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Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*)

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Abstract

Insect distributions are shifting rapidly in response to climate change and are undergoing rapid evolutionary change. We investigate the molecular signatures underlying local adaptation in the range-expanding damselfly, *Ischnura elegans*. Using a landscape genomic approach combined with generalized dissimilarity modelling (GDM), we detect selection signatures on loci via allelic frequency change along environmental gradients. We analyse 13,612 single nucleotide polymorphisms (SNPs), derived from restriction site-associated DNA sequencing (RADseq), in 426 individuals from 25 sites spanning the *I. elegans* distribution in Sweden, including its expanding northern range edge. Environmental association analysis (EAA) and the magnitude of allele frequency change along the range expansion gradient revealed significant signatures of selection in relation to high maximum summer temperature, high mean annual precipitation and low wind speeds at the range edge. SNP annotations with significant signatures of selection revealed gene functions associated with ongoing range expansion, including heat shock proteins (*HSP40* and *HSP70*), ion transport (V-ATPase) and visual processes (*long-wavelength-sensitive opsin*), which have implications for thermal stress response, salinity tolerance and mate discrimination, respectively. We also identified environmental thresholds where climate-mediated selection is likely to be strong, and indicate that *I. elegans* is rapidly adapting to the climatic environment during its ongoing range expansion. Our findings empirically validate an integrative approach for detecting spatially explicit signatures of local adaptation along environmental gradients.

KEY WORDS

environmental association analysis, insects, *Ischnura*, landscape genomics, local adaptation, range expansion

1 | INTRODUCTION

Adaptation is driven by the interaction between heritable phenotypes and local selective environments, and the outcomes of this process vary along species' ranges and are shaped by spatial variation in selection pressures, standing genetic diversity and demographic potential (Bridle & Vines, 2006). Theory and some empirical evidence suggest that directional selection may be particularly

pronounced at species' range limits where environments tend to be less optimal for growth and reproduction (Kirkpatrick & Barton, 1997; Lancaster, 2016; Warren et al., 2001). In addition to lower habitat suitability, range limits are typically characterized by stochastic genetic and population dynamics due to lower effective population sizes (N_e), which might increase genetic drift and thereby among-population genetic differentiation (Swaegers et al., 2013; Trumbo et al., 2016). Due to gene flow from populations adapted to

conditions in the range core, peripheral, range limit populations are expected to be maladapted relative to core populations (Bridle & Vines, 2006; Kirkpatrick & Barton, 1997). However, with adequate genetic variation, maladaptation in peripheral populations may be counteracted by rapid adaptive evolution to novel environmental pressures, which can facilitate species' range expansions and their future persistence (Colautti & Barrett, 2013).

Evolutionary and landscape genomics approaches have recently enabled the characterization of the role of environmental variables in explaining signatures of local adaptation at the molecular level (Ahrens et al., 2018; Hoban et al., 2016; Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015). Searching for loci underpinning local adaptation is a formidable challenge that has become increasingly accessible via new analytical tools that identify loci with higher than expected genetic divergence among populations (F_{ST} outlier tests, e.g., Foll & Gaggiotti, 2008; Whitlock & Lotterhos, 2015) or exhibit high correlation with spatially explicit environmental variables (environmental association analysis; EAA; Rellstab et al., 2015), while accounting for neutral genetic structure. However, identifying a few specific loci that differ dramatically among populations in allele frequencies under putative locally divergent selection regimes is but one part of the question, while we should also strive to understand how the strength of selection operates across many loci along environmental gradients, and the functional significance of such loci. For species undergoing range expansion in response to climate change functional loci that respond with shifts in allele frequencies along environmental gradients will ultimately determine the capacity of a species to adapt and persist.

Genes that are relevant for local adaptation are expected to predictably change their allele frequency along environmental gradients. Such adaptive molecular differentiation can be quantified via changes in allele frequency among locations across environmental gradients (hereafter "allelic turnover"; Fitzpatrick & Keller, 2015). Signatures of local adaptation can then be teased apart across species distributions. Analytical tools to translate genomic information into signatures of local adaptation have only recently been developed and few empirical applications have been presented (Creech et al., 2017; Fitzpatrick & Keller, 2015; Landguth, Bearlin, Day, & Dunham, 2017). This may be partially due to a lack of data sets with appropriate sampling designs at both the genomic and the spatial scales that are needed to test for selection processes along environmental gradients (Ahrens et al., 2018; Hoban et al., 2016; Rellstab et al., 2015). However, characterizing variation in selection and local adaptation across environmental gradients is a necessary next step in evolutionary and landscape genomics, which will inform conservation management of biodiversity (Hoffmann & Sgro, 2011; Hoffmann et al., 2015). For example, selection on candidate genes may be monitored spatially and temporally as climate change proceeds, revealing "hot and cold spots" of local adaptation (Hansen, Olivieri, Waller, Nielsen, & Ge, 2012).

Insect distributions are currently experiencing pronounced shifts in response to climate change (Lancaster, 2016; Sánchez-Guillén, Muñoz, Rodríguez-Tapia, Arroyo, & Córdoba-Aguilar, 2013), and

insects also exhibit altered physiological (Advani et al., 2016; Lancaster, Dudaniec, Hansson, & Svensson, 2015; Lancaster et al., 2016) and phenological trait changes (Arribas, Abellán, Velasco, Millán, & Sánchez-Fernández, 2017; Sánchez-Guillén et al., 2013) associated with range shifts. Aquatic and semi-aquatic insects may be among the first organisms to suffer from ongoing climate change due to exposure to anthropogenic stressors (e.g., habitat degradation), and dependence on climate-mediated water temperatures (Woodward, Perkins, & Brown, 2010). This makes freshwater insects appropriate models to investigate microevolutionary responses to climate change (Bybee et al., 2016). Here, we use a landscape genomics approach to investigate genomic signatures of local adaptation along environmental gradients in the blue-tailed damselfly, *Ischnura elegans* (Odonata; Vander Linden 1820). We sample the distribution of *I. elegans* in southern Sweden—a gradient where mean annual temperature varies substantially and rapid range expansions in ectotherms are occurring (Jaenson, Jaenson, Eisen, Petersson, & Lindgren, 2012). Damselfly distributions are shifting globally (Swaegegers et al., 2015; Takahashi et al., 2016; Watts, Keat, & Thompson, 2010), and for *I. elegans* in the United Kingdom, the northern range limit was extended by 143 km between two 10-year survey periods of 1960–1970 and 1985–1995 (Hickling, Roy, Hill, & Thomas, 2005). In Sweden, our recent discovery of populations beyond the known range limit, with shifts in thermal niche breadth (Lancaster et al., 2015, 2016) that interact with social feedback mechanisms (Lancaster, Dudaniec, Hansson, & Svensson, 2017), supports a recent and ongoing rapid range expansion in *I. elegans*. In particular, strong selection on cold tolerance was documented in range margin populations based on phenotypic and gene expression responses to thermal challenges, indicating an important role of the thermal stress response on adaptive processes during range expansion (Lancaster et al., 2015, 2016).

Using genomewide data from restriction site-associated DNA sequencing (RADseq) and gene annotation, we identify candidate single nucleotide polymorphisms (SNPs) under selection in relation to environmental gradients from southern "core" populations of *I. elegans* (Le Rouzic, Hansen, Gosden, & Svensson, 2015; Svensson & Abbott, 2005; Svensson, Abbott, & Härdling, 2005) up to populations at the expanding northern range margin ("edge" populations). Covering a five degree latitudinal gradient with high resolution genomic and spatial sampling, we test for (i) signatures of selection on SNP loci (i.e., via F_{ST} outlier analysis, EAA and annotation) that associate with temperature, habitat and climate-related variables; and (ii) significant allele frequency changes in candidate SNPs that track environmental gradients towards the range limit, and evidence for environmental thresholds of selection. We corroborate our findings with prior observations of latitudinal shifts in thermal tolerance phenotypes and gene expression profiles (Lancaster et al., 2015, 2016). We apply a novel, three-tiered analytical approach to identify environmental variables driving local selection on alleles that are putatively adaptive or neutral along a range expansion gradient, revealing highly resolved spatial variation in local adaptation. Our results reveal patterns of spatially explicit adaptive genetic variation

during a climate change-induced range shift, which has significant implications for understanding the future distribution of this species and the structure of biodiversity more generally.

2 | MATERIALS AND METHODS

2.1 | Approach

We implement a three-tiered analytical approach to identify genes under putative selection in response to environmental gradients along a range expansion zone in *I. elegans* (Figure 1). First, (i) candidate SNPs being under putative selection are identified using two F_{ST} outlier approaches (Foll & Gaggiotti, 2008; Whitlock & Lotterhos, 2015) and one environmental association analysis (EAA) approach (Frichot, Schoville, Bouchard, & Francois, 2013). Second, (ii) generalized dissimilarity modelling (GDM) is applied to these identified candidate SNPs, to determine relationships of SNP allelic turnover magnitude in relation to environmental gradients and geographic distance (Fitzpatrick & Keller, 2015). Finally, (iii) signatures of local adaptation are identified via SNP mapping to an annotated *I. elegans* transcriptome (Chauhan, Wellenreuther, & Hansson, 2016; Chauhan et al., 2014), and interpretations about adaptive variation are then based on gene function, experimental gene expression data (e.g., Lancaster et al., 2016), SNP \times environment associations and the

pattern of allelic turnover observed (Figure 1). Our analysis provides fine-scale characterization of SNP-specific genetic gradients of genome-wide selection signatures.

2.2 | Sampling and study area

Ischnura elegans is common across Europe and Asia, with its northern range extending to the southern coastal areas of Scandinavia and the northern United Kingdom (Dijkstra & Lewington, 2006). Our study area spans a latitudinal gradient of five degrees in Sweden (range: 55.64° to 60.57°; Supporting Information Table S1), extending 583 km from the southern populations to the northern range edge (see Lancaster et al., 2015, 2016, 2017). Between the summer months of June and August 2013, we sampled 25 sites throughout the Swedish distribution of *I. elegans* following a paired-gradient sampling design, encapsulating both coastal and inland sites and the northern range edge (Figure 2). Adult *I. elegans* were caught near to reed beds and vegetation using sweep nets within 10 m of water bodies including ponds, lakes and coastal inlets. We implemented a paired-gradient sampling design to the best of our ability (i.e., approximately two samples per latitudinal sampling interval), as this approach has improved power to detect local adaptation at weakly selected loci using EAA in range expansion models, as opposed to random or transect designs (Lotterhos & Whitlock,

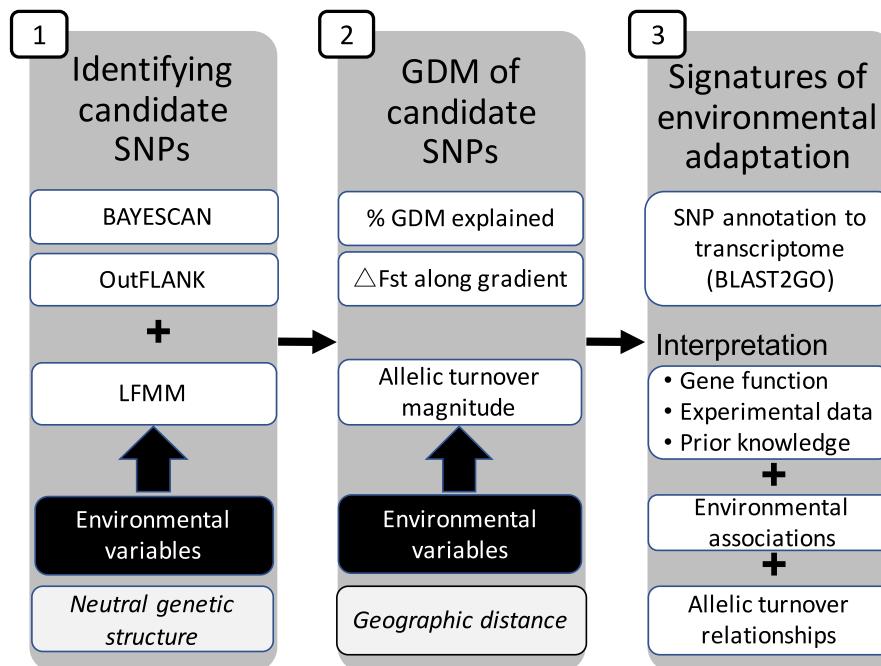


FIGURE 1 Flow chart of analytical approach. (1) Candidate SNPs under putative selection are identified using two F_{ST} outlier approaches (BAYESCAN, OUTFLANK) and one environmental association analysis approach (LFMM). LFMM incorporates a prior estimate of neutral genetic structure and environmental variables from each sampling location. (2) Generalized dissimilarity modelling (GDM) is applied to each candidate SNP to determine relationships between SNP allelic turnover magnitude and environmental gradients, and geographic distance. SNP response is assessed via the maximum change in F_{ST} between sampling locations (ΔF_{ST}), and the explanatory power of the SNP in the GDM via percentage deviance explained (% GDM explained). SNPs with a % GDM explained \leq that of the reference SNP group were excluded as potential false positives (3) Signatures of environmental adaptation are characterized via annotation of SNPs to a transcriptome and interpreted based on gene function, prior knowledge, experimental data, SNP \times environment associations and the allelic turnover relationships observed [Colour figure can be viewed at wileyonlinelibrary.com]

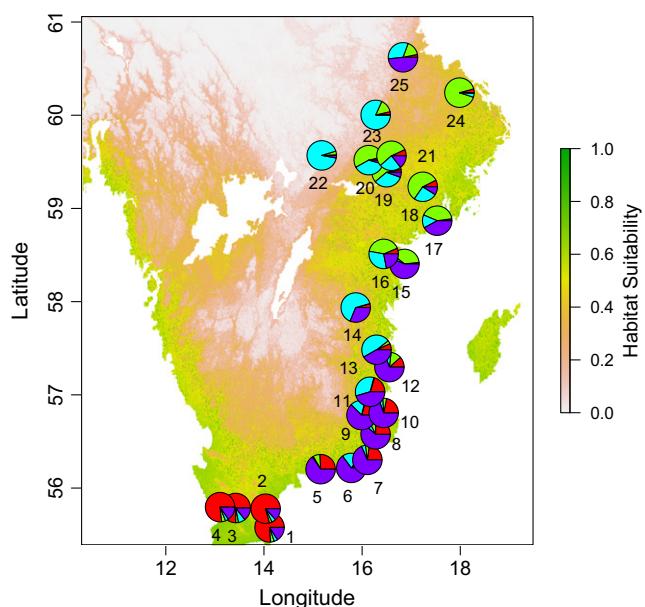


FIGURE 2 Genetic structure of *Ischnura elegans* across the environmental gradient. Probability of *I. elegans* genetic cluster assignment ($K = 4$) is shown at the population level (with population names from Table 1) on a habitat suitability map in Sweden (from Lancaster et al., 2015). The proportion of each colour within each pie chart indicates the mean assignment probability of individuals to a genetic cluster in that population, displayed for 426 individuals across 25 populations [Colour figure can be viewed at wileyonlinelibrary.com]

2015). We performed all procedures in accordance with the ethical guidelines of Lund University in Sweden and obtained sampling permissions from local authorities and landholders.

2.3 | RAD sequencing, bioinformatics and SNP characterization

We extracted DNA from 432 *I. elegans* from 25 sites (10–20 individuals per site, mean 17.04 ± 0.72 ; Supporting Information Table S1) using the head, thorax and legs from each individual with a DNeasy Blood and Tissue extraction kit (Qiagen). We quantified extracted genomic DNA using a QUBIT 2.0 Fluorometer (Life Technologies) that was then processed into paired-end RAD libraries according to the protocol implemented in Etter, Bassham, Hohenlohe, Johnson, and Cresko (2011), and as described in the Supplementary Material 1.0. Each RAD library was sequenced on a separate lane of an Illumina HiSeq 2000 or 2500 at the Beijing Genomics Institute, Shenzhen, China yielding 20–30GB of data per library. Adapter sequences and low-quality bases below a Phred score of 20 were trimmed from raw reads according to standard quality control protocols (to 100 bp read length).

Raw sequences from each RAD library were quality checked visually using FASTQC (Andrews, 2010) and each library was processed using pipelines within STACKS v.1.40 (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011; Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Methods used in STACKS are described in more detail in the Supplementary Material 1.1. Samples were processed in STACKS

using the *process_radtags* with a mean of 105 million reads (± 13.36 M) per library, followed by the *clone_filter* program to remove PCR duplicates, resulting in a mean of 36 million reads (± 6.4 M) per library (Supporting Information Table S1). The final sample size of individuals retained for analyses was 426 across the 25 populations, as six samples were excluded due to low coverage. Deduplicated reads were aligned to an *I. elegans* draft genome assembly (version 12-2015) by P. Chauhan et al.; Supplementary Information) using BOWTIE2 v.2.2.5 (Langmead & Salzberg, 2012). Aligned reads from BOWTIE2 were analysed in the *ref_map* program in STACKS to build the initial consensus catalogue of SNPs, resulting in 3,452,911 loci. SNPs were further filtered using the *rxstacks* corrections model, which removes excess haplotypes and confounded loci (Catchen et al., 2013).

The final set of SNP markers was determined within the *populations* program in STACKS, which was run twice: first, including all SNPs on each RAD tag, and second, including only the first SNP on each RAD tag to create a data set without closely linked loci (using the *write_single_snp* option in STACKS). We specified an initial minimum depth of coverage of 5 \times for each SNP-containing RAD locus with a minor allele frequency (MAF) of 0.05. Additionally, a locus was only included if it occurred in 22/25 populations and in at least 80% of individuals within each population to ensure wide representation of data for each SNP across all samples and sampling locations (recommended by Paris, Stevens, & Catchen, 2017). After filtering loci using the STACKS POPULATIONS program, 13,612 SNPs (including linked SNPs, used for F_{ST} outlier, EAA and GDM analyses) and 3,809 SNPs (excluding closely linked SNPs, used for genetic structure analysis) were retained for analysis. Depth of coverage per SNP varied between 8 \times and 23 \times (mean 15.3 \times ; Supporting Information Figure S1).

2.4 | Environmental data

Variables used in environmental association analysis (EAA) and generalized dissimilarity modelling (GDM) were chosen from those previously identified in species distribution modelling (SDM) for *I. elegans* within the same study area (Lancaster et al., 2015). Lancaster et al. (2015) identified 12 variables that predicted the distribution of *I. elegans* that all had a pairwise Pearson correlation coefficient (r) less than 0.8 in a prior habitat suitability model. Of these 12 variables, we chose five (described in Table 1) that varied widely over the sampling gradient (Supporting Information Figure S2): (i) mean annual temperature (BIO1, "Annual Temp"; 62.1% contribution to SDM), (ii) the maximum temperature of the warmest month (BIO5, "Max Temp"; 0.1% contribution to SDM), (iii) mean annual precipitation (BIO12, "Annual Rain" 0.1% contribution to SDM) and (iv) percentage tree cover ("Tree Cover," 0.4% contribution to SDM). We also included a fifth variable that was not examined by Lancaster et al. (2015), (v) mean summer wind speed ("Wind Speed," averaged for June–August; metres per second, measured at 80 m height; Supporting Information Table S1, Figure S2). These chosen variables were selected due to explicit biological predictions regarding their effects on adult fitness during the short adult reproductive and dispersal period, which is a critical period for selection processes in odonates

TABLE 1 Numbers of loci under putative selection detected via F_{ST} outlier and EAA approaches. Overlapping and unique (i.e., nonoverlapping) F_{ST} outliers or SNP \times environment associations are shown across the 1,758 candidate SNPs, identified using BAYESCAN (diversifying SNPs only), OUTFLANK and LFMM, broken down into the five tested environmental variables (Annual Temp: mean annual temperature, BIO1; max Temp: mean maximum summer temperature, BIO5; Annual Rain: mean annual precipitation, BIO12; Wind Speed: mean summer wind speed; and Tree Cover: percentage tree cover). Shown are the total number of significant SNPs and the number of uniquely associated SNPs per method and environmental variable. The number of SNPs in common with the total number of SNPs ("Total SNPs") is shown in matrix form. Uniquely associating SNPs ("Unique SNPs") were those found to be specific to the method used or the environmental variable tested

Approach		Total SNPs	Unique SNPs	BAYESCAN	OUTFLANK	BIO1	BIO5	BIO12	Wind Speed	Tree Cover
F_{ST} outlier	BAYESCAN	391	360	—	—	5	7	11	13	3
	OUTFLANK	188	138	9	—	11	19	14	13	19
LFMM	BIO1	374	75	5	11	—	172	116	211	97
	BIO5	566	114	7	19	172	—	292	146	174
	BIO12	500	65	11	14	116	292	—	117	182
	WS	416	114	13	13	211	146	117	—	86
	TC	471	183	3	19	97	174	182	86	—
	ALL ^a	1251	1,188	22 ^b	41 ^b					

^aRefers to all SNPs identified by LFMM with significant associations to environmental variables. ^bUnique SNPs.

(discussed in Wellenreuther, Larson, & Svensson, 2012; Supplementary Information). Although the larval period is longer than the adult period in many insects including *I. elegans*, it is proposed that genetic variation for fitness is primarily expressed in the adult phase of insects (e.g., in *Drosophila*; Chippindale, Gibson, & Rice, 2001). Therefore, we selected climate and landscape variables that are most likely to be relevant for evolutionary processes during the adult period (e.g., Max Temp, Tree Cover, Wind Speed), but also those that may act as selection pressures over longer developmental periods (e.g., Annual Temp, Annual Rain). Further justification of the environmental variables is given in the Supplementary Information (1.3).

The Pearson correlation coefficients (r) between the five environmental variables taken from each site were less than 0.4 except for Annual Temp and Wind Speed ($r = 0.75$), and Annual Temp and Max Temp ($r = -0.48$; Supporting Information Table S3). Therefore, our ability to separate Annual Temp from Wind Speed and Max Temp was limited (Supporting Information Table S3). We calculated geographic distance (km) between sites using the R package ECODIST (Goslee & Urban, 2007). All environmental variables were extracted at a 1 km cell resolution from BIOCLIM variables within the WORLDCLIM Version 1.4 database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) except wind speed data that were extracted from WORLDCLIM Version 2.0 (Fick & Hijmans, 2017), and percentage tree cover data that were obtained from the Global Land Cover Facility (Defries, Hansen, Townshend, Janetos, & Loveland, 2000).

2.5 | Outlier SNP detection and genetic structure

Detection of outlier SNPs (i.e., loci putatively under divergent selection) was performed on the complete data set (13,612 SNPs) using two contrasting F_{ST} -based approaches implemented in BAYESCAN 2.1 (Foll & Gaggiotti, 2008) and OUTFLANK (Whitlock & Lotterhos, 2015). Two approaches were used to maximize the identification of potential loci under selection for exclusion from genetic structure analysis,

and to identify common significant SNPs across methods. The false discovery rate (FDR) was set at 0.05 and the number of populations (K) was set to 25 in both programs. The Bayesian likelihood approach implemented in BAYESCAN compares population allele frequencies with a common migrant gene pool, which allows for different migration rates and acts to account for effects of neutral genetic structure, reducing the proportion of false positives (Narum & Hess, 2011). OUTFLANK (Whitlock & Lotterhos, 2015) identifies outliers by first inferring the distribution of F_{ST} for loci that are unlikely to be under selection, and only attempts to identify loci under positive selection. This method performs well under diverse demographic history scenarios, including range expansion (Whitlock & Lotterhos, 2015). Further details are in the Supplementary Material 1.3.

To minimize the inclusion of putative loci under selection and linked loci from analyses of neutral genetic structure, F_{ST} outlier loci identified using BAYESCAN and OUTFLANK analyses were removed from the "unlinked" SNP data set (i.e., single SNP per RAD tag), resulting in 3554 SNPs. Genetic structure was estimated with the program ADMIXTURE (Alexander, Novembre, & Lange, 2009), which uses a cross-validation procedure to determine genetic structure in large autosomal SNP data sets. ADMIXTURE was run for 1–25 potential ancestral populations (K) with a fivefold cross-validation (CV) error and K was chosen where the cross-validation error was minimized. The probability of individual assignment to each genetic cluster (Q) was graphically displayed and plotted in R (Figure 1, Figures S4 and S5).

2.6 | Environmental association analysis

Environmental association analysis (EAA) was performed using Latent Factor Mixed Modeling (LFMM), implemented with in the R package LEA (Frichot & François, 2015) using all 13,612 SNPs. LFMM uses a stochastic Monte Carlo Markov Chain algorithm and tests for associations between environmental or ecological variables and allele

frequencies while estimating unobserved latent factors that model confounding effects of genetic structure, which may be due to shared demographic history or background genetic variation (Frichot et al., 2013). LFMM was run with the number of latent factors set to the number of genetic clusters (K) obtained via ADMIXTURE (see below; K was equal to four) with five repetitions, and 10,000 iterations with a 5,000 burn-in. The z-scores over the five runs were combined and p -values adjusted as recommended by Frichot and François (2015). To include SNPs that were highly significantly correlated with the environmental variables, we applied a conservative Benjamini–Hochberg p -value cut-off $<\log_{10}^{-6}$. We ran LFMM to find SNP-by-environment associations for all five environmental variables (e.g., Annual Temp, Max Temp, Annual Rain, Tree Cover, Wind Speed). Shared and unique SNP \times environment associations were quantified across the five environmental variables and their overlap with F_{ST} outlier results examined (Table 1). The genomic inflation factor (GIF) described by Devlin and Roeder (1999) was calculated for each environmental variable from the z-scores derived from LFMM and was assessed for its closeness to the recommended value of 1.0 (Frichot & François, 2015). The GIF across four of the variables ranged from 1.04 to 1.48, but Annual Temp had a GIF = 2.34. This indicates that FDRs are likely to be higher for Annual Temp than the other variables analysed due to poor statistical calibration. Given the high GIF, the high correlation of Annual Temp with both Max Temp and Wind Speed, and the relevance of Max Temp to the adult flying period, we chose to exclude Annual Temp from further analyses.

2.7 | Generalized dissimilarity modelling of candidate SNPs

We examine spatially explicit selection processes for each SNP found to be under putative selection using a modified generalized dissimilarity modelling (GDM) approach described in Fitzpatrick and Keller (2015), implemented using the R package gdm (Ferrier, Manion, Elith, & Richardson, 2007; Manion et al., 2017). The approach is adapted from the use of GDMs in biodiversity modelling to examine nonlinear turnover in community-level composition (Ferrier et al., 2007), but uses large numbers of loci (instead of species) to find both linear or nonlinear responses of loci to environmental gradients (Fitzpatrick & Keller, 2015). The approach takes the pairwise F_{ST} of SNPs across sample sites and models the rate and magnitude of “allelic turnover” (i.e., change in allele frequency represented as a genetic distance measure) in relation to the distribution of an environmental variable along a spatial sampling gradient, using a site-by-SNP matrix (Fitzpatrick & Keller, 2015). This is achieved using permutation on distance matrices to perform model and variable significance testing and to estimate variable importance. By identifying functions of allelic turnover according to environmental gradients, the approach offers a means of scaling from population-level genomic variation to predictions of landscape scale adaptive variation, which are both subject to ongoing environmental change (Fitzpatrick & Keller, 2015).

Using the GDM approach, we identify thresholds on the landscape where signatures of local adaptation in *I. elegans* increase or decrease in relation to the five environmental gradients we examined using EAA. We conducted GDM for a candidate set of SNPs identified as being putatively under selection using either BAYESCAN, OUTFLANK or LFMM (total SNPs = 1,758). SNPs identified in BAYESCAN with significantly negative F_{ST} values (i.e., under potentially balancing selection) were excluded from the candidate set as these loci are likely to have a very high FDR (Whitlock & Lotterhos, 2015). The complete set of F_{ST} outliers identified from both BAYESCAN and OUTFLANK were included in the GDM because each program implements a uniquely valid statistical approach to detect selection, and we observed little lack of overlap in significant SNPs between the approaches. We modified the approach of Fitzpatrick and Keller (2015) by taking a “single-SNP” approach with each putatively selected SNP modelled independently, regardless of annotation, as opposed to selecting specific, annotated SNPs or grouping related SNPs for GDM.

Additionally, as in Fitzpatrick and Keller (2015), we integrate a random sample of 200 SNPs of the 13,612 available SNPs, which act as a “reference group” in the GDM to test whether allelic turnover at a given candidate SNP differs from that expected in a random sample of the genetic data. Further, geographic distance (Euclidean) was incorporated as a sixth variable in the GDM to test whether allelic turnover across environmental gradients was better explained by distance, which effectively acts as a second screening (i.e., after F_{ST} outlier and EAA tests) for loci that may respond predominantly to neutral genetic processes (i.e., those influenced by genetic structure, including isolation by distance), and may therefore have been falsely identified in outlier tests, or have lower confidence to be identified as candidate SNPs involved in adaptation. Although geographic distance alone does not incorporate other demographic effects associated with range expansion that can influence selection detection (e.g., founder effects, allele surfing), we attempt to control for false positives by, (i) comparing outcomes with relationships with geographic distance and, (ii) by comparing allelic turnover responses of the random “reference” SNP group with that of each locus to test if its response is more or just as likely in a random sample of genetic variation.

Genetic distance matrices between the 25 sample sites were calculated for each of the 1,758 candidate SNPs, and for the reference group based on Nei’s pairwise F_{ST} (Nei, 1987) using the R package HIERFSTAT (Goudet, 2005), and were rescaled between 0 and 1 within the GDM analysis. To assess the role of each SNP in selection processes in relation to each environmental variable examined, we ranked the allelic turnover functions of each SNP and for each environmental variable using two different methods: (i) within each SNP: ranking was based on the magnitude of allelic turnover at a given SNP (i.e., change in F_{ST} along a specific environmental gradient) relative to its turnover magnitude for other environmental variables in the model; and (ii) across all SNPs: ranking was based on the percentage deviance explained by each SNP relative to all SNPs in the GDM model (using the permutation procedure of the R function gdm.varImp), which gives an indication of selection strength for each

SNP relative to the whole data set. For (i), the top 250 SNPs with the highest magnitude of allelic turnover are plotted for each environmental variable (Figure 3). The second ranking (ii) was used as a secondary assessment of the overall selection signature of the SNP within the entire GDM model. GDM results for all 1,758 SNP responses and tests are in the Supplementary Material.

2.8 | Gene annotation

To identify functional genes, RAD tags containing one or more of the 1,758 candidate SNPs were mapped against the annotated transcriptome for *I. elegans* (Chauhan et al., 2014, 2016) using BLASTN with an *e*-value cut-off of 1×10^{-5} . All BLASTN results were imported into the BLAST2GO Web version for further annotation (Conesa et al., 2005). INTERPROSCAN was used for identifying conserved protein domains in the assembly (Jones et al., 2014), and GO annotations were performed on the BLASTN and INTERPROSCAN annotated transcripts (Ashburner et al., 2000). Gene Ontology (GO) annotations and GO Slim reductions were applied to categorize transcripts into major GO categories, Biological Processes, Cellular Components and Molecular

Functional annotations using second-level database functions (Ashburner et al., 2000). Finally, enzymes and their corresponding biological pathways were identified using the BLAST2GO integrated KEGG database (Conesa et al., 2005). All analyses were performed using default settings. Gene functions were identified from those previously annotated in Chauhan et al. (2014, 2016), those with expression levels associated with thermal challenge treatments in *I. elegans* performed by Lancaster et al. (2016), or were identified directly from the NCBI database (Table 2). Gene functions were only considered for those with an annotation match of $\geq 70\%$ (Supplementary Material). Transcripts with SNP annotations were mapped to an assembled genome (Supplementary Material) using BLAST and the positions where transcripts mapped were recorded (i.e., scaffold ID and base pair position on RAD tag).

2.9 | Mapping adaptive genetic variation over the temperature gradient

To examine how adaptive variation changes in *I. elegans* along its current Swedish distribution, we mapped allelic turnover functions

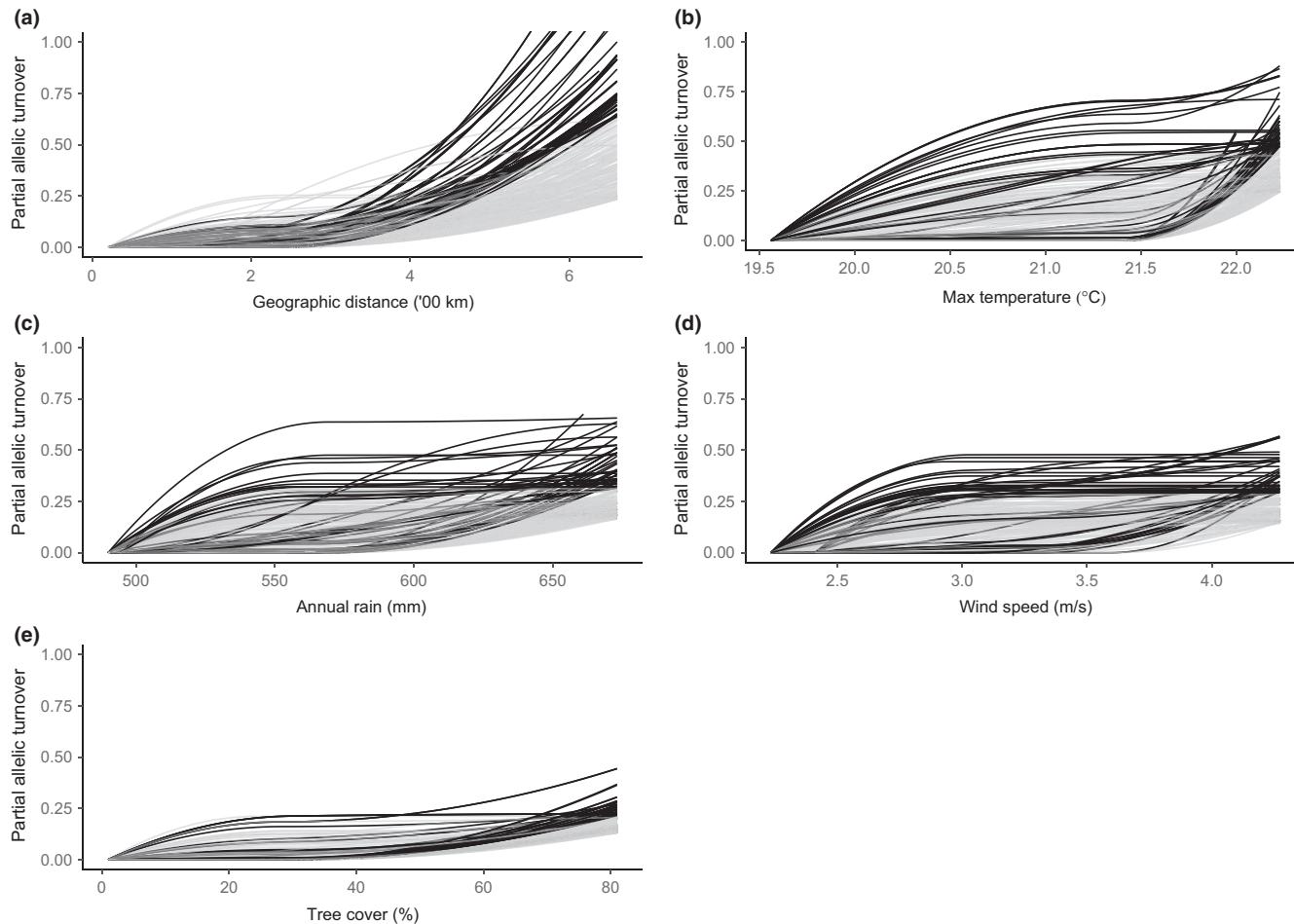


FIGURE 3 Allelic turnover relationships for each environmental variable. Allelic turnover functions for the top 250 SNPs (grey) and top 50 SNPs (black) that showed the highest generalized dissimilarity modelling (GDM) partial allelic turnover in relation to each environmental variable and geographic distance: (a) Geographic distance (km), (b) Max Temperature (°C; BIO5), (c) Annual Rain (mm; BIO12), (d) Tree Cover (%) and (e) Wind Speed (m/s)

for selected candidate SNPs that (i) were annotated to genes associated with thermal tolerance or other phenotypic traits previously identified (e.g., Chauhan et al., 2014, 2016; Lancaster et al., 2016) and (ii) had a higher explanatory power in the GDM than the reference “random” SNP group. In addition to the above, we focused on SNPs that (iii) had the highest allelic turnover in relation to Max Temp in the GDM or (iv) showed a large change in F_{ST} along the sampled gradient (Figure 1). This resulted in a list of 23 SNPs, and allele frequencies and turnover functions were mapped for four of these SNPs to reveal spatially explicit selection gradients. All maps were produced in R using the GDM, RASTER and GGPlot packages (Ferrier et al., 2007; Hijmans & van Etten, 2012; Wickham, 2009).

3 | RESULTS

3.1 | F_{ST} outlier detection and genetic structure

BAYESCAN identified 688 SNPs (5% of 13,612 SNPs) under putative selection across the 25 sites. There was a distinct split among the outliers with divergent selection being represented in 57% ($n = 391$) of SNPs and potentially balancing selection being represented in 43% ($n = 297$) of SNPs. Using OUTFLANK, 188 outliers (1.4%) were detected, which were all under putative positive selection. Nine SNPs were commonly identified in BAYESCAN (diversifying only) and OUTFLANK. All SNPs identified as an F_{ST} outlier in either BAYESCAN or OUTFLANK were removed for genetic structure analysis. Notably, removing even the least conservatively estimated loci under putative selection can minimize false estimates of genetic structure, and therefore, we attempt to address this risk of false positives by removing all candidates from both programs. ADMIXTURE analysis showed a cross-validation (CV) error that was minimized at four genetic clusters ($K = 4$, using 3,554 SNPs; Figure 2, Supporting Information Figure S3, Table S2). A high proportion (39%) of individuals showed ancestry to more than one cluster (Supporting Information Figure S3), although probabilities of ancestry were overall higher to a given cluster for populations in the southern region (Figure 2). There was greater variability in assignment probabilities towards the range limit, but a larger number of distinct genetic clusters represented (i.e., 3–4; Figure 1 and Supporting Information Figure S6, Table S2) while all four sites in the southern region belonged to a single cluster (Figure 2).

3.2 | Environmental association analysis

A total of 2,327 significant SNP associations were identified across the five environmental variables analysed using LFMM (with a $<\log_{10}^{-6}$ p-value significance cut-off), with a similar number of SNP associations for each variable (mean = 465 SNPs; range = 374–566; see Table 1 and Supporting Information Figure S4). However, these associations were attributed to 451 unique SNPs, and none of the SNPs were significantly associated across all five environmental variables. Very few SNPs identified as F_{ST} outliers were also found in the EAA associations using LFMM with 22 SNPs (5%) overlapping with

BAYESCAN outliers and 41 SNPs (9%) overlapping with OUTFLANK outliers, yet none across all three approaches (Table 1). Of the EAA associations, between 18.3% and 58.4% (mean = 35%) of the associations were shared across more than one environmental variable (Table 1). Annual Temp shared 30.4% and 50.7% of its associations with Max Temp and Wind Speed, respectively (Table 1).

3.3 | Patterns of selection signatures along environmental gradients

Including all significant associations across all tests, a total of 1,758 unique SNPs were identified as being under putative selection (Table 1) and all were analysed using GDM. A large proportion of putatively adaptive SNPs (60%) were identified via at least one F_{ST} outlier test (i.e., BAYESCAN, OUTFLANK) or were associated with a single environmental variable using LFMM ($n = 5$ environmental variables tested). SNPs identified with two ($n = 381$; 22%), three ($n = 236$; 13%), four ($n = 73$; 4%) or five ($n = 19$; 1%) tests were less common. We present GDM results for the top 250 SNPs with the highest magnitude of allelic turnover in relation to each environmental variable (Figure 3). A wide F_{ST} distribution was observed for these top ranking SNPs, which was similar to the shapes of the F_{ST} distribution for all 1,758 candidate SNPs (Supporting Information Figure S5). The allelic turnover for each of the top 250 SNPs according to each environmental variable (Figure 3) indicates differing gradients and strengths of selection across loci. Despite being associated with an environmental variable using LFMM, the SNPs with the highest allelic turnovers were associated with geographic distance (and noted as possible false positives), which was followed by (in decreasing order of allelic turnover magnitude) Max Temp, Annual Rain, Wind Speed and Tree Cover (Figure 3). The shapes of the allelic turnovers across SNPs ranged from distinct “plateaus” at a given position on the gradient, to positive and almost exponential allelic turnover responses at particular gradient positions. For example, the top 50 SNPs for geographic distance appeared to mostly reach fixation at the largest distances (Figure 3a), while most SNPs associated with Wind Speed ceased allelic turnover beyond a wind speed threshold of 3.0 m/s (Figure 3d). Max Temp (Figure 3b) and Annual Temp (Figure 3c) drove the strongest and most variable allelic turnover magnitudes of the environmental variables, with distinct turnover thresholds identifiable for each associated SNP.

3.4 | Allelic turnover responses and annotation

For 206 of 1,758 SNPs (11.7%), there was no significant allelic turnover response associated with geographic distance or any of the environmental gradients analysed using GDM, and these SNPs were not interpreted further. Selective neutrality in relation to environmental gradients was assessed via SNP allelic turnover response to geographic distance vs. environmental variables within our GDM (Fitzpatrick & Keller, 2015). Geographic distance had the highest magnitude in allelic turnover response for 372 of the 1,758 SNPs analysed (21%), relative to the other environmental variables. The

TABLE 2 Gene annotations and associated environmental variables for SNPs under putative selection. Transcript IDs, gene function, genome scaffold ID ("Scaff") and SNP ID on the *Ischnura elegans* draft genome are shown. The difference between the highest and lowest population F_{ST} value is shown for each annotated SNP (ΔF_{ST}). SNPs presented had (i) a $\geq 70\%$ BLAST match rate, (ii) a higher % of the GDM explained than the reference SNP group (% GDM) and (iii) a prior annotation in Lancaster et al. (2016) and Chauhan et al. (2016, 2014). Environmental variables are BIO5; maximum temperature of the warmest month ("Max Temp"); BIO12; mean annual precipitation ("Annual Rain"); TC: Tree Cover; and WS: Wind Speed. Allelic turnover is shown for each SNP relative to each environmental variable. SNP rank per environmental variable is the magnitude of allelic turnover ranked relative to the other environmental variables in the GDM. This provides a measure of the relative explanatory power of each environmental variable on allelic turnover. Bold SNPs are those for which spatial allelic turnover was mapped (53905_36 and 35404_9 in Supporting Information Figures S8 and S9). Transcripts for SNP 3504_9 are in the Supplementary Data

Transcript ID	Gene function description	Function	Scaff	SNP ID	ΔF_{ST}	% GDM	Partial allelic turnover by variable						SNP rank in GDM by variable
							GEOG	BIO5	BIO12	WS	TC	GEOG	
c9603_g1_i1	Heat shock protein 70	HSP70	4300	53905_36^a	0.40	19.26	0.33	0.12	0.10	0.00	0.14	171	551
c48098_g1_i1	Heat shock cognate protein 70	HSP70	4300	53905_36^a	0.40	19.26	0.33	0.12	0.10	0.00	0.14	171	551
c42128_g1_i1	Heat shock cognate protein 70	HSP70	4300	53905_36^a	0.40	19.26	0.33	0.12	0.10	0.00	0.14	171	551
c42128_g2_i1	Heat shock cognate protein 70	HSP70	4300	53905_36^a	0.40	19.26	0.33	0.12	0.10	0.00	0.14	171	551
c36939_g1_i1	Heat shock protein 40	HSP40	2	39733_28^a	0.14	20.02	0.00	0.41	0.00	0.08	0.07	1,065	75
c36939_g1_i1	Heat shock protein 40	HSP40	2	39594_49^a	0.23	33.21	0.43	0.00	0.09	0.41	0.03	114	1,251
c36939_g1_i1	Heat shock protein 40	HSP40	2	39519_58	0.20	16.12	0.09	0.12	0.17	0.08	0.12	540	566
c36939_g1_i1	Heat shock protein 40	HSP40	2	39594_63^a	0.16	18.83	0.00	0.06	0.52	0.00	0.05	1,064	798
c36939_g1_i1	Heat shock protein 40	HSP40	2	39519_36^a	0.19	22.80	0.03	0.01	0.15	0.31	0.03	788	1,126
c36939_g1_i1	Heat shock protein 40	HSP40	2	39692_78	0.16	23.16	0.04	0.00	0.34	0.00	0.12	708	1,639
c36939_g1_i1	Heat shock protein 40	HSP40	2	39648_74	0.32	21.82	0.18	0.07	0.14	0.05	0.12	331	745
c36939_g1_i1	Heat shock protein 40	HSP40	2	39594_35^a	0.21	16.00	0.06	0.24	0.07	0.00	0.14	643	260
c36939_g1_i1	Heat shock protein 40	HSP40	2	39648_33	0.24	14.25	0.13	0.10	0.01	0.13	0.08	430	646
c36939_g1_i1	Heat shock protein 40	HSP40	2	39648_19	0.24	14.54	0.13	0.10	0.01	0.14	0.08	442	645
c36939_g1_i1	Heat shock protein 40	HSP40	2	39692_51	0.19	21.25	0.00	0.05	0.28	0.00	0.12	1,611	858
c43579_g4_i1	Long-wavelength-sensitive opsin 3b	Visual	6	73426_72	0.21	14.00	0.05	0.24	0.08	0.15	0.00	703	266
c43579_g4_i1	Long-wavelength-sensitive opsin 3b	Visual	6	73426_69	0.19	18.48	0.62	0.21	0.08	0.15	0.03	53	317
c43579_g4_i1	Long-wavelength-sensitive opsin 3b	Visual	6	73426_85	0.19	21.31	0.74	0.21	0.07	0.14	0.03	34	329
c22378_g1_i1	tpa_exp: pteropsin	Visual	47	57982_91	0.15	34.07	0.11	0.71	0.00	0.02	0.07	497	8
c39329_g1_i1	Rhodopsin-specific isozyme-like	Visual	38855	50006_70	0.18	25.26	0.00	0.12	0.31	0.00	0.13	1,577	553
c4570_g1_i1	Histone-lysine n-methyltransferase	Epigenetics	102	1735_72	0.18	12.79	0.01	0.00	0.16	0.14	0.12	887	1,443
c26924_g1_i2	Histone-lysine n-methyltransferase	Epigenetics	102	1735_72	0.18	12.79	0.01	0.00	0.16	0.14	0.12	887	1,443
c4570_g1_i1	Histone-lysine n-methyltransferase	Epigenetics	102	1803_8^a	0.17	17.18	0.12	0.02	0.03	0.11	0.23	460	1,043
c26924_g1_i2	Histone-lysine n-methyltransferase	Epigenetics	102	1803_8^a	0.17	17.18	0.12	0.02	0.03	0.11	0.23	460	1,043
c4570_g1_i1	Histone-lysine n-methyltransferase	Epigenetics	102	1735_84	0.13	27.06	0.00	0.01	0.49	0.02	0.14	1,365	1,088

(Continues)

TABLE 2 (Continued)

Transcript ID	Gene function description	Function	Scaffold	SNP ID	ΔF_{ST}	% GDM	Partial allelic turnover by variable						SNP rank in GDM by variable			
							GEOG	BIO5	BIO12	WS	TC	GEOG	BIO5	BIO12	WS	TC
c26924_g1_i2	Histone-lysine n-methyltransferase	Epigenetics	102	1735_84	0.13	27.06	0.00	0.01	0.49	0.02	0.14	1,365	1,088	18	208	861
c28633_g2_i1	Histone-lysine n-methyltransferase	Epigenetics	383	49386_86	0.09	15.91	0.22	0.36	0.00	0.01	0.03	260	106	1,247	982	918
c28633_g1_i2	Histone-lysine n-methyltransferase	Epigenetics	383	49386_86	0.09	15.91	0.22	0.36	0.00	0.01	0.03	260	106	1,247	982	918
c3122_g1_i1	Vacuolar H ⁺ ATPase ^c	Proton pump	28	37543_9 ^b	0.50	47.31	0.00	0.63	0.06	0.06	0.22	987	10	643	575	44
10 matches	Intracellular processes ^c	Various	26	35404_9 ^b	0.38	42.05	0.031	0.83	0.16	0	0.01	781	3	254	1,049	1,179

^a F_{ST} outlier using BAYESCAN (diversifying) or ^bOutFLANK. ^cAnnotation previously unpublished in *C. elegans*.

reference ("random") SNP group explained 11.8% of the GDM deviance for the entire model, and SNPs that did not exceed 11.8% were also considered to be potential false positives.

Of the 1,758 candidate SNPs (located on 640 different scaffolds), 1,196 (68%) were annotated to the *C. elegans* transcriptome, and of these, 50 SNPs (located on 13 scaffolds) were located on transcripts previously identified in gene expression analyses by Chauhan et al. (2014, 2016) and Lancaster et al. (2016) (see Supplementary Material). After additional filtering of SNPs that had greater explanatory power in the GDM than the reference SNP group, 21 of 50 previously annotated SNPs (located on 7 scaffolds) were retained, with some occurring on the same RAD tag (i.e., tightly linked SNPs) or having more than one matching transcript, isoform or annotation (Table 1). An additional two SNPs (on 2 scaffolds) with annotations of relevance to environmental adaptation (although not previously reported) were also retained. These two SNPs were in the top 10 SNPs with respect to the percentage of the GDM explained, allelic turnover magnitude with respect to Max Temp, and had the highest change in F_{ST} along the sampled gradient.

We focus on these 23 annotated SNPs from here forward as they exhibited the most significant selection signatures in tandem with annotations that can be linked to processes during environmental adaptation. The 23 SNPs spanned five key functional groups relevant for thermal stress (i.e., 11 SNPs for HSP40 and one for HSP70, represented across six RAD tags), visual processes (5 SNPs spanning rhodopsin, pteropsin and long-wavelength-sensitive opsin across three RAD tags) epigenetic modification (4 SNPs for histone-lysine n-methyltransferase across three RAD tags), ion transport (1 SNP for vacuolar H⁺ proton pump) and varied cellular processes (1 SNP with multiple annotations; Table 2; Supplementary Data). One isoform was found for each gene function except for one epigenetic modification gene that contained two isoforms (Table 2). Seven of the annotated SNPs were identified as significant outliers using BAYESCAN and one SNP using OUTFLANK (Table 2). All annotations are provided in the Supplementary Material.

3.5 | Environmental associations and allelic turnover of annotated SNPs

Of the 23 focal SNPs, five showed the greatest allelic turnover magnitude with respect to geographic distance, although one SNP was equal or within 0.02 magnitude to Annual Rain (SNP 39648_74; Table 1). These SNPs are considered to be less likely to be under selection by the environmental variables analysed per se, despite showing significant changes in allele frequencies according to geographic distance. For the 23 SNPs, the magnitude of allelic turnover was highest for those that associated with Max Temp (mean = 0.42 ± 0.08 ; 9 SNPs), followed by Annual Rain (mean = 0.30 ± 0.05 ; 8 SNPs), Wind Speed (mean = 0.25 ± 0.07 ; 4 SNPs) and Tree Cover (mean = 0.185 ± 0.05 , 2 SNPs; Table 1, Figure 3). Allelic turnovers of the 23 SNPs (Table 1) in response to each environmental gradient were highly variable, both within and across gene functions (Figure 3). Generally, the locations at which rates of allelic

turnover changed the most (i.e., where one allele was selected for most strongly) were observed between sites with the greatest geographic distance apart (Figure 3a), at upper latitudes where summer temperature was high (Max Temp; Figure 3b), at lower latitudes where rainfall was lower (Annual Rain; Figure 3c) and wind speed was higher (Figure 3d). Although weak, locations with higher tree cover also showed some allelic turnover (Figure 3e). Max Temp and Annual Rain both increase with latitude (Figure S2) and their associated SNPs showed polarized patterns of selection, with some showing strong allelic turnover at lower values before stabilizing and others becoming strong only at high gradient values (Figure 3).

3.6 | SNP-specific signatures of local adaptation

We examined spatial genetic gradients over the study area by quantifying allele frequency changes in four selected SNPs that were selected based on (i) the SNP's functional annotation, (ii) its statistical association with Max Temp (both magnitude of allelic turnover and ranking of turnover), (iii) its change in F_{ST} along the gradient and (iv) the percentage of the GDM model the SNP explained. We first examined SNP 37543_9, which was annotated to *vacuolar H⁺ ATPase*, which is involved in proton pump activity to regulate pH in eukaryotic cellular compartments that affect important cellular processes (Nishi & Forgac, 2002). This SNP had the highest magnitude of allelic turnover in relation to Max Temp (0.63), a high change in F_{ST} along the gradient ($\Delta F_{ST} = 0.50$) and the highest ranking in the GDM model for Max Temp (10) and was also identified as an F_{ST} outlier using OUTFLANK (Table 2, Figure 4). Second, we examined SNP

73426_72, which was annotated to a *long-wavelength-sensitive opsin 3b*, involved in visual processes. This SNP had the highest magnitude of allelic turnover in relation to Max Temp (0.24) and a high change in F_{ST} along the gradient ($\Delta F_{ST} = 0.21$) and was ranked highly in the GDM model for Max Temp (266) (Table 2, Figure 5). The allelic turnover functions for the above two SNPs are shown in relation to Max Temp (Figures 4 and 5).

Third, we examined SNP 53905_36, which was annotated to *HEAT SHOCK PROTEIN 70* (HSP70; Table 2, Supporting Information Figure S6), a gene that is involved in the thermal stress response (Lancaster et al., 2016; Sørensen, Kristensen, & Loeschke, 2003). This SNP had the highest magnitude of allelic turnover in relation to geographic distance (0.33), but had a high change in F_{ST} along the gradient ($\Delta F_{ST} = 0.40$), and was identified as an F_{ST} outlier in BAYESCAN. Finally, we examined SNP 35404_9, which had the highest magnitude of allelic turnover in relation to Max Temp (0.83) and a high change in F_{ST} along the gradient ($\Delta F_{ST} = 0.38$) and was identified as an F_{ST} outlier in OUTFLANK (Table 2, Supporting Information Figure S7). This SNP was annotated to 10 transcripts that annotated to various proteins and enzymes (see Supplementary Data), including Pellino proteins, which are involved in the immune response via the Toll-like receptor pathway (Schauvliege, Janssens, & Beyaert, 2007), and PACS2 (phosphofurin acidic cluster sorting protein), which is involved in cell apoptosis (Simmen et al., 2005). The above four SNPs showed spatial patterns of allelic turnover along the core to range limit gradient that varied in magnitude and linearity, indicating differential selection on particular alleles along the *I. elegans* expansion axis in relation to latitude

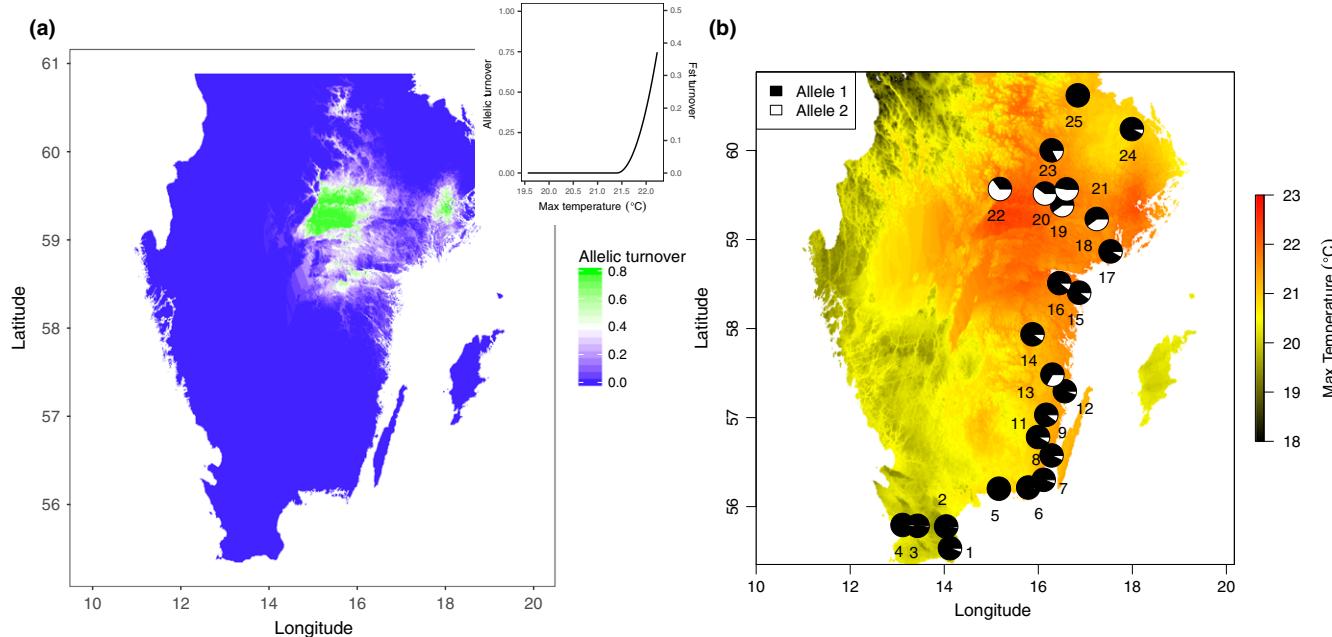


FIGURE 4 Allelic turnover of SNP ID 37543_9, shown as (a) the allelic turnover response curve in relation to BIO5 and Partial F_{ST} change, and (b) allele frequency for each sampling location (black = high-frequency allele 1; white = low-frequency allele 2) mapped on BIO5 (mean maximum summer temperature). Allele 2 undergoes substantial change in frequency from south to north, increasing in warmer inland sites, before becoming less frequent at the cooler extreme range edge. This SNP was annotated to a gene for *vacuolar H⁺ ATPase*, involved in proton pump activity, and was associated most strongly with BIO5, with a maximum F_{ST} change of 0.50 across the sampling gradient [Colour figure can be viewed at wileyonlinelibrary.com]

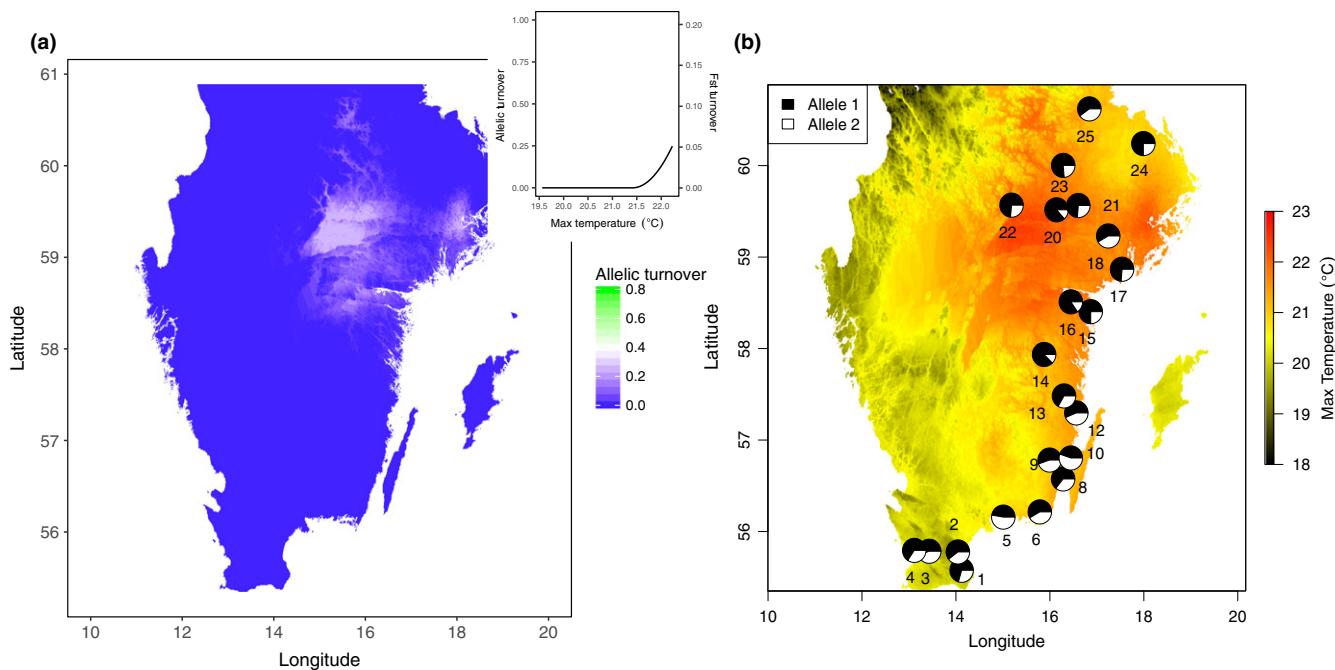


FIGURE 5 Allevic turnover of SNP ID 73426_72, shown as (a) the allelic turnover response curve in relation to BIO5 and Partial F_{ST} change, and (b) allele frequency for each sampling location (black = high-frequency allele 1; white = low-frequency allele 2) mapped on BIO5 (mean maximum summer temperature). Alleles 1 and 2 have comparable frequencies up to the mid-north latitudes, beyond which allele 1 increases in frequency towards the inland and coastal range limit. This SNP was annotated to a long-wavelength-sensitive opsin gene 3b, involved in visual processing, and was associated most strongly with BIO5, with a maximum F_{ST} change of 0.21 across the sampling gradient [Colour figure can be viewed at wileyonlinelibrary.com]

and Max Temp (Figures 4 and 5 and Supporting Information Figures S8 and S9).

4 | DISCUSSION

We characterize genetic signatures of local adaptation to environment along a climate-mediated range expansion in a species exhibiting rapid response to shifting temperature regimes (Hickling et al., 2005; Jaeschke, Bittner, Reineking, & Beierkuhnlein, 2013; Lancaster et al., 2015, 2016; Swaegers et al., 2013, 2015; Watts et al., 2010). Among four environmental variables tested, the strongest driver of allelic turnover along the *I. elegans* expansion gradient was maximum summer temperature (Max Temp), followed by mean annual precipitation (Annual Rain), wind speed, and to a much lesser extent, % tree cover (Table 1, Figure 3). The greatest allele frequency changes in *I. elegans* were in localities spanning low to mid-latitudes (i.e., from Scania to further north), where Max Temp shifts most dramatically (~1.2°C; Figures 4 and 5 and Supporting Information Figure S1, Table S2), rainfall is lower and more variable, and wind speeds are higher than in the northern range edge (Supporting Information Figure S2). Selected annotated SNPs exhibited allele-specific patterns of selection along the core to edge sampling gradient (Figures 4 and 5 and Supporting Information Figures S8 and S9), with wide variation in the magnitudes of allelic turnover across SNPs (Table 2). SNP annotations indicated that genes involved in the thermal stress response, visual processes, epigenetic modification and ion regulation

may play significant roles in adaptation during this climate-mediated range expansion in *I. elegans*. Our multilayered approach (Figure 1) validates a “bottom-up” approach for detecting signatures of local adaptation from reduced representation genomic data, in which a group of SNP candidates is first identified, followed by SNP-specific modelling of genetic gradients, supported by gene annotation and prior experimental knowledge of gene functional response (e.g., Chauhan et al., 2014, 2016; Lancaster et al., 2016).

4.1 | Detection of putative SNPs under selection

F_{ST} outlier analysis and EAA are increasingly popular methods for identifying SNPs under putative selection (Hoban et al., 2016; Rellstab et al., 2015). One notable aspect of our F_{ST} outlier and EAA results is their lack of overlap in terms of the number and identity of SNPs (Table 1). Not only did the SNPs identified by our two F_{ST} outlier approaches overlap by just 1.5%, but F_{ST} outliers overlapped with just 0.6%–4.0% of SNPs identified using EAA (Table 1). This does not necessarily indicate a lack of power in the analysis and is consistent with findings that EAA performs better than F_{ST} outlier tests in detecting weak or polygenic selection signatures (Frichot & François, 2015; De Villemereuil et al., 2014). The minimal overlap and difference in numbers of SNPs identified between F_{ST} outlier approaches identified may indicate different sensitivities of each approach to the effects of genetic drift and structure. Notably, studies comparing OUTFLANK and BAYESCAN have found little overlap between the approaches (e.g., Bernatchez, Laporte, Perrier, Sirois, &

Bernatchez, 2016; Chen, Farrell, Matala, & Narum, 2018; Micheletti, Matala, Matala, & Narum, 2018). The significant SNP associations using EAA were unique to each environmental variable in 41%–79% of cases (Table 1). Concordantly, F_{ST} distributions were negatively skewed and variable across all 1,758 candidate SNPs (Supporting Information Figure S5a), which was mirrored when examining SNPs according to the environmental variable they were associated with (Supporting Information Figure S5c–f). The dominance of low F_{ST} values indicates that many SNPs show weak selection signatures along the sampling gradient.

Notably, the F_{ST} changes observed in the 23 annotated and most highly supported SNPs from the GDM were not biased towards higher F_{ST} values (F_{ST} range = 0.09–0.5; Figure 2, Table 2). Overall, the results indicate that an increased change in F_{ST} along a sampling gradient of a SNP does not correlate with a greater likelihood of identifying that SNP as being under selection using EAA. This lack of correlation has similarly been observed in a recent meta-analysis of studies using F_{ST} outlier tests and EAA (Ahrens et al., 2018). This observation indicates that adaptation to environmental conditions is polygenic and involves many interacting loci of both small and large effect (e.g., Lee & Mitchell-Olds, 2012).

4.2 | Accounting for neutral genetic structure

Detecting genetic selection signatures is riddled with the issue of separating true adaptive genetic responses from neutral genetic structure (Hoban et al., 2016), which is particularly relevant when neutral structure mirrors sampled environmental gradients (Lotterhos & Whitlock, 2015). Range expansion processes can result in patterns of selection on loci that mirror neutral genetic structure, for example, via allele surfing mechanisms, whereby rare alleles become more frequent at range expansion fronts according to the process of genetic drift rather than selection. Allele surfing can therefore increase population genetic differentiation and confound signatures of local adaptation (Klopfenstein, Currat, & Excoffier, 2006) but might also affect adaptation when either beneficial or deleterious alleles are “surfed” on the wave of expansion (Gralka et al., 2016; Travis et al., 2007). Such processes make teasing apart adaptive and neutral processes in range-expanding species a challenge.

Genetic admixture was greatest within sites at the low to mid-latitudes and declined towards the range limit in *I. elegans*, where sites were comprised of individuals assigned to multiple or unique clusters (Figure 2). Given this tracking of genetic structure with latitude, it was particularly important to account for false positive SNPs in our data. At each step of our analysis we applied approaches to avoid false positives. First, we selected only putative SNPs under selection using F_{ST} outlier tests (diversifying only) and EAA and excluded SNPs associating with geographic distance in our EAA. In addition, we implemented two additional approaches to avoid the inclusion of false positives using GDM by (i) including a randomly selected “reference” SNP group to compare with each SNP and (ii) including geographic distance as a predictor in the GDM to identify selection signatures correlating with geography. Finally, SNP

annotations to gene functions involved in thermal stress response and other ecologically relevant genes indicated climate-mediated local selection on some candidate SNPs along the range expansion gradient (Table 2, Figures 4 and 5). Despite expectations that gene flow will have a constraining effect on adaptive divergence (discussed in Smadja & Butlin, 2011), the relationship between gene flow and local adaptation is increasingly found to be positive (Jacob et al., 2017; Moody et al., 2015), including at species’ range edges (Halbritter, Billeter, Edwards, & Alexander, 2015). Further analysis of how neutral genetic connectivity and landscape features are related to the pattern of adaptive genetic variation in *I. elegans* is needed to address this.

4.3 | Broad allelic frequency changes across the range expansion

The contrasting steepness of the environmental gradients we sampled (Supporting Information Figure S2) appeared to correspond with the magnitudes of allelic turnover observed across SNPs using GDM (Figure 3), which is in contrast to the lack of an environmental “steepness” effect on selection detection across studies using EAA (reviewed in Ahrens et al., 2018). For example, percentage tree cover was highly variable according to latitude (Supporting Information Figure S2) and attracted the lowest allelic turnovers (Figure 3). In contrast, Max Temp showed the steepest environmental gradient and correspondingly high allelic turnovers (Figure 3). Pronounced allele frequency changes in relation to Max Temp between low to mid-latitudes, indicate a “transition area” of local adaptation (Figure 1 and Supporting Information Figure S9) where the greatest shifts in environmental conditions are present. In this area, Max Temp increases by approximately 2°C, mean annual precipitation decreases by 170 mm and wind speed decreases by 1.9 m/s within an approximate 3-degree shift in latitude (Supporting Information Table S2, Figure S2). At the range edge, sites are located further inland and conditions are less variable (e.g., only 0.57°C maximum difference in Max Temp between sites). A second area that exhibited high allelic turnover was at the northern range limit, where distinct changes in allele frequencies were evident that were often correlated with the warmer Max Temp at sites in this region (Figures 3–5 and Supporting Information Figure S8).

Our “bottom-up” approach of screening RAD-derived SNPs for environmental selection signatures is an alternative to when dense genomic resources are available (e.g., using GWAS; Berg & Coop, 2014) or preidentified candidate genes are targeted (e.g., Fitzpatrick & Keller, 2015; Hoekstra, Hirschmann, Bundey, Insel, & Crossland, 2006; Sork et al., 2016), and is informed largely by the spatial heterogeneity of both environmental and adaptive variation within the data set. One important caveat of the EAA approach is that some loci may only show a weak association with environmental variables when the locus is simultaneously advantageous across a diversity of environments (Frichot et al., 2013). Our GDM approach is complementary in this case, as it allows for relative allelic responses to be simultaneously characterized across predictor variables. Approaches that characterize gene interactions may further

elucidate the polygenic basis of environmental adaptation (e.g., Herold et al., 2012; Lee & Mitchell-Olds, 2012).

4.4 | Signatures of environmental selection on annotated genes

The response curves of the annotated candidate SNPs to the tested environmental variables (using GDM) indicate that allele frequencies are tracking environmental gradients along the *I. elegans* range expansion (e.g., Figures 4 and 5 and Supporting Information Figures S8 and S9). A variety of gene functions were represented with a diversity of environmental associations (Table 2). Our annotations of candidate SNPs matched gene functions associated with thermal tolerance in a gene expression study by Lancaster et al. (2016) along the *I. elegans* range expansion. Three major gene functions were previously identified from gene expression experiments (Table 2) in both Lancaster et al. (2016) (thermal stress and epigenetic modification) and Chauhan et al. (2014, 2016) (visual processing and thermal stress), while we found additional support for strong selection on genes involved in ion transport (V-ATPase) and other cellular processes.

Eleven candidate SNPs annotated to the HSP40 gene. All of these SNPs were located on the same genome scaffold and showed significant environmental associations with Max Temp and other variables (Table 2). HSP40 was not differentially expressed in *I. elegans* in Lancaster et al. (2016) in response to thermal tolerance treatments, which may be indicative of the different mechanisms involved in gene expression. However, HSP70 that was included among our candidate genes, showed greater upregulation in gene expression in response to heat stress in the core compared with edge populations (Lancaster et al., 2016). Only a single SNP was annotated to HSP70 (Table 2) and showed a large change in F_{ST} and allelic turnover along the sampled environmental gradient (Supporting Information Figure S6). HSP70 is a highly conserved, ATP-dependent molecular chaperone that facilitates protein homoeostasis under a variety of conditions including thermal stress (Beere, 2004; King & MacRae, 2015). The allelic turnover of the SNP annotated to HSP70 was strongest in relation to geographic distance (Table 2; Supporting Information Figure S6), which indicates a lack of power to detect environmental selection on this SNP using the GDM. Notably, the reduced differential gene expression in *I. elegans* in response to heat shock at the sampled range edge compared to the core indicates a possible loss of gene function at the range edge (discussed in Lancaster et al., 2016), which is to be further examined.

We detected a strong selection signature for the vacuolar H⁺ ATPase (V-ATPase) gene (Figure 4), which is noteworthy as the activity of this gene has pleiotropic effects on both cold tolerance and salinity. V-ATPase is an ion transporter and aids in sodium (Na⁺) modulation at the Malpighian tubules of insects by energizing fluid secretion while coupled to an H⁺/K⁺ exchanger, modulating pH and salinity (Beyenbach, Skaer, & Dow, 2010). V-ATPases may also play a role in cold tolerance in insects, which is related to body-ion

gradients regulated by water loss. The inability of insects to maintain ion gradients at low (i.e., $\leq 0^\circ\text{C}$) temperatures may be an important cause of mortality from cold exposure and influence cold tolerance (e.g., in bugs: Koštál & Vambera, 2004; in *Drosophila*: MacMillan et al., 2015; in crickets: MacMillan & Sinclair, 2011). Lancaster et al. documented phenotypic (2015) and gene expression (2016) changes in relation to cold tolerance in *I. elegans* from Sweden, with faster cold acclimation rates and unique cold-response gene expression profiles at the range edge compared to the core. This evidence for selection on cold tolerance and the decrease in minimum temperature along our sampled gradient suggests a cold tolerance benefit for selection on V-ATPase in *I. elegans*. Notably, changes in V-ATPase activity in the optic lobe during circadian cycles has also been found in flies, indicating a role in visual processes (Górnska-Andrzejak, Damulewicz, & Pyza, 2015). Our sampled gradient also exhibits variation in water body salinity, with many sites within coastal areas and others within inland freshwater lakes and ponds, some closed, and others open to the Baltic Sea. This variation in salinity may impose further selection pressure on V-ATPase genes during the aquatic larval stage of *I. elegans*. Our findings suggest that vacuolar H⁺ ATPase contributes to local adaptation in *I. elegans* during its poleward range expansion, which is observed as a shift in allele frequency towards colder, range limit sites sampled from noncoastal, low-salinity sites (Figure 2).

Although weaker than for V-ATPase, we detected a strong selection signature for long-wavelength-sensitive (LWS) opsin (annotated to SNP 73426_72; Table 2, Figure 5), which is a phylogenetically diverse class of opsins in Odonata (Suvorov et al., 2017) that have previously been identified in transcriptomic analyses of *I. elegans* in Chauhan et al. (2014, 2016). Odonates have between three and five classes of photoreceptors (Futahashi et al., 2015) that are involved in visual processes that may play roles in food acquisition, mate choice (e.g., in cichlids; Terai, Mayer, Klein, Tichy, & Okada, 2002), development (in odonates; Futahashi et al., 2015) and sex-specific behaviours (in *I. elegans*; Chauhan et al., 2016). The importance of colour discrimination in sexual selection and sexual conflict in Odonata is well known (e.g., in *I. elegans*; Gosden & Svensson, 2009; Le Rouzic et al., 2015; Svensson et al., 2005). Further, within our study area, the frequency of *I. elegans* gynochromes (female-specific female morphs) increases with latitude and shows a frequency-dependent fitness benefit with respect to cold tolerance that may facilitate range shifts (Lancaster et al., 2017). It is possible that selection on LWS opsins through its cascading effects in sexual interactions may contribute to climate adaptation during range expansion, via social feedback mechanisms, thermal conditions and their possible interactions.

As genomic resources improve for *I. elegans* (e.g., transcriptome: Chauhan et al., 2014, 2016; genome: P. Chauhan et al. unpublished), candidate gene regions identified in this study may be more closely examined for soft selective sweeps and their emergence according to climate change (Messer and Petrov, 2013). Previous studies on other coenagrionid damselflies have identified putatively adaptive traits in range-expanding populations,

for example, increased flight ability and enhanced immune function in *Coenagrion scitulum* (Therry, Lefevre, Bonte, & Stoks, 2014; Therry, Nilsson-Örtman, Bonte, & Stoks, 2014) and identification of candidate genes associated with increased flight performance (Swaegers et al., 2015). Future studies may take advantage of both phenotypic measurements and high-quality genomic resources to disentangle multiple functional genetic changes that occur during Odonata range expansions.

5 | CONCLUSION

With maximum summer temperatures (Max Temp) in our study area projected to increase up to 4°C by 2050 (under RCP8.5 data from BIOCLIM; Hijmans et al., 2005), and with similar trends occurring worldwide, the development of effective approaches for measuring and predicting species' adaptive responses, and thus future biodiversity structure under environmental change, is crucial. Our findings empirically validate a multitiered statistical approach for uncovering spatial heterogeneity in signatures of local adaptation along environmental gradients (Figure 1). Our results reveal environmental thresholds where climate-mediated selection indicates that *I. elegans* is currently in the process of evolving local adaptation along its range, with selection on genes that show functional relevance with respect to environmental variation and stressors. The effects of plasticity and ensuing genetic assimilation of adaptive traits in augmenting the persistence of *I. elegans* during range expansion require further investigation (e.g., Lande, 2009), as well as how intra- and inter-specific competition might also influence local adaptation (Case & Taper, 2000; Price & Kirkpatrick, 2009). Further, the parallel environmental gradients where *I. elegans* is subject to range limit processes in northern Europe offer future opportunities for a replicated investigation of parallel signatures of adaptation, which may reveal common adaptive processes that apply to ectotherms more generally.

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DATA ACCESSIBILITY

All supplementary files are deposited on DRYAD at <https://doi.org/10.5061/dryad.8s449qb>, including the *I. elegans* draft genome used in the manuscript, SNP data sets, environmental data and R code for the GDM.

AUTHOR CONTRIBUTION

R.Y.D., B.H., E.S. and L.L. conceptualized the study design. R.Y.D. and L.L. collected samples in the field. R.Y.D. prepared genomic libraries and conducted bioinformatics analyses with assistance from B.H. R.Y.D. and C.Y. analysed the data. C.Y. performed statistical modelling and prepared figures. R.Y.D. wrote the manuscript. All authors edited the final manuscript.

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SUPPORTING INFORMATION

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