over *D* for identifying candidate regions because they are better suited to detecting local introgression, and because *Z*-scores for individual *D* values cannot be robustly estimated from single sliding windows (Martin et al. 2015; Malinsky et al. 2021). Window-based *D* values were therefore used only to assess the direction of allele-sharing asymmetry. Candidate introgressed windows were defined as those in which fd and fdM fell within the upper 5% of their genome-wide empirical distributions, with *D* indicating excess allele sharing between *D. cucullata* from heated lakes and *D. galeata*.

To further evaluate whether candidate windows were consistent with introgression rather than stochastic variation in allele sharing, we combined *D*, fd, and fdM with topology weighting and absolute sequence divergence (dxy). Topology weighting was performed with Twisst2 (Martin and Van Belleghem 2017) and Sticcs (https://github.com/simonhmartin/sticcs) based on *SNP_whole* dataset, quantifying support for alternative local phylogenetic relationships among unheated-lake *D. cucullata*, heated-lake *D. cucullata*, *D. galeata*, and *D. longispina*. We specifically examined whether candidate windows showed increased support for a topology grouping *D. cucullata* from heated-lake population with *D. galeata*. Absolute divergence (dxy) between heated-lake *D. cucullata* and *D. galeata* was calculated using pixy, combining *SNP_whole* dataset with invariant sites as recommended (Korunes and Samuk 2021), to test for reduced absolute divergence between the putative recipient and donor lineages that would reflect an introgression event (Martin et al. 2015).

We interpreted introgression signals conservatively: a window was considered to provide evidence for introgression only when multiple lines of evidence were concordant, namely an elevated fd and/or fdM, *D* in the expected direction, increased support for a grouping of *D. cucullata* from heated lakes with *D. galeata*, and reduced dxy between heated-lake *D. cucullata* and *D. galeata*.

Results

We sequenced 92 *Daphnia cucullata* individuals and 14 *D. cucullata* × *D. galeata* hybrids, achieving a minimum coverage of 22.7 × per individual. Together with 17 publicly available whole-genome datasets from the SRA, this yielded 3,791,331 high-confidence biallelic SNPs (*SNP_whole* dataset) after filtering. Fourteen individuals were removed from the dataset due to high missing ratios, resulting in 109 individuals in the *SNP_whole* dataset (per-lake breakdown in Supporting Information Table S1). PCA revealed that samples clustered by population identity and thermal regime rather than read length, indicating no detectable batch effects associated with the change in sequencing mode from PE150 bp to PE100 bp reads (interactive PCA plot in Data File 3).

Diversity and structure of populations

Genome-wide diversity (observed heterozygosity, proportion of polymorphic loci and nucleotide diversity π) was comparable between unheated-lake and heated-lake populations (Fig. 1b). However, heated-lake populations contained significantly fewer private alleles ($p = 0.03$, df = 10), with an average of 10,875 private alleles compared to 27,537 in unheated-lake populations. This pattern persisted after excluding Mik and Sle, the two populations with the fewest sequenced individuals. Discriminant analysis of principal components (DAPC;

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Fig. 2) revealed a clear separation between heated and unheated-lake populations, with heated-lake populations forming a more tightly clustered group, whereas unheated-lake populations were more dispersed. This pattern was supported by moderate genome-wide differentiation between lake types as detected by AMOVA ($F_{ST} = 0.0801$), with 8% of the total genetic variation attributable to lake type ($p < 0.01$; Supporting Information Table S4).

Genomic signals of thermal adaptation (H1)

From the *SNP_HighQuality* dataset (560,144 SNPs from 75 *D. cucullata* individuals), the HMM classified 52.4% of SNPs to low, 39.2% to intermediate, and 8.4% to high differentiation states (emission curve in Supporting Information Fig. S2). We identified 4910 genomic intervals containing at least three consecutive highly differentiated SNPs. Of these, 3364 intervals (58.5%) showed significant divergence ($p < 0.01$) and were designated HDGIs. Among HDGIs, 119 overlapped with and 365 fell within 10 kb of thermal candidate genes. These regions are associated with 87 and 188 candidate thermal genes (Data File 4), out of the 481 candidate thermal genes identified, respectively. Genome-wide, 2199 of 17,514 annotated genes (12.6%) fell within 10 kb of HDGIs, providing a baseline proximity rate. Of the 481 thermal candidate genes, 188 (39.1%) fell within this distance—a 3.11-fold enrichment over the genome-wide baseline. Randomization (1000 iterations of 3364 genomic intervals matching the size profile of the HDGIs) confirmed that the observed count of 188 exceeded the expectation by chance (mean: 113.8; 95% randomization interval: 100–127; empirical $p = 0$). Similarly, 12 of the 250 BayEnv2 outlier loci overlapped with HDGIs that were located within 10 kb of thermal candidate genes. Randomization again showed that this overlap was greater than expected by chance (mean: 1.9; 95% randomization interval: 0–5; empirical $p = 0$), supporting the presence of temperature-associated divergent selection within the genomic regions differentiating populations from heated and unheated lakes.

Distribution of adaptive genomic variation (H2)

To assess genomic diversity at putatively adaptive loci, we quantified the percentage of polymorphic loci, observed heterozygosity, and nucleotide diversity for outlier loci near or overlapping with thermal candidate genes (*SNP_thermal_outlier* dataset). All three metrics were significantly higher in the heated-lake populations (Fig. 3a). No population-specific private alleles were detected.

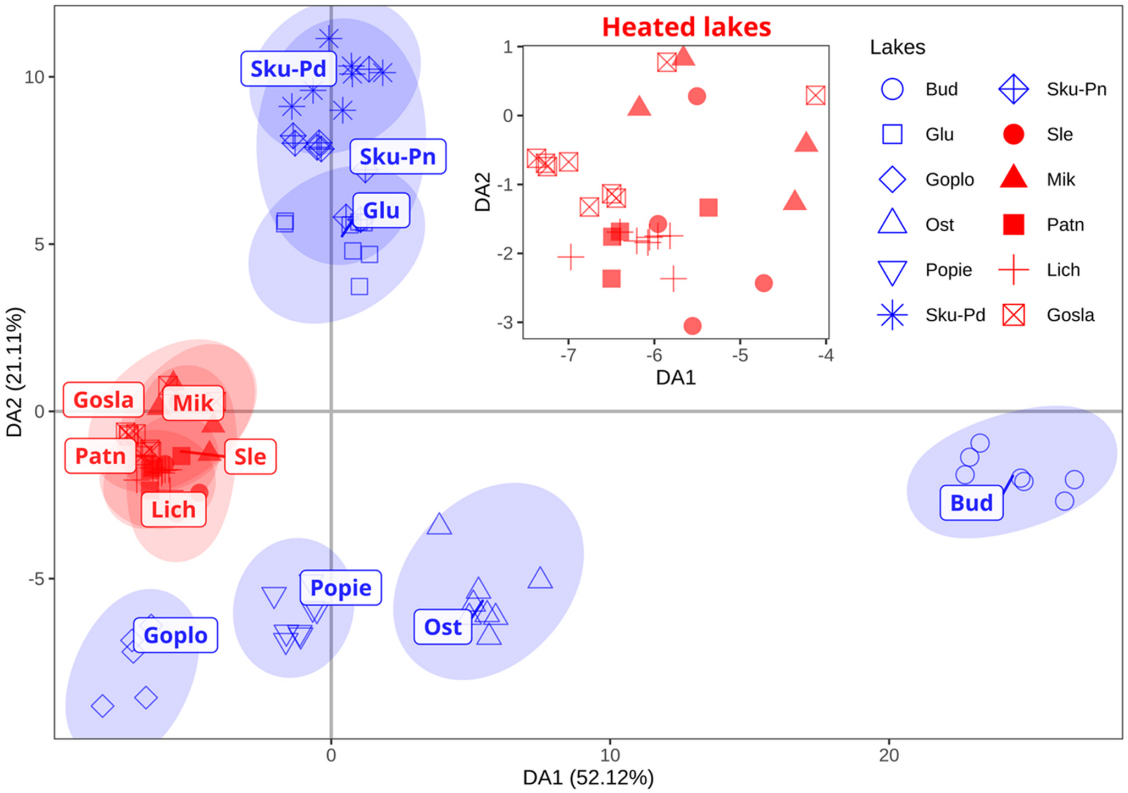

Fig. 2. Discriminant analysis of principal components (DAPC) based on neutral SNPs (*SNP_4dtv_dcucu* dataset) for 12 *Daphnia cucullata* populations. Individuals from the same population are enclosed within ellipses. Populations from heated lakes form a distinct cluster, clearly separated from those inhabiting unheated lakes. The inset displays the clustering of individuals from heated lakes. Eigenvalue ratios indicate the relative discriminatory power of the first two discriminant axes: DA1 = 52.12% and DA2 = 21.11%.

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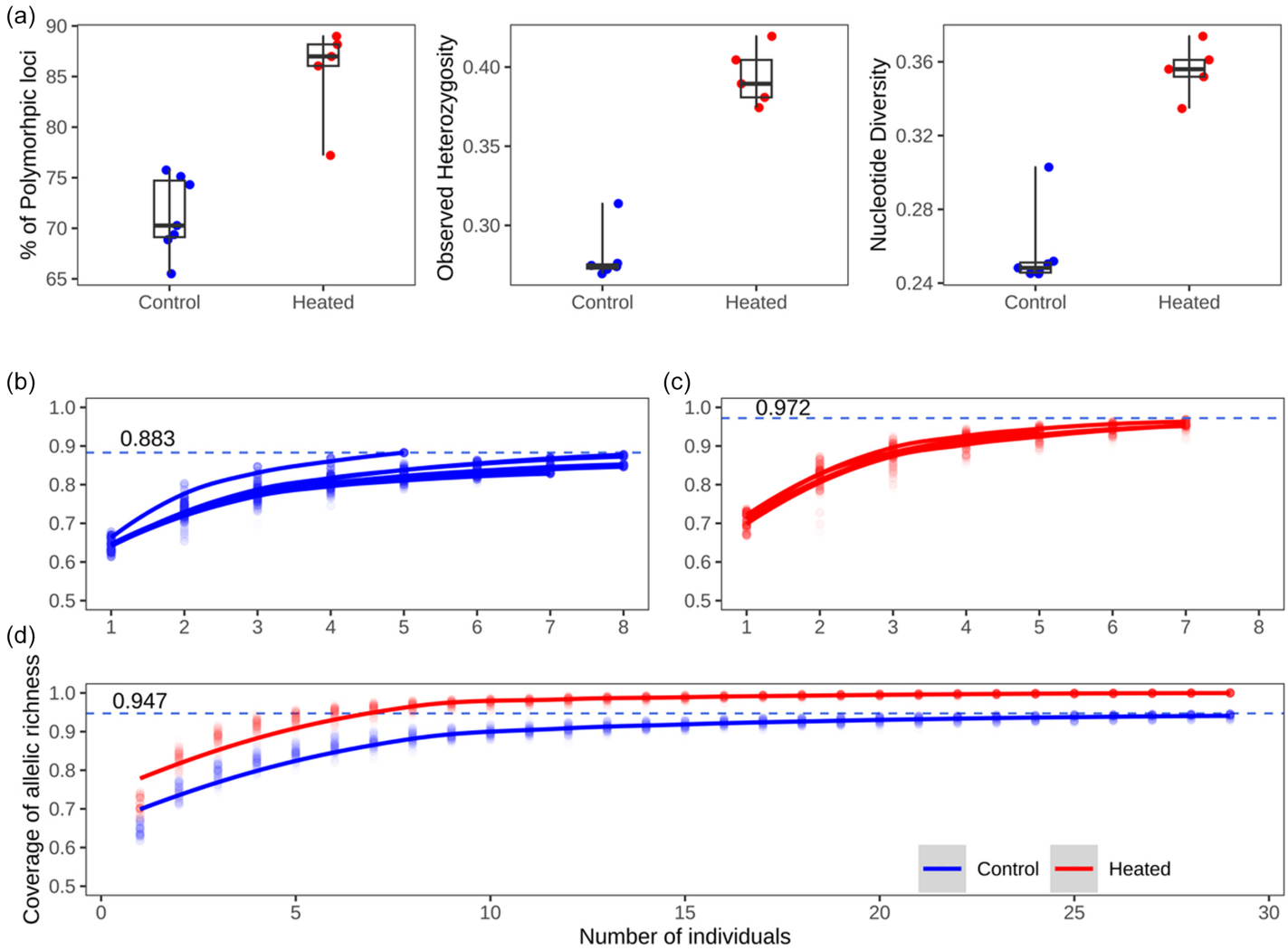

Fig. 3. Genetic diversity and rarefaction-based recovery of cumulative allelic richness across adaptive loci in unheated-lake vs. heated-lake populations. (**a**) Comparison of genetic diversity based on SNPs (*SNP_thermal_outlier* dataset) located within 10 kb of putative thermal adaptation genes and falling within highly differentiated genomic intervals (HDGIs). Metrics shown are proportion of polymorphic loci (percentage of variable sites), observed heterozygosity (frequency of heterozygous individuals), and nucleotide diversity (π). The p values from Mann–Whitney U tests indicate significant differences between heated and unheated lakes ($p = 0.02$, $p < 0.01$, and $p < 0.01$ from left to right). (**b**) Rarefaction curves illustrating the recovery rate of putatively adaptive alleles as a function of sample size of randomly picked individuals from a given lake or lake group. Top panels: individual unheated-lake and heated-lake populations; bottom panels: combined unheated-lake and heated-lake populations (metapopulations). Asymptotic lines indicate estimated upper bounds of the recovery rate. Note that the asymptotic line is omitted for the heated metapopulation, as a 100% recovery rate is achieved with 29 sampled individuals.

Rarefaction analysis indicated substantial sharing of alleles at putatively adaptive loci between populations from unheated and heated lakes. Sampling five individuals from a single unheated-lake population recovered 81.0%–88.3% of alleles present at these putatively adaptive loci in populations from heated lakes, whereas five randomly sampled individuals from a single heated-lake population recovered 92.4–93.9%. Increasing sample size improved recovery: 29 individuals sampled across unheated lakes recovered 94.7% of the putatively adaptive alleles identified in heated-lake populations, while 15 individuals sampled across heated lakes recovered 98.8%.

Tajima's D at candidate loci was significantly higher in populations from heated lakes. A linear mixed-effects model confirmed an effect of lake type on Tajima's D after accounting for population and contig effects (estimate = 0.256, SE = 0.070, $t = 3.662$, $p < 0.01$). The proportion of loci exhibiting significant heterozygosity excess did not differ significantly between heated-lake and control-lake populations ($p = 0.303$).

Hybridization and introgression (H3)

The discordance between mitochondrial and nuclear phylogenies was consistent with hybridization between

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D. cucullata and *D. galeata* in the heated lakes (Fig. 4). In the nuclear tree, hybrid individuals were placed between the two parental species, whereas mitochondrial haplotypes were mostly assigned to the *D. cucullata* clade, except for one individual (Patn_H11) that grouped with *D. galeata*. Supervised ADMIXTURE analysis supported this pattern, identifying putative hybrid individuals as approximately equal mixtures of *D. galeata* and *D. cucullata* ancestry (Supporting Information Fig. S3). All other individuals that we sampled from both unheated-lake and heated-lake populations were assigned to *D. cucullata*.

Despite evidence of hybridization, sliding-window analyses (topological weighting, *D*, fd, fdM, and dxy) provided no compelling evidence of adaptive introgression from *D. galeata* into *D. cucullata* from heated lakes (*see* window-based statistics for all contigs in Data File 5). We required concordant signals across multiple statistics to identify introgression (*see* Methods section), and no genomic region met these criteria. No HDGI consistently showed elevated allele-sharing statistics across all six trios. Although 824 windows fell within the upper 5th percentile of fd or fdM in at least one trio, this number dropped to only two when requiring elevated values in at least two trios, indicating a lack of repeatable signals. For example, windows around 1 Mb on CAKKLH010000035.1 showed elevated allele-sharing statistics between *D. cucullata* from heated lakes and *D. galeata* in a single trio, but these windows did not exhibit reduced dxy between *D. cucullata* from heated lakes and *D. galeata*, nor did topology weighting consistently favor the introgression topology. This region was therefore considered inconclusive (Supporting Information Fig. S4). Importantly, a comparison of *D*, fd, and fdM distributions between the genomic background and windows near HDGIs revealed no enrichment of introgression signals near highly differentiated regions, indicating that adaptive introgression has not detectably contributed to thermal adaptation in populations from heated lakes (Data File 6).

Functional annotation and enrichment

The 188 diverging thermal candidate genes located within or near 10 kb of thermal candidate genomic intervals were enriched in four KEGG pathways: cGMP-PKG signaling (ko04022), transcription factors (ko03000), apelin signaling (ko04371), and hypertrophic cardiomyopathy (ko05410). No GO terms were enriched in the Molecular Function category, while 13 Cellular Component and 30 Biological Process terms were significantly enriched (Data Files 7–8). Enriched Biological Process terms included metamorphosis (GO:0007552), appendage morphogenesis (GO:0035107), regulation of circulatory system process (GO:1903522), and response to toxic substance (GO:0009636), among others (*see* Data Files 7–8 for the full list).

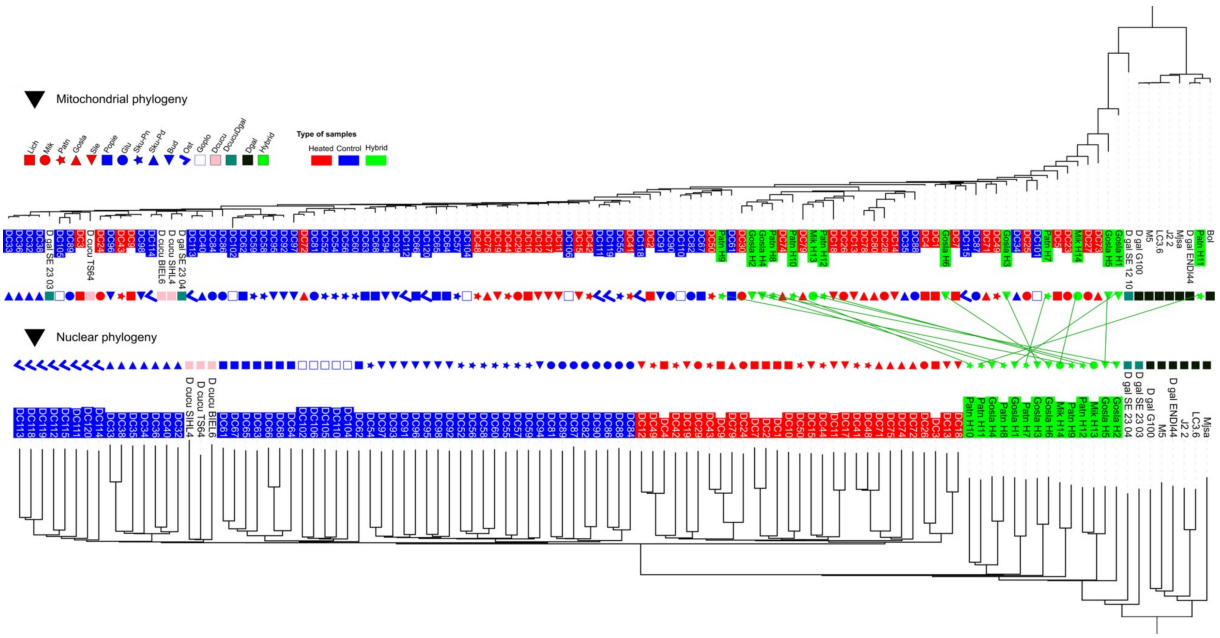

Fig. 4. Phylogenies and mitochondrial haplotype distribution among populations of *Daphnia cucullata*. Phylogenetic trees including all individuals analyzed in this study: 92 *D. cucullata*, 14 putative hybrid individuals, and 13 whole-genome sequencing (WGS) datasets retrieved from the Sequence Read Archive (SRA). *D. longispina* was used as the root, but its terminal branches were removed for clarity. The mitochondrial genome-based phylogeny is shown at the top, while the phylogeny based on neutral nuclear loci is shown at the bottom. Icons adjacent to the sample labels indicate sample origin—either the lake of origin for newly generated samples or species identity for SRA data. Background colors of labels denote the three major sample categories: hybrids, clones from heated lakes, and clones from control (unheated) lakes.

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Discussion

Our study uncovered pronounced temperature-associated genomic differentiation between *Daphnia cucullata* populations exposed to contrasting thermal regimes for more than six decades and identified multiple genomic intervals and genes associated with thermal responses. Importantly, our results provide compelling evidence that thermal adaptation in these natural populations is underpinned by a polygenic genetic architecture characterized by numerous loci distributed across the genome. Moreover, selection potentially imposed by elevated water temperatures did not lead to a loss of genetic diversity at loci relevant for thermal responses; instead, this functionally important genetic variation is consistently retained across spatially distributed populations.

Population genomic structure

We observed strong genomic differentiation between populations inhabiting heated and unheated lakes. Although such differentiation could result from genetic drift, differences in founding populations, or divergent selection, several lines of evidence indicate that genetic drift alone is unlikely to fully explain the observed patterns. First, overall genetic diversity metrics were similar between heated and unheated-lake populations. While comparable diversity does not exclude demographic effects, it argues against scenarios in which only populations inhabiting one lake type underwent a strong bottleneck or founder effect. Populations inhabiting heated lakes contained fewer private alleles, but this likely reflects greater connectivity among heated lakes rather than reduced genetic diversity. The heated lakes are connected by canals that might facilitate dispersal. Individuals from heated lakes indeed formed a relatively tight cluster in the DAPC analysis, whereas unheated lakes showed stronger lake-specific differentiation (Fig. 2). Similarly, the ADMIXTURE analysis showed highly similar ancestry profiles across populations inhabiting heated lakes (Supporting Information Fig. S5). Together, DAPC and ADMIXTURE patterns are consistent with higher gene flow among populations inhabiting heated lakes than among populations from unheated lakes, and this gene flow reduces differentiation within the heated lake population network while maintaining genetic diversity within each of the individual populations of this network. In addition, the large population sizes and extensive sediment egg banks characteristic of *Daphnia* populations are expected to buffer against diversity loss and support long-term maintenance of genetic variation, consistent with observations from resurrection genomic studies (Chaturvedi et al. 2021).

Second, mitochondrial genomes do not recombine and therefore preserve signatures of colonization history (Haileselasie et al. 2016). The absence of distinct mitochondrial clades separating the heated and unheated-lake populations (Fig. 4) indicates that their founding populations were not strongly differentiated. This makes founder effects an unlikely primary explanation for the observed genome-wide divergence.

Finally, the strong association between HDGIs and thermal-candidate genes provides additional support for temperature-driven divergence. Although genetic drift and demographic history undoubtedly contribute to genomic structure, they alone are unlikely to explain the concentration of differentiation around genes previously linked to thermal responses.

We acknowledge that most populations were represented by only six to eight individuals, limiting precision for population-specific estimates, particularly for rare variants. We therefore interpret lake-specific patterns cautiously and focus mainly on replicated differences between heated and unheated lake groups. The consistency of patterns across multiple analytical approaches nevertheless suggests that the major genomic patterns and analyses are robust.

Thermal adaptation hypothesis (H1)

Several lines of evidence support the hypothesis that long-term warming generated genomic changes consistent with thermal adaptation. First, HDGIs were significantly enriched for thermal candidate genes (empirical $p = 0$), consistent with temperature-associated selection acting on loci involved in thermal responses. Despite limited overlap between the HMM-based and BayEnv2 approaches, the observed overlap also exceeded random expectations (empirical $p = 0$), supporting the presence of temperature-associated divergent selection despite strong population structure.

Second, our genomic results are consistent with previous experimental work in the same lake system: Dziuba et al. (2021) demonstrated adaptive responses to long-term warming in the populations inhabiting the heated lakes through life-table experiments. Although genomic differentiation alone cannot confirm adaptation, its concordance with experimentally documented phenotypic divergence strengthens the interpretation that temperature-mediated selection has contributed to evolutionary change.

Third, several enriched pathways and biological processes detected in the present study are plausibly linked to thermal stress and physiological responses to warming. The evolutionarily conserved cGMP–PKG pathway is involved in stress responses, and in *Drosophila melanogaster*, PKG inhibition enhances thermo-protection under high temperatures (Krill and Dawson-Scully 2016); in *Daphnia*, where oxidative stress is a central mechanism of thermal-induced toxicity (Samanta et al. 2020), this pathway may contribute to protection through its known regulation of antioxidative processes. Likewise, enrichment in the Apelin signaling pathway—a key regulator of energy metabolism and cardiovascular function—is consistent with the increased metabolic and circulatory demands experienced by *Daphnia* at elevated temperatures (Paul et al. 2004).

While the temperature gradient is clear, the heated lakes differ from unheated lakes in ecological dimensions beyond

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temperature alone. Long-term warming has altered plankton communities, fish predation regimes, and host–parasite interactions in the Konin lake system (Kapusta and Bogacka-Kapusta 2015; Dziuba et al. 2023; Beng et al. 2025). These ecological changes might contribute to some of the observed genomic divergence either directly or through interactions with thermal selection—particularly given that most HDGIs did not coincide with thermal candidate genes. Therefore, our results should be interpreted as genomic responses associated with long-term warming and its ecological consequences, rather than as responses to temperature in isolation.

A limitation of our study is that we used contemporary samples only and therefore inferred thermal adaptation indirectly from spatial comparisons between heated and unheated lakes, which cannot fully reconstruct temporal allele-frequency changes since the onset of artificial heating. In *Daphnia*, sediment dormant egg banks offer a powerful opportunity to directly assess evolutionary change through time by comparing historical and contemporary genotypes (Brendonck and De Meester 2003). Resurrection-genomic studies have shown that such temporal samples can directly reveal genome-wide allele-frequency changes associated with rapid adaptation in natural *Daphnia* populations (Chaturvedi et al. 2021). Future resurrection-genomic approaches using dormant eggs from different sediment layers could therefore provide a more direct test of whether the candidate loci identified here changed in frequency during long-term warming.

Standing genetic variation hypothesis (H2)

Our results strongly support the hypothesis that adaptation to long-term warming primarily drew on standing genetic variation. Rarefaction analyses showed that most genetic variation enabling *D. cucullata* to adapt to the heated lakes (Dziuba et al. 2021) can be recovered from as few as five founding individuals from neighboring populations. More than 90% of the allelic richness observed in heated-lake populations at thermal-related loci was recovered with just eight individuals. Notably, a single unheated-lake population already harbored high allelic richness at thermal-associated loci, comparable to that of the full regional metapopulation (Supporting Information Fig. S6). These results echo Chaturvedi et al. (2021), who reported that a small number of *Daphnia* from the regional pool can supply ample standing variation for rapid adaptation to fish predation.

Strikingly, when averaged across the most strongly differentiated thermal-associated loci (SNP_thermal_outlier dataset), genetic diversity in populations from heated lakes was not merely retained but higher than in populations from control lakes (Fig. 3a). Polygenic adaptation from standing genetic variation predicts the retention of variation summed across many loci: selection acting on alleles already segregating at appreciable frequency before the onset of warming generates soft, partial sweeps that proceed through modest allele-frequency shifts at many loci and leave surrounding diversity largely intact, whereas selection on rare de novo mutations tends to drive hard sweeps that fix beneficial alleles and erode linked variation (Hermisson and Pennings 2005, 2017; Barghi et al. 2020). Polygenic adaptation itself, however, does not predict the increase in diversity we observed. At any individual locus, selection redistributes alleles that are already segregating rather than adding new ones, so the number of alleles present cannot increase. The elevated diversity we recover must therefore arise from frequency changes among alleles that were already present.

In our system, many favored alleles appear to have started at low frequencies: in heated-lake populations the allele-frequency spectrum at these loci is depleted at both extremes (Supporting Information Fig. S7), with variants that remain rare in unheated lakes segregating at intermediate frequencies. Such a shift raises average heterozygosity and nucleotide diversity across the locus set and is also reflected in the elevated Tajima's *D* values in heated-lake populations. An excess of intermediate-frequency alleles is the signature expected under soft or partial sweeps rather than the strong allele-frequency skews characteristic of hard sweeps (Messer and Petrov 2013). The same shift also raises count-based measures of diversity, although through a different route: alleles segregating below the frequency detectable at our rarefied sample size are not scored, so loci at which such alleles rise to intermediate frequency cross the threshold at which they are counted as polymorphic. The percentage of polymorphic loci and rarefied allelic richness therefore increase without any allele being newly gained. The large effective population sizes characteristic of *Daphnia* (Chaturvedi et al. 2021) and the extensive sediment egg banks that buffer populations against environmental fluctuations (Brendonck and De Meester 2003) sustain a large reservoir of standing genetic variation, ultimately providing the genetic resource on which the shifts we observed acted (Barrett and Schluter 2008).

Several non-mutually exclusive processes could also maintain or increase diversity at adaptive loci of populations inhabiting heated lakes. First, gene flow among heated lakes, which are hydrologically connected through canals, may replenish genetic variation and counteract local diversity loss (Lenormand 2002). Second, spatially varying selection—arising from heterogeneous thermal and ecological conditions across seasons, microhabitats, or individual lakes—could maintain polymorphism through migration–selection balance, particularly given the broader thermal niche documented for heated lakes (Dziuba et al. 2021; Sakamoto and Yeaman 2026). Third, temporal environmental fluctuations, including seasonal temperature cycles and food-web dynamics, may impose temporally variable selection that favors different alleles at different times, thereby preventing fixation (Bergland et al. 2014). The last two processes fall under the broad umbrella of balancing selection, which can elevate Tajima's *D* and generate an excess of intermediate-frequency variants, and thus an elevated genetic diversity (Charlesworth 2006)—the same signature we

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attribute above to partial sweeps from standing variation. However, our data do not allow us to distinguish among these mechanisms. Future work combining temporal genomic data from dormant egg banks, more comprehensive population-level sampling, and direct fitness assays across thermal and ecological conditions could help disentangle their relative contributions.

Adaptive introgression hypothesis (H3)

Hybridization within the *D. longispina* complex is well-documented, particularly under environmental change (Keller et al. 2008; Brede et al. 2009). Hybrids can outperform parental species under certain conditions, for example under simulated winter temperatures (Griebel et al. 2015) or low-quality food (Seidendorf et al. 2007). Dziuba et al. (2020) reported an increase in the relative abundance of *D. cucullata* in upper sediment layers of heated lakes, following its earlier decline after the introduction of thermal effluent. Adaptive introgression from *D. galeata* into *D. cucullata* could therefore be a possible explanation for its reestablished dominance.

The mito-nuclear discordance observed among putative hybrid individuals (Fig. 4), together with their mixed *D. cucullata* and *D. galeata* ancestry inferred from supervised ADMIXTURE analysis (Supporting Information Fig. S3), confirms hybridization between these two species in the heated lakes. Although mitochondrial and nuclear phylogenies were reconstructed from different data types and using different phylogenetic methods, our inference does not rely solely on tree topology. The same putative hybrid individuals were independently identified as approximately equal mixtures of *D. cucullata* and *D. galeata* ancestry based on genome-wide nuclear SNPs, indicating that the observed discordance reflects biological hybridization rather than methodological artifacts. Equal ancestries from both parental species are consistent with hybrids of mixed ancestry (potentially F1), which are often abundant in the active population in the *Daphnia longispina* complex when parental species co-occur (Keller and Spaak 2004; Griebel et al. 2018). However, F2 and later-generation hybrids may show the same ancestry proportions, and additional analyses would be required to resolve the exact nature of the hybrids, which were beyond the scope of the present study.

Despite the presence of hybrid individuals, our analyses did not identify genomic regions in which topology weighting, *D*, *fd*, *fdM*, and *dxy* provided concordant support for introgression from *D. galeata* into heated-lake *D. cucullata*, including in regions associated with thermal candidate genes. We therefore interpret hybridization and adaptive introgression as distinct processes in this system: hybridization occurs, but introgressed *D. galeata* alleles did not detectably contribute to the genomic signatures of thermal adaptation in *D. cucullata*.

Several non-mutually exclusive mechanisms could explain why occasional hybridization did not translate into detectable adaptive introgression. Hybridization can generate F1 individuals without necessarily leading to sustained introgression, because introgression requires hybrid fertility, repeated backcrossing, and the persistence of introgressed alleles across generations (Mallet 2005). Hybrid or backcrossed individuals may also experience reduced fitness because of intrinsic genomic incompatibilities or because recombinant genotypes are mismatched to local ecological conditions. In the *D. longispina* species complex, the relative fitness of hybrids and parental taxa is known to be strongly environment-dependent, varying with factors such as food availability, fish predation, parasite pressure, and seasonal conditions (Spaak and Hoekstra 1997; Wolinska et al. 2004). Thus, under the combined ecological conditions of the heated lakes, hybrids or backcrossed individuals may not have persisted or contributed substantially to the *D. cucullata* gene pool. Our data do not allow us to distinguish among these mechanisms, but they suggest that the presence of hybrid individuals alone does not necessarily imply that introgression contributes to thermal adaptation. Nevertheless, because rare, spatially heterogeneous, or early-stage introgression events may be difficult to detect with modest sampling, we interpret the absence of introgression signals conservatively as no conclusive evidence for adaptive introgression, rather than as definitive evidence that introgression did not contribute at all to adaptive thermal evolution.

Because our introgression hypothesis specifically tested for genomic regions of *D. galeata* origin in *D. cucullata*, using a *D. galeata* reference genome should, if anything, increase sensitivity for detecting such regions, making it unlikely that reference choice generated a false absence of introgression signals. Nevertheless, we acknowledge that mapping to a heterospecific reference may reduce sensitivity in highly divergent genomic regions, and that a chromosome-level *D. cucullata* reference genome would improve introgression detection as well as all other analyses in this study.

Notwithstanding all limitations, the Konin lake system offers a unique opportunity to observe genomic responses to multi-decadal warming in natural populations. Taking advantage of this long-term experiment, we showed that the resident populations of the key zooplankton species *D. cucullata* exposed to warming for multiple decades exhibit strong genetic differentiation in many genomic islands of divergence, without any net loss of genetic diversity across the set of loci central to adaptation. Despite strong divergence between heated and control lakes, the two groups shared many genetic variants; furthermore, fewer than 10 immigrants from any control lake population would collectively harbor approximately 90% of the genetic variation relevant to thermal adaptation. These findings highlight two key insights: (1) Thermal adaptation in *D. cucullata* is primarily driven by selection on pre-existing standing genetic variation, not hybridization or de novo mutations; and (2) Regional lakes harbor sufficient adaptive potential to respond to future warming, thanks to large effective population sizes and the buffering effect of sediment egg banks. Together, these results demonstrate that long-term warming can drive extensive genomic change

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without depleting adaptive potential, and they underscore standing genetic variation as a powerful substrate for rapid evolutionary responses to climate change. More specifically, this adaptive potential is expressed under complex natural conditions rather than in laboratory settings in which only temperature is manipulated. While laboratory settings offer greater causal clarity, whether the same adaptive potential is expressed under more complex natural conditions requires empirical validation. Our results confirm this, and support earlier phenotypic observations on responses to warming obtained on the same populations (Dziuba et al. 2021) and in a resurrection ecological study on a lake in the United Kingdom (Geerts et al. 2015). Our results further show that the earlier observed adaptive differences between heated-lake and control-lake populations using quantitative genetics reflect polygenic inheritance involving many loci of small effect rather than a few genes with major effects. Given that many plankton species (e.g., ciliates, phytoplankton, rotifers, cladocerans) share the mixed sexual–asexual reproductive strategy of *Daphnia* and generally have very large population sizes, we expect our observations to hold for many zooplankton species, although future genomic studies across additional taxa will be needed to corroborate this.

Author Contributions

Shaolin Xu, Luc De Meester and Justyna Wolinska conceived and designed the study. Sample collection was conducted by Justyna Wolinska and Slawek Cerbin. Shaolin Xu carried out the morphological identification, performed DNA extractions, and conducted the data analyses with input from Markus Möst and Luc De Meester. All authors contributed to the development of the discussion. Shaolin Xu drafted the initial version of the manuscript, and all authors contributed to its revision and approved the final version for submission.

Acknowledgments

Shaolin Xu was supported by the Sino-German (CSC-DAAD) Postdoctoral Scholarship Program, 2022 (57607866). Sequencing costs were covered by an IGB starting grant to Luc De Meester. Luc De Meester also acknowledges support from KU Leuven Research Council project C16/2023/003. Justyna Wolinska and Slawek Cerbin were supported by a joint Beethoven Life-1 grant from the German Research Foundation (WO 1587/9-1) and the National Science Centre, Poland (2018/31/F/NZ8/01986). This research was funded in whole or in part by the Austrian Science Fund FWF (10.55776/Y1442). For open access purposes, the author has applied a CC BY public copyright license to any author-accepted manuscript version arising from this submission. The authors thank all members of the participating laboratories for their valuable assistance and constructive discussions throughout the course of this study. During the preparation of this manuscript, the authors used ChatGPT to refine the English grammar, syntax, and clarity. This tool was used solely for linguistic improvement and did not contribute to the conceptual design, data analysis, or scientific conclusions of the study. The authors have critically reviewed the output and assume full accountability for the text. The authors declare no conflicts of interest. Open Access funding enabled and organized by Projekt DEAL.

Conflicts of Interest

None declared.

Data Availability Statement

Raw NGS data were deposited in the SRA database under project PRJNA1416968 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1416968). Scripts and data files used in the analyses are available through Zenodo under DOI: https://doi.org/10.5281/zenodo.20840623 (https://zenodo.org/records/20840623).

References

Alexander, D. H., J. Novembre, and K. Lange. 2009. "Fast Model-Based Estimation of Ancestry in Unrelated Individuals." *Genome Research* 19, no. 9: 1655–1664. https://doi.org/10.1101/gr.094052.109.

Barghi, N., J. Hermisson, and C. Schlötterer. 2020. "Polygenic Adaptation: A Unifying Framework to Understand Positive Selection." *Nature Reviews Genetics* 21, no. 12: 769–781. https://doi.org/10.1038/s41576-020-0250-z.

Barrett, R., and D. Schluter. 2008. "Adaptation From Standing Genetic Variation." *Trends in Ecology & Evolution* 23, no. 1: 38–44. https://doi.org/10.1016/j.tree.2007.09.008.

Beng, K. C., S. Cerbin, M. T. Monaghan, and J. Wolinska. 2025. "Long-Term Changes to Plankton Communities in Artificially Heated Lakes." *Limnology and Oceanography* 70: 3029–3042. https://doi.org/10.1002/lno.70192.

Benzie, J. A. H. 2005. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World: Cladocera: The Genus Daphnia (Including Daphniopsis). SPB Academic Publishing.

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt, and D. A. Petrov. 2014. "Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*." *PLoS Genetics* 10: e1004775. https://doi.org/10.1371/journal.pgen.1004775.

Brans, K. I., M. Jansen, J. Vanoverbeke, N. Tüzün, R. Stoks, and L. De Meester. 2017. "The Heat Is on: Genetic Adaptation to Urbanization Mediated by Thermal Tolerance and Body Size." *Global Change Biology* 23, no. 12: 5218–5227. https://doi.org/10.1111/gcb.13784.

Brede, N., C. Sandrock, D. Straile, et al. 2009. "The Impact of Human-Made Ecological Changes on the Genetic Architecture of *Daphnia* Species." *Proceedings of the National Academy of Sciences* 106, no. 12: 4758–4763. https://doi.org/10.1073/pnas.0807187106.

19395590, 2026, 9, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lno.70498 by Luc De Meester - Leibniz Institut Fuer Gewaesserokologie , Wiley Online Library on [04/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Brendonck, L., and L. De Meester. 2003. "Egg Banks in Freshwater Zooplankton: Evolutionary and Ecological Archives in the Sediment." *Hydrobiologia* 491, no. 1–3: 65–84. https://doi.org/10.1023/A:1024454905119.

Camacho, C., G. Coulouris, V. Avagyan, et al. 2009. "BLAST+: Architecture and Applications." *BMC Bioinformatics* 10, no. 1: 421. https://doi.org/10.1186/1471-2105-10-421.

Carlson, M., and H. Pagès. 2019. AnnotationForge: Tools for Building SQLite-Based Annotation Data Packages. R Package Version 1.26.0. Bioconductor. https://doi.org/10.18129/B9.bioc.AnnotationForge.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. "Stacks: An Analysis Tool Set for Population Genomics." *Molecular Ecology* 22, no. 11: 3124–3140. https://doi.org/10.1111/mec.12354.

Charlesworth, D. 2006. "Balancing Selection and Its Effects on Sequences in Nearby Genome Regions." *PLoS Genetics* 2: e64. https://doi.org/10.1371/journal.pgen.0020064.

Chaturvedi, A., J. Zhou, J. A. Raeymaekers, et al. 2021. "Extensive Standing Genetic Variation from a Small Number of Founders Enables Rapid Adaptation in *Daphnia*." *Nature Communications* 12, no. 1: 1–9. https://doi.org/10.1038/s41467-021-24581-z.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. "fastp: An Ultra-Fast All-in-One FASTQ Preprocessor." *Bioinformatics* 34, no. 17: i884–i890. https://doi.org/10.1093/bioinformatics/bty560.

Choi, T.-J., T. D. Do, J.-I. Kim, and C.-B. Kim. 2023. "Analysis of the Complete Mitogenome of *Daphnia galeata* from the Han River, South Korea: Structure Comparison and Control Region Evolution." *Functional & Integrative Genomics* 23, no. 1: 65. https://doi.org/10.1007/s10142-023-00986-5.

Cingolani, P., A. Platts, L. L. Wang, et al. 2012. "A Program for Annotating and Predicting the Effects of Single Nucleotide Polymorphisms, SnpEff: SNPs in the Genome of *Drosophila melanogaster* Strain w1118; Iso-2; Iso-3." *Fly* 6, no. 2: 80–92. https://doi.org/10.4161/fly.19695.

Danecek, P., A. Auton, G. Abecasis, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Danecek, P., J. K. Bonfield, J. Liddle, et al. 2021. "Twelve Years of Samtools and Bcftools." *GigaScience* 10, no. 2: giab008. https://doi.org/10.1093/gigascience/giab008.

Dayan, D. I., X. Du, T. Z. Baris, D. N. Wagner, D. L. Crawford, and M. F. Oleksiak. 2019. "Population Genomics of Rapid Evolution in Natural Populations: Polygenic Selection in Response to Power Station Thermal Effluents." *BMC Evolutionary Biology* 19, no. 1: 61. https://doi.org/10.1186/s12862-019-1392-5.

De Meester, L., W. Van Doorslaer, A. Geerts, L. Orsini, and R. Stoks. 2011. Thermal Genetic Adaptation in the Water Flea Daphnia and Its Impact: An Evolving Metacommunity Approach. Oxford University Press. https://academic.oup.com/icb/article-abstract/51/5/703/627136.

DePristo, M. A., E. Banks, R. Poplin, et al. 2011. "A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data." *Nature Genetics* 43, no. 5: 491–498. https://doi.org/10.1038/ng.806.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. "Testing for Ancient Admixture Between Closely Related Populations." *Molecular Biology and Evolution* 28: 2239–2252. https://doi.org/10.1093/molbev/msr048.

Dziuba, M. K., M. Herdegen-Radwan, E. Pluta, Ł. Wejnerowski, W. Szczuciński, and S. Cerbin. 2020. "Temperature Increase Altered *Daphnia* Community Structure in Artificially Heated Lakes: A Potential Scenario for a Warmer Future." *Scientific Reports* 10, no. 1: 13956. https://doi.org/10.1038/s41598-020-70294-6.

Dziuba, M. K., L. Kuczyński, Ł. Wejnerowski, S. Cerbin, and J. Wolinska. 2021. "Countergradient Variation Concealed Adaptive Responses to Temperature Increase in *Daphnia* From Heated Lakes." *Limnology and Oceanography* 66, no. 4: 1268–1280. https://doi.org/10.1002/lno.11680.

Dziuba, M. K., F. Manzi, S. Cerbin, and J. Wolinska. 2023. "Can Climate Warming Save *Daphnia* From Parasites? Reduced Parasite Prevalence in *Daphnia* Populations From Artificially Heated Lakes." *Limnology and Oceanography* 68: 181–191. https://doi.org/10.1002/lno.12257.

Ebert, D. 2022. "*Daphnia* as a Versatile Model System in Ecology and Evolution." *EvoDevo* 13, no. 1: 16. https://doi.org/10.1186/s13227-022-00199-0.

Edwards, A. 1971. "Distances Between Populations on the Basis of Gene Frequencies." *Biometrics* 27, no. 4: 873–881. https://doi.org/10.2307/2528824.

Fan, Z., B. Yue, X. Zhang, L. Du, and Z. Jian. 2017. "CpGIScan: An Ultrafast Tool for CpG Islands Identification From Genome Sequence." *Current Bioinformatics* 12, no. 2: 181–184. https://doi.org/10.2174/1574893611666160907111325.

Faust, G. G., and I. M. Hall. 2014. "SAMBLASTER: Fast Duplicate Marking and Structural Variant Read Extraction." *Bioinformatics* 30, no. 17: 2503–2505. https://doi.org/10.1093/bioinformatics/btu314.

Flynn, J. M., R. Hubley, C. Goubert, et al. 2020. "RepeatModeler2 for Automated Genomic Discovery of Transposable Element Families." *Proceedings of the National Academy of Sciences* 117, no. 17: 9451–9457. https://doi.org/10.1073/pnas.1921046117.

Francis, R. M. 2016. "Pophelper: An R Package and Web App to Analyse and Visualize Population Structure." *Molecular Ecology Resources* 17, no. 1: 27–32. https://doi.org/10.1111/1755-0998.12509.

Geerts, A. N., J. Vanoverbeke, B. Vanschoenwinkel, et al. 2015. "Rapid Evolution of Thermal Tolerance in the Water Flea *Daphnia*." *Nature Climate Change* 5, no. 7: 665–668. https://doi.org/10.1038/nclimate2628.

19395590, 2026, 9, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lno.70498 by Luc De Meester - Leibniz Institut Fuer Gewaesserokologie , Wiley Online Library on [04/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Goudet, J. 2004. "Hierfstat, a Package for r to Compute and Test Hierarchical *F*-Statistics." *Molecular Ecology Notes* 5, no. 1: 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Griebel, J., S. Gießler, M. Poxleitner, A. N. Faria, M. Yin, and J. Wolinska. 2015. "Extreme Environments Facilitate Hybrid Superiority—The Story of a Successful *Daphnia galeata* × *longispina* Hybrid Clone." *PLoS One* 10, no. 10: e0140275. https://doi.org/10.1371/journal.pone.0140275.

Griebel, J., M. Utz, J. Hermisson, and J. Wolinska. 2018. "The Establishment of Hybrids of the *Daphnia longispina* Complex Explained by a Mathematical Model Incorporating Different Overwintering Life History Strategies." *PLoS One* 13, no. 7: e0200802. https://doi.org/10.1371/journal.pone.0200802.

Günther, T., and G. Coop. 2013. "Robust Identification of Local Adaptation from Allele Frequencies." *Genetics* 195: 205–220. https://doi.org/10.1534/genetics.113.152462.

Haileselasie, T. H., J. Mergeay, L. J. Weider, E. Jeppesen, and L. De Meester. 2016. "Colonization History and Clonal Richness of Asexual *Daphnia* in Periglacial Habitats of Contrasting Age in West Greenland." *Journal of Animal Ecology* 85, no. 4: 1108–1117. https://doi.org/10.1111/1365-2656.12513.

Hedrick, P. W. 2013. "Adaptive Introgression in Animals: Examples and Comparison to New Mutation and Standing Variation as Sources of Adaptive Variation." *Molecular Ecology* 22, no. 18: 4606–4618. https://doi.org/10.1111/mec.12415.

Hemstrom, W., and M. Jones. 2023. "snpR: User Friendly Population Genomics for SNP Data Sets With Categorical Metadata." *Molecular Ecology Resources* 23, no. 4: 962–973. https://doi.org/10.1111/1755-0998.13721.

Hermisson, J., and P. S. Pennings. 2005. "Soft Sweeps." *Genetics* 169: 2335–2352. https://doi.org/10.1534/genetics.104.036947.

Hermisson, J., and P. S. Pennings. 2017. "Soft Sweeps and Beyond: Understanding the Patterns and Probabilities of Selection Footprints Under Rapid Adaptation." *Methods in Ecology and Evolution* 8: 700–716. https://doi.org/10.1111/2041-210X.12808.

Herrmann, M., and L. Y. Yampolsky. 2021. "False and True Positives in Arthropod Thermal Adaptation Candidate Gene Lists." *Genetica* 149, no. 3: 143–153. https://doi.org/10.1007/s10709-021-00122-w.

Huerta-Cepas, J., D. Szklarczyk, D. Heller, et al. 2019. "eggNOG 5.0: A Hierarchical, Functionally and Phylogenetically Annotated Orthology Resource Based on 5090 Organisms and 2502 Viruses." *Nucleic Acids Research* 47, no. D1: D309–D314. https://doi.org/10.1093/nar/gky1085.

IPCC. 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. https://doi.org/10.1017/9781009157896.

Jansen, M., A. N. Geerts, A. Rago, et al. 2017. "Thermal Tolerance in the Keystone Species *Daphnia magna*—A Candidate Gene and an Outlier Analysis Approach." *Molecular Ecology* 26, no. 8: 2291–2305. https://doi.org/10.1111/mec.14040.

Jombart, T., S. Devillard, and F. Balloux. 2010. "Discriminant Analysis of Principal Components: A New Method for the Analysis of Genetically Structured Populations." *BMC Genetics* 11, no. 1: 94. https://doi.org/10.1186/1471-2156-11-94.

Kalyaanamoorthy, S., B. Q. Minh, T. K. Wong, A. Von Haeseler, and L. S. Jermiin. 2017. "ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates." *Nature Methods* 14, no. 6: 587–589. https://doi.org/10.1038/nmeth.4285.

Kapusta, A., and E. Bogacka-Kapusta. 2015. "Non-Native Fish Species in Heated Lakes: Origins and Present Status." *Archives of Polish Fisheries* 23: 121–129. https://doi.org/10.1515/aopf-2015-0014.

Keller, B., and P. Spaak. 2004. "Nonrandom Sexual Reproduction and Diapausing Egg Production in a *Daphnia* Hybrid Species Complex." *Limnology and Oceanography* 49, no. 4part2: 1393–1400. https://doi.org/10.4319/lo.2004.49.4_part_2.1393.

Keller, B., J. Wolinska, M. Manca, and P. Spaak. 2008. "Spatial, Environmental and Anthropogenic Effects on the Taxon Composition of Hybridizing *Daphnia*." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 363, no. 1505: 2943–2952. https://doi.org/10.1098/rstb.2008.0044.

Korunes, K. L., and K. Samuk. 2021. "pixy: Unbiased Estimation of Nucleotide Diversity and Divergence in the Presence of Missing Data." *Molecular Ecology Resources* 21, no. 4: 1359–1368. https://doi.org/10.1111/1755-0998.13326.

Krill, J. L., and K. Dawson-Scully. 2016. "cGMP-Dependent Protein Kinase Inhibition Extends the Upper Temperature Limit of Stimulus-Evoked Calcium Responses in Motoneuronal Boutons of *Drosophila melanogaster* Larvae." *PLoS One* 11, no. 10: e0164114. https://doi.org/10.1371/journal.pone.0164114.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82, no. 13: 1–26. https://doi.org/10.18637/jss.v082.i13.

Lenormand, T. 2002. "Gene Flow and the Limits to Natural Selection." *Trends in Ecology & Evolution* 17: 183–189. https://doi.org/10.1016/S0169-5347(02)02497-7.

Letunic, I., and P. Bork. 2021. "Interactive Tree of Life (iTOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation." *Nucleic Acids Research* 49, no. W1: W293–W296. https://doi.org/10.1093/nar/gkab301.

Li, H. 2013. "Aligning Sequence Reads, Clone Sequences and Assembly Contigs With BWA-MEM." arXiv. arXiv: 1303.3997. http://arxiv.org/abs/1303.3997.

Lotterhos, K. E., and M. C. Whitlock. 2014. "Evaluation of Demographic History and Neutral Parameterization on the Performance of FST Outlier Tests." *Molecular Ecology* 23: 2178–2192. https://doi.org/10.1111/mec.12725.

Malinsky, M., M. Matschiner, and H. Svardal. 2021. "Dsuite—Fast *D*-Statistics and Related Admixture Evidence From VCF

19395590, 2026, 9, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lno.70498 by Luc De Meester - Leibniz Institut Fuer Gewaesserokologie , Wiley Online Library on [04/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Files." *Molecular Ecology Resources* 21, no. 2: 584–595. https://doi.org/10.1111/1755-0998.13265.

Mallet, J. 2005. "Hybridization as an Invasion of the Genome." *Trends in Ecology & Evolution* 20: 229–237. https://doi.org/10.1016/j.tree.2005.02.010.

Manichaikul, A., J. C. Mychaleckyj, S. S. Rich, K. Daly, M. Sale, and W.-M. Chen. 2010. "Robust Relationship Inference in Genome-Wide Association Studies." *Bioinformatics* 26, no. 22: 2867–2873. https://doi.org/10.1093/bioinformatics/btq559.

Martin, S. H., J. W. Davey, and C. D. Jiggins. 2015. "Evaluating the Use of ABBA–BABA Statistics to Locate Introgressed Loci." *Molecular Biology and Evolution* 32, no. 1: 244–257. https://doi.org/10.1093/molbev/msu269.

Martin, S. H., and S. M. Van Belleghem. 2017. "Exploring Evolutionary Relationships Across the Genome Using Topology Weighting." *Genetics* 206, no. 1: 429–438. https://doi.org/10.1534/genetics.116.194720.

McKenna, A., M. Hanna, E. Banks, et al. 2010. "The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data." *Genome Research* 20, no. 9: 1297–1303. https://doi.org/10.1101/gr.107524.110.

Messer, P. W., and D. A. Petrov. 2013. "Population Genomics of Rapid Adaptation by Soft Selective Sweeps." *Trends in Ecology & Evolution* 28: 659–669. https://doi.org/10.1016/j.tree.2013.08.003.

Miao, B., Z. Wang, and Y. Li. 2016. "Genomic Analysis Reveals Hypoxia Adaptation in the Tibetan Mastiff by Introgression of the Grey Wolf From the Tibetan Plateau." *Molecular Biology and Evolution* 34, no. 3: 734–743. https://doi.org/10.1093/molbev/msw274.

Minh, B. Q., H. A. Schmidt, O. Chernomor, et al. 2020. "IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era." *Molecular Biology and Evolution* 37, no. 5: 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Nickel, J., T. Schell, T. Holtzem, et al. 2021. "Hybridization Dynamics and Extensive Introgression in the *Daphnia longispina* Species Complex: New Insights from a High-Quality *Daphnia galeata* Reference Genome." *Genome Biology and Evolution* 13, no. 12: evab267. https://doi.org/10.1093/gbe/evab267.

Norris, L. C., B. J. Main, Y. Lee, et al. 2015. "Adaptive Introgression in an African Malaria Mosquito Coincident With the Increased Usage of Insecticide-Treated Bed Nets." *Proceedings of the National Academy of Sciences* 112, no. 3: 815–820. https://doi.org/10.1073/pnas.1418892112.

Paul, R. J., B. Zeis, T. Lamkemeyer, M. Seidl, and R. Pirow. 2004. "Control of Oxygen Transport in the Microcrustacean *Daphnia*: Regulation of Haemoglobin Expression as Central Mechanism of Adaptation to Different Oxygen and Temperature Conditions." *Acta Physiologica Scandinavica* 182: 259–275. https://doi.org/10.1111/j.1365-201X.2004.01362.x.

Pritchard, J. K., J. K. Pickrell, and G. Coop. 2010. "The Genetics of Human Adaptation: Hard Sweeps, Soft Sweeps, and Polygenic Adaptation." *Current Biology* 20, no. 4: R208–R215. https://doi.org/10.1016/j.cub.2009.11.055.

Purcell, S., B. Neale, K. Todd-Brown, et al. 2007. "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses." *The American Journal of Human Genetics* 81, no. 3: 559–575. https://doi.org/10.1086/519795.

Reid, A. J., A. K. Carlson, I. F. Creed, et al. 2019. "Emerging Threats and Persistent Conservation Challenges for Freshwater Biodiversity." *Biological Reviews* 94: 849–873. https://doi.org/10.1111/brv.12480.

Sakamoto, T., and S. Yeaman. 2026. "Maintenance of Polymorphism in Spatially Heterogeneous Environments." *Genetics* 232: iyaf229. https://doi.org/10.1093/genetics/iyaf229.

Samanta, P., H. Im, T. Shim, J. Na, and J. Jung. 2020. "Linking Multiple Biomarker Responses in *Daphnia magna* under Thermal Stress." *Environmental Pollution* 263: 114432. https://doi.org/10.1016/j.envpol.2020.114432.

Scheffers, B. R., L. De Meester, T. C. L. Bridge, et al. 2016. "The Broad Footprint of Climate Change From Genes to Biomes to People." *Science* 354, no. 6313: aaf7671. https://doi.org/10.1126/science.aaf7671.

Seefeldt, L., and D. Ebert. 2019. "Temperature- Versus Precipitation-Limitation Shape Local Temperature Tolerance in a Holarctic Freshwater Crustacean." *Proceedings of the Royal Society B* 286: 20190929. https://doi.org/10.1098/rspb.2019.0929.

Seidendorf, B., M. Boersma, and K. Schwenk. 2007. "Evolutionary Stoichiometry: The Role of Food Quality for Clonal Differentiation and Hybrid Maintenance in a *Daphnia* Species Complex." *Limnology and Oceanography* 52, no. 1: 385–394. https://doi.org/10.4319/lo.2007.52.1.0385.

Soria-Carrasco, V., Z. Gompert, A. A. Comeault, et al. 2014. "Stick Insect Genomes Reveal Natural Selection's Role in Parallel Speciation." *Science* 344, no. 6185: 738–742. https://doi.org/10.1126/science.1252136.

Spaak, P., and J. R. Hoekstra. 1997. "Fish Predation on a *Daphnia* Hybrid Species Complex: A Factor Explaining Species Coexistence?" *Limnology and Oceanography* 42: 753–762. https://doi.org/10.4319/lo.1997.42.4.0753.

Stoks, R., A. N. Geerts, and L. De Meester. 2014. "Evolutionary and Plastic Responses of Freshwater Invertebrates to Climate Change: Realized Patterns and Future Potential." *Evolutionary Applications* 7, no. 1: 42–55. https://doi.org/10.1111/eva.12108.

Tarailo-Graovac, M., and N. Chen. 2009. "Using RepeatMasker to Identify Repetitive Elements in Genomic Sequences." *Current Protocols in Bioinformatics* 25, no. 1: 4–10. https://doi.org/10.1002/0471250953.bi0410s25.

Visser, I., and M. Speekenbrink. 2010. "depmixS4: An *R* Package for Hidden Markov Models." *Journal of Statistical*

19395590, 2026, 9, Downloaded from https://anthrosource.onlinelibrary.wiley.com/doi/10.1002/ece3.70498 by Luc De Meester - Leibniz Institut Fuer Gewaesserokologie , Wiley Online Library on [04/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Software 36, no. 7: 1–21. https://doi.org/10.18637/jss.v036.i07.

Wolinska, J., B. Keller, K. Bittner, S. Lass, and P. Spaak. 2004. "Do Parasites Lower *Daphnia* Hybrid Fitness?" *Limnology and Oceanography* 49: 1401–1407. https://doi.org/10.4319/lo.2004.49.4_part_2.1401.

Wu, T., E. Hu, S. Xu, et al. 2021. "clusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data." *The Innovation* 2, no. 3: 100141. https://doi.org/10.1016/j.xinn.2021.100141.

Yang, R.-C. 1998. "Estimating Hierarchical f-Statistics." *Evolution: International Journal of Organic Evolution* 52, no. 4: 950. https://doi.org/10.2307/2411227.

Zdanowski, B., A. Napiórkowska-Krzebietke, K. Stawecki, et al. 2020. "Heated Konin Lakes: Structure, Functioning, and Succession." In Polish River Basins and Lakes—Part I, edited by E. Korzeniewska and M. Harnisz, vol. 86, 321–349. Springer International Publishing. https://doi.org/10.1007/978-3-030-12123-5_16.

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Submitted 13 January 2026
Revised 26 August 2026
Accepted 27 August 2026

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