Received: 15 February 2022 | Revised: 21 September 2022 | Accepted: 21 October 2022

DOI: 10.1111/mec.16748

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

# Selective effects of a short transient environmental fluctuation on a natural population

Markus Pfenninger[1,2,3] | Quentin Foucault[1] | Ann-Marie Waldvogel[4] | Barbara Feldmeyer[1]

[1]Department of Molecular Ecology, Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

[2]LOEWE Centre for Translational Biodiversity Genomics, Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

[3]Institute for Molecular and Organismic Evolution, Johannes Gutenberg University, Mainz, Germany

[4]Department of Ecological Genomics, Institute of Zoology, University of Cologne, Köln, Germany

**Correspondence**
Markus Pfenninger, Department of Molecular Ecology, Senckenberg Biodiversity and Climate Research Centre, Georg-Voigt-Str. 14-16, D-60325, Frankfurt am Main, Germany.
Email: markus.pfenninger@senckenberg.de

**Funding information**
Deutsche Forschungsgemeinschaft, Grant/Award Number: PF390/8-1

**Handling Editor:** Kathryn Hodgins

## Abstract

Natural populations experience continuous and often transient changes of environmental conditions. These in turn may result in fluctuating selection pressures leading to variable demographic and evolutionary population responses. Rapid adaptation as short-term response to a sudden environmental change has in several cases been attributed to polygenic traits, but the underlying genomic dynamics and architecture are poorly understood. In this study, we took advantage of a natural experiment in an insect population of the non-biting midge *Chironomus riparius* by monitoring genome-wide allele frequencies before and after a cold snap event. Whole genome pooled sequencing of time series samples revealed 10 selected haplotypes carrying ancient polymorphisms, partially with signatures of balancing selection. By constantly cold exposing genetically variable individuals in the laboratory, we could demonstrate with whole genome resequencing (i) that among the survivors, the same alleles rose in frequency as in the wild, and (ii) that the identified variants additively predicted fitness (survival time) of its bearers. Finally, by simultaneously sequencing the genome and the transcriptome of cold exposed individuals we could tentatively link some of the selected SNPs to the *cis*- and *trans*-regulation of genes and pathways known to be involved in cold response of insects, such as cytochrome P450 and fatty acid metabolism. Altogether, our results shed light on the strength and speed of selection in natural populations and the genomic architecture of its underlying polygenic trait. Population genomic time series data thus appear as promising tool for measuring the selective tracking of fluctuating selection in natural populations.

**KEYWORDS**
fluctuating selection, genotype–phenotype map, selective tracking

## 1 | INTRODUCTION

Adaptation in natural populations occurs when selection acts on variable phenotypic traits with a heritable basis. There is a general agreement that selection in the wild is intense (Hoekstra et al., 2001; Kingsolver et al., 2001). It is also variable in space and time (Bell, 2010; Price et al., 1984; Siepielski et al., 2011), even though there is some debate whether changes in the direction of selection are frequent or not (Kingsolver et al., 2012; Kingsolver & Pfennig, 2007; Siepielski et al., 2009). Recent theoretical (Messer &

Petrov, 2013) and empirical work (Bitter et al., 2019) has shown that selection in natural populations can lead to rapid adaptation, in particular of polygenic traits (Barghi et al., 2020; Jain & Stephan, 2017). If the rate of environmental change is not too fast and the population characteristics allows for effective selection, adaptation from standing genetic variation to track moving phenotypic optima is theoretically possible nearly in real-time (Matuszewski et al., 2015). It is, therefore, possible that at least some organisms, for example multivoltine species with large population sizes, adaptively track their fluctuating environment (Bell, 2010).

This theoretical basis, however, has currently little empirical support from natural populations. While there are, on the one hand, examples of selective tracking of the fluctuating environment for phenotypic traits (de Villemereuil et al., 2020; Grant & Grant, 1989; Marrot et al., 2017) and on the other demonstrations of rapid selectively driven changes on the molecular level (Margres et al., 2017; Yang et al., 2016; Zong et al., 2021), we are not aware of any studies bringing together the observed temporal fitness differences among different phenotypes with the underlying molecular variants in natural populations.

In this study, we took advantage of a natural experiment to tackle the above question. We studied the genomic response of natural population of a nonbiting midge to a short-term weather event, in this case a cold snap. This promised the opportunity to study the selective effects of a defined transient event as is typical for the selective regime of fluctuating environments (Bell, 2010). Nonbiting midges of the Chironomid family are widely distributed aquatic insects and have a crucial role in freshwater benthic ecosystems serving as a basis of benthic food webs (Horváth et al., 2011; Oppold et al., 2016; Pfenninger & Nowak, 2008). *Chironomus riparius* (Meigen, 1803) is a multivoltine species with up to 15 generations per year in Europe (Oppold et al., 2016). Therefore, the different generations are subjected to widely varying environmental conditions. Accordingly, extensive research on temperature and photoperiod has shown that several traits can and do adapt locally (Waldvogel et al., 2018), and temporally among seasons (Doria, Caliendo, et al., 2022; Foucault et al., 2018). However, other factors are also known to act as selection pressures on this species, for example, organic load (Kraak et al. 2000), conductivity (Pfenninger & Nowak, 2008), nitrogen (Nemec et al., 2012), temperature (Nemec et al., 2013) and anthropogenic substances (Nowak et al., 2009). The high effective and demographic population size (>1,000,000, Waldvogel et al., 2018) and the very high number of offspring per breeding pair (400–800) allows for rapid adaptation (Pfenninger & Foucault, 2020). Since genomic resources and parameters are available (Oppold et al., 2016; Schmidt et al., 2020) and the species is amenable for evolutionary experiments in the laboratory (Foucault et al., 2019), the species is increasingly becoming a model for molecular ecology and the emerging field of evolutionary ecotoxicology (Doria et al., 2021; Doria, Hannappel, et al., 2022).

In this study we focussed on three research questions. Does normal, transient environmental variation like a cold snap trigger measurable molecular selection in a natural population? Are the putatively selected SNP-loci linked to longer survival also under experimental cold exposure conditions? And finally, can we link the identified variants to lower level phenotypic changes, that is, gene expression differences?

## 2 | MATERIALS AND METHODS

### 2.1 | Temporal sampling of natural population

In the course of routine sampling for another project (Pfenninger & Foucault, 2022), we sampled larvae of the species *Chironomus riparius* on 15 February 2018 with a sieve at a single site situated in a small river (Hasselbach, Hessen, Germany 50.167562°N, 9.083542°E) following the protocol of Foucault et al. (2019). The sampling site is located close to a wastewater treatment plant (Abwasserverband Freigericht) that continuously monitors physical and chemical water parameters, which they generously provided. A few days after the sampling, the air temperature in the region fell substantially below zero for a couple of days, which eventually drove the water temperatures at the sampling site from the long-term average of 9–10°C during this time of the year down to about 5°C for two consecutive days (Figure 1a). We seized the opportunity to obtain another sample of 80 individuals from the same site. Please note that no reproduction takes place in this species at temperatures below ~12–14°C and thus the same generation was sampled. A third sample from the same site was obtained in September 2018, about 6–7 generations later (Oppold et al., 2016). The taxonomic identity of the larvae was ascertained by DNA-barcoding of a mitochondrial (COI) and a nuclear locus (L44). Eighty thus identified *C. riparius* were pooled and subjected to pool-sequencing (see below).

### 2.2 | Population genomic analyses

DNA was extracted for the three pools from the field using the Quiagen blood and tissue extraction kit on pooled samples of 80 larval head capsules, respectively. Integrity and quality of extracted DNA was controlled using electrophoresis, and the DNA concentration for each sample was measured with a Qubit fluorimeter (Invitrogen).

Whole genome pool-sequencing was carried out on an Illumina MiSeq with 250 bp paired end reads. Reads were trimmed using the wrapper tool Autotrim (Waldvogel et al., 2018) that integrates Trimmomatic (Bolger et al., 2014) for trimming and FastQC (Andrews, 2010) for quality control. The trimmed reads were then mapped on the latest *C. riparius* reference genome (Schmidt et al., 2020) using the BWA mem algorithm (Li & Durbin, 2009). Low quality reads were subsequently filtered and SNPs were initially called using Samtools (Li et al., 2009). The pipelines PoPoolation1 version 1.2.2 and PoPoolation2 version 1.201 (Kofler et al., 2011) were used to call SNPs and remove indels. Allele frequencies for all SNPs with coverage between 15× and 70× were estimated with the R library poolSeq (Taus et al., 2017).

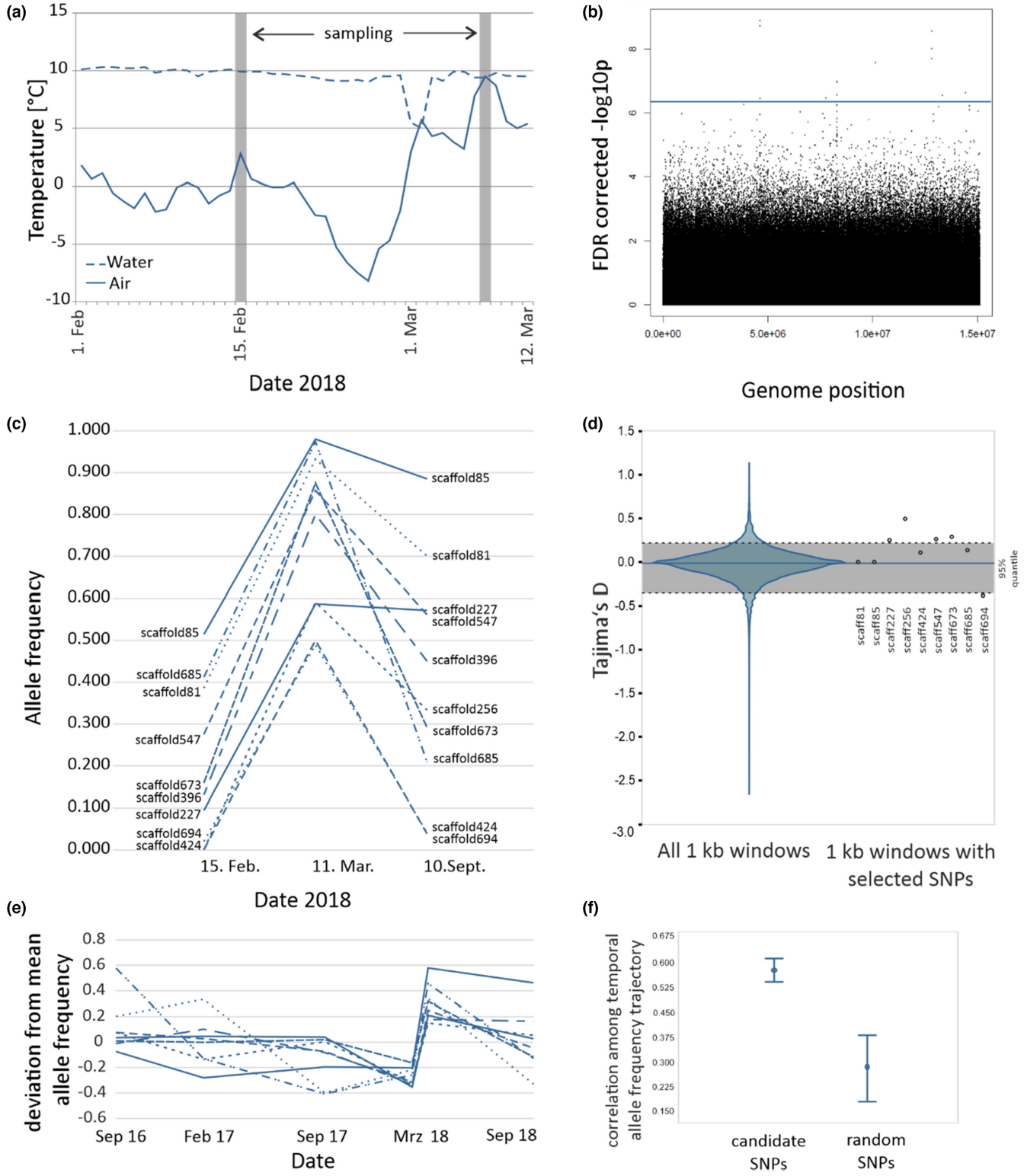


FIGURE 1 Field data. (a) Air and water temperature curves at the sampling site with sampling dates (grey bars). (b) Manhattan plot of the genome-wide SNPs FDR-corrected $-\log_{10} p$ values, contrasting the population pools sampled before and after the cold snap. The horizontal line shows the inferred threshold. (c) Allele frequency trajectories at potentially selected loci before and after the cold snap and 6–7 generations later in September 2018. (d) Left: Violin-plot of the distribution of Tajima's D for all 177,185 1 kb windows in the genome The mean is indicated by a blue horizontal line, the grey area indicates the 90% quantile around the mean. The dashed lines mark the beginning of the upper, respectively lower 5% quantile. Right: Tajima's D for the 1 kb windows harbouring the candidate SNPs. For one window (scaff396) Tajima's D could not be computed. (e) Long term allele frequency trajectories of the candidate loci. (f) Comparison of the mean pairwise correlation coefficient of the candidate loci (left) and random SNPs (right).

Selected SNP loci were identified by their allele-frequency change (AFC) larger than expected by sampling variance. Neutral simulations were used to compute false discovery rate *q*-values < 0.001 with parameters (number of SNPs, starting allele frequencies matching the ancestral population, sequence coverage, number of generations) matching those of the respective samples. To be conservative, we calculated the drift for one generational passage. As effective population size, we used 15,000, which constitutes a very conservative estimate as well (see Waldvogel et al., 2018). The resulting −log10 *p*-value includes an assessment of the sampling depth at the respective locus. All calculations and simulations were performed with the R-library poolSeq (Taus et al., 2017).

To infer the selection regime acting in the long-term at the identified loci and linked sites, we calculated Tajima's D as a summary statistic of the site frequency spectrum (Hohenlohe et al., 2010). As this statistic is insensitive to short-term changes (Hohenlohe et al., 2010), only a single pool was used for the analysis. We used Popoolation1 to calculate Tajima's D for all nonoverlapping 1 kb windows in the genome. We then compared Tajima's D from the windows harbouring the candidate loci with the distribution of this statistics in all other windows (Schreiber & Pfenninger, 2021). The window size was chosen based on the short average LD (<150 bp) in this species (Pfenninger & Foucault, 2022).

The long-term behaviour of the candidate loci was investigated using data from Pfenninger and Foucault (2022). The mean allele frequency for each locus was determined for seven time points and the deviation from this mean was plotted for each time point. In addition, we determined the average pairwise correlation of these loci over time and compared it with that of the same number of randomly selected, uncoupled SNPs to see whether the candidate loci tend evolve in concert over longer time periods as well.

## 2.3 | Experimental confirmation

To verify the association of the SNPs to survival under cold stress, we exposed 160 fourth instar larvae from a genetically variable laboratory population initially gained from the same population (Pfenninger & Foucault, 2022) to 4°C until they died or survived for at least 28 days. The individuals were kept separately in 2 cm well plates with at least 1 cm water column in a normal fridge. We checked daily whether they were still alive by touching them to see if they still moved. Dead larvae or larvae still alive on the 28th day were individually transferred to tubes filled with 70% alcohol and the day of their death recorded. Of these we chose 30 individuals which died early and 30 individuals which died late or even survived until the end of the 28th days for individual whole genome resequencing (see below).

To link genotype to gene expression, we were not able to use the dead individuals from the survival experiment mentioned above. We therefore performed a corresponding short-term experiment exposing another set of 54 fourth instar larvae from the same laboratory population to 4°C, this time for 3 days only in order to guarantee for survival. After these 3 days, 36 living individuals were cut into three pieces on a −80°C cool pad. We cut two segments from a mid-body segment from each larva for subsequent DNA isolation and resequencing, and the rest of the individual was stored at −80°C for later RNA-isolation. We chose the two mid-body segments for DNA extraction, because these segments are most redundant with other such segments in terms of specialized organs or tissues, thus preventing bias or omission in RNA analysis as far as possible.

## 2.4 | DNA/RNA isolation and sequencing

In total, DNA was isolated from 96 individuals (60 from the long-term, and 36 from the short-term 4°C exposure experiments) using the Qiagen blood and tissue kit. RNA was extracted using the Quick-RNA Miniprep kit (Zymo Research) from 36 single individuals. Library preparation and 150 bp paired-end sequencing was conducted on a NovaSeq platform at Novogene.

## 2.5 | Identification of individual genotypes

Quality trimming and mapping of reads was conducted similar to the approach outlined above. Genotypes at the SNP positions identified in the PoolSeq approach were called with bcftools version 1.10.2 (Li, 2011). The genotypes were cross-checked manually for a random subsample of individuals with IGV viewer version 2.8.2 (Thorvaldsdóttir et al., 2013). We calculated the mean number of potentially adaptive alleles (i.e., those that rose in frequency in the natural population) per variable locus (MNAA) for each resequenced individual as quantitative measure of the multilocus genotype at the respective loci, thereby assuming an additive genotype-to-phenotype relationship (Sella & Barton, 2019).

## 2.6 | Association of survival times with genotypes

Under the assumption that alleles identified to rise in frequency during the cold snap indeed conferred collectively a fitness advantage (i.e., polygenic trait of longer survival) to its bearers, we expected that individuals carrying more of these alleles should also tend to survive longer in the experiment. We used a Bayesian implementation of a Pearson's correlation analysis (Bååth, 2014) to test for a correlation between MNAA and survival times. Instead of testing the biologically implausible assumption of no association between variables, the Bayesian approach evaluates support for a positive or negative correlation in the data and provides high density intervals, corresponding to confidence limits, for the Pearson correlation parameter estimate (Bååth, 2014).

### 2.7 | RNA-seq analysis of cold-exposed individuals and co-expression networks

Three samples were of bad quality and thus not sequenced. For the other 33 samples, we obtained between 28 to 69 Mio reads per individual, of which ~0.1% of bp were trimmed and 100% of the reads remained after trimming (Table S1). Adapters were trimmed and quality checked with TrimGalore (Krueger, 2016). HiSat2 version 2.1.0 (Kim et al., 2019) was used to map the reads to the *C. riparius* genome (Schmidt et al., 2020). The counts table (Table S2) was created with HTSeq (Anders et al., 2015). To prevent spurious results due to low read counts, we removed genes with less than 10 reads in at least four samples, and samples C4 and C6 due to missing allele frequency information. The differential gene expression analysis was conducted using DESeq2 (Love et al., 2014) with the mean number of potentially adaptive alleles per variable locus (MNAA) as continuous variable. We tested for association of genotypes at the identified variable SNP positions and normalized gene expression for genes within ±200 kb on the same scaffold with the Bayesian correlation test described above.

To identify networks of coexpressed genes (modules), we constructed a weighted gene coexpression network analysis using the R package WGCNA (Langfelder & Horvath, 2008), based on the genes that had passed the quality filtering step for the expression analysis ($N$ = 8264). Gene counts were normalized using the varianceStabilizingTransformation function from DESeq2 (Love et al., 2014). Following the WGCNA guidelines, we chose a soft-thresholding power of 6 for adjacency calculation. To associate modules to the MNAA polygenic score, we first calculated the modules' eigengene using the moduleEigengenes function and tested for module trait correlation using the *corPvalueStudent* function. To obtain up-to-date annotations, we ran a local blastp (Altschul et al., 2008) of the *C. riparius* proteins versus the nonredundant protein database (version January 2022). We ran Interproscan version 5.53–87.0 (Jones et al., 2014) locally to obtain GO information using the *C. riparius* predicted proteome. The GO enrichment analysis on genes within the significant module was performed with the R package TopGO (Alexa & Rahnenführer, 2016), using the "parentchild" algorithm and the Fisher's exact test for significance.

## 3 | RESULTS

### 3.1 | Cold snap and sampling

From 21 February 2018, the air temperature at the sampling site dropped for 10 consecutive days below zero, with a minimum daily average of −8°C on 27 February 2018. The water temperature, usually fluctuating around 10°C in winter, started to fall slowly as well. At the end of this period, the water temperature dropped steeply to 5°C for two consecutive days. We sampled the population 6 days after the water temperatures were back to about 10°C (Figure 1a). Sampling the required number of larvae after the cold snap took considerably more time than before.

### 3.2 | Large allele frequency changes within a single generation

The allele frequency changes of 19 SNPs between before and after the cold snap could not be explained by sampling variance (Figure 1b). These SNPs were therefore considered as candidates for selection (Table 1). Some of these SNPs occurred on the same scaffold in close spatial proximity to other such SNPs (within 40–750 bp, Table 1). As resequencing data showed, the rising alleles at these SNPs were linked to haplotypes (data not shown). We considered the regions with several SNPs therefore as a single locus and the SNP with the largest AFC was used as marker SNP for these linked haplotypes. Taking this into account, 10 loci, each on a different scaffold of the reference genome and thus most likely physically unlinked (Pfenninger & Foucault, 2022), were potentially affected by selection. We refer to these loci by their scaffold numbers hereafter (e.g., scaffold 227; Table 2).

The starting frequencies of the rising alleles at these candidate loci before the cold snap were highly variable, spanning the range from undetectably rare (scaffold 424 and scaffold 674) to the majority allele (scaffold85, Figure 1c). All candidate alleles rose in frequency by at least about 0.5 (0.467–0.781, Table 1, Figure 1c). In September 2018, the allele frequencies of all but one locus (scaffold 227) dropped back towards the level they had before the cold snap event (Figure 1c, Table 3).

The effect of the cold snap on allele frequency spectra varied between candidate loci. Comparing Tajima's D in the 1 kb windows encompassing the selected regions with the distribution of all 1 kb windows showed that four selected regions had a Tajima's D in the upper 5% quantile (scaffold 227, scaffold 256, scaffold 547, scaffold 673), indicating balancing selection. One value (scaffold 694) was in the lower 5% tail, suggesting a recent selective sweep. The remaining values were inconspicuous (four) or not calculable (one, Figure 1d).

### 3.3 | Validation experiment

Of the 160 larvae constantly exposed to 4°C, the first larvae died after 15 days. Mortality on day 21 was extraordinarily high (53 individuals, 34%). After 28 days, 17 (11%) larvae were still alive. This was a much higher mortality than the usually observed 10%–25% from hatching to eclosion in experimental settings at normal temperatures (Foucault et al., 2019). For seven individuals, the dying day could not be clearly determined. The distribution of survival over time can be found in Figure S1.

### 3.4 | Genotyping of experimental individuals

For 59 of the 60 randomly selected individuals from the experiment, resequencing was successful. The individuals could be genotyped on average at 9.02 out of the 10 candidate loci. Two loci were fixed for

**TABLE 1** Outlier SNPs in field data

| Scaffold | Position | Reference allele | Alternate allele | Rising allele | Start allele freq | End allele freq | AFC | $-\log_{10}p$ | *p* fdr-corrected | Tajima's D 1 k |
|---|---|---|---|---|---|---|---|---|---|---|
| Scaffold 81 | 616771 | T | C | T/ref | 0.386 | 0.932 | 0.545 | 6.636 | .035 | −0.0050318 |
| Scaffold 85 | 970786 | G | C | G/ref | 0.514 | 0.980 | 0.467 | 6.224 | .046 | −0.0162351 |
| Scaffold 227 | 185498 | A | G | G/alt | 0.094 | 0.587 | 0.493 | 6.267 | .046 | 0.2564445 |
| Scaffold 256 ~750 bp | 10257 | C | G | G/alt | 0.000 | 0.591 | 0.591 | 8.734 | .001 | 0.4939933 |
| | 10279 | A | G | G/alt | 0.000 | 0.765 | 0.765 | 8.897 | .001 | |
| | 11003 | G | C | C/alt | 0.086 | 0.867 | 0.781 | 6.455 | .037 | |
| Scaffold 396 | 51484 | A | G | A/ref | 0.130 | 0.800 | 0.670 | 6.466 | .037 | NA |
| Scaffold 424 ~400 bp | 450404 | C | G | G/alt | 0.000 | 0.557 | 0.557 | 6.266 | .046 | 0.1154956 |
| | 450407 | G | T | T/alt | 0.000 | 0.557 | 0.557 | 6.266 | .046 | |
| | 450623 | G | A | A/alt | 0.000 | 0.500 | 0.500 | 6.561 | .035 | |
| | 450627 | A | C | C/alt | 0.000 | 0.534 | 0.534 | 6.967 | .019 | |
| | 450663 | C | T | T/alt | 0.000 | 0.500 | 0.500 | 6.236 | .046 | |
| | 450800 | C | T | T/alt | 0.000 | 0.533 | 0.533 | 6.993 | .019 | |
| Scaffold 547 | 301068 | T | C | T/ref | 0.275 | 0.857 | 0.582 | 7.582 | .006 | 0.2763451 |
| Scaffold 673 ~40 bp | 15211 | G | T | T/alt | 0.140 | 0.857 | 0.718 | 8.015 | .003 | 0.3208233 |
| | 15229 | A | G | G/alt | 0.163 | 0.862 | 0.699 | 7.706 | .005 | |
| | 15257 | C | T | T/alt | 0.159 | 0.875 | 0.716 | 8.569 | .001 | |
| Scaffold 685 | 402992 | T | C | T/ref | 0.412 | 0.974 | 0.562 | 6.190 | .047 | 0.1319014 |
| Scaffold 694 | 289921 | T | G | T/ref | 0.020 | 0.489 | 0.469 | 6.552 | .035 | −0.3782241 |

**TABLE 2** Nearest genes to potentially selected loci

| Scaffold | Position | Distance to closest gene | Gene start | Gene end | Annotation | Annotation spatially most proximate gene |
|---|---|---|---|---|---|---|
| Scaffold 81 | 616771 | 5044 | 621815 | 629417 | Scaffold81-snap-gene-1.377 | Uncharacterized protein LOC119074020 |
| Scaffold 85 | 970786 | 120 | 970906 | 973122 | Scaffold85-snap-gene-1.251 | |
| Scaffold 227 | 185498 | 6810 | 194991 | 196989 | Scaffold227-augustus-gene-0.165 | cytochrome P450 28a5 |
| Scaffold 256 | 10257 | 1802 | 6914 | 8455 | Scaffold256-snap-gene-0.15 | Delta-aminolevulinic acid dehydratase-like |
| Scaffold 396 | 51484 | 24,716 | 12088 | 26768 | Scaffold396-processed-gene-0.5 | trichohyalin isoform X3 |
| Scaffold 424 | 450800 | 265 | 451065 | 456420 | Scaffold424-augustus-gene-0.206 | Uncharacterized protein LOC109541342 isoform X2 |
| Scaffold 547 | 301068 | 1357 | 297539 | 302425 | Scaffold547-processed-gene-0.68 | Protein dachsous |
| Scaffold 673 | 15257 | 208 | 15465 | 17951 | Scaffold673-augustus-gene-0.265 | Histone acetyltransferase p300 |
| Scaffold685 | 402992 | 42,771 | 358566 | 360221 | Scaffold685-processed-gene-0.94 | – |
| Scaffold 694 | 289921 | 16,169 | 306317 | 308300 | Scaffold694-snap-gene-0.260 | – |

**TABLE 3** Allele frequencies in the experiment

| Scaffold | Position | Reference allele | Alternate allele | Rising allele | AF Feb18 | AF Mar18 | AF Sep18 | AF in exp pop |
|---|---|---|---|---|---|---|---|---|
| Scaffold 81 | 616771 | T | C | T/ref | 0.386 | 0.932 | 0.700 | 1.000 |
| Scaffold 85 | 970786 | G | C | G/ref | 0.514 | 0.980 | 0.885 | 0.907 |
| Scaffold 227 | 185498 | A | G | G/alt | 0.094 | 0.587 | 0.571 | 0.339 |
| Scaffold 256 | 10257 | C | G | G/alt | 0.000 | 0.591 | 0.333 | 1.000 |
| Scaffold 396 | 51484 | A | G | A/ref | 0.130 | 0.800 | 0.450 | 0.894 |
| Scaffold 424 | 450623 | G | A | A/alt | 0.000 | 0.500 | 0.038 | 0.724 |
| Scaffold 547 | 301068 | T | C | T/ref | 0.275 | 0.857 | 0.561 | 0.839 |
| Scaffold 673 | 15257 | C | T | T/alt | 0.159 | 0.875 | 0.291 | 0.902 |
| Scaffold 685 | 402992 | T | C | T/ref | 0.412 | 0.974 | 0.208 | 0.991 |
| Scaffold 694 | 289921 | T | G | T/ref | 0.020 | 0.489 | 0.041 | 0.414 |

Abbreviation: AF, allele frequency.

the rising allele in the experimental sample (Table 3) and were thus not further considered.

In the 59 individuals used in the experiment, all candidate alleles had a considerably higher start frequency than in the natural population before the cold snap (Table 3). Nevertheless, among the survivors at day 25, the frequency of all candidate alleles rose between 0.04 and 0.22 in the course of the experiment with moderate (60.6%) to very high (98.4%) posterior probability (Figure 2a). The mean number of potentially adaptive alleles per variable locus (MNAA) locus ranged between 0.50 and 1.38 among the individuals in the experiment with a mean of 0.98 (s.d. 0.18). Individuals with genotypes containing more potentially adaptive alleles (MNAA) survived the cold stress conditions longer. The association between these variables was moderately strong (most likely estimate of $r = .33$, 95% high density interval between 0.08 and 0.55, Figure 2b). The correlation of MNAA with the length of survival was positive with almost certainty (posterior probability 99.3%) and also significantly different from zero in a frequentist approach ($p < .01$).

### 3.5 | Genotype associations with local and global gene expression data

For 31 individuals with genotype information, we obtained gene expression data for 11,386 of the 13,449 annotated genes (85%). Only two loci showed all possible genotypes at the candidate loci in the individuals used for transcription analysis (scaffold 227 and scaffold 694). On their scaffolds, the genotypes were strongly associated with the expression levels of one (scaffold 227, Figure 3a), or respectively two (scaffold 694, Figure 3b) genes. On scaffold 227, the selected SNP was in an exon of the gene to which it was associated via the genotype specific expression levels. Specifically, the candidate allele was associated to a higher expression rate. The gene (scaffold227_gene0.177) is annotated as cytochrome P450, family 6 (CYP6). The two genes with expression levels strongly associated to genotypes on scaffold 694 were both roughly 100 kb away from the selected SNP. The first gene, scaffold694_gene0.209 codes for protein (RFT1) that is essential for protein N-glycosylation and here the candidate allele was associated with an increase of transcription.

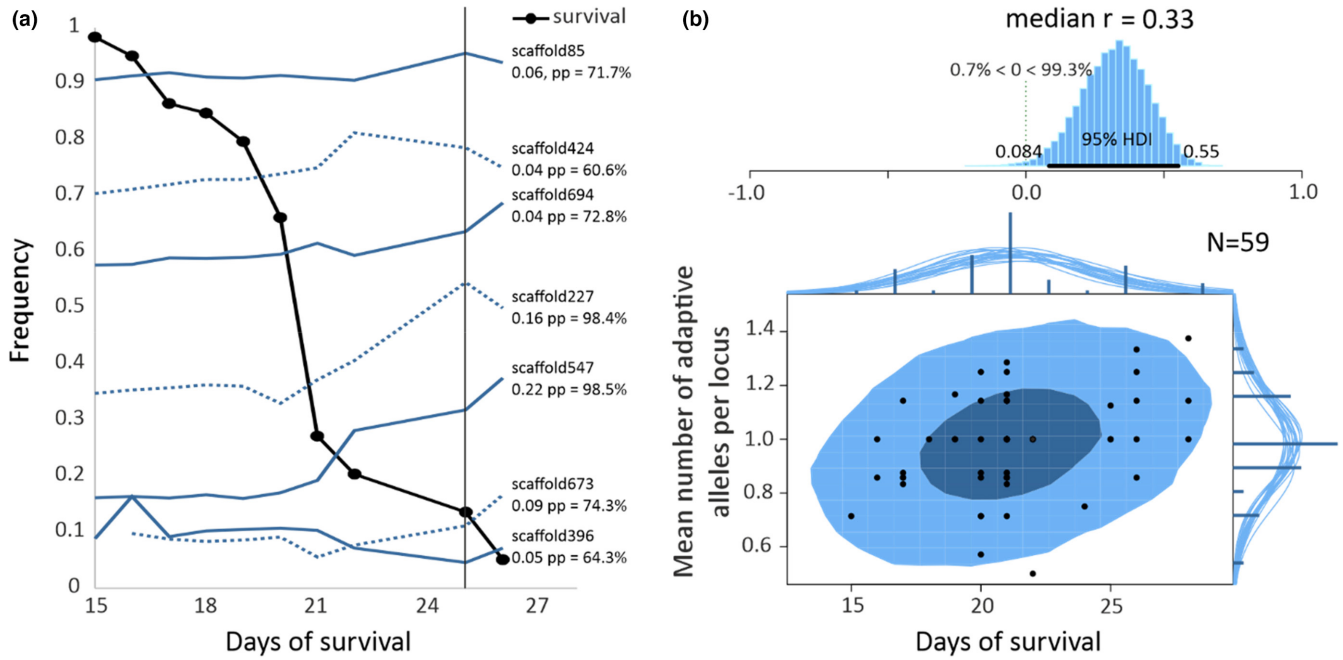


FIGURE 2 Experimental data. (a) Temporal course of allele frequency changes at polymorphic candidate loci and survival of individuals during the experiment. Shown in blue are the frequency trajectories of the selected alleles in the natural population. The values to the right show the median Bayesian estimate of increase and the associated posterior probability (pp) of this value being larger than zero for the respective alleles. (b) Bayesian estimate of correlation between the mean number of potentially adaptive alleles per locus and the survival time in the experiment.

In contrast, for the second gene scaffold694_gene0.209, a transmembrane receptor protein tyrosine phosphatase (DEP1), a lower transcription level was associated with the rising candidate allele.

In total, we found the expression of 28 genes to be significantly associated with the MNAA. Nine out of these (32%) belong to genes of (larval) cuticule proteins, endocuticule proteins or endochitinase. One of the 20 distinct coexpressed modules identified, module-cyan (containing 169 genes Table S3), covaried substantially with MNAA (Figure 4a; $r = .41$, $p = .03$, Table S4). A GO-enrichment analysis revealed that gene functions related to fatty acid metabolism were overrepresented in this module (Figure 4b, Table S3). The cold snap candidate loci themselves were not part of module cyan.

## 4 | DISCUSSION

### 4.1 | Natural experiment

In this study, we took advantage of a natural experiment. Following a sampling routine, we coincidently sampled a population pool from a natural *C. riparius* population in late winter just before a cold snap (Pfenninger & Foucault, 2020). Such cold snaps at this time of the year are not the rule in Germany, but also not uncommon. The drop of temperature was marked, but not extreme. Likewise, the duration of the snap was relatively short, at least with regard to the temperature drop in the water. It was therefore an event with the potential to leave a selective mark, but it was not an extreme weather event, let alone a catastrophe. This promised the opportunity to study the selective effects of a defined transient event as is typical for the selective regime of fluctuating environments (Bell, 2010). To infer allele frequency changes potentially driven by selection, we therefore sampled another pool from the same site directly after the cold snap.

*Chironomus riparius* does not reproduce at temperatures below 10°C and larval development is nearly stalled. We can therefore rule out selection based on differential reproductive success over the time-span of the experiment (Reznick, 2016). Any potential selection must have occurred in the same generation by differential mortality of different genotypes. The substantially increased time to collect sufficient specimen second sampling despite digging deeper indeed indicated that the cold snap most likely resulted in significant mortality in the population. As chironomids tend to dwell as deep as the interplay between their haemoglobin concentration and oxygen-availability in the environment allows (Panis et al., 1996), it was unlikely that the differential catch-rate was, for example, due to deeper burrowing larvae. Without being able to precisely quantify the demographic decline, this indicates a much smaller population size after the cold snap, as experience has shown that the duration of sampling for a predefined number of individuals at a known occurrence site depends on the population density.

At the same time this meant that genetic drift, defined as sampling variance among generations (Wright, 1948), could not have played a role here, since there was no generational passage involved. We have nevertheless included one generation of drift in the calculation of the selection threshold. This threshold is therefore particularly conservative which increases our certainty that the observed allele frequency change beyond this threshold is not due to random

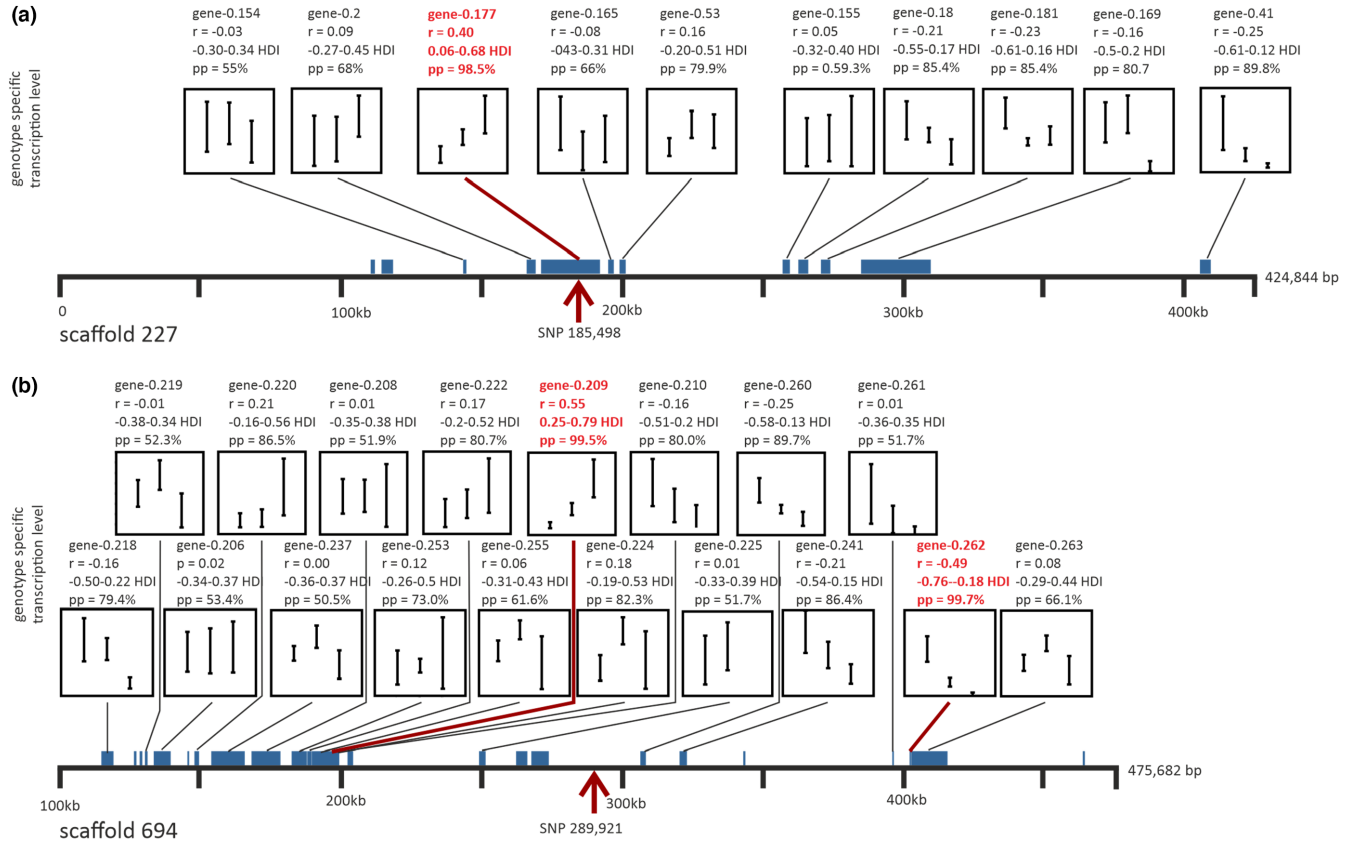


**FIGURE 3** Associations of genotype at selected sites with gene expression levels of genes within +/− 200 kb on the same scaffold. The position of the selected SNP is indicated with a red arrow. Annotated genes are indicated as blue bars, whose length is proportional to the length of the gene. For genes with expression data available, the genotype specific transcription level is given in a plot. Within plot, the bars represent the standard deviation range of gene expression variation for the possible genotypes, from left to right: homozygous falling allele, heterozygous, homozygous rising allele. Above the panels, the Bayesian statistics for association are given. r, coefficient of association; HDI, 95% high density interval; pp, posterior probability for the association coefficient being larger or smaller than zero, respectively. Highlighted in red are genes with a HDI not comprising zero and pp > 95%. (a) Scaffold 227 with selected SNP at position 185,498 with an upregulation associated with the rising allele at gene 0.177. The SNP is positioned in an exon of the gene, (b) Scaffold 694 with selected SNP at position 289,921. One gene (0.209), situated about 100 kb from the SNP showed a strong upregulation associated with the rising allele. Another gene (0.262), more than 100 kb away showed a strong downregulation.

processes (Barghi et al., 2019; Hohenlohe et al., 2010). On the other hand, we have certainly missed smaller, yet selection driven allele frequency changes.

## 4.2 | Signs of selection in the natural population

In total, 10 regions in the genome showed signs of selection in the data. Given our rather conservative threshold, which required a large change in allele frequency (~0.5) for detection, we assume that many loci with less pronounced changes remained undetected. The candidate haplotypes were very short, often a single SNP, which indicated that they are relatively ancient polymorphisms long since segregating in the population (Nordborg & Tavaré, 2002) and thus separated by recombination from the background in which they arose. Analysis of Tajima's D for the 1 kb windows the selected haplotypes resided in showed that nine out of 10 had a positive D, four even in the upper 5% quantile. This indicated that the respective polymorphisms could be regularly under differential, balancing selection (Fijarczyk & Babik, 2015). Given that the presumed selection pressure was a seasonal event, a more or less regularly fluctuating environment with opposite selection pressures in winter and summer appeared plausible. Alternatively, the return to initial frequencies may be due to costs associated with antagonistic pleiotropy (Marden et al., 2021). Both can lead to a long-term maintenance of the polymorphism under biologically plausible scenarios (Wittmann et al., 2017). A recent study suggested that balancing selection could be more widespread than previously thought (Gloss & Whiteman, 2016).

The view that seasonal fitness is related to the different alleles at the identified loci was confirmed by the observation that allele frequencies at the candidate loci, with one exception (scaffold 227), returned to near their original frequencies a few generations later. This remarkable correlation between the temporal allele frequency trajectories suggested that the same selection pressure with changing signs among seasons was acting. Seasonally selected polymorphisms with correlated allele frequency trajectories were

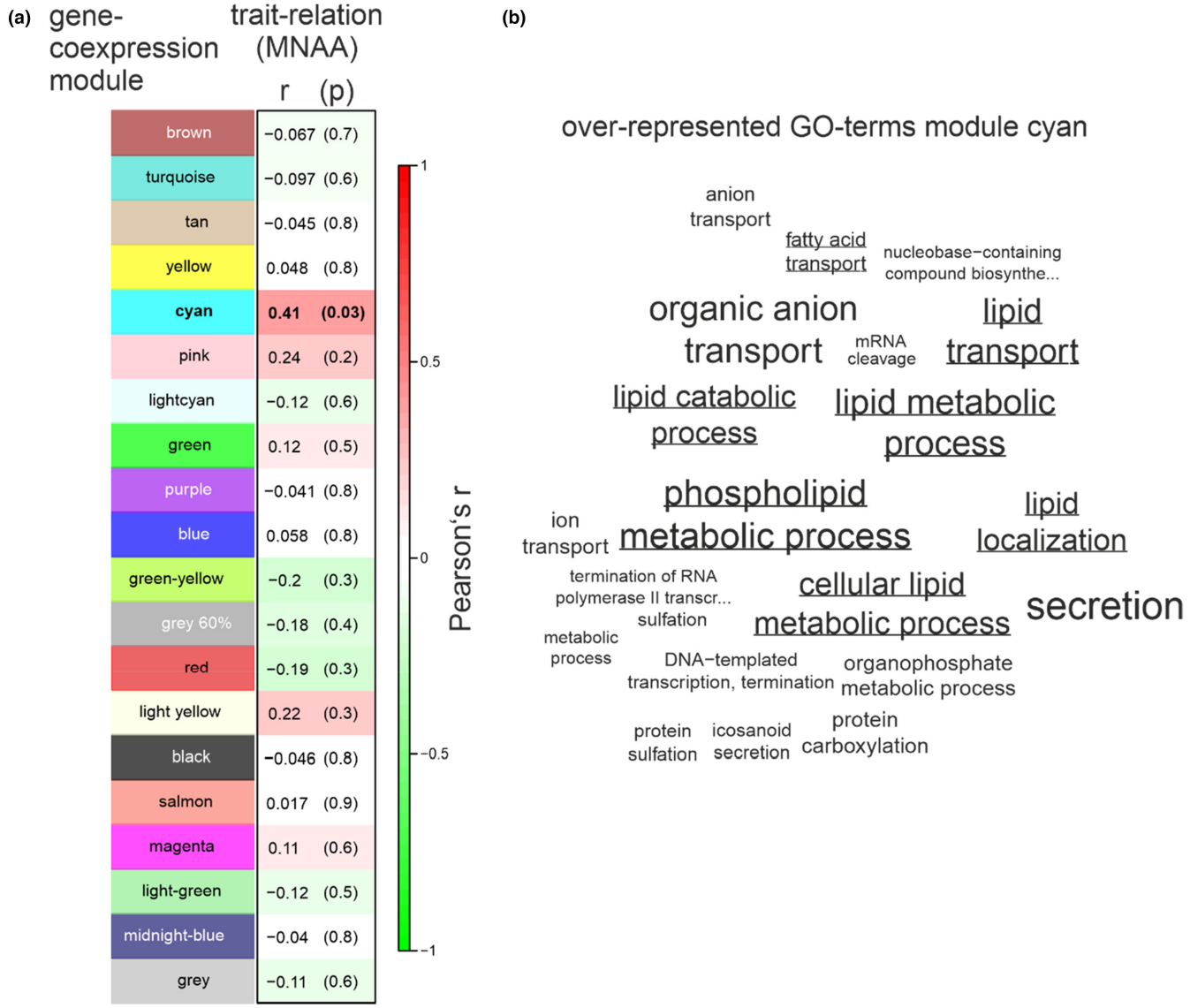


**FIGURE 4** Association between mean number of adaptive alleles (MNAA) and gene coexpression modules. (a) Correlation of MNAA with inferred gene coexpression modules. The modules carry arbitrary colour names. In the right column, the Pearson correlation coefficient between individual transcription levels and the polygenic score and its probability of being identical to zero (in brackets) for the respective module is given. For immediate visual recognition, the correlation coefficient was also translated into a heatmap from green (−1) over white (0) to red (+1). (b) Word-cloud of significantly over-represented gene ontology (GO) terms in the cyan gene coexpression module. The font size is proportional to the number of genes. GO terms with relation to fatty acid metabolism are underlined.

also observed in natural populations of another dipteran species, *Drosophila melanogaster* (Bergland et al., 2014; Croze et al., 2017; but see Buffalo & Coop, 2020). But also the selection-driven beak variability of Galapagos finches in response to different weather conditions in different years, taking into account the different generation times, are a classic example of very rapid evolutionary adaptations to a variable environment (Boag & Grant, 1981). Interestingly, we found a strong negative correlation between the start frequency of the selected SNPs and the absolute deviation from neutrality as measured by Tajima's D (Figure S2). Theory predicts balanced allele frequencies for overdominance (i.e., by heterozygote advantage [Slatkin & Muirhead, 1999]). However, we are not aware of theoretical predictions for expected allele frequencies due to balancing selection by temporally changing selection pressures.

## 4.3 | Validation experiment

We observed a substantial variation in survival time in the validation experiment. Compared to the field observations, it took quite a long time (15 days) until the first larvae started to die. In the field, the temperature dropped for 2 days only and this was obviously long enough to trigger a substantial mortality. This discrepancy could have several, mutually not exclusive explanations.

First, the laboratory population had already quite high allele frequencies at the loci in question. If these loci were indeed responsible for the longer survival, the laboratory population could have been *a priori* better protected against the cold exposure. This shift in allele frequency relative to the natural population might be due to random drift in the relatively large but nevertheless demographically necessarily restricted laboratory population. However, the high allele frequencies could also be a tribute to the practice of storing egg ropes at 4°C for a few days prior to initial population set-up and experiments to synchronize their development (Foucault et al., 2019). This could have involuntarily preselected the laboratory population.

Second, the larvae in the experiment came from normal development at benign temperatures (~20°C) and were well-fed before they were exposed to 4°C. The larvae in the field likely hatched in autumn and had passed already several months at about 10°C before the cold snap set in. This resulted in substantial "treatment" differences. Initially, the experimental midges experienced a veritable cold-shock, while the natural population was comparatively slowly cooled down only a few degrees. In *Drosophila*, it was shown that the rate of temperature change may well have an impact on the organismal reaction to cold exposure (Gerken et al., 2018). However, the cold shock did not cause any mortality in the experimental population, so that the effect of long-term exposure to low temperatures was probably the major cause for mortality in both experimental and natural population. Probably more importantly, the level of internal resources the two groups could draw upon were likely very different. The laboratory population could draw on their fat reserves, while the natural population had probably nearly exhausted this resource by the end of the winter and thus died faster when exposed to increased cold stress. Lastly, it is likely that the laboratory experiment did not cover all selection factors that were acting in the field. Given the simplified experimental environment, it is almost certain that the set of selection factors acting on the larvae in the field was different from those in the experiment (Pfenninger & Foucault, 2020).

Despite the reduced complexity of the validation experiment, it confirmed nevertheless the hypothesis that the candidate alleles identified in the natural population conferred longer survival during the experimental cold exposure. The same selection pressure triggered an overall increase in frequency of the same alleles in the experiment by differential mortality as was observed in nature. This is strong evidence that the candidate alleles indeed played a role in the selection process.

The positive correlation between a straightforward polygenic score (mean number of adaptive alleles per locus involved) and the survival time strongly indicated a relatively simple relation: the more of these alleles are present in an individual, the better are the chances of its longer survival under cold stress. Given the likely involvement of more, but yet unidentified loci, nonlinear interactions among loci and non-quantified environmental components (Sella & Barton, 2019), the degree of determination found here appeared quite substantial. Additional evidence that these loci are coselected by the same selection pressures came from a strong temporal covariance in allele frequencies, also over longer time scales.

### 4.4 | Single and multilocus genotype associations with gene transcription data

None of the putatively selected SNPs was within the coding region of an annotated gene. We therefore expected phenotypic effects rather due to changes in the transcription regulation of spatially more or less proximate genes than in structural protein changes.

In an attempt to link the identified loci with basal phenotypic aspects, we sequenced a set of non-lethally cold-exposed individuals for both the genome and the transcriptome. We found transcripts for a substantial proportion of the annotated genes. This is similar to results found in *Drosophila* (Brown et al., 2014). With a what could be called "inversed eQTL approach" (Gilad et al., 2008; Majewski & Pastinen, 2011), we explored spatially proximate and thus putative *cis*-interactions between identified selected sites and gene expression levels. By analysing only loci shown to be involved in phenotypic variation and restricting the spatial extent of the search to a plausible range (Schoenfelder & Fraser, 2019), we retained sufficient statistical power even with our relatively limited sample size. Since only two of the identified SNP loci (scaffold 227 and scaffold 694) showed all possible three genotypes in the sample for which both genotype and transcription data was available, the search for associated *cis*-regulated genes was necessarily restricted to these.

The gene associated to the selected site on scaffold 227 was identified as cytochrome P450, family 6, a gene-family that is characteristic for insects (Lewis et al., 1998). This gene was already several times implicated in the reaction to cold stress (Huang et al., 2017; Lv et al., 2020; Zhang et al., 2015; Zhou et al., 2019) with an increased expression level under cold conditions. It is therefore plausible that an allele associated with an inherently increased expression level as observed here is under positive selection under cold stress conditions. While the selected marker SNP on scaffold 227 was spatially closely linked to the gene with the associated transcription regulation, the genes most credibly associated to the SNP genotype on scaffold 694 were both roughly 100 kb up-, respectively downstream. Both genes were also not the closest neighbouring genes, but the fourth, respectively fifth transcribed gene up- respectively downstream on the same scaffold. These observations are compatible with recent models of gene-regulation by long range interactions (Schoenfelder & Fraser, 2019) that were also observed in insects (Dorsett, 1999). The gene situated upstream was identified as RFT1 homologue. This protein of the endoplasmatic reticulum membrane appears to be necessary for glycolipid translocation and normal protein N-glycosylation, but its exact function is unknown (Gottier et al., 2017). The associated gene downstream was most similar to a transmembrane receptor protein tyrosine phosphatase DEP1. The selected allele was associated to the downregulation of the respective transcripts. We could not find any studies that have previously linked this gene to cold stress.

The polygenic score as a proxy for survival time on the individual level was associated to 28 differentially expressed genes. One third of these (nine) belong to (larval) cuticule proteins, endocuticule proteins or endochitinase, suggesting a role for these genes in differential

survival. Moreover, the polygenic score as a proxy for survival time on the individual level revealed a moderately strong correlation ($r = .4$) to a coexpression module. The coexpression module was statistically enriched for genes involved in fatty acid metabolism. The fatty acid metabolism is known to be crucial for the overwintering of insects (Sinclair & Marshall, 2018; Storey & Storey, 2013; Toprak et al., 2020), that is, under cold stress, for example, by providing the necessary energy storage resources or keeping membranes subtle by changing their fatty acid composition (Overgaard et al., 2005). There are many examples showing that unsaturated fatty acids increase under cold temperatures in insects (reviewed in Clark & Worland, 2008; Teets & Denlinger, 2013). Moreover, fat content was one among other phenotypes linked to exposure to a new temperature in *Drosophila*, which also appears to be a polygenic trait (Barghi et al., 2019).

The association suggested that at least some of the identified loci may be directly or indirectly involved in *trans*-regulation of the transcription of these genes. A potential candidate for such a *trans*-regulation was one of the spatially closest genes to a selected site; a histone acetyltransferase p300 on scaffold 673. These genes are high level switches that regulate broad scale gene transcription pattern via chromatin remodelling (Tropberger et al., 2013). Among many other biological functions, it has been shown that the gene is responsible for the differentiation of fat cells (Gesta et al., 2007). The transcription level of this gene was significantly associated with the observed genotypes (Figure S3), suggesting that a *trans*-regulated altered transcription of this gene could provide a link between the multilocus genotype and the fatty acid metabolism genes.

This finding allowed us to hypothesise that the identified selected SNPs could, among others, contribute to the observed variation in survival time via particular physiological trait(s). Individuals with a high polygenic score for the loci involved could, for example, accumulate more fat reserves, which increased survival time under prolonged cold conditions. This clearly beneficial fitness effect during the winter season could be reversed in summer, when the accumulation of then unnecessary fat reserves deviates resources from reproduction or increases attractivity for predators. Such a more or less regularly fluctuating selection regime on a polygenic trait could well conform to the theoretical preconditions necessary to maintain the respective polymorphisms over longer periods (Wittmann et al., 2017). Whether there is really a link between the extent of the individual fat reserves and the observed survival time under cold stress conditions, however, remains to be tested, just like a causal relation between the respective multilocus genotype and the regulation of the fatty acid metabolism. However, many more intermediate level phenotypic traits, for example, changes in cuticule composition likely contributed to survival under constant cold exposure.

## 5 | CONCLUSIONS

In this study we could show that normal, short term environmental variability can lead to measurable natural selection on a polygenic trait in a natural population. Time series population genomic analyses from field samples obviously have the power to pick up such transient signals, even if they consist rather of moderate to strong changes in allele frequencies than in fixation of loci. The observed high temporal correlation of the allele-frequency changes of the loci involved holds the promise that changes in the selective regime of natural populations may be identified from population genomic time series data.

## AUTHOR CONTRIBUTIONS

Markus Pfenninger, Ann-Marie Waldvogel and Barbara Feldmeyer conceptualized the study, Quentin Foucault carried out the fieldwork, Markus Pfenninger and Ann-Marie Waldvogel performed experiments, Markus Pfenninger, Quentin Foucault and Barbara Feldmeyer analysed the data, and Markus Pfenninger drafted the manuscript with contributions from all coauthors.

## ACKNOWLEDGEMENTS

We thank Andreas Wieser for help with sampling and the Abwasserverband Freigericht for generously providing the environmental Hasselbach data. The work was partially funded by DFG (grant no. PF390/8-1). Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All sequencing data is publicly available on ENA. PoolSeq data: (project ERP115516, samples ERS4040036–ERS4040041). Individual resequencing data: (project PRJEB56138). Transcriptome data: (project PRJEB56138).

## BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the research, sharing of our data and results on public databases as described above.

## ORCID

*Markus Pfenninger* https://orcid.org/0000-0002-1547-7245
*Ann-Marie Waldvogel* https://orcid.org/0000-0003-2637-0766
*Barbara Feldmeyer* https://orcid.org/0000-0002-0413-7245

## REFERENCES

Alexa, A., & Rahnenführer, J. (2016). *Gene set enrichment analysis with topGO*. R package version 2.24. 0.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (2008). BLAST: Basic local alignment search tool. In *Encyclopedia of genetics, genomics, proteomics and informatics* (p. 221). Springer Netherlands.

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—A python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169.

Andrews, S. (2010). *FastQC-A quality control application for FastQ files*. Babraham Bioinformatics.

Bååth, R. (2014). Bayesian first aid: A package that implements Bayesian alternatives to the classical*. test functions in R. *Proceedings of UseR*, *2014*, 2.

Barghi, N., Hermisson, J., & Schlötterer, C. (2020). Polygenic adaptation: A unifying framework to understand positive selection. *Nature Reviews Genetics*, *21*(12), 769–781.

Barghi, N., Tobler, R., Nolte, V., Jakšić, A. M., Mallard, F., Otte, K. A., Dolezal, M., Taus, T., Kofler, R., & Schlötterer, C. (2019). Genetic redundancy fuels polygenic adaptation in *Drosophila*. *PLoS Biology*, *17*(2), e3000128.

Bell, G. (2010). Fluctuating selection: The perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *365*(1537), 87–97.

Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S., & Petrov, D. A. (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. *PLoS Genetics*, *10*(11), e1004775.

Bitter, M. C., Kapsenberg, L., Gattuso, J.-P., & Pfister, C. A. (2019). Standing genetic variation fuels rapid adaptation to ocean acidification. *Nature Communications*, *10*(1), 1–10.

Boag, P. T., & Grant, P. R. (1981). Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galapagos. *Science*, *214*(4516), 82–85.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120.

Brown, J. B., Boley, N., Eisman, R., May, G. E., Stoiber, M. H., Duff, M. O., Booth, B. W., Wen, J., Park, S., Suzuki, A. M., Wan, K. H., Yu, C., Zhang, D., Carlson, J. W., Cherbas, L., Eads, B. D., Miller, D., Mockaitis, K., Roberts, J., … Suzuki, A. M. (2014). Diversity and dynamics of the *Drosophila* transcriptome. *Nature*, *512*(7515), 393–399.

Buffalo, V., & Coop, G. (2020). Estimating the genome-wide contribution of selection to temporal allele frequency change. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(34), 20672–20680.

Clark, M. S., & Worland, M. R. (2008). How insects survive the cold: Molecular mechanisms—a review. *Journal of Comparative Physiology B*, *178*(8), 917–933.

Croze, M., Wollstein, A., Božičević, V., Živković, D., Stephan, W., & Hutter, S. (2017). A genome-wide scan for genes under balancing selection in Drosophila melanogaster. *BMC Evolutionary Biology*, *17*(1), 15.

de Villemereuil, P., Charmantier, A., Arlt, D., Bize, P., Brekke, P., Brouwer, L., Cockburn, A., Côté, S. D., Dobson, F. S., Evans, S. R., Festa-Bianchet, M., Gamelon, M., Hamel, S., Hegelbach, J., Jerstad, K., Kempenaers, B., Kruuk, L. E. B., Kumpula, J., Kvalnes, T., … Chevin, L. M. (2020). Fluctuating optimum and temporally variable selection on breeding date in birds and mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(50), 31969–31978.

Doria, H. B., Caliendo, C., Gerber, S., & Pfenninger, M. (2022). Photoperiod is an important seasonal selection factor in Chironomus riparius (Diptera: Chironomidae). *Biological Journal of the Linnean Society*, *135*(2), 277–290.

Doria, H. B., Hannappel, P., & Pfenninger, M. (2022). Whole genome sequencing and RNA-seq evaluation allowed to detect Cd adaptation footprint in Chironomus riparius. *Science of the Total Environment*, *819*, 152843.

Doria, H. B., Waldvogel, A.-M., & Pfenninger, M. (2021). Measuring mutagenicity in ecotoxicology: A case study of Cd exposure in Chironomus riparius. *Environmental Pollution*, *272*, 116004.

Dorsett, D. (1999). Distant liaisons: Long-range enhancer–promoter interactions in *Drosophila*. *Current Opinion in Genetics & Development*, *9*(5), 505–514.

Fijarczyk, A., & Babik, W. (2015). Detecting balancing selection in genomes: Limits and prospects. *Molecular Ecology*, *24*(14), 3529–3545.

Foucault, Q., Wieser, A., Waldvogel, A. M., Feldmeyer, B., & Pfenninger, M. (2018). Rapid adaptation to high temperatures in Chironomus riparius. *Ecology and Evolution*, *8*(24), 12780–12789. https://doi.org/10.1002/ece3.4706

Foucault, Q., Wieser, A., Waldvogel, A.-M., & Pfenninger, M. (2019). Establishing laboratory cultures and performing ecological and evolutionary experiments with the emerging model species *Chironomus riparius*. *Journal of Applied Entomology*, *143*(5), 584–592.

Gerken, A. R., Eller-Smith, O. C., & Morgan, T. J. (2018). Speed of exposure to rapid cold hardening and genotype drive the level of acclimation response in Drosophila melanogaster. *Journal of Thermal Biology*, *76*, 21–28.

Gesta, S., Tseng, Y.-H., & Kahn, C. R. (2007). Developmental origin of fat: Tracking obesity to its source. *Cell*, *131*(2), 242–256.

Gilad, Y., Rifkin, S. A., & Pritchard, J. K. (2008). Revealing the architecture of gene regulation: The promise of eQTL studies. *Trends in Genetics*, *24*(8), 408–415.

Gloss, A. D., & Whiteman, N. K. (2016). Balancing selection: Walking a tightrope. *Current Biology*, *26*(2), R73–R76.

Gottier, P., Gonzalez-Salgado, A., Menon, A. K., Liu, Y.-C., Acosta-Serrano, A., & Bütikofer, P. (2017). RFT1 protein affects glycosylphosphatidylinositol (GPI) anchor glycosylation. *Journal of Biological Chemistry*, *292*(3), 1103–1111.

Grant, B. R., & Grant, P. R. (1989). Natural selection in a population of Darwin's finches. *The American Naturalist*, *133*(3), 377–393.

Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E., Beerli, P., & Kingsolver, J. G. (2001). Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(16), 9157–9160.

Hohenlohe, P. A., Phillips, P. C., & Cresko, W. A. (2010). Using population genomics to detect selection in natural populations: Key concepts and methodological considerations. *International Journal of Plant Sciences*, *171*(9), 1059–1071.

Horváth, G., Móra, A., Bernáth, B., & Kriska, G. (2011). Polarotaxis in non-biting midges: Female chironomids are attracted to horizontally polarized light. *Physiology and Behavior*, *104*(5), 1010–1015. https://doi.org/10.1016/j.physbeh.2011.06.022

Huang, H.-J., Xue, J., Zhuo, J.-C., Cheng, R.-L., Xu, H.-J., & Zhang, C.-X. (2017). Comparative analysis of the transcriptional responses to low and high temperatures in three rice planthopper species. *Molecular Ecology*, *26*(10), 2726–2737.

Jain, K., & Stephan, W. (2017). Modes of rapid polygenic adaptation. *Molecular Biology and Evolution*, *34*(12), 3169–3175.

Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A. F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S. Y., Lopez, R., Hunter, S., & Nuka, G. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, *30*(9), 1236–1240.

Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*, *37*(8), 907–915.

Kingsolver, J. G., Diamond, S. E., Siepielski, A. M., & Carlson, S. M. (2012). Synthetic analyses of phenotypic selection in natural populations: Lessons, limitations and future directions. *Evolutionary Ecology*, *26*(5), 1101–1118.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., & Beerli, P. (2001). The strength of phenotypic selection in natural populations. *The American Naturalist*, *157*(3), 245–261.

Kingsolver, J. G., & Pfennig, D. W. (2007). Patterns and power of phenotypic selection in nature. *Bioscience*, *57*(7), 561–572.

Kofler, R., Orozco-terWengel, P., De Maio, N., Pandey, R. V., Nolte, V., Futschik, A., Kosiol, C., & Schlötterer, C. (2011). PoPoolation: A toolbox for population genetic analysis of next generation sequencing data from pooled individuals. *PLoS ONE*, *6*(1), e15925.

Kraak, M. H., Stuijfzand, S. C., & Admiraal, W. (2000). Interacting effects of toxicants and organic matter on the midge *Chironomus riparius* in polluted river water. *Ecotoxicology and Environmental Safety*, *46*(3), 351–356.

Krueger, F. (2016). TrimGalore: A wrapper around Cutadapt and FastQC to consistently apply adapter and quality trimming to FastQ files, with extra functionality for RRBS data. *TrimGalore*.

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*, 559. https://doi.org/10.1186/1471-2105-9-559

Lewis, D. F. V., Watson, E., & Lake, B. G. (1998). Evolution of the cytochrome P450 superfamily: Sequence alignments and pharmacogenetics. *Mutation Research*, *410*(3), 245–270.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079.

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, *27*(21), 2987–2993.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-014-0550-8

Lv, B., Wang, J., Zhuo, J., Yang, H., Yang, S., Wang, Z., & Song, Q. (2020). Transcriptome sequencing reveals the effects of cadmium toxicity on the cold tolerance of the wolf spider Pirata subpiraticus. *Chemosphere*, *254*, 126802.

Majewski, J., & Pastinen, T. (2011). The study of eQTL variations by RNA-seq: From SNPs to phenotypes. *Trends in Genetics*, *27*(2), 72–79.

Marden, J. H., Langford, E. A., Robertson, M. A., & Fescemyer, H. W. (2021). Alleles in metabolic and oxygen-sensing genes are associated with antagonistic pleiotropic effects on life history traits and population fitness in an ecological model insect*. *Evolution*, *75*(1), 116–129. https://doi.org/10.1111/evo.14095

Margres, M. J., Wray, K. P., Hassinger, A. T., Ward, M. J., McGivern, J. J., Moriarty Lemmon, E., Lemmon, A. R., & Rokyta, D. R. (2017). Quantity, not quality: Rapid adaptation in a polygenic trait proceeded exclusively through expression differentiation. *Molecular Biology and Evolution*, *34*(12), 3099–3110.

Marrot, P., Garant, D., & Charmantier, A. (2017). Multiple extreme climatic events strengthen selection for earlier breeding in a wild passerine. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *372*(1723), 20160372.

Matuszewski, S., Hermisson, J., & Kopp, M. (2015). Catch me if you can: Adaptation from standing genetic variation to a moving phenotypic optimum. *Genetics*, *200*(4), 1255–1274.

Messer, P. W., & Petrov, D. A. (2013). Population genomics of rapid adaptation by soft selective sweeps. *Trends in Ecology & Evolution*, *28*(11), 659–669.

Nemec, S., Heß, M., Nowak, C., & Pfenninger, M. (2012). Experimental evidence for niche segregation in a sister species pair of non-biting midges. *Hydrobiologia*, *691*(1), 203–212.

Nemec, S., Patel, S., Nowak, C., & Pfenninger, M. (2013). Evolutionary determinants of population differences in population growth rate$\times$ habitat temperature interactions in *Chironomus riparius*. *Oecologia*, *172*(2), 585–594.

Nordborg, M., & Tavaré, S. (2002). Linkage disequilibrium: What history has to tell us. *Trends in Genetics*, *18*(2), 83–90.

Nowak, C., Vogt, C., Pfenninger, M., Schwenk, K., Oehlmann, J., Streit, B., & Oetken, M. (2009). Rapid genetic erosion in pollutant-exposed experimental chironomid populations. *Environmental Pollution*, *157*(3), 881–886.

Oppold, A. M., Pedrosa, J. A. M., Bálint, M., Diogo, J. B., Ilkova, J., Pestana, J. L. T., & Pfenninger, M. (2016). Support for the evolutionary speed hypothesis from intraspecific population genetic data in the non-biting midge hironomus riparius. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1825), 20152413. https://doi.org/10.1098/rspb.2015.2413

Oppold, A.-M., & Pfenninger, M. (2017). Direct estimation of the spontaneous mutation rate by short-term mutation accumulation lines in *Chironomus riparius*. *Evolution Letters*, *1*(2), 86–92.

Overgaard, J., Sørensen, J. G., Petersen, S. O., Loeschcke, V., & Holmstrup, M. (2005). Changes in membrane lipid composition following rapid cold hardening in Drosophila melanogaster. *Journal of Insect Physiology*, *51*(11), 1173–1182.

Panis, L. I., Goddeeris, B., & Verheyen, R. (1996). On the relationship between vertical microdistribution and adaptations to oxygen stress in littoral Chironomidae (Diptera). *Hydrobiologia*, *318*(1), 61–67.

Pfenninger, M., & Foucault, Q. (2020). Genomic processes underlying rapid adaptation of a natural *Chironomus riparius* population to unintendedly applied experimental selection pressures. *Molecular Ecology*, *29*(3), 536–548.

Pfenninger, M., & Foucault, Q. (2022). Population genomic time series data of a natural population suggests adaptive tracking of fluctuating environmental changes. *Integrative and Comparative Biology*. icac098. https://doi.org/10.1101/2020.06.16.154054

Pfenninger, M., & Nowak, C. (2008). Reproductive isolation and ecological niche partition among larvae of the morphologically cryptic sister species *Chironomus riparius* and C piger. *PLoS One*, *3*(5), e2157. https://doi.org/10.1371/journal.pone.0002157

Price, T. D., Grant, P. R., Gibbs, H. L., & Boag, P. T. (1984). Recurrent patterns of natural selection in a population of Darwin's finches. *Nature*, *309*(5971), 787–789.

Reznick, D. (2016). Hard and soft selection revisited: How evolution by natural selection works in the real world. *Journal of Heredity*, *107*(1), 3–14.

Schmidt, H., Hellmann, S. L., Waldvogel, A.-M., Feldmeyer, B., Hankeln, T., & Pfenninger, M. (2020). A high-quality genome assembly from short and long reads for the non-biting midge *Chironomus riparius* (Diptera). *G3: Genes, Genomes Genetics*, *10*(4), 1151–1157.

Schoenfelder, S., & Fraser, P. (2019). Long-range enhancer–promoter contacts in gene expression control. *Nature Reviews Genetics*, *20*(8), 437–455.

Schreiber, D., & Pfenninger, M. (2021). Genomic divergence landscape in recurrently hybridizing Chironomus sister taxa suggests stable steady state between mutual gene flow and isolation. *Evolution Letters*, *5*(1), 86–100.

Sella, G., & Barton, N. H. (2019). Thinking about the evolution of complex traits in the era of genome-wide association studies. *Annual Review of Genomics and Human Genetics*, *20*, 461–493.

Siepielski, A. M., DiBattista, J. D., & Carlson, S. M. (2009). It's about time: The temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, *12*(11), 1261–1276.

Siepielski, A. M., DiBattista, J. D., Evans, J. A., & Carlson, S. M. (2011). Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1711), 1572–1580.

Sinclair, B. J., & Marshall, K. E. (2018). The many roles of fats in overwintering insects. *Journal of Experimental Biology*, *221*(Suppl_1), jeb161836.

Slatkin, M., & Muirhead, C. A. (1999). Overdominant alleles in a population of variable size. *Genetics*, *152*(2), 775–781.

Storey, K. B., & Storey, J. M. (2013). Molecular biology of freezing tolerance. *Comprehensive Physiology*, *3*(3), 1283–1308.

Taus, T., Futschik, A., & Schlötterer, C. (2017). Quantifying selection with pool-seq time series data. *Molecular Biology and Evolution*, *34*(11), 3023–3034.

Teets, N. M., & Denlinger, D. L. (2013). Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiological Entomology*, *38*(2), 105–116.

Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): High-performance genomics data

visualization and exploration. *Briefings in Bioinformatics*, *14*(2), 178–192.

Toprak, U., Hegedus, D., Doğan, C., & Güney, G. (2020). A journey into the world of insect lipid metabolism. *Archives of Insect Biochemistry and Physiology*, *104*(2), e21682.

Tropberger, P., Pott, S., Keller, C., Kamieniarz-Gdula, K., Caron, M., Richter, F., Li, G., Mittler, G., Liu, E. T., Bühler, M., & Margueron, R. (2013). Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. *Cell*, *152*(4), 859–872.

Waldvogel, A. M., Wieser, A., Schell, T., Patel, S., Schmidt, H., Hankeln, T., … Pfenninger, M. (2018). The genomic footprint of climate adaptation in *Chironomus riparius*. *Molecular Ecology*, *27*(6), 1439–1456. https://doi.org/10.1111/mec.14543

Wittmann, M. J., Bergland, A. O., Feldman, M. W., Schmidt, P. S., & Petrov, D. A. (2017). Seasonally fluctuating selection can maintain polymorphism at many loci via segregation lift. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(46), E9932–E9941.

Wright, S. (1948). On the roles of directed and random changes in gene frequency in the genetics of populations. *Evolution*, *2*, 279–294.

Yang, J. I., Li, W.-R., Lv, F.-H., He, S.-G., Tian, S.-L., Peng, W.-F., Sun, Y. W., Zhao, Y. X., Tu, X. L., Zhang, M., Xie, X. L., Wang, Y. T., Li, J. Q., Liu, Y. G., Shen, Z. Q., Wang, F., Liu, G. J., Lu, H. F., Kantanen, J., … Liu, M. J. (2016). Whole-genome sequencing of native sheep provides insights into rapid adaptations to extreme environments. *Molecular Biology and Evolution*, *33*(10), 2576–2592.

Zhang, Y., Wu, H., Xie, J., Jiang, R., Deng, C., & Pang, H. (2015). Transcriptome responses to heat-and cold-stress in ladybirds (Cryptolaemus montrouzieri Mulasnt) analyzed by deep-sequencing. *Biological Research*, *48*(1), 1–14.

Zhou, X.-R., Shan, Y.-M., Tan, Y., Zhang, Z.-R., & Pang, B.-P. (2019). Comparative analysis of transcriptome responses to cold stress in Galeruca daurica (Coleoptera: Chrysomelidae). *Journal of Insect Science*, *19*(6), 8.

Zong, S.-B., Li, Y.-L., & Liu, J.-X. (2021). Genomic architecture of rapid parallel adaptation to fresh water in a wild fish. *Molecular Biology and Evolution*, *38*(4), 1317–1329.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Pfenninger, M., Foucault, Q., Waldvogel, A.-M., & Feldmeyer, B. (2023). Selective effects of a short transient environmental fluctuation on a natural population. *Molecular Ecology*, *32*, 335–349. https://doi.org/10.1111/mec.16748