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FROM THE COVER

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Ecological gradients drive insect wing loss and speciation: The role of the alpine treeline

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Abstract

Alpine ecosystems are frequently characterized by an abundance of wing-reduced insect species, but the drivers of this biodiversity remain poorly understood. Insect wing reduction in these environments has variously been attributed to altitude, temperature, isolation, habitat stability or decreased habitat size. We used fine-scale ecotypic and genomic analyses, along with broad-scale distributional analyses of ecotypes, to unravel the ecological drivers of wing reduction in the wing-dimorphic stonefly *Zelandoperla fenestrata* complex. Altitudinal transects within populations revealed dramatic wing reduction over very fine spatial scales, tightly linked to the alpine treeline. Broad biogeographical analyses confirm that the treeline has a much stronger effect on these ecotype distributions than altitude per se. Molecular analyses revealed parallel genomic divergence between vestigial-winged (high altitude) and full-winged (low altitude) ecotypes across distinct streams. These data thus highlight the role of the alpine treeline as a key driver of rapid speciation, providing a new model for ecological diversification along exposure gradients.

KEYWORDS

altitude, biodiversity, dispersal, gene flow, insect, speciation, treeline

1 | INTRODUCTION

The evolution of flight ~400 million years ago (Misof et al., 2014) is thought to have underpinned the astonishing ecological diversity of insects, by enhancing mate and resource location, dispersal and predator evasion (Wagner & Liebherr, 1992). Subsequently, however, wing loss has occurred repeatedly across nearly all winged insect orders (Roff, 1990). Although this secondary wing reduction eliminates the advantages of insect flight, recent studies have suggested that wing loss can increase the rate of species diversification (Ikeda, Nishikawa, & Sota, 2012; Vogler & Timmermans, 2012). Additionally, while numerous insect taxa exhibit wing-length polymorphism, the ecological drivers of this diversity have often remained unclear.

Wingless insect lineages are over-represented in alpine ecosystems (Hodkinson, 2005). Wing loss in high-altitude environments has been attributed to the increased stability (Denno et al., 1996; Roff, 1994) and/or isolation of alpine ecosystems (Roff, 1990; Wagner & Liebherr, 1992). By contrast, other studies have suggested that increasing exposure (i.e., high winds) and reduced temperature associated with increasing altitude may underpin alpine wing reduction (Roff, 1990). Specifically, it has been argued that the consistent, wind-driven loss of flighted emigrants should directly select locally against flight (Darwin, 1859; Roff, 1990), while others have suggested that insects are likely to invest more resources into reproduction rather than producing wings when conditions for flight are unfavourable (e.g., where there are low temperatures or high winds; Zera and Harshman 2001). Intriguingly, recent distributional analyses of full-winged versus wing-reduced stonefly assemblages suggest that wing reduction may be more tightly associated with the position of the alpine treeline, rather than altitude per se (McCulloch, Foster, Ingram, & Waters, 2019), suggesting that increased exposure above the treeline drives wing reduction. However, this hypothesis of exposure-driven ecotypic shifts has yet to be tested at the microevolutionary (intraspecific) level.

Insect species exhibiting intraspecific wing-length polymorphism provide ideal systems for understanding the ecological drivers and evolutionary consequences of wing reduction. One well-studied system involves the widespread New Zealand stonefly *Zelandoperla fenestrata* complex (McLellan, 1999; McCulloch, Wallis, & Waters, 2009; Dussex, Chuah, & Waters, 2016; Veale, Foster, Dearden, & Waters, 2018). This species complex contains five distinct genetic (and geographical) clades, with wing reduction only occurring in two of these groups (Clades 1 and 5; Figure S1a; McCulloch et al., 2009). Within Clade 1, full-winged ecotypes are particularly common at low altitude, while wing-reduced ecotypes are associated with alpine habitats (Dussex et al., 2016; McCulloch et al., 2009). Recent genomic studies imply that vestigial-winged ecotypes in this clade may have evolved repeatedly via selection at one or a few loci (Dussex et al., 2016; McCulloch et al., 2009; Veale et al., 2018), but the forces underpinning this apparent convergent evolution have remained unclear.

Gradual (clinal) variation in ecotype frequencies over environmental gradients has previously been used to infer the role of natural selection (Haldane, 1948; Savolainen, Lascoux, & Merilä, 2013). Indeed, numerous biological studies have detected clinal variation in traits across altitudinal gradients (Barber & Jackson, 1957; Pitchers, Pool, & Dworkin, 2013). Recent studies have further suggested that the presence of replicated clines across independent geographical systems can strengthen inferences regarding the “agents and targets” of natural selection (Kooyers & Olsen, 2013).

Here we combine fine-scale ecotypic and genomic analyses, with broad-scale distributional modelling, to test the hypothesis that the alpine treeline is a key driver of insect wing reduction and diversification in mid- to high-latitude alpine ecosystems. Specifically, we use population genomic analysis to reveal the role of wing loss in driving rapid insect diversification within stream populations. In addition, we use a binomial generalized linear model to test the effects of altitude and the alpine treeline on the broad distributions of winged versus wing-reduced insect ecotypes.

2 | METHODS

2.1 | Sampling and morphological characterization

A total of 166 *Zelandoperla fenestrata* complex specimens were collected from two parallel streams (Lug Creek, Six Mile Creek) on the Rock and Pillar Range, South Island, New Zealand (Figure S2a, Table S1). These neighbouring streams are ~2 km apart, both flowing into the Taieri River. Specimens were collected from several discrete altitudinal zones within each stream (ranging from ~300 to 1,200 m), with altitudinal intervals of ~100–200 m. Recently emerged adults were collected from stones, wood and moss in rapids, and immediately preserved in absolute ethanol. Late-instar nymphs were also collected from wood or stones in rapids, and reared in Styrofoam cups at 11°C in water from their natal stream until emergence. To avoid potential sampling of close relatives (i.e., family groups), samples from within a locality were obtained from numerous different rocks across each sampling location. The forewing lengths of 119 adults originating from Lug Creek and Six Mile Creek were measured from stereo microscope images in IMAGEJ (Schindelin et al., 2012). Wing-length was highly dimorphic (Figure 1a), and varied equally among sexes, meaning individuals could definitively be categorized as either full-winged or vestigial-winged.

2.2 | DNA extraction and sequencing

DNA was extracted from head, pronotum and femur tissue using DNeasy kits (Qiagen) according to the manufacturer's protocols. Genotyping-by-sequencing (GBS) was conducted by AgResearch. DNA was fragmented with restriction enzyme *Pst*I, and sample-specific barcode adaptors ligated to the sticky ends of the fragments after digestion. Individually barcoded samples were pooled into two separate libraries. Libraries were column purified, then size-selected (193–500 bp range) using a Pippin device (Sage Science). Each library was sequenced on a single lane of a HiSeq 2500 for 101 cycles in high-output mode.

2.3 | Bioinformatics and genotyping

The sequence data were quality assessed using FASTQC (Andrew, 2010), and single nucleotide polymorphisms (SNPs) were extracted using STACKS 2.0 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Raw sequence reads were demultiplexed according to barcode and filtered for quality using the *process_radtags* module in STACKS. All reads were trimmed to 100 bp, with low-quality reads, and reads that did not contain the enzyme recognition site, removed. As there is no reference genome, the cleaned reads were assembled de novo using the default parameters. Following assembly and genotyping, the *populations* module was used to further filter the data. We retained only those loci that were genotyped in at least 80% of samples and had a minor allele frequency of ≥0.05 (to minimize the inclusion of false SNPs caused by sequencing errors). Only the first SNP in each stack was kept for subsequent analysis, as other SNPs within the same read would be in strong linkage disequilibrium.

The parameters used in de novo assembly, genotyping and filtering may significantly impact downstream analyses (see Mastretta-Yanes et al., 2015). We therefore conducted additional, more stringent filtering, to assess whether this would alter our results. This filtering included the removal of sites that were tri-allelic, sites that were significantly out of Hardy–Weinberg equilibrium, linked sites ($r^2 > 0.5$), and SNPs with more than 0.65 heterozygosity.

2.4 | Population genomic structuring

Genetic similarity among specimens was assessed using principal component analysis (PCA) in the R package ADEGENET version 2.1.1

(Jombart, 2008). Pairwise F_{ST} values were calculated among collection sites within each stream, and among vestigial-winged and full-winged ecotypes, using ARLEQUIN version 3.5 (Excoffier & Lischer, 2010). The significance of these values was assessed with 10,000 permutations, with Bonferroni corrections applied to account for multiple tests. Individual- and population-level heterozygosity was estimated in the R package ADEGENET version 2.1.1. Population structuring within each stream was further assessed using STRUCTURE version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The analysis was run for 500,000 iterations (after an initial burn-in of 50,000 iterations) using the admixture model with allele frequencies correlated. We ran five independent runs from $K = 1$ to $K = 6$, with the most likely number of clusters assessed using CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). NEWHYBRIDS version 1.1 (Anderson & Thompson, 2002) was used to compute the posterior probability that an individual fell into one of six categories: pure vestigial-winged, pure full-winged, F_1 hybrid, F_2 hybrid, first-generation backcross to vestigial-winged, or first-generation backcross to full-winged. Posterior distributions were evaluated after 100,000 iterations (after a burn-in period of 10,000 iterations), with default priors for mixing proportions and allele frequencies.

To estimate the centre and width of the observed clines we fitted classical cline models to our data using the R pacakage HZAR (Derryberry, Derryberry, Maley, & Brumfield, 2013). This software fits genetic or morphological data to classical equilibrium cline models using the Metropolis–Hastings Markov chain Monte Carlo algorithm. For each stream we fitted a cline to both morphological data (proportion of vestigial-winged specimens) and genetic data (the mean proportion of cluster membership [Q] for each site along the transect, as inferred by STRUCTURE). We ran the analysis for 500,000 iterations (following a burn-in of 50,000 iterations). Fifteen distinct models were fitted to each data set (see Derryberry et al., 2013), with the best cline model for each selected based on the lowest Akaike's information criterion score. Observed frequency data were plotted over the associated fuzzy cline regions (95% credible cline regions).

2.5 | Ecotype distributional analyses

To assess the biogeographical distribution of vestigial-winged populations within *Z. fenestrata* Clade 1 (see Figure S1a; McCulloch et al., 2009) we measured forewing length for an additional 312 adult specimens from the Auckland Museum Entomology Collection (Auckland), Canterbury Museum (Christchurch), Museum of New Zealand Te Papa Tongarewa (Wellington), New Zealand Arthropod Collection (Auckland) and Otago Museum (Dunedin). The majority of these specimens had forewing lengths conforming to the discrete

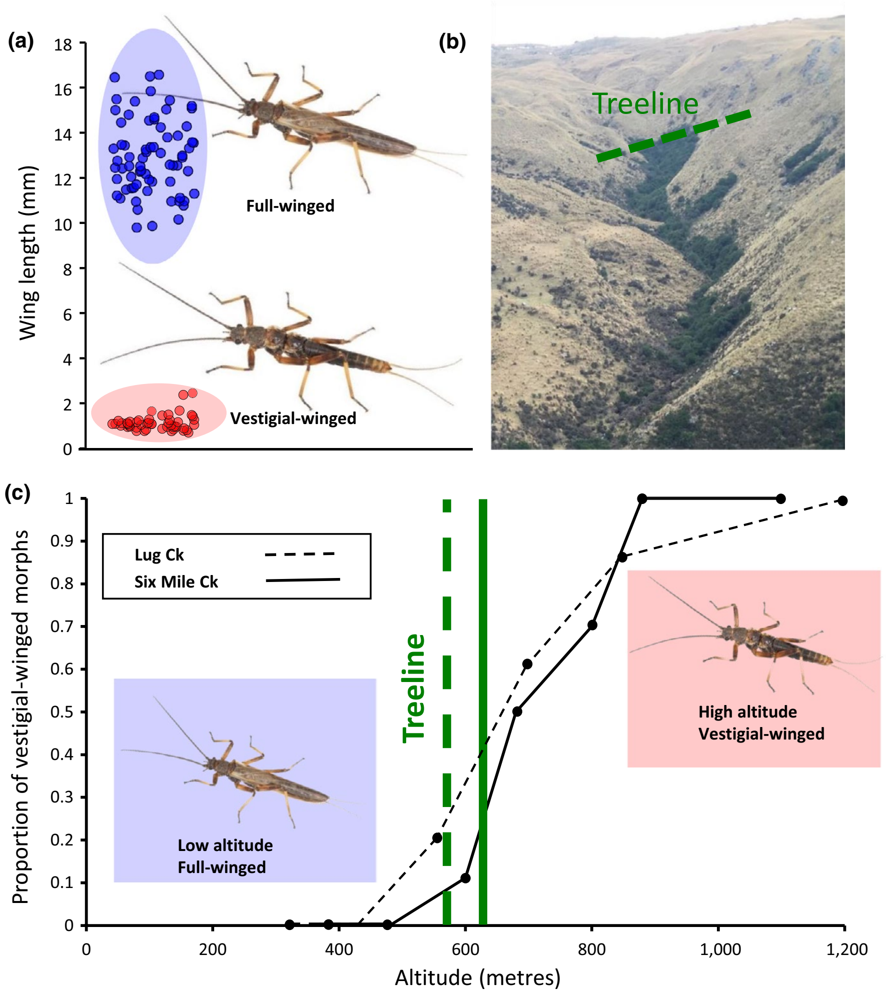


FIGURE 1 The alpine treeline governs the distribution of full-winged versus vestigial-winged stoneflies. (a) Jitter plot illustrating the bimodal wing-length data associated with distinct *Zelandoperla fenestrata* ecotypes collected from two parallel streams (Lug Creek, Six Mile Creek) on the Rock and Pillar Range, South Island, New Zealand. Inset: dorsal view of full-winged (above) and vestigial-winged (below) *Z. fenestrata* ecotypes (body length 15 mm). (b) The alpine treeline in Lug Creek, where riparian tree cover rapidly gives way to tussock grassland. (c) Altitudinal clines in relative proportions of wing ecotypes in Lug Creek (dashed line) and Six Mile Creek (solid line). Vertical green lines indicate the altitude of the alpine treeline for each stream (Lug Creek = dashed line, Six Mile Creek = solid line)

(bimodal) wing phenotypes consistently observed on the Rock and Pillar Range (Figure 1a; see also Veale et al., 2018), although specimens with intermediate wing-size (wings slightly reduced; brachyptery) were identified at a relatively small number of locations. Such brachypterous specimens were generally found at higher altitudes, but were not found at the same locations as vestigial-winged specimens, perhaps suggesting there are multiple distinct forms of altitude-associated wing reduction in this group. These specimens were removed from subsequent biogeographical analyses given the uncertain ecological consequences of minor reductions in wing size. This left a subset of 249 adults with forewing lengths conforming to the discrete forms of wing phenotypes present in the study populations. These data were combined with distributional records from McCulloch et al. (2009) and included in subsequent biogeographical analyses. Records from duplicate locations were removed, along with records from which accurate collection localities could not be determined. The altitude of each record, and the altitude of the local treeline, were assessed by mapping collection coordinates onto the NZ Topo Map (http://www.nztopomap.co.nz).

Before testing for relationships between wing loss and treeline or altitude, we assessed if intraspecific genetic structure needed to be taken into account when comparing wing loss to elevation and to the treeline. We lacked genomic or multilocus data to estimate the phylogenetic relationships or degrees of isolation and migration between all relevant populations (see Stone, Nee, & Felsenstein, 2011). Instead, we assessed the level of phylogenetic signal in wing phenotypes using a cytochrome oxidase I (*COI*) phylogeny for the *Z. fenestrata* complex from McCulloch et al. (2009) (Figure S1b), made ultrametric using the “chronos” (Paradis, Claude, & Strimmer, 2004; https://CRAN.R-project.org/package=ape) function in R with a smoothing parameter of 1. This tree includes a subset of 127 individuals from the complete data set of 322 individuals. As existing methods for evaluating phylogenetic signal can handle binary but not binomial data, six haplotypes possessed by both full-winged and vestigial-winged ecotypes were excluded, leaving 36 haplotypes in the tree (24 full-winged and 12 vestigial-winged). These were then tested for phylogenetic signal using the *D* statistic for binary traits (Fritz & Purvis, 2010), implemented in the R package “caper” (Orme et al., 2011). *D* is expected to be close to 1 if traits are random with regard to phylogeny, close to 0 if binary traits are determined by an underlying continuous trait following a Brownian motion random walk model of evolution, and <0 if traits are highly structured in the phylogeny.

The relationship between wing ecotype and altitude or position relative to the alpine treeline was analysed with the assumption that each population (sampling site) consisted of an independent replicate (with wing ecotypes for 1–16 individuals per site). Wing ecotypes were therefore analysed using a binomial generalized linear model with a logit link, with two predictors: position relative to the treeline (binary: above or below) and altitude (divided by two standard deviations to facilitate comparison between predictors; see Gelman, 2008). Effect sizes of the two predictors were compared using the generalized partial coefficient of determination computed in the R package “rsq” (Zhang, 2017; https://CRAN.R-project.org/package=rsq).

3 | RESULTS

3.1 | Fine-scale ecotypic and genomic analyses

Parallel altitudinal transects of *Zelandoperla fenestrata* from adjacent alpine streams revealed independent ecotypic clines, with low-altitude samples fixed for the full-winged phenotype and high-altitude samples fixed for vestigial wings (Figure 1). Sudden transitions from full-winged to vestigial-winged ecotypes in each stream occurred around the alpine treeline, where trees abruptly give way to tussock grassland (580–620 m a.s.l.; see Figure 1b). Narrow zones of ecotypic overlap were identified directly above the alpine treeline in each stream (Figure 1c).

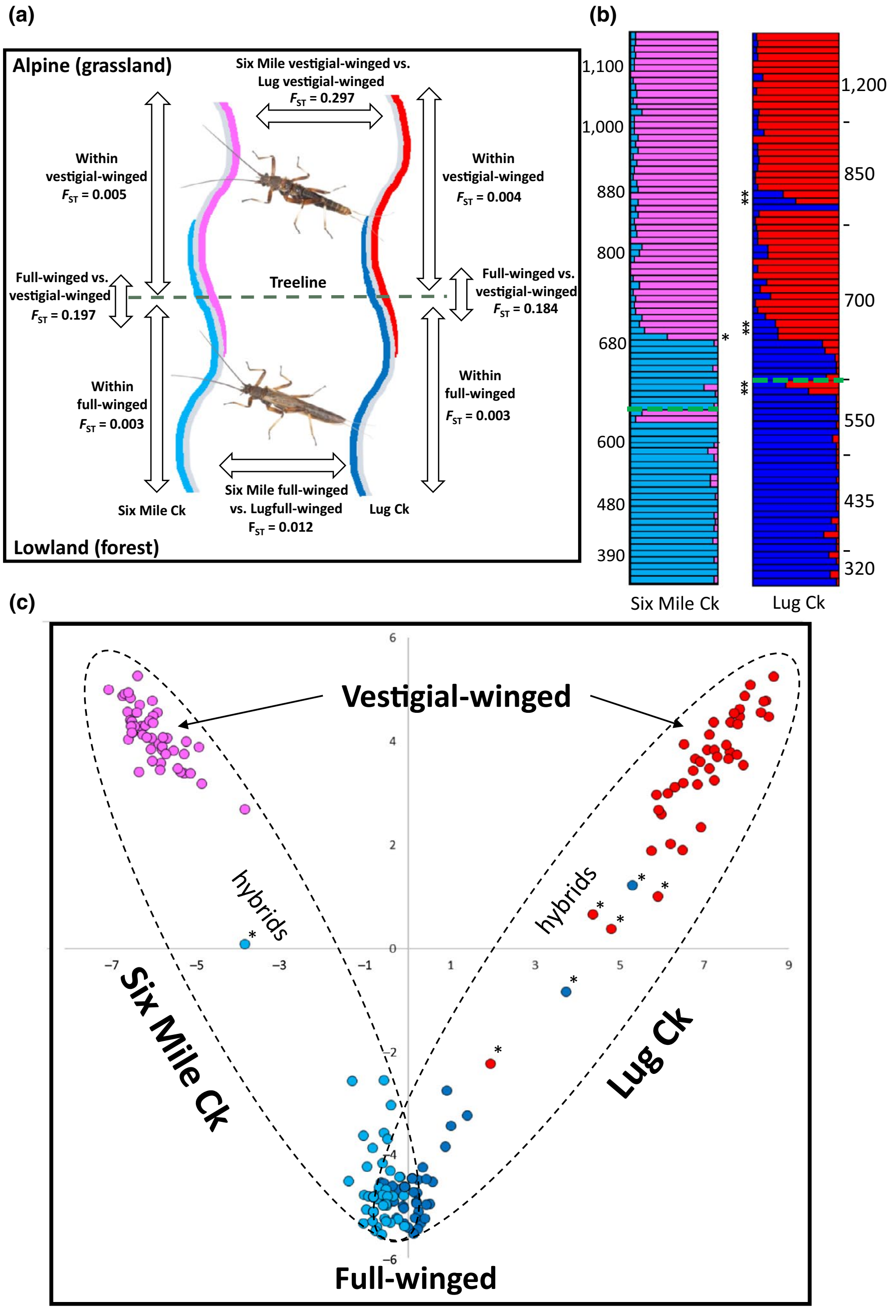
Population genomic analyses of 166 *Z. fenestrata*, using 4,633 SNP loci, revealed substantial genome-wide divergence between vestigial-winged (high altitude) and full-winged (low altitude) ecotypes within each stream (Figure 2; Figure S2b). More stringent filtering of the data set produced concordant results (data not shown). Indeed, there was an almost complete concordance between ecotype and genotype within each stream (Figure 2). No within-stream genomic differentiation was detected for either ecotype across a range of altitudes, and full-winged populations from adjacent streams exhibited only minimal differentiation (pairwise $F_{ST} = 0.012$; $p = 0.023$; Figure 2a, c). By contrast, vestigial-winged populations from the two streams were genomically highly distinct from one another (pairwise $F_{ST} = 0.297$; $p < 0.001$; Figure 2a), each being substantially more closely related to its corresponding full-winged population (pairwise $F_{ST} = 0.184$ and 0.197; $p < 0.001$; Figure 2a, c).

STRUCTURE results were congruent with the results of the PCA, with the delta *K* method indicating that $K = 2$ was the most likely number of population clusters within each stream (Figure 2b). Narrow hybrid zones were detected in the vicinity of the treeline in each stream, with NEWHYBRIDS analysis confirming that there were F_1 and F_2 hybrids (four vestigial-winged; three full-winged; Figure 2b, c).

Steep and concordant clines were identified for the molecular and morphological data sets (Figure 3). The centre of the cline at Six Mile Creek (based on the morphological data) was estimated to be at an altitude of 675 m (670 m based on genetic data), ~100 m above the local treeline (Figure 3a, b). The centre of the cline at Lug Creek was similarly estimated to be at an altitude of 700 m (based on both morphological data and molecular data), ~80 m above the local treeline (Figure 3c, d). The width of the cline at Six Mile Creek was estimated to cover an altitude of 343 m based on morphological data (284 m based on molecular data), with the beginning of the cline starting ~50–80 m below the local treeline (Figure 3a, b). The cline at Lug Creek was estimated to be slightly narrower (260 m based on both morphological and molecular data), with this cline starting ~40 m below the local treeline (Figure 3c, d).

Average heterozygosity did not differ across the two streams (Lug Creek = 0.0959, Six Mile Creek = 0.0976). Likewise, this measure did not differ significantly across sampling sites within each stream, but was generally highest in the lowest altitude sites, and lowest in the highest altitude sites (Table S1). Individuals from Lug Creek identified as hybrids from the STRUCTURE and NEWHYBRIDS analysis had

FIGURE 2 Genome-wide divergence between full-winged and vestigial-winged *Zelandoperla fenestrata* ecotypes within Six Mile Creek and Lug Creek, Rock and Pillar Range, New Zealand. (a) Schematic of the population-genomic differentiation among *Z. fenestrata* ecotypes and streams. Grey lines represent two streams flowing down the Rock and Pillar Range from the alpine grasslands to lowland forests. Coloured lines alongside streams represent the approximate proportion of vestigial-winged (red/magenta) and full-winged (blue/cyan) ecotypes at each altitude. Pairwise F_{ST} values between distinct ecotypes (or streams) are indicated. (b) STRUCTURE plot (K = 2) for Six Mile Creek (left) and Lug Creek (right). Each horizontal bar represents an individual, with colour representing the inferred genomic cluster. Numbers indicate sample altitudes. The dashed green line indicates the position of the treeline. *Specimens identified as hybrids between vestigial-winged and full-winged ecotypes using NEWHYBRIDS. (c) Principal component analysis demonstrating the genetic differentiation between vestigial-winged and full-winged ecotypes within Six Mile Creek (left) and Lug Creek (right). *Specimens identified as hybrids between vestigial-winged and full-winged ecotypes using NEWHYBRIDS



significantly higher heterozygosity (0.1205) than the remaining samples from the stream (0.0959; p = 0.02).

3.2 | Broad-scale distributional analyses

The vestigial-winged and full-winged ecotypes were widespread throughout the geographical range of Clade 1 *Z. fenestrata* (Figure 4a). Biogeographical analysis demonstrated a clear association between wing ecotype and altitude, demarcated by the treeline (Figure 4b). Indeed, at the majority of sites above the treeline only vestigial-winged specimens were found (62/72 sites; 86%). Conversely, at almost all of the sites below the treeline only full-winged specimens were recorded (58/61 sites; 95%; Figure 4b). A mixture of both ecotypes was identified at seven sites, with six of these seven sites above the treeline (Figure 4b). Overall, at sites above the treeline, 195/210 (93%) of the specimens were vestigial-winged, while below the treeline 106/112 (95%) of the specimens were full-winged.

There was only very weak intraspecific phylogenetic signal in wing ecotype. The D statistic was estimated to be 0.88, which was not significantly different from the expectation if traits were random with regard to the phylogeny (randomization test: p = 0.279), and was significantly higher (weaker phylogenetic signal) than the expectation under Brownian motion (simulation test: p = 0.011). As phylogenetic signal was absent, we used the binomial generalized linear model without any phylogenetic correction. There was only moderate overdispersion in the fitted model (Φ = 1.46), and the two predictors were not strongly collinear (variance inflation factor = 1.77). Both altitude and position relative to the treeline were associated with a higher

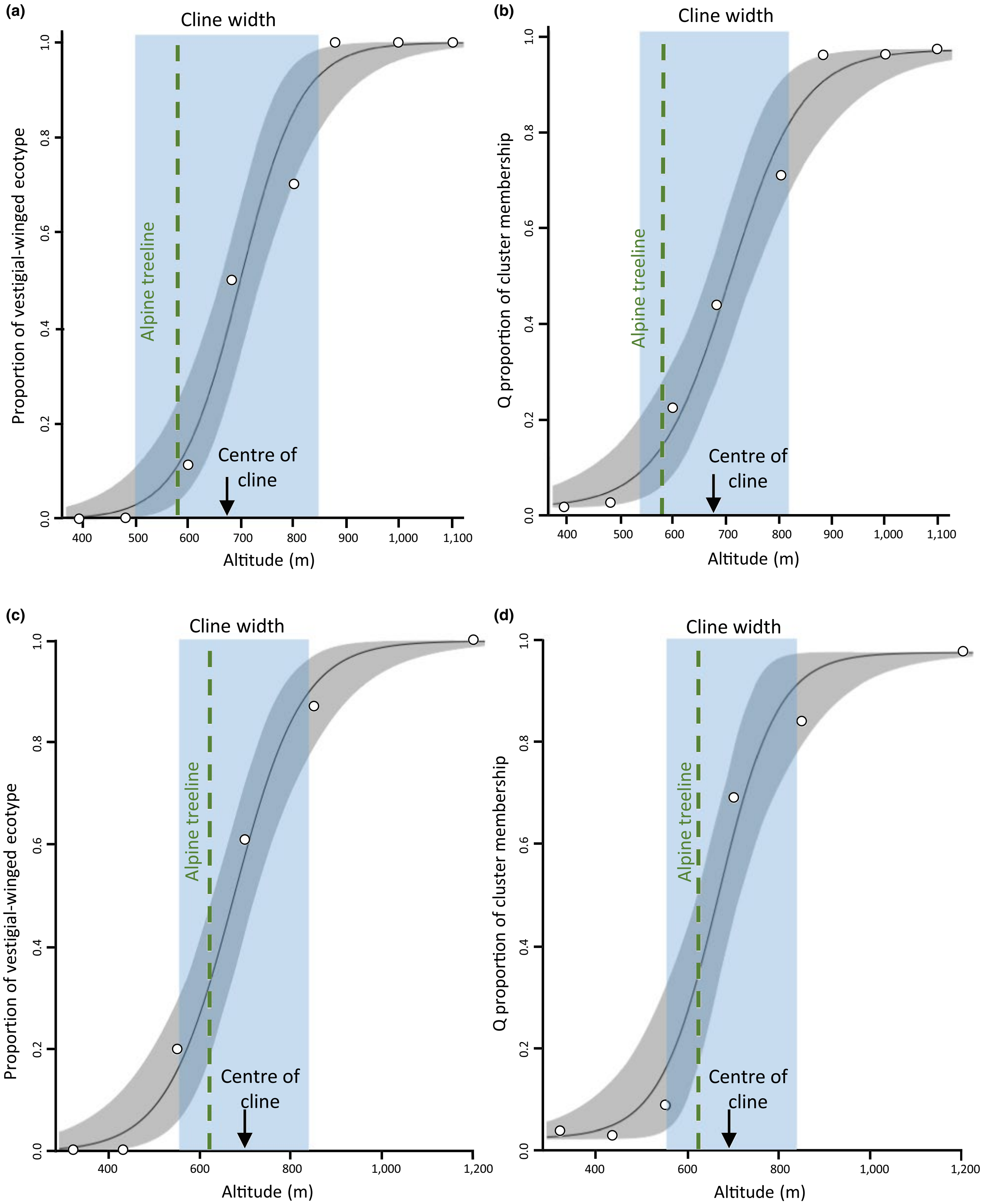


FIGURE 3 Plots of maximum-likelihood cline and observed frequency data over the associated fuzzy cline (95% credible cline region). (a) Six Mile Creek morphological data set. (b) Six Mile Creek molecular data set. (c) Lug Creek morphological data set. (d) Lug Creek molecular data set. The centre and width of the cline are indicated

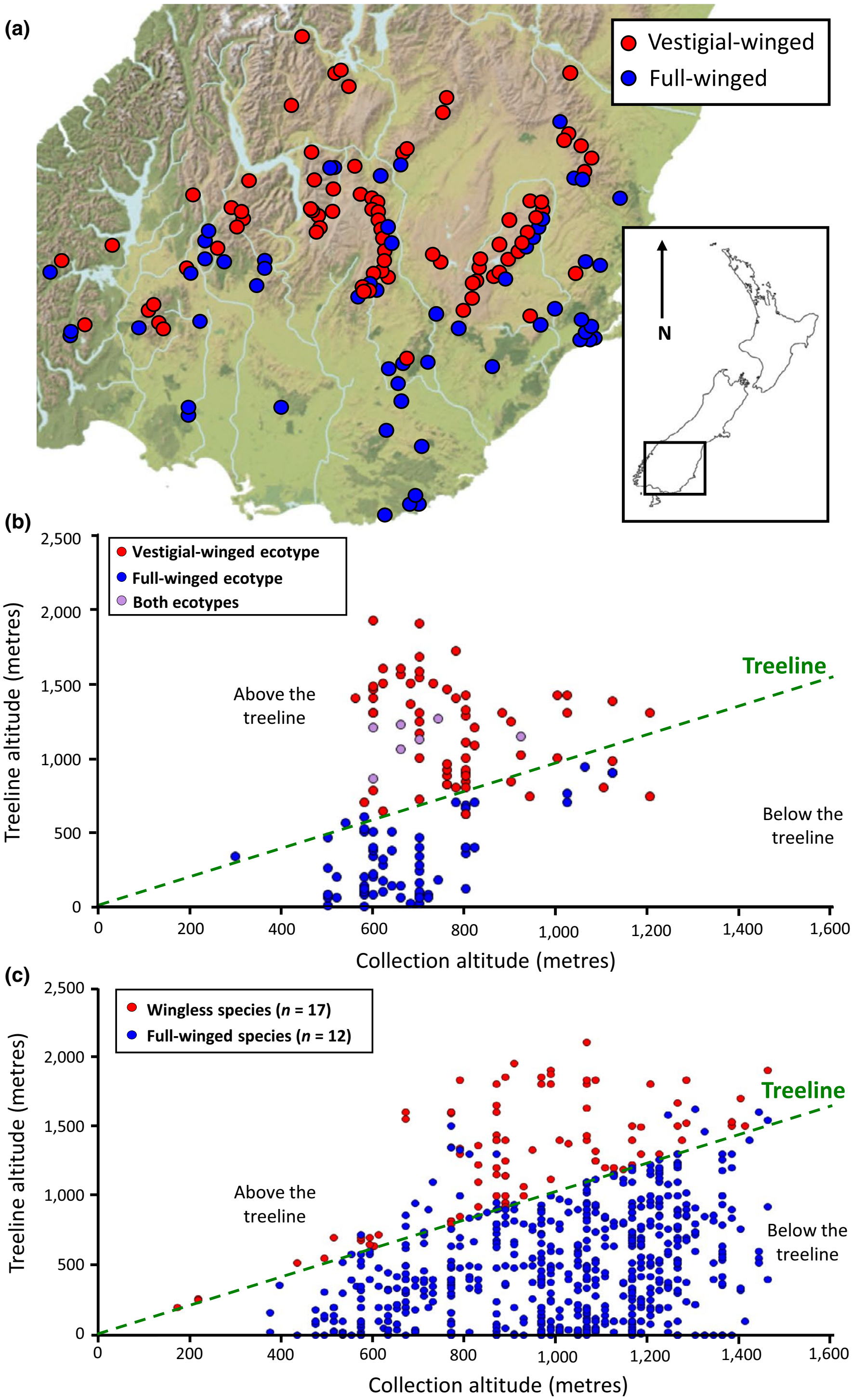


FIGURE 4 Distributional analyses demonstrating that intraspecific wing loss is tightly linked to the alpine treeline. (a) Distribution records of vestigial-winged (red circles) and full-winged (blue circles) *Zelandoperla fenestrata* Clade 1 ecotypes. (b) Intraspecific biogeographical data showing collection altitude relative to the altitude of the local treeline (dashed line) for Clade 1 *Z. fenestrata*. Sites where only full-winged ecotypes were identified are indicated with blue circles, sites with only vestigial-winged ecotypes identified are indicated with red circles, while sites where both ecotypes were identified are indicated with purple circles. (c) Multispecies biogeographical data showing stonefly collection altitude relative to the altitude of the local treeline (dashed line), for 12 full-winged (blue circles) versus 17 wing-reduced (red circles) New Zealand gripopterygid stonefly species (modified from McCulloch, Foster, Ingram, et al., 2019)

incidence of the vestigial-winged ecotype. However, the treeline had a much stronger effect (β = 4.068 ± 0.67 *SE*, partial R^2 = 0.43) than altitude per se (β = 1.82 ± 0.72 *SE*, partial R^2 = 0.01).

4 | DISCUSSION

Both fine- and broad-scale biogeographical analyses of stonefly ecotypes reveal dramatic ecological shifts associated with the alpine treeline. Specifically, parallel altitudinal transects detected striking wing reduction within *Zelandoperla fenestrata* populations, demarcated by the treeline. These fine-scale patterns are mirrored by the findings of a broad biogeographical analysis of *Z. fenestrata* ecotypes, which confirms that the treeline has a much stronger effect on ecotype distributions than altitude perse. Additionally, parallel molecular analyses demonstrated repeated genomic differentiation between high-altitude (wing-reduced) and low-altitude (full-winged) ecotypes, highlighting links between the treeline, wing reduction and ecological speciation.

4.1 | Ecological effects of alpine treeline

Although researchers have long debated the causes of wing reduction in alpine insects, ours is the first study to show the role of the alpine treeline in driving wing reduction, and in constraining the distributions of winged versus wing-reduced populations over both fine and broad spatial scales. Specifically, the sudden increase in wing-reduced ecotypes detected at the treeline over fine spatial scales within streams (Figures 1, 2 and 3) is mirrored by the results of generalized linear modelling analyses of broad ecotype distributional data (Figure 4a, b), confirming that the treeline has a substantially stronger effect on ecotype distributions compared with altitude alone. These findings are further supported by a recent interspecific study of stonefly assemblages (Figure 4c; McCulloch, Foster, Ingram, et al., 2019), which similarly showed the role of the treeline in partitioning full-winged versus wingless taxa.

While alpine treelines have long been recognized as crucial in the structuring of botanical assemblages (Batllori, Blanco-Moreno, Ninot, Gutiérrez, & Carrillo, 2009), our study may be the first to reveal their important effects on animal populations. These data also add to accumulating evidence that natural selection can facilitate dramatic cases of local adaptation in wild populations (Barrett & Hoekstra, 2011), and drive differentiation over fine spatial scales, even with ongoing gene flow. Moreover, as the altitude of the alpine treeline has been artificially reduced by recent deforestation across much of southern New Zealand (including the current study region), we suspect that the altitudes of associated wing loss clines will have been similarly lowered, in cases of rapid anthropogenic evolution (see Kettlewell, 1973; Cook & Saccheri, 2013).

4.2 | Rapid ecological speciation

The near-identical genetic clines (Figures 2a, b and 3) detected in two spatially and genomically divergent (Figure 2c) stream populations, in the absence of physical barriers, support a common driver of evolutionary divergence (Kooyers & Olsen, 2013). Ecotypic clines can be either formed in situ by ecological speciation, or by secondary contact following allopatric speciation, although distinguishing between these competing hypotheses can be challenging (see Mayr, 1942; Slatkin, 1973, Van Belleghem et al., 2018). Several lines of evidence suggest that the ecotypic clines observed in this study were the result of in situ ecological divergence. Notably, the vestigial-winged populations from the two streams were genomically highly distinct from one another, each being substantially more closely related to their corresponding full-winged population (Figure 2b, c), suggesting independent wing loss events for each stream. More broadly, the finding that both mitochondrial and nuclear genetic variation within Clade 1 are strongly structured by geography rather than by wing phenotype (McCulloch et al., 2009), but with both full-winged and wing-reduced phenotypes being widespread (Figure 4a), strongly suggests that wing reduction has occurred locally and independently on multiple occasions. Additionally, while studies elsewhere have suggested that wing reduction can be a slow process (Donald, 1985), the presence of wing-reduced lineages in some formerly glaciated regions of southwestern New Zealand (e.g., Fiordland; Figure 4) suggests that wing reduction can evolve rapidly.

The narrow hybrid zones detected within each transect (Figure 3) suggest ongoing gene flow between the ecotypes in each stream. This finding implies that within-stream genomic divergence between parapatric ecotypes in the current study is driven by selection against full-winged individuals above the treeline (due to exposure and its covariates), and apparent selection against vestigial-winged individuals below the treeline. The narrow zones of hybridity contrast with evidence for high gene flow within streams and ecotypes, implying strong selection against hybrids with unsuitable phenotypes. Indeed, fewer hybrids were identified in the primary zone of mixture in each stream (just above the treeline; Figure 2; Table S1) than would have been expected if mating between ecotypes occurred at random. This suggests that the genomic divergence among ecotypes may be reinforced by assortative mating based on wing morphology (as has been observed in other systems; Langellotto, Denno, & Ott, 2000), while potential reductions in hybrid fertility and/or differences in reproductive timing (see McCulloch & Waters, 2018) may also contribute to the genome-wide differentiation (see Nosil, Vines, & Funk, 2005).

Despite their substantial genome-wide differentiation, previous analyses detected no mitochondrial or nuclear phylogeographical differentiation among these sympatric ecotypes (Figure S1b; McCulloch et al., 2009), suggesting that they evolved only recently (e.g., since the Last Glacial Maximum). Moreover, the widespread occurrence of similarly wing-reduced upland populations across southern New Zealand (Figure 4a) suggests that natural selection may have underpinned multiple independent wing reduction events across different regions. This recently evolved, diverse species complex thus provides a potential new system for elucidating the mechanisms and dynamics of rapid divergence and speciation (Roff, 1990; Shapiro et al., 2004), and in particular the genomic basis of reductive evolution (Porter & Crandall, 2003; Protas et al., 2006; Sackton et al., 2019).

The current study demonstrates that disruptive natural selection can play a key role in the earliest stages of biological speciation (Schluter, 2001, 2009). Wing loss has previously been linked to increased speciation rates in insects, although this effect had been widely assumed to result from the physical isolation of wingless populations (i.e., allopatric speciation; Ikeda et al., 2012; Vogler & Timmermans, 2012). Crucially, the results of our current study may provide a new evolutionary explanation for the global phenomenon of wingless alpine insect assemblages. Specifically, we demonstrate how differential natural selection (constrained by the treeline) drives wing loss and rapid genomic differentiation over fine spatial scales, even with ongoing gene flow. We thus provide a new model for ecological speciation of wingless alpine insects along exposure gradients.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

J.W. and G.M. conceptualized the study. B.F. and J.W. undertook fieldwork, with B.F. conducting the lab rearing and morphological analyses. A.V., E.H., P.D. and J.W. undertook genetic analyses. L.D. and G.M. analysed the GBS data. G.M., B.F. and T.I. conducted the distributional analyses. G.M. drafted the manuscript, with significant input from J.W. All authors contributed to editing of the manuscript, and gave final approval for publication.

DATA AVAILABILITY

All sequencing data used in this study can be found (demultiplexed by sample) on the NCBI Sequence Read Archive PRJNA530622. The detailed SNP calling procedures and the processed genotypes are available at the Dryad Digital Repository. https://doi.org/10.5061/dryad.c56kb12

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SUPPORTING INFORMATION

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

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