Genomic signatures of local adaptation across parasitised cricket populations

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Abstract

Host-parasite interactions are predicted to exhibit geographic heterogeneity, creating the opportunity for local adaptation. This is difficult to detect because it requires knowledge of selection pressures and fitness consequences of phenotypic variation. We explored the adaptive landscape of Hawaiian cricket populations which are parasitised by larvae of a fly that targets singing male crickets, in which multiple protective male-silencing morphs have recently spread. We resequenced cricket genomes from several locations across three islands, finding genetic variation is dominated by massive inversions, and observing sharp declines in effective population size following the fly's introduction. We find flies continue to impose extreme but heterogeneous selection on cricket populations, influencing the distribution of a recently discovered male-silencing phenotype and allelic variation at genes with functions related to evading or resisting larval infestation. Our study illuminates the extreme conditions contributing to and resulting from recurrent and remarkably rapid adaptive evolution in these wild populations.

1. Introduction

Coevolutionary interactions, such as those between host and parasite, are predicted to vary across landscapes due to geographical factors, creating hot- and cold-spots of coevolution and the potential for local adaptation (*1*, *2*). This is challenging to document in wild populations because it requires detailed knowledge of variation in selection pressure and fitness consequences of phenotypic variation. Studies often make assumptions about the traits under selection, e.g., based on trait-correlations between interacting species (*3*), which might be biased towards pronounced morphological variation. This in turn could bias investigations of local adaptation towards large effect loci (*4*), contributing to an ascertainment bias influencing our understanding of evolutionary dynamics in wild populations (*5*). These biases can be mitigated by complementing findings from genetic and phenotypic data with tests for signatures of selection (*6–8*) or, ideally, directly incorporating knowledge of geographical variation in selection pressure. Studies of local adaptation that combine these approaches might find that selection-informed analyses support the overarching importance of candidate adaptive phenotypes, or suggest they offer an incomplete picture.

The host-parasite interaction between Oceanic field crickets, *Teleogryllus oceanicus*, and an eavesdropping parasitoid fly, *Ormia ochracea,* is one system in which: 1) we can confidently estimate specific selection pressures imposed on crickets by measuring fly attack rates, and 2) we have prior knowledge of phenotypic variation that directly influences crickets' likelihood of attracting the parasitoid fly. These species are only known to co-occur in Hawaii, to which both were introduced. *T. oceanicus* is the primary host for *O. ochracea* in Hawaii; although the fly is also attracted to the songs of other co-occurring cricket species, these species are either infrequently observed (e.g., *Gryllus bimaculatus*) or offer less viable hosts for larval development (e.g., *G. sigillatus*) (*9*). Within the last 25 years, multiple mutant wing morphs have emerged and spread in Hawaiian *T. oceanicus* populations, each of which strongly reduce the amplitude of males' song. This protects males from being located by gravid female *O. ochracea* (*10–15*), which use cricket song as a phonotactic cue (*16*) and are remarkably accurate in pinpointing the source (*17*, *18*), upon which they deposit planidia that burrow into the cricket host. The best characterized of these male-silencing cricket morphs, 'flatwing', was discovered in 2003 in a population on the island of Kauai, in which it subsequently spread very rapidly, but has since been

observed on two more islands (*11*, *19*). However, at least four more alternative male-silencing morphologies have now been described in Hawaiian *T. oceanicus*, and these exist in a heterogeneous mixture of abundances and combinations across a geographic mosaic of small, fragmented populations across the Hawaiian archipelago (*14*, *15*, *20*, *21*). To date, research into the genetic basis of adaptive responses to selection from *O. ochracea* has focused on identifying loci underlying these mutant wing morphologies (but see Zhang *et al.*, 2024), repeatedly finding evidence of large-effect loci or QTL regions (*10*, *20*, *23*). Yet, while adaptive wing morphs have repeatedly emerged and spread in populations across the Hawaiian archipelago, they have not spread to fixation in most locations. This is likely due in part to strong countervailing fitness benefits of song in the context of attracting and courting females (*24*), suggesting that the fitness benefits of songlessness are contingent on the relative risk of attracting *O. ochracea*. However, the prediction that the distribution and spread of male-silencing phenotypes is influenced by local differences in selection from *O. ochracea* has not been tested.

Our aims in the current study were to characterise the genomic landscape of recent and ongoing adaptation across multiple cricket populations, and to evaluate support for behavioural observations indicating adaptive mutant wing morphologies spread following the introduction of the fly (*11*, *19*, *21*). To this end, we surveyed locations across three islands of the Hawaiian archipelago for *T. oceanicus* populations. From each location, we resequenced genomes of male crickets and recorded the number of *O. ochracea* that were attracted to cricket song playback as a measure of local selection pressure. We combine these data for a comprehensive study of the genomic landscape of local adaptation in wild cricket populations, illuminating the conditions that have underpinned this remarkable example of rapid, recurrent adaptation. Our empirical test of the presumed overarching importance of male-silencing cricket wing morphologies will offer important insights for this and other natural systems characterised by adaptive morphs, which include several of the most compelling examples of genetic adaptation in the wild (*25*–*29*).

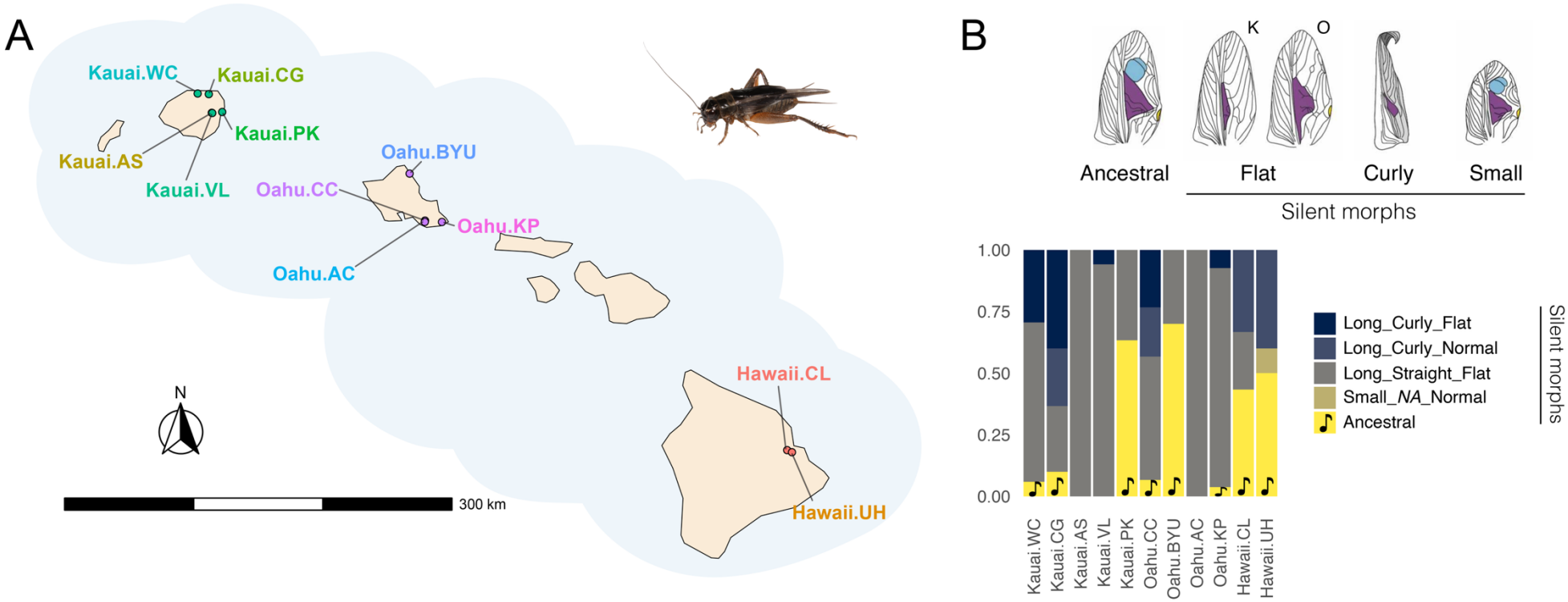


Figure 1. Sampling locations and morphological wing variants in Hawaiian *T. oceanicus*.

A) Map of Hawaii showing locations from which crickets and flies were sampled. Note that some sampling locations are nearby, but all are separated by urban or geographic barriers and multiple nearby locations show fixed differences in the presence/absence of various wing phenotypes (*20*). **B)** Proportions of different wing phenotypes (illustrated above) represented in genome-sequencing samples from each patch. Bright yellow bars represent singing-capable males, i.e., those with typical ancestral phenotypes: long wings, straight wing morphology, and normal-wing venation. Small-wing males are assigned 'NA' values for wing shape because it is difficult to confidently assess. Line drawings of wing phenotypes are adapted from (*15*). K and O labels above flatwing illustrations represent variants typically found on islands of Kauai and Oahu, respectively.

2. Results

We assayed variation in selection, via the likelihood of male cricket song attracting gravid female flies. We conducted 489 ten-minute cricket song playback trials (on 42 nights) in 11 locations (on three islands) at which we located cricket populations (Fig. 1A). We then resequenced whole genomes from hindleg samples of 300 male crickets from the same locations to ca. 15x depth, having recorded whether each male expressed common, pronounced male-silencing wing morphs: flatwing (N = 187, across 10 locations) and curly-wing (N = 63, across 7 locations) (*11*, *14*, *30*) (Fig. 1B). We also recorded a third silencing morph, 'small-wing', but at very low frequency (N = 3 males at a single location).

2.1. Polymorphic structural variants dominate genetic and transcriptomic variation

Initial exploratory analyses revealed genetic variation of Hawaiian *T. oceanicus* to be dominated by large, polymorphic structural variants (SVs). These were inferred from the presence of discrete linked genomic regions (Fig 2A) that contribute disproportionately to heterogeneous patterns of population structure across the genome based on windowed principal component analysis (PCA) (Fig. 2A) (*31*), and lead samples to cluster into discrete karyotypes in chromosome-wide PCA (Fig. 2B) (*32*). Based on these criteria, we observed evidence of at least one large (> 10Mb) SV on each of 11 of the 14 chromosomes, and evidence of two large SVs on Chrs 2, 4 and 11. These include two recently described inversions on Chr1 (the X) and Chr2 (*20*, *33*), suggesting the other SVs we observe are also likely to be inversions, and we did not observe clear differences in read depth in these regions (Fig. S1). Linked regions exhibited significantly elevated nucleotide diversity and Tajima's D values (Wilcoxon rank-sum tests: $P < 0.05$), and were polymorphic on at least two islands, and in most cases on all three (Fig. 2B; Fig. S2), suggesting they represent balanced polymorphisms. We observed very strong genetic divergence between alternate karyotypes (F_{ST} at or approaching 1) across the length of these regions, with the exception of a linked region on Chr3 which showed a U-shaped pattern and stronger linkage disequilibrium towards and between the distal edges, symptomatic of an older SV (*34*) (Fig. S3). Based on discrete regions of elevated F_{ST} between inferred karyotypes, we conservatively estimate polymorphic SVs cover at least 40% of filtered variants identified in our data. With the possible exception of an SV on Chr6, we did not observe clear evidence of these SVs in genome sequencing data from seven *T. oceanicus* from the ancestral Australian mainland (Fig. S4), suggesting SVs have proliferated more recently.

The size of these SVs and their persistence as polymorphisms in small, fragmented cricket populations on multiple islands, presumably reflecting balancing selection, suggests they are likely to have important phenotypic effects (*35*). We tested this prediction in existing RNA-sequencing data from neural tissue samples collected from 48 lab-reared crickets of both sexes (*36*). We again found evidence of SVs dominating genetic variation on several chromosomes, and we observed from variance partitioning and differential expression analyses that these strong cis-regulatory effects on gene expression. For example, the large SV observed on Chr6 was associated with differential expression ($P_{adj.} < 0.05$) of 795 genes, 69.8% of which are located on Chr6. (Fig. S5)

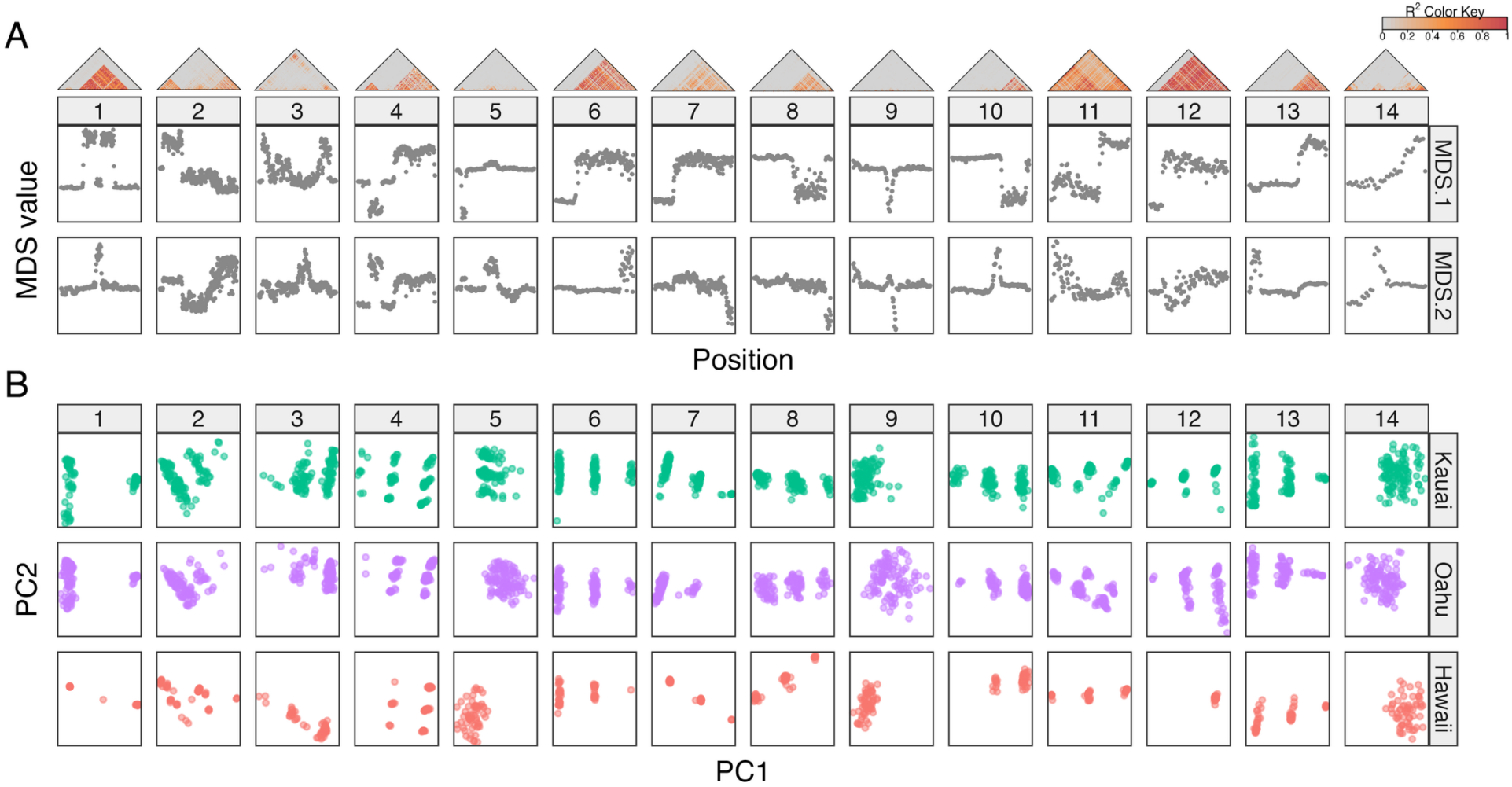


Figure 2. Large, polymorphic structural variants abound in Hawaiian *T. oceanicus*.

A) Triangles show observed patterns of linkage across 14 chromosomes. Panels below show MDS values, with discrete tracts of divergence supporting evidence of large structural variants (*31*). **B)** PCA plots by chromosome, separated by island, with discrete clustering of inferred karyotypes into two or three groups illustrating structural variants are typically polymorphic on each island.

136 ***2.2. The parasitoid fly is implicated in recent cricket population declines***

137 Because patterns of genomic variation were strongly influenced by SVs, which would likely
138 confound analyses of population structure and demographic inference, we restricted these
139 analyses to linkage-pruned variants from chromosomes 5, 9 and 14, on which we did not
140 observe evidence of large structural variants. Principal component analysis (PCA),
141 approximate maximum-likelihood phylogenetic trees (*37*) and pairwise F_{ST} analyses all
142 indicated cricket population structure emerged largely at the level of the three islands:
143 Kauai, Oahu, or Hawaii (Fig. 3). There was evidence of finer-scale population structure
144 between sampling locations on the island of Oahu, on which the crickets appear to have
145 originally been first introduced to the archipelago (Zhang *et al.*, 2021). F-branch statistics
146 (*38*, *39*) supported excess allele sharing and thus evidence of gene flow between multiple
147 locations on Kauai, and between locations in Kauai and Hawaii, but not between locations
148 on Oahu (Figs. 3C, S6-7).

149 *T. oceanicus* appears to have been introduced to Hawaii by Polynesian settlers ca. 800
150 years ago (*10*), whereas the parasitoid fly was introduced more recently, likely from North
151 America, having been documented in Hawaii since 1989 (*40*, *41*). Historical changes in
152 effective population size inferred via Bayesian inference of coalescent times (*42*) supported
153 a long-term decline in effective population size of Hawaiian *T. oceanicus*, likely reflecting
154 repeated bottlenecks associated with divergence from the ancestral Australian mainland
155 population (Fig. 3D). There was a high degree of uncertainty in estimates of effective
156 population size in the interval 100 and 700 generations before 2022, potentially influenced
157 by fluctuating population dynamics following colonisation of Hawaii due to factors such as
158 initial population expansion, increasing anthropogenic activity, and the recent introduction of
159 *O. ochracea*.

160 Assuming an average of 3.5 cricket generations per year (*43*) indicates the fly became
161 prevalent within the 150 cricket generations prior to sample collection. The flatwing morph,
162 adaptive under selection imposed by the fly, was first observed in 2003 (ca. 67 generations
163 prior to sample collection) (*11*) and other male-silencing phenotypes have emerged and
164 spread in the years since (*15*). Linkage-based inference of changes in effective population
165 size over the last 150 generations indicated precipitous recent declines in all populations,
166 fitting expectations following the first documented observation of *O. ochracea* in Hawaii
167 (Fig. 3E). Interestingly, this inferred decline began earliest in the Wailua population of

Kauai, at which flatwing was first observed (*11*), suggesting the early emergence of flatwing at this site could have been influenced by unusually strong selection. This is supported by observations from 1993 that crickets at this location were more than twice as likely to be infested by flies larvae compared with those in Oahu or Hawaii (*44*, *45*). While it is intuitive that recent declines in effective population size of Hawaiian crickets were precipitated by the introduction of the fly, it is possible that the spread of songless phenotypes also influenced these declines by reducing reproductive output due to the reduced frequency of an important sexually selected signal. However, we observed no evidence that populations that have retained a high frequency of singing-capable morphs showed any less drastic declines (Fig. 1B cf. Fig. 3E), supporting the primary influence of the parasitoid fly.

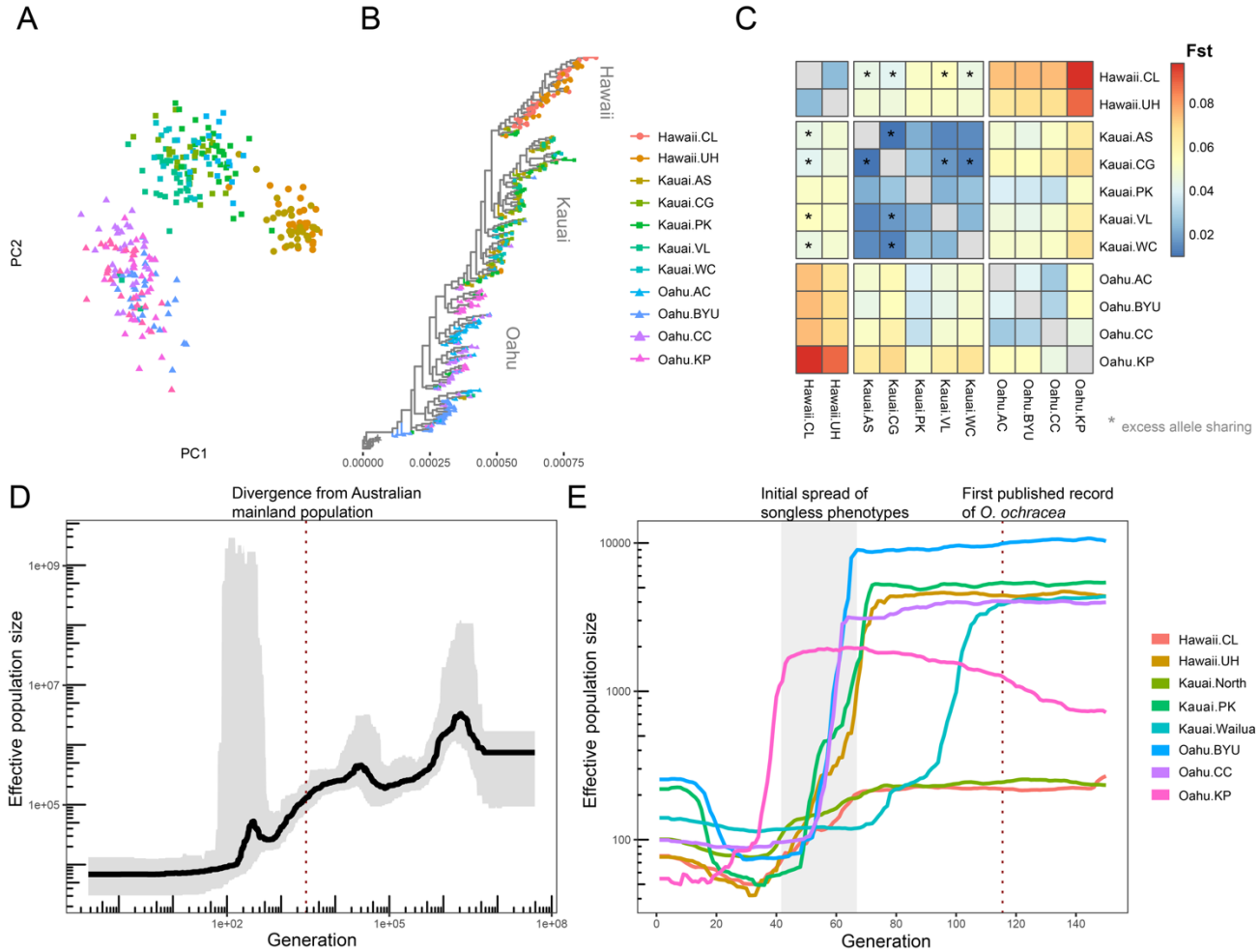


Figure 3. Cricket population structure and changes in effective population size.

A) PCA of genetic variation across 300 *T. oceanicus* males, with points coloured according to sampling location. **B)** Phylogenetic tree of all Hawaiian genomes, rooted by 3 samples from a closely related sister species *T. commodus* and 7 samples from *T. oceanicus* from ancestral Australian populations. **C)** Pairwise mean F_{ST} values across Hawaiian populations. Pairs of locations with excess allele-sharing according to F-branch statistics are identified by asterisks (Fig. S5-6). **D)** Long-term changes in effective population size inferred by coalescent-based analysis. The black line shows the median estimated effective population size, while the ribbon illustrates the posterior 95% confidence interval. The dotted line represents the estimated time of divergence between Australian and Hawaiian populations from (*10*). **E)** Short-term changes in effective population size inferred by linkage-based analysis. Lines are coloured according to geographic location of samples. The dotted vertical line indicates the first observation of the parasitoid fly in the Hawaiian archipelago, and the grey rectangle indicates the interval in which the first male-silencing variants were observed to emerge and spread.

2.3. Strong geographical variation in the strength of selection by parasitoid flies

We next investigated how selection imposed on crickets by the fly varies across locations. We set out fly traps, consisting of 2L bottles with the funnel section inverted, and the base replaced by a speaker. In 489 replicate 10-minute trials with eight such traps (four of which broadcasted cricket song), cricket song playbacks attracted 415 female flies, whereas an equal number of silent traps attracted just a single fly. While males with mutant wing morphs such as flatwing, curly-wing and small-wing are technically capable of producing sound through stridulation, the amplitude of this 'song' is reduced to near-ambient noise levels and prior research has repeatedly shown that fly traps broadcasting their song attract no more flies than those not broadcasting song (*14*, *17*, *46*). Our observations therefore reiterate that males expressing these mutant wing morphs are very strongly protected from parasitism, resembling findings from dissections of wild-caught Hawaiian *T. oceanicus*, in which fewer than 1% of flatwing males harboured parasitoid larvae contrasting with > 30% of singing-capable males (*11*).

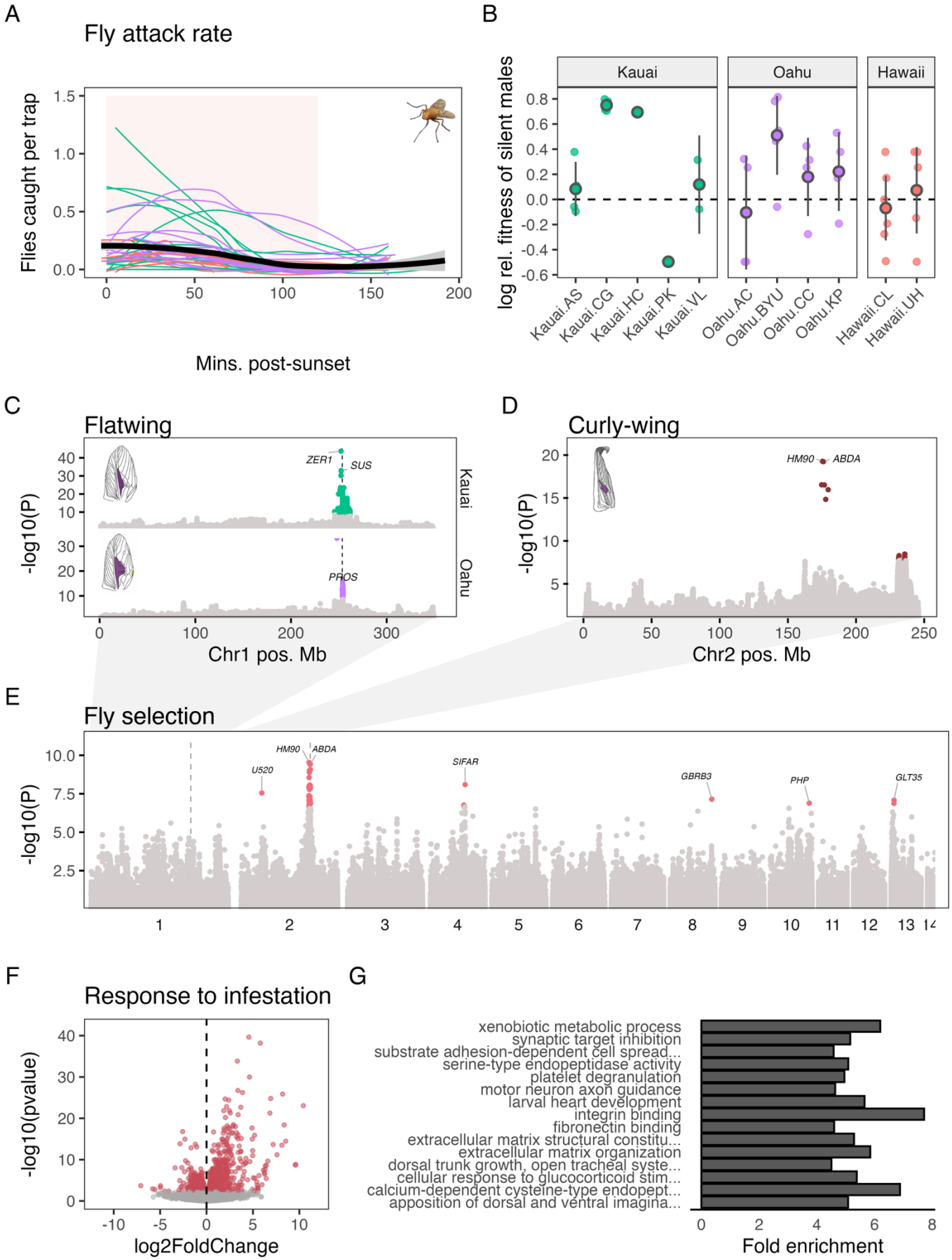
Fly attack rates varied greatly across time and locations. Rates were highest in the first hour following sunset, after which they steadily declined (Fig. 4A), consistent with observations from populations from North American mainland populations (*47*). Across nights, the likelihood of a song playback attracting one or more flies in the two hours after sunset was significantly repeatable with respect to location (linear mixed model with log-transformed response: $R = 0.575$, 95% CI = [0.178, 0.801], $P < 0.001$). Moreover, across 10 locations sampled four months apart, (Jan/Feb and June/July), the average number of flies captured showed evidence of being correlated across sites (Spearman's rho = 0.616, $P = 0.058$) despite a small sample (N = 10 sites sampled in Jan/Feb and June/July). Fly attack rates were positively associated with daily precipitation (linear regression: $F_{1,29} = 12.370$, $P = 0.001$) but did not show a relationship with temperature ($P > 0.40$), and differed significantly between locations (location nested within island: $F_{8,29} = 9.826$, $P < 0.001$; full model $R^2 = 0.807$). Interestingly, at one location with a high proportion of singing male crickets (Kauai.PK), song playback attracted just a single fly in 36 trials across three nights. Conversely, song playback attracted substantial numbers of flies at all three locations in which singing male cricket phenotypes are absent (Fig. 4B), consistent with previous anecdotal observations (*9*).

227 Attracting a single fly has drastic fitness costs, as infestation by *O. ochracea* larvae is very
228 often fatal and gravid females are remarkably accurate in locating the source of cricket
229 song (*17*, *18*) even after the cessation of singing behaviour (*48*). To estimate the expected
230 adult lifespan of singing-capable males at different locations, from which to infer the
231 strength of selection against song, we made the following assumptions based on existing
232 data. Assumption 1: we estimated the typical adult lifespan in the wild of reduced-song
233 (e.g., expressing curly-wing or flatwing morphs) males at 29 days, based on longevity
234 records from a wild population of a related field cricket, *Gryllus campestris* (average
235 longevity of 28.9 days) (*49*) and from *T. oceanicus*' sister species, *T. commodus*, reared
236 under semi-natural conditions (29.07 days) (*50*). Assumption 2: males sing for an average
237 of 7.6 minutes per night in the two hours following sunset, as observed in a prior study (*51*),
238 and begin singing shortly after adult eclosion (singing behaviour was recorded at 0-3 days
239 post-eclosion by (*52*)). Assumption 3: phonotaxis by gravid female *O. ochracea* has fatal
240 consequences for *T. oceanicus* males 61% of the time, based on findings from a related
241 cricket host, *Gryllus texensis*, in which deposited planidia successfully established in,
242 emerged from and killed 61% of males (*53*). Finally, assumption 4: Cessation of
243 reproductive viability occurs at six days post-infestation; Adamo et al. (*54*) found
244 reproductive viability strongly declines after five days infestation, with larvae emerging and
245 killing crickets 7-10 days post-infestation.

246 From these assumptions, we estimated the average reproductive lifespan of singing-
247 capable males at 16.514 days ± 7.054 SD across nights/locations, indicating average
248 relative fitness of reduced-song males in the context of fly parasitism is high but greatly
249 variable across sites, with mean 2.082 ± 0.841 SD. However, silent males are
250 disadvantaged in the context of attracting female mates, and a previous study estimated the
251 relative fitness of silent males in the context of reduced mate attraction at 0.609 (*24*). Thus,
252 we multiplied silent males' relative fitness in the context of selection from the fly by that in
253 the context of sexual selection to estimate their net relative fitness at 1.268 ± 0.512 SD
254 (minimum = 0.609 at Kauai.PK, maximum = 2.257 at Kauai.CG) across locations (Fig. 4B).
255 Geographical variation in net fitness indicated that male-silencing phenotypes are strongly
256 favoured at three locations (Kauai.CG, Kauai.WC, Oahu.BYU) and strongly disfavoured at
257 one location (Kauai.PK), with the pattern variable across nights at the other seven locations
258 (Fig. 4B).

2.4. Fly selection has influenced the spread of a protective curly-wing morph

A motivating question of our study is whether male-silencing morphs, flatwing and curly-wing, would show evidence of selection due to fly attack rates. Testing this requires knowledge of genomic regions underpinning each morph's expression. Both flatwing and curly-wing phenotypes have previously been subjected to genome-wide association studies, but with smaller sample sizes (or reduced representation sequencing) and earlier genome assemblies for *T. oceanicus* (*10*, *13*, *20*, *23*), so we reperformed these analyses. A complication is that at least two and perhaps three convergent flatwing phenotypes appear to have arisen independently via independent mutations in the region of the gene encoding doublesex (*10*, *13*) on Chr1 (the X chromosome). We cannot readily distinguish these convergent phenotypes by eye, but prior work has indicated they are stratified by island. These independent architectures could obscure evidence of selection on independent flatwing variants across the three islands, so we identified genetic variants that are consistently flatwing-associated on both Kauai and Oahu (with too few flatwing phenotypes surveyed from Hawaii for a similar analysis), among which it is reasonable to expect signatures of selection across all locations. This analysis highlighted 10 Fw-associated variants shared across islands ($P_{adj.} < 0.05$ in Kauai and Oahu), all of which were annotated for the nearest gene *prospero*, adjacent to the gene encoding doublesex, at 254Mb on Chr1 (Fig. 4C). Curly-wing associated loci were located on Chr2, an autosome, near the locations of two homeobox genes at 176Mb (Fig. 4D) including *ABDA*, which encodes abdominal-A, a transcription factor involved in positioning cells on the anterior-posterior axis (*55*).


A
Fly attack rate
Flies caught per trap
Mins. post-sunset
B
Kauai
Oahu
Hawaii
log rel. fitness of silent males
Kauai.AS
Kauai.CG
Kauai.HC
Kauai.PK
Kauai.VL
Oahu.AC
Oahu.BYU
Oahu.CC
Oahu.KP
Hawaii.CL
Hawaii.UH
C
Flatwing
ZER1
SUS
PROS
Kauai
Oahu
-log10(P)
Chr1 pos. Mb
D
Curly-wing
HM90
ABDA
-log10(P)
Chr2 pos. Mb
E
Fly selection
U520
HM90
ABDA
SIFAR
GBRB3
PHP
GLT35
-log10(P)
F
Response to infestation
-log10(pvalue)
log2FoldChange
G
xenobiotic metabolic process
synaptic target inhibition
substrate adhesion-dependent cell spread...
serine-type endopeptidase activity
platelet degranulation
motor neuron axon guidance
larval heart development
integrin binding
fibronectin binding
extracellular matrix structural constitu...
extracellular matrix organization
dorsal trunk growth, open tracheal syste...
cellular response to glucocorticoid stim...
calcium-dependent cysteine-type endopept...
apposition of dorsal and ventral imagina...
Fold enrichment

280

281 **Figure 4. Variants associated with song-loss phenotypes and selection strength.**

282 **A)** Numbers of flies caught per 10-minute playback over time. Each coloured line shows
283 loess regression from a single night's trials, coloured by island, with the black line
284 representing the average number of flies caught across all nights ± 95% confidence
285 intervals. Photograph adapted from **B)** Log-transformed relative fitness of silent males at
286 each site (see main text). Small transparent points show estimates per night, whereas
287 larger points show averages across nights ± 2 standard errors. **C,D)** Variants and the
288 statistical strength of their association with flatwing and curly-wing phenotypes. The dashed
289 line in panel C indicates the position of *DSX*. Significant ($P_{adj.} < 0.05$) Fw-associated
290 variants are highlighted in green and purple for Kauai and Oahu flatwing phenotypes,
291 respectively, and Cw-associated variants in dark red. Illustrations of flatwing and curly-wing
292 phenotypes are adapted from (*15*). **E)** Distribution of variants and the strength of their
293 association with the strength of selection based on fly capture rates, with genes near
294 variants with $P_{adj.} < 0.05$ highlighted in red. Flatwing and curly-wing associated regions are
295 indicated by dashed vertical lines. **F)** Differential expression of genes between body tissues
296 of crickets that were uninfested or 4-days post-infestation with fly larvae. Significantly
297 differentially expressed genes are highlighted in red. **G)** The top 15 overrepresented gene
298 ontology categories among differentially expressed genes.

299

300 To investigate genomic regions showing evidence of responding to selection from the fly,
301 we compared genetic variation across the 300 cricket genomes from all 11 locations with
302 the nightly probability of a singing male becoming fatally infested using a latent factor mixed
303 model (LFMM) (*56*). QQ-plots of observed versus expected p-values indicated a very strong
304 overrepresentation of low p-values in the empirical data (Fig. S8). Of 40 significantly ($P_{adj.}$ <
305 0.05) selected variants identified in this analysis, 33 (82.5%) were within 5 Mb of the
306 consistently curly-wing-associated locus at 176 Mb on Chr2, with the top selected variants
307 annotated for proximity to curly-wing associated genes *ABDA* and *HM90* (Fig. 4E).
308 Interestingly, the flatwing-associated region was not strongly highlighted, perhaps
309 influenced by its multiple genetic architectures or because it spread much less recently than
310 curly-wing (*11*, *19*). Nevertheless, there were several variants within 5Mb the region that
311 had p-values lower than predicted by chance ($P_{unadj.}$ < 1e-4; Fig. 4E cf. Fig. S8).

312 Recent research into genetic adaptation by Hawaiian populations of *T. oceanicus* under
313 selection from the fly has focused heavily on male-silencing phenotypes, which is at least
314 partly validated by the findings above. However, other selected variants were within or
315 nearby five more genes distributed across the rest of the genome—annotated for similarity
316 with *Drosophila* genes *SIFAR*, *U520*, *GBRB3, GLT35* and *PHP*—which might reflect
317 selection on other traits important in evading or suppressing larval infestation. Of potential
318 interest, the gene *SIFAR* influences wing extension behaviour during male courtship

319 displays in *Drosophila* (*57*); in *T. oceanicus*, changes to wing-generated singing behaviour
320 could influence the likelihood of singing-capable males attracting flies. Second, *GLT35*
321 influences tracheal development, with mutations in this gene leading to malformed tracheal
322 tubes in *Drosophila* (*58*). Tracheal development is implicated in cricket responses to larval
323 infestation, due to the apparent ability of *O. ochracea* larvae to co-opt host's immune
324 encapsulation responses during infestation to construct trachea that they use to avoid
325 suffocating (*63*). To explore this further, we interrogated publicly available RNA-sequencing
326 data from body tissue of Hawaiian *T. oceanicus* that were uninfested or had been infested
327 for four days (*59*). We found 1,099 genes (of 13,374 in the analysis) significantly differed in
328 expression after 4 days' infestation (Fig. 4F). None were highlighted by our selection
329 analysis above, however, differentially expressed (Padj. < 0.05) genes were
330 overrepresented in gene ontology categories 'serine-type endopeptidase activity' and
331 'integrin binding', both of which are involved in encapsulation responses (*60*, *61*), and for
332 genes involved in 'dorsal trunk growth, open tracheal system' (all $P \leq 3.10e^{-5}$) (Fig. 4G). The
333 overrepresentation of these genes supports the view that encapsulation and tracheal
334 development play important roles in larval infestation, and could present a potential target
335 for selection.

3. Discussion

We investigated the geographic landscape of local adaptation in Hawaiian cricket populations that are subject to strong selection by flies, which use cricket song to locate hosts for their endoparasitic larvae. Host cricket populations have repeatedly adapted to the introduction of the parasitoid fly via the spread of male-silencing wing phenotypes (*11*, *15*) and recent studies of adaptive responses to selection imposed by the fly have overwhelmingly focused on these phenotypes. However, the association between fly attack rate and the frequency of male-silencing phenotypes has not previously been tested. We observed clear evidence of selection associated with fly attack rate in genomic regions underpinning the recently emerged, male-silencing curly-wing morph. This finding validates prior speculation that this phenotype has spread due to protective benefits in evading detection by the fly. Perhaps surprisingly, we did not find strong evidence of selection in the genomic region underpinning flatwing phenotypes, but this could be due to multiple, independent genetic architectures (*10*). Another possibility is that, while curly-wing spread very recently, having first been recorded in 2017, flatwing was observed in 2003 and rapidly spread to near-fixation in multiple populations (*11*, *19*) in some of which it subsequently fixed (*14*, *62*), suggesting its frequency may not reflect contemporary selection pressures.

Parasitoid flies and their cricket hosts exert reciprocal selection pressures, and our observations fit predictions of the geographic mosaic theory of coevolution (*2*). First, we observe geographical influences on fly abundance and spatial variation in the strength of the host-parasite interaction. Fly attack rates were positively associated with daily precipitation, whereas Hawaiian *T. oceanicus* are restricted to a fragmented landscape of urban lawns, indicating geographical factors influence each species' distribution. Fly attack rates varied greatly across locations, with the average of number of flies attracted per 10-minute song bout ranging from 0.009 (at *Kauai.PK*) to 0.789 (at *Kauai.CG*). The former location is a notable outlier, with playback at all other sites attracting an average of 0.1 or more flies, representing strong selection. Songless male morphs are disadvantaged in the context of sexual selection, and our estimates of net selection indicate that while silent males are strongly favoured at three of the 11 locations surveyed, they are strongly selected against at *Kauai.PK*. Across the remainder, the direction of selection varied nightly, though these include three locations at which singing-capable males are absent (*14*, *62*). Another prediction of the geographic mosaic theory of evolution is that trait remixing

368 between locally adapted populations could lead to maladaptation. The strongest evidence
369 of this came from *Kauai.PK*, at which songless males are strongly disadvantaged, yet a
370 third of surveyed males carried male-silencing phenotypes. This might be influenced by
371 gene flow from nearby populations at which flies are more abundant—though we did not
372 observe evidence of this in our analysis, there are likely nearby populations we did not
373 discover. We also observed non-negligible fly attack rates at three locations in which no
374 singing-capable male morphs remain, which would be expected to greatly reduce parasite
375 populations (*63*). This is curious, but a recent study showed flies are attracted to the song
376 of other co-occurring Grylline crickets that offer less viable hosts (*9*), raising the intriguing
377 prospect that *O. ochracea* are able to co-opt the acoustic signals of other species to
378 parasitise silent *T. oceanicus* populations, complicating the coevolutionary interaction. An
379 alternative possibility is that flies are itinerant and were attracted from nearby locations. We
380 have not heard singing males in recent surveys of areas surrounding these sites, but the
381 likely migration asymmetry between flies and crickets represents an important direction for
382 future research, as this is key to predictions of local adaptation (*64*).

383 The retention of singing-capable males at multiple locations, partly due to their advantages
384 in the context of mate attraction, suggests the opportunity for selection from flies to act on
385 other traits involved in evading, impeding, or overcoming infestation. We detected further
386 evidence of selection on variants within genes in regions that do not, to our knowledge,
387 correspond male-silencing phenotypes, but do correspond to traits that attracted the
388 interest of early behavioural studies testing evidence of adaptive responses to the
389 parasitoid fly (*44*, *65*, *66*)—in some cases prior to the emergence of silent male morphs.
390 One selected variant was within the gene *SIFAR,* which in *Drosophila* influences variation in
391 wing extension index during male courtship (*57*). Prior work in Hawaiian *T. oceanicus*
392 explored whether singing behaviour differs across populations (*44*, *45*, *51*), with an early
393 study reporting evidence that singing-capable males in parasitised populations reduced
394 calling effort during the interval of peak fly activity (*44*). More recently, the prediction that
395 selection would act to modulate the singing behaviour has been largely overlooked
396 following the spread of adaptive male-silencing phenotypes. We also observed selection on
397 the gene *GLT35*, which influences tracheal development; mutations in this gene lead to
398 malformed tracheal tubes in *Drosophila* (*58*). This, too, relates to prior research in this
399 system. As in other insect hosts of parasitoids, crickets mount an immune encapsulation
400 response against infesting *O. ochracea* larvae (*65*). However, *O. ochracea* larvae appear to

401 co-opt this encapsulation response to construct a tracheal funnel which attaches to the
402 cricket's abdominal wall, allowing developing larvae to avoid suffocation (63). Our re-
403 analysis of differences in gene expression between uninfested and infested cricket body
404 tissues (*59*) showed infestation-responsive genes are overrepresented not just for functions
405 involved in larval encapsulation, but also tracheal development. The apparent trade-off
406 between host encapsulation response and larval co-option has led to speculation that
407 selection might act on attenuated encapsulation responses (*65*), but it could also feasibly
408 act on genes involved in tracheal development. Whether evidence of selection on genes
409 implicated in singing behaviour and tracheal development reflects adaptive variation in
410 these traits represents an interesting avenue for further research.

411 We previously reported evidence that the co-occurrence of multiple male-silencing wing
412 morphs could act to impede any one of them from spreading to fixation (*20*). In addition,
413 genomic regions underlying flatwing and curly-wing phenotypes are nearby but not
414 intrinsically linked with large segregating inversions (*20*, *33*). We were surprised to find
415 evidence of similarly large segregating structural variants (many if not all of which are likely
416 to be inversions) across nearly all *T. oceanicus* chromosomes, most of which were
417 polymorphic on all three islands, indicating they may be maintained as balanced
418 polymorphisms. These structural variants account for a massive proportion of the genetic
419 variation and gene expression in these populations, suggesting they have substantial
420 phenotypic effects. As well impeding recombination between potentially adaptive loci,
421 selection acting upon these large SVs could be a source of interference that impedes the
422 spread of adaptive variants (*67*). The maintenance of inversions at intermediate frequencies
423 has received considerable recent interest (*68*), but the sources of balancing selection on
424 these large regions are still not well understood (*34*). A combination of theoretical and
425 empirical studies have implicated various factors influencing balancing selection in large
426 linked regions, including overdominance or heterokaryotype advantage (*69*, *70*), epistasis
427 and antagonistic pleiotropy (*71*), and sexual antagonism (*72*). Hawaiian *T. oceanicus*
428 appear remarkable for the number of large potentially balanced structural polymorphisms
429 that are maintained at intermediate frequencies in several populations, and could offer a
430 compelling system to comprehensively test the sources of balancing selection maintaining
431 variation in these regions.

4. Methods

4.1. Selection assays

We performed 489 fly capture trials starting at sunset on 42 nights at 11 patches, and were performed in January-February and June-July in 2022. Each fly capture trial involved eight traps, half of which broadcasted a cricket song model based on average song parameters across several Hawaiian populations of *T. oceanicus* (*73*), and lasted 10 minutes, with song broadcast at 85dB at a distance of 5cm using a speaker (Jam HX-P303BK) attached to an mp3 player (SanDisk SDMX26-008G-E46P). Control traps were identical to those broadcasting song, but without playback. Traps were set out in a 3 x 3 square, with 1.5m between traps and an alternating order of control and song traps, which were swapped each trial. Trials began at sunset, with twelve trials per night (unless interrupted by heavy rain). After trials, captured flies were released 10 metres from the trap, after which we waited for three minutes before resuming trials. We recorded the number of flies attracted to each trap. One site, Kauai.WC, was only assayed on a single night in October 2022. Because fly abundance was repeatable across nights at other sites (see main text), did not appear to differ between surveys performed in early and mid-2022, and because assays at this site reported similar rates of fly attack as a nearby site, Kauai.CG, we retained this site in our analysis.

Repeatability of fly attack rates across nights were estimated using rptR v0.9.22 (*74*) by running linear mixed models with a response variable of the log-adjusted fly attack rate between within 2 hours of sunset, averaged across nights. Data for average daily temperature and precipitation at each location were retrieved using the nasapower R package.

4.2. Estimating expected lifespan

Following the assumptions stated above, we calculated the number of flies a singing-capable male cricket attracts per day as N_{fly} = mean number of flies attracted per 10-minute playback * 0.76, based on prior observations of nightly singing effort (*51*). We then assume:

$$P(infestation) = 1 - (1 - 0.61)^{N_{fly}}$$

Where *P(infestation)* is the daily probability of becoming fatally infested, given that fly planidia successfully infest and emerge as larvae from 61% of male *G. texensis*, another

462 cricket host (*53*). We further assume that the lifespan of a cricket follows a geometric
463 distribution, with a time lag *L* between infection and loss of reproductive viability (6 days –
464 see above). Under this assumption, a singing male's expected reproductive lifespan is:

465 $$E(reproductive\ lifespan) = \frac{1}{P(infestation)} + L$$

466 On top of this, we imposed a maximum expected reproductive lifespan of 29 days, the
467 same as that of silent males.

468 ***4.3. Sample collection, extraction, sequencing***

469 Tissue samples were collected from wild-caught males at 11 locations between July 2021
470 and June 2022 by removing a single hindleg. Samples from one population (Oahu.AC)
471 were supplemented with samples collected in October 2018 (N = 15 of 30). This population
472 was therefore not included in analysis of recent changes in effective population size. After
473 tissue collection, animals were released, and crickets missing a hindleg were not sampled
474 to avoid resampling. Legs were stored in 100% ethanol at -80C until DNA extraction using a
475 CTAB-chloroform protocol. Libraries were prepared using NEBNext Ultra II FS Kits with
476 ~300bp inserts, and sequenced at the University of Liverpool Centre for Genomic Research
477 on an Illumina NovaSeq S4, generating paired-end 150bp reads. Ninety of these
478 sequenced samples were used in a previously published analysis (*20*).

479 ***4.4. Variant identification, calling, filtering***

480 Reads were provided by the facility after initial quality and adapter trimming using Cutadapt
481 v1.2.1104 (*75*) and Sickle v1.200, with a minimum window quality score of 20. Further
482 trimming was performed using FastP v.0.24.0 with default parameters. Reads were aligned
483 to the *T. oceanicus* genome v3 (*33*) using BWA-mem v.0.7.19 with default parameters,
484 following which PCR duplicates were flagged using SAMBAMBA v.1.0.1. We performed
485 variant discovery from aligned reads of 55 samples, 5 per location, selecting samples with
486 high sequencing depth and which represented all morphological variants recorded at the
487 respective location. Variant discovery and calling of samples was performed in Freebayes
488 v1.3.6 (*76*). Variants were initially filtered using vcftools v0.1.16 (*77*) to retain genotype calls
489 with: minimum sequencing depth greater than 5x and less than 60x; minimum genotype
490 quality 20; minimum quality 30; minimum genotype call rate across samples > 0.75;
491 mapping quality >= 30; biallelic variants (N = 75,180,819 variants). For most analyses,

492 variants with minor allele frequency < 0.05 (or < 0.1 in genetic association and selection
493 analyses) were also removed (N = 48,363,493 variants), although not when quantifying
494 nucleotide diversity or Tajima's D. Tajima's D, nucleotide diversity and Weir and
495 Cockerham's F_{ST} were calculated in 50Kb windows with 10Kb step size in vcftools.

496 ***4.5. Windowed PCA and linkage analysis***

497 We used the Lostruct R package to run windowed PCA (*31*), often used to identify putative
498 chromosomal inversions, across the genome. We first further filtered the full VCF for SNPs
499 with less than 1% missing genotypes, samples missing genotypes at less than 20% of loci,
500 and retained only one variant per 50Kb (N = 149,673; 294 samples). We converted the VCF
501 to TPED format and implemented PCA in 50-SNP windows across the genome. To visualise
502 linkage across chromosomes, we thinned the set of SNPs using a window of 200Kb, filtered
503 for minor allele frequency > 0.2, then calculated and plotted r^2 values in LDheatmap (*78*).

504 ***4.6. Population structure and demographic inference***

505 Inferences of population structure and demographic history were performed using linkage-
506 pruned (plink: indep-pairwise 50 10 0.1) variants genotyped in >=90% of samples and
507 located on chromosomes 5, 9 and 14 (those not showing evidence of massive structural
508 variants) (N = 73,674 variants). Phylogenetic relationships were explored using FastTree
509 (*37*), and were plotted using the R package ggtree (*79*). F-branch statistics were calculated
510 in Dsuite (*39*).

511 Repetitive regions identified by (*33*) were excluded before running analyses to estimate
512 historical changes in effective population size. Long-term changes in effective population
513 size were inferred using all samples collected from Oahu (to which *T. oceanicus* were first
514 introduced) in 2022. This analysis was performed in Phlash (*42*). To explore more recent
515 shifts in effective population size, we used GONE2, a method based on patterns of linkage
516 disequilibrium (*80*). For this analysis, we pooled geographically nearby populations
517 (Kauai.CG and Kauai.WC; and Kauai.AS and Kauai.VL) in an effort to avoid confounding
518 effects of possible recent gene flow between these locations.

519 ***4.7. Genome-wide association and selection tests***

520 Genome-wide association tests for curly-wing and flatwing phenotypes were implemented
521 in GEMMA (*81*), using linear mixed models with a genetic relatedness matrix included as a

522 random effect, and tested using likelihood ratio tests. Tests for loci that differed in frequency
523 in association with fly abundance were implemented using latent factor mixed models that
524 account for unmeasured variation (i.e., hierarchical population structure) implemented in the
525 R package LFMM (*56*) with ridge penalty estimates and a K value (reflecting the number of
526 populations) of 3, which was determined from inspection of PCA screeplots and the number
527 of islands. P-values were calibrated by a genomic inflation factor. Input data were linkage-
528 pruned in 50-variant windows using Plink (*--indep-pairwise 50 10 0.1*), variants missing in >
529 90% of individuals or with minor allele frequency < 0.1 were removed, and missing
530 genotypes were imputed using the *impute* function in the R package LEA (*82*). Gene
531 annotations were retrieved from (*33*).

532 ***4.8. Gene expression analyses***

533 Trimmed, paired-end RNA-sequencing data collected from neural tissue of 48 lab-reared *T.*
534 *oceanicus* of both sexes (*36*), and body tissues from 18 *T. oceanicus* that were or were not
535 infected with *O. ochracea* larvae (*59*), were downloaded from the NCBI Sequence Read
536 Archive. In each case, reads filtered with FastP and default parameters, then aligned to the
537 *T. oceanicus* genome using HISAT2 with default parameters (*83*), after which gene counts
538 were estimated using StringTie (*84*). In addition, for the 48 samples from (*36*), variant
539 calling was performed using bcftools v.1.21, and filtered for genotype quality (> 20),
540 missingness (< 0.25) and minor allele frequency (> 0.05), before running chromosome-level
541 PCA in Plink (*85*). Variance partitioning of gene expression counts was performed using the
542 R package variancePartition v1.34.0 (*86*), and differential expression analyses were
543 performed in DESeq2 v1.44.0 (*87*). Gene ontology overrepresentation tests for biological
544 processes were performed using topGO v2.56.0 (*88*) using gene ontology annotations
545 retrieved from (*33*).

546

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6. Author contributions

NWB, JGR and OEG conceived of the study and planned experiments and analyses. JGR, OEG, NWB, LHY and RZ performed data collection. SZ contributed genomic resources. JGR led analysis and writing the manuscript with input from all authors.

7. Data and code availability

Newly published genome sequencing data are available at the NCBI Sequence Read Archive under accession PRJNA948842. Previously published genome sequencing data from Hawaiian *T. oceanicus* are at the NCBI SRA under accession PRJNA1019311, and for Australian populations of *T. commodus* and *T. oceanicus* are available from the ENA under accession PRJEB39125, previously published RNA-sequencing datasets are available from the NCBI SRA under accession PRJNA344019 (song experiment) and the NCBI Gene Expression Omnibus under accession GSE15139 (infestation experiment). Scripts and smaller data files used in analyses are available at https://github.com/jackgrayner/cricket_popgen and will be made permanently available upon publication.

8. References

1. R. Gomulkiewicz, J. N. Thompson, R. D. Holt, S. L. Nuismer, M. E. Hochberg, Hot Spots, Cold Spots, and the Geographic Mosaic Theory of Coevolution. *Am. Nat.* **156**, 156–174 (2000).

2. J. N. Thompson, The geographic mosaic of coevolution. (2005).

3. S. L. Nuismer, R. Gomulkiewicz, B. J. Ridenhour, When Is Correlation Coevolution? *Am. Nat.* **175**, 525–537 (2010).

4. C. Pardo-Diaz, C. Salazar, C. D. Jiggins, Towards the identification of the loci of adaptive evolution. *Methods Ecol. Evol.* **6**, 445–464 (2015).

5. W. R. Milligan, L. K. Hayward, G. Sella, When should adaptation arise from a polygenic response versus few large effect changes? *bioRxiv*, 2025.05.15.654234 (2025).

6. J. R. Stinchcombe, H. E. Hoekstra, Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity* **100**, 158–170 (2008).

7. R. D. H. Barrett, H. E. Hoekstra, Molecular spandrels: tests of adaptation at the genetic level. *Nat. Rev. Genet.* **12**, 767–780 (2011).

8. K. Bomblies, C. L. Peichel, Genetics of adaptation. *Proc. Natl. Acad. Sci.* **119**, e2122152119 (2022).

9. E. D. Broder, J. H. Gallagher, A. W. Wikle, G. T. Welsh, D. M. Zonana, T. J. Firneno Jr., R. M. Tinghitella, A well-studied parasitoid fly of field crickets uses multiple alternative hosts in its introduced range. *Evol. Ecol.* **37**, 477–492 (2023).

10. X. Zhang, J. G. Rayner, M. L. Blaxter, N. W. Bailey, Rapid parallel adaptation despite gene flow in silent crickets. *Nat. Commun.* **12**, 50 (2021).

11. M. Zuk, J. T. Rotenberry, R. M. Tinghitella, Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biol. Lett.* **2**, 521–524 (2006).

12. R. M. Tinghitella, Rapid evolutionary change in a sexual signal: genetic control of the mutation “flatwing” that renders male field crickets (Teleogryllus oceanicus) mute. *Heredity* **100**, 261–267 (2008).

13. S. Pascoal, T. Cezard, A. Eik-Nes, K. Gharbi, J. Majewska, E. Payne, M. G. Ritchie, M. Zuk, N. W. Bailey, Rapid convergent evolution in wild crickets. *Curr. Biol.* **24**, 1369–1374 (2014).

14. J. G. Rayner, S. Aldridge, F. Montealegre-Z, N. W. Bailey, A silent orchestra: convergent song loss in Hawaiian crickets is repeated, morphologically varied, and widespread. *Ecology* **100**, e02694 (2019).

15. N. W. Bailey, M. Zuk, R. M. Tinghitella, Quiet but not forgotten: Insights into adaptive evolution and behavior from 20 years of (mostly) silent Hawaiian crickets. *Adv. Study Behav.* (2024).

16. W. Cade, Acoustically Orienting Parasitoids: Fly Phonotaxis to Cricket Song. *Science* **190**, 1312–1313 (1975).

17. E. D. Broder, J. H. Gallagher, A. W. Wikle, C. P. Venable, D. M. Zonana, S. J. Ingley, T. C. Smith, R. M. Tinghitella, Behavioral responses of a parasitoid fly to rapidly evolving host signals. *Ecol. Evol.* **12**, e9193 (2022).

18. R. N. Miles, D. Robert, R. R. Hoy, Mechanically coupled ears for directional hearing in the parasitoid fly Ormia ochracea. *J. Acoust. Soc. Am.* **98**, 3059–3070 (1995).

19. M. Zuk, N. W. Bailey, B. Gray, J. T. Rotenberry, Sexual signal loss: The link between behaviour and rapid evolutionary dynamics in a field cricket. *J. Anim. Ecol.* **87**, 623–633 (2018).

20. J. G. Rayner, F. Eichenberger, J. V. A. Bainbridge, S. Zhang, X. Zhang, L. H. Yusuf, S. Balenger, O. E. Gaggiotti, N. W. Bailey, Competing adaptations maintain nonadaptive variation in a wild cricket population. *Proc. Natl. Acad. Sci.* **121**, e2317879121 (2024).

21. J. H. Gallagher, E. D. Broder, A. W. Wikle, H. O'Toole, C. Durso, R. M. Tinghitella, Surviving the serenade: how conflicting selection pressures shape the early stages of sexual signal diversification. *Evolution*, qpae035 (2024).

22. X. Zhang, M. Blaxter3, J. Wood, A. Tracey, S. McCarthy, P. Thorpe, J. G. Rayner, S. Zhang, K. L. Sikkink, S. L. Balenger, N. W. Bailey, Temporal genomics in Hawaiian crickets reveals compensatory intragenomic coadaptation during adaptive evolution. *Nat. Commun.* **15** (2024).

23. S. Pascoal, J. E. Risse, X. Zhang, M. Blaxter, T. Cezard, R. J. Challis, K. Gharbi, J. Hunt, S. Kumar, E. Langan, X. Liu, J. G. Rayner, M. G. Ritchie, B. L. Snoek, U. Trivedi, N. W. Bailey, Field cricket genome reveals the footprint of recent, abrupt adaptation in the wild. *Evol. Lett.*, doi: 10.1002/evl3.148 (2020).

24. J. C. Tanner, E. Swanger, M. Zuk, Sexual signal loss in field crickets maintained despite strong sexual selection favoring singing males. *Evolution* **73**, 1482–1489 (2019).

25. C. R. Linnen, Y.-P. Poh, B. K. Peterson, R. D. H. Barrett, J. G. Larson, J. D. Jensen, H. E. Hoekstra, Adaptive Evolution of Multiple Traits Through Multiple Mutations at a Single Gene. *Science* **339**, 1312–1316 (2013).

26. K. Kunte, W. Zhang, A. Tenger-Trolander, D. H. Palmer, A. Martin, R. D. Reed, S. P. Mullen, M. R. Kronforst, doublesex is a mimicry supergene. *Nature* **507**, 229–232 (2014).

27. A. E. van't Hof, P. Campagne, D. J. Rigden, C. J. Yung, J. Lingley, M. A. Quail, N. Hall, A. C. Darby, I. J. Saccheri, The industrial melanism mutation in British peppered moths is a transposable element. *Nature* **534**, 102–105 (2016).

646 28. K. T. Xie, G. Wang, A. C. Thompson, J. I. Wucherpfennig, T. E. Reimchen, A. D. C.
647 MacColl, D. Schluter, M. A. Bell, K. M. Vasquez, D. M. Kingsley, DNA fragility in the
648 parallel evolution of pelvic reduction in stickleback fish. *Science* **363**, 81–84 (2019).

649 29. C.-J. Rubin, E. D. Enbody, M. P. Dobreva, A. Abzhanov, B. W. Davis, S. Lamichhaney,
650 M. Pettersson, A. T. Sendell-Price, C. G. Sprehn, C. A. Valle, K. Vasco, O. Wallerman,
651 B. R. Grant, P. R. Grant, L. Andersson, Rapid adaptive radiation of Darwin's finches
652 depends on ancestral genetic modules. *Sci. Adv.* **8**, eabm5982 (2022).

653 30. R. Zhang, J. G. Rayner, N. W. Bailey, Rapid sexual signal diversification is facilitated
654 by permissive females. *Curr. Biol.* **34**, 403-409.e3 (2024).

655 31. H. Li, P. Ralph, Local PCA Shows How the Effect of Population Structure Differs Along
656 the Genome. *Genetics* **211**, 289–304 (2019).

657 32. J. Ma, C. I. Amos, Investigation of Inversion Polymorphisms in the Human Genome
658 Using Principal Components Analysis. *PLOS ONE* **7**, e40224 (2012).

659 33. S. Zhang, J. Rayner, L. Knowles, N. Bailey, Inversions accelerate adaptation by
660 creating a permissive genetic background. (In prep).

661 34. M. Kapun, T. Flatt, The adaptive significance of chromosomal inversion polymorphisms
662 in Drosophila melanogaster. *Mol. Ecol.* **28**, 1263–1282 (2019).

663 35. M. Turelli, N. H. Barton, Polygenic variation maintained by balancing selection:
664 pleiotropy, sex-dependent allelic effects and G x E interactions. *Genetics* **166**, 1053–
665 1079 (2004).

666 36. S. Pascoal, X. Liu, Y. Fang, S. Paterson, M. G. Ritchie, N. Rockliffe, M. Zuk, N. W.
667 Bailey, Increased socially mediated plasticity in gene expression accompanies rapid
668 adaptive evolution. *Ecol. Lett.* **21**, 546–556 (2018).

669 37. M. N. Price, P. S. Dehal, A. P. Arkin, FastTree 2 – Approximately Maximum-Likelihood
670 Trees for Large Alignments. *PLOS ONE* **5**, e9490 (2010).

671 38. M. Malinsky, H. Svardal, A. M. Tyers, E. A. Miska, M. J. Genner, G. F. Turner, R.
672 Durbin, Whole-genome sequences of Malawi cichlids reveal multiple radiations
673 interconnected by gene flow. *Nat. Ecol. Evol.* **2**, 1940–1955 (2018).

674 39. M. Malinsky, M. Matschiner, H. Svardal, Dsuite - Fast D-statistics and related
675 admixture evidence from VCF files. *Mol. Ecol. Resour.* **21**, 584–595 (2021).

676 40. L. G. Eldredge, N. L. Evenhuis, *Hawaii's Biodiversity: A Detailed Assessment of the*
677 *Numbers of Species in the Hawaiian Islands* (Bishop Museum Press, 2003).

678 41. D. A. Gray, H. D. Kunerth, M. Zuk, W. H. Cade, S. L. Balenger, Molecular biogeography
679 and host relations of a parasitoid fly. *Ecol. Evol.* **9**, 11476–11493 (2019).

680 42. J. Terhorst, Accelerated Bayesian inference of population size history from recombining
681 sequence data. *bioRxiv*, 2024.03.25.586640 (2024).

43. R. M. Tinghitella, M. Zuk, Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. *Evolution* **63**, 2087–2098 (2009).

44. M. Zuk, L. Simmons, L. Cupp, Calling characteristics of parasitized and unparasitized populations of the field cricket Teleogryllus oceanicus. *Behav. Ecol. Sociobiol.* **33**, 339–343 (1993).

45. D. A. Lewkiewicz, M. Zuk, Latency to resume calling after disturbance in the field cricket, Teleogryllus oceanicus, corresponds to population-level differences in parasitism risk. *Behav. Ecol. Sociobiol.* **55**, 569–573 (2004).

46. R. M. Tinghitella, E. D. Broder, J. H. Gallagher, A. W. Wikle, D. M. Zonana, Responses of intended and unintended receivers to a novel sexual signal suggest clandestine communication. *Nat. Commun.* **12**, 797 (2021).

47. W. H. Cade, M. Ciceran, A.-M. Murray, Temporal patterns of parasitoid fly (Ormia ochracea) attraction to field cricket song (Gryllus integer). *Can. J. Zool.* **74**, 393–395 (1996).

48. P. Müller, D. Robert, A shot in the dark: the silent quest of a free-flying phonotactic fly. *J. Exp. Biol.* **204**, 1039–1052 (2001).

49. R. Rodríguez-Muñoz, J. J. Boonekamp, D. Fisher, P. Hopwood, T. Tregenza, Slower senescence in a wild insect population in years with a more female-biased sex ratio. *Proc. R. Soc. B Biol. Sci.* **286**, 20190286 (2019).

50. F. Zajitschek, R. Bonduriansky, S. R. K. Zajitschek, R. C. Brooks, Sexual Dimorphism in Life History: Age, Survival, and Reproduction in Male and Female Field Crickets Teleogryllus commodus under Seminatural Conditions. *Am. Nat.* **173**, 792–802 (2009).

51. J. G. Rayner, W. T. Schneider, N. W. Bailey, Can behaviour impede evolution? Persistence of singing effort after morphological song loss in crickets: Singing effort in mute crickets. *Biol. Lett.* **16** (2020).

52. M. L. Westwood, Q. Geissmann, A. J. O'Donnell, J. Rayner, W. Schneider, M. Zuk, N. W. Bailey, S. E. Reece, Machine learning reveals singing rhythms of male Pacific field crickets are clock controlled. *Behav. Ecol.* **35**, arad098 (2024).

53. Thomson, IR, Vincent, CM, Bertram, SM, Success of the Parasitoid Fly Ormia ochracea (Diptera: Tachinidae) on Natural and Unnatural Cricket Hosts. *Fla. Entomol.* **95**, 43–48 (2012).

54. S. A. Adamo, D. Robert, J. Perez, R. R. Hoy, The response of an insect parasitoid, Ormia ochracea (Tachinidae), to the uncertainty of larval success during infestation. *Behav. Ecol. Sociobiol.* **36**, 111–118 (1995).

55. F. Karch, W. Bender, B. Weiffenbach, abdA expression in Drosophila embryos. *Genes Dev.* **4**, 1573–1587 (1990).

56. E. Frichot, S. D. Schoville, G. Bouchard, O. François, Testing for Associations between Loci and Environmental Gradients Using Latent Factor Mixed Models. *Mol. Biol. Evol.* **30**, 1687–1699 (2013).

721 57. A. Sellami, J. A. Veenstra, SIFamide acts on fruitless neurons to modulate sexual
722 behavior in Drosophila melanogaster. *Peptides* **74**, 50–56 (2015).

723 58. E. Tian, K. G. T. Hagen, A UDP-GalNAc:Polypeptide N-
724 Acetylgalactosaminyltransferase Is Required for Epithelial Tube Formation *. *J. Biol.*
725 *Chem.* **282**, 606–614 (2007).

726 59. K. L. Sikkink, N. W. Bailey, M. Zuk, S. L. Balenger, Immunogenetic and tolerance
727 strategies against a novel parasitoid of wild field crickets. *Ecol. Evol.* **10**, 13312–13326
728 (2020).

729 60. M. Shakeel, X. Xu, S. D. Mandal, F. Jin, Role of serine protease inhibitors in insect-
730 host-pathogen interactions. *Arch. Insect Biochem. Physiol.* **102**, e21556 (2019).

731 61. S. Kausar, M. N. Abbas, I. Gul, Y. Liu, B.-P. Tang, I. Maqsood, Q.-N. Liu, L.-S. Dai,
732 Integrins in the Immunity of Insects: A Review. *Front. Immunol.* **Volume 13-2022**
733 (2022).

734 62. R. M. Tinghitella, E. D. Broder, G. A. Gurule-Small, C. J. Hallagan, J. D. Wilson,
735 Purring crickets: the evolution of a novel sexual signal. *Am. Nat.* **192**, 773–782 (2018).

736 63. P. Sriramulu, D. Schluter, D. I. Bolnick, Dynamics of infection and immunity over 50
737 years as marine stickleback adapt to freshwater. [Preprint] (2025).
738 https://doi.org/10.1101/2025.02.04.636402.

739 64. S. Gandon, Local adaptation and the geometry of host–parasite coevolution. *Ecol. Lett.*
740 **5**, 246–256 (2002).

741 65. N. W. Bailey, M. Zuk, Changes in immune effort of male field crickets infested with
742 mobile parasitoid larvae. *J. Insect Physiol.* **54**, 96–104 (2008).

743 66. M. Zuk, L. W. Simmons, J. T. Rotenberry, A. M. Stoehr, Sex differences in immunity in
744 two species of field crickets. *Can. J. Zool.* **82**, 627–634 (2004).

745 67. W. G. Hill, A. Robertson, The effect of linkage on limits to artificial selection. *Genet.*
746 *Res.* **8**, 269–294 (1966).

747 68. E. L. Berdan, N. H. Barton, R. Butlin, B. Charlesworth, R. Faria, I. Fragata, K. J.
748 Gilbert, P. Jay, M. Kapun, K. E. Lotterhos, C. Mérot, E. Durmaz Mitchell, M. Pascual, C.
749 L. Peichel, M. Rafajlović, A. M. Westram, S. W. Schaeffer, K. Johannesson, T. Flatt,
750 How chromosomal inversions reorient the evolutionary process. *J. Evol. Biol.* **36**,
751 1761–1782 (2023).

752 69. L. Zhao, B. Charlesworth, Resolving the Conflict Between Associative Overdominance
753 and Background Selection. *Genetics* **203**, 1315–1334 (2016).

754 70. M. Paris, E. Durmaz Mitchell, E. Kerdaffrec, D. Rubin, C. Spichtig, F. Zurbriggen, J.
755 Becker, H. Augustijnen, H. Thyagarajan, E. Zinn, F. Gagliardi, E. Gobet, T. Rey, Y.
756 Rime, S. Ribeiro Machado, J. Bachmann, N. Sgammeglia, P. Schmidt, T. Flatt, Multiple
757 forms of balancing selection maintain inversion polymorphism. *Heredity*, doi:
758 10.1038/s41437-025-00780-y (2025).

71. C. Mérot, V. Llaurens, E. Normandeau, L. Bernatchez, M. Wellenreuther, Balancing selection via life-history trade-offs maintains an inversion polymorphism in a seaweed fly. *Nat. Commun.* **11**, 670 (2020).

72. C. S. McAllester, J. E. Pool, Inversions Can Accumulate Balanced Sexual Antagonism: Evidence from Simulations and Drosophila Experiments. doi: 10.7554/elife.93338.2 (2024).

73. J. C. Tanner, E. R. Johnson, M. Zuk, Is plasticity in field cricket mating behaviour mediated by experience of song quality? *Anim. Behav.* **187**, 253–262 (2022).

74. M. A. Stoffel, S. Nakagawa, H. Schielzeth, rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* **8**, 1639–1644 (2017).

75. M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**, 10 (2011).

76. E. Garrison, G. Marth, Haplotype-based variant detection from short-read sequencing (2012). https://arxiv.org/abs/1207.3907.

77. P. Danecek, A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, 1000 Genomes Project Analysis Group, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).

78. J.-H. Shin, S. Blay, B. McNeney, J. Graham, LDheatmap: An R Function for Graphical Display of Pairwise Linkage Disequilibria Between Single Nucleotide Polymorphisms. *J. Stat. Softw. Code Snippets* **16**, 1–9 (2006).

79. G. Yu, D. K. Smith, H. Zhu, Y. Guan, T. T.-Y. Lam, ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol. Evol.* **8**, 28–36 (2017).

80. E. Santiago, I. Novo, A. F. Pardiñas, M. Saura, J. Wang, A. Caballero, Recent Demographic History Inferred by High-Resolution Analysis of Linkage Disequilibrium. *Mol. Biol. Evol.* **37**, 3642–3653 (2020).

81. X. Zhou, M. Stephens, Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.* **44**, 821–824 (2012).

82. E. Frichot, O. François, LEA: An R package for landscape and ecological association studies. *Methods Ecol. Evol.* **6**, 925–929 (2015).

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

84. M. Pertea, D. Kim, G. M. Pertea, J. T. Leek, S. L. Salzberg, Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.* **11**, 1650–1667 (2016).

85. S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly, P. C. Sham, PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

86. G. E. Hoffman, E. E. Schadt, variancePartition: interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics* **17**, 483 (2016).

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

88. A. Alexa, J. Rahnenfuhrer, topGO: enrichment analysis for gene ontology. (2010).

9. Supporting Information

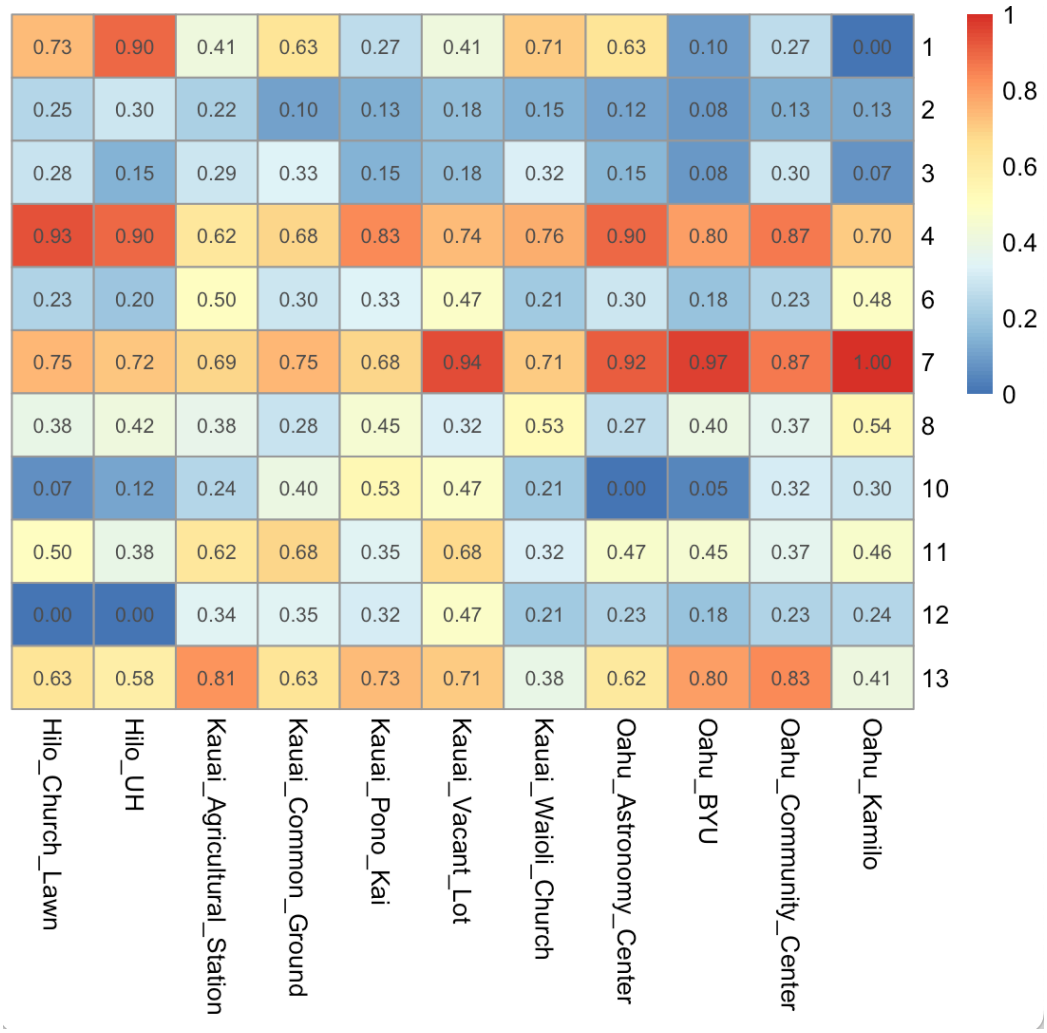

Figure S1. Inferred frequencies of large structural variants inferred from principal component axis 1 (see Fig. 2) on each of 11 chromosomes (rows) in each of 11 locations. Note that this does not account for all of the large SVs we observed (e.g., we observed two large SVs on Chr2), but only those that contribute most strongly to variance on PC1.

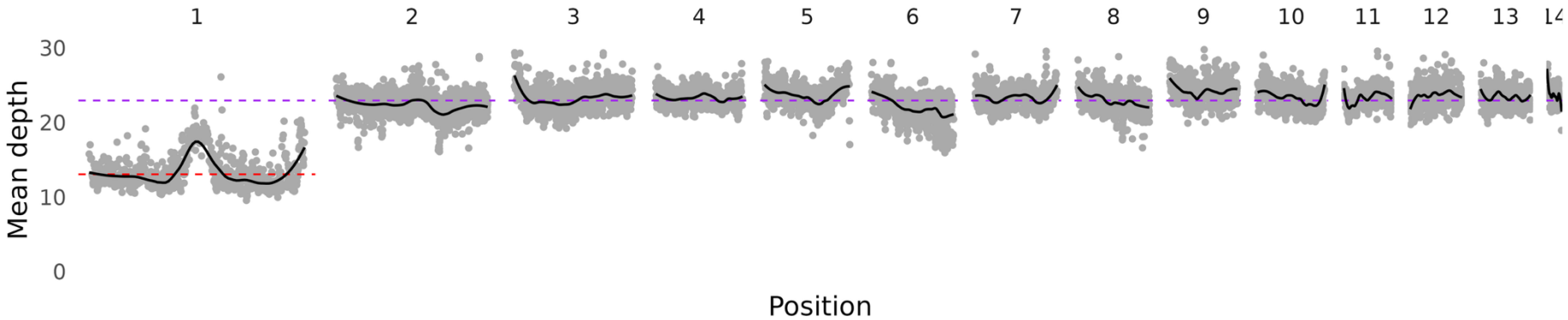

Figure S2. Mean sequencing depth along each chromosome. Black lines show trends from LOESS regression. Purple lines show average read depth across filtered autosomal variants. The red line shows average read depth on Chr1, the X chromosome.

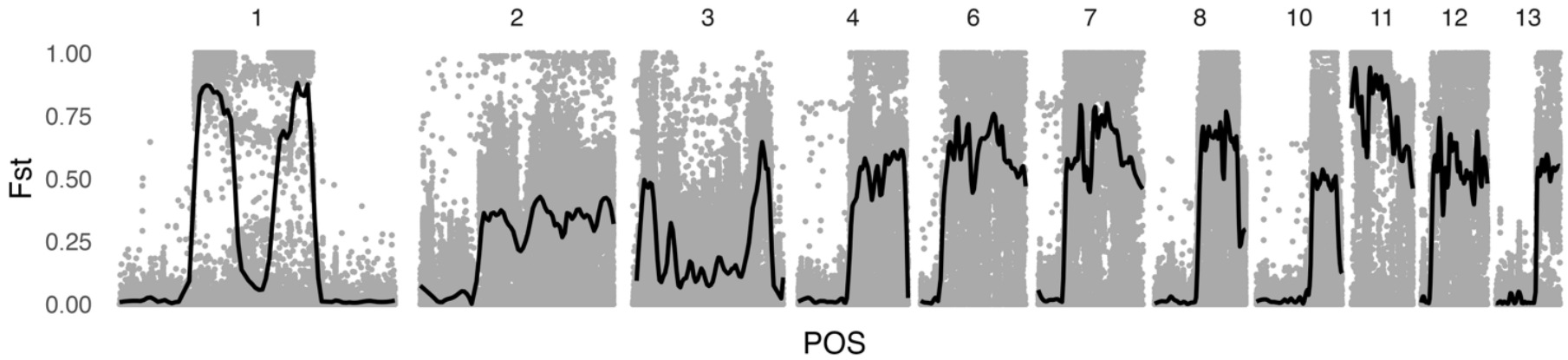

Figure S3. F_{ST} values between alternative homokaryotypes are consistently high in structural variants, with the exception of Chr3, which appears to show a U-shaped pattern with higher divergence near the presumed edges of the variant. F_{ST} values were calculated using just cricket samples from Kauai to avoid confounding effects of genetic divergence between island populations. As above, this does not account for all of the large SVs we observed (e.g., we observed two large SVs on Chr2), but only those that contributed most strongly to variance on PC1. Lines illustrate LOESS trends.

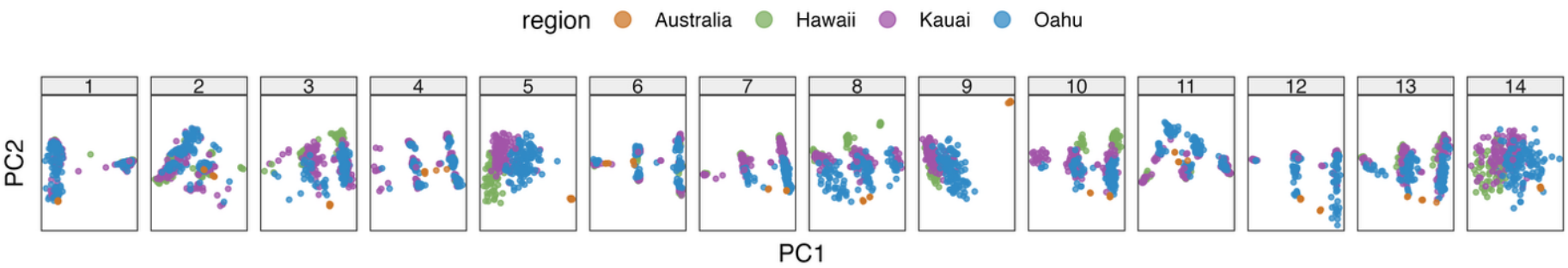

Figure S4. Chromosome-wide PCA projections for samples from three Hawaiian islands, alongside seven further *T. oceanicus* samples from mainland Australian populations.

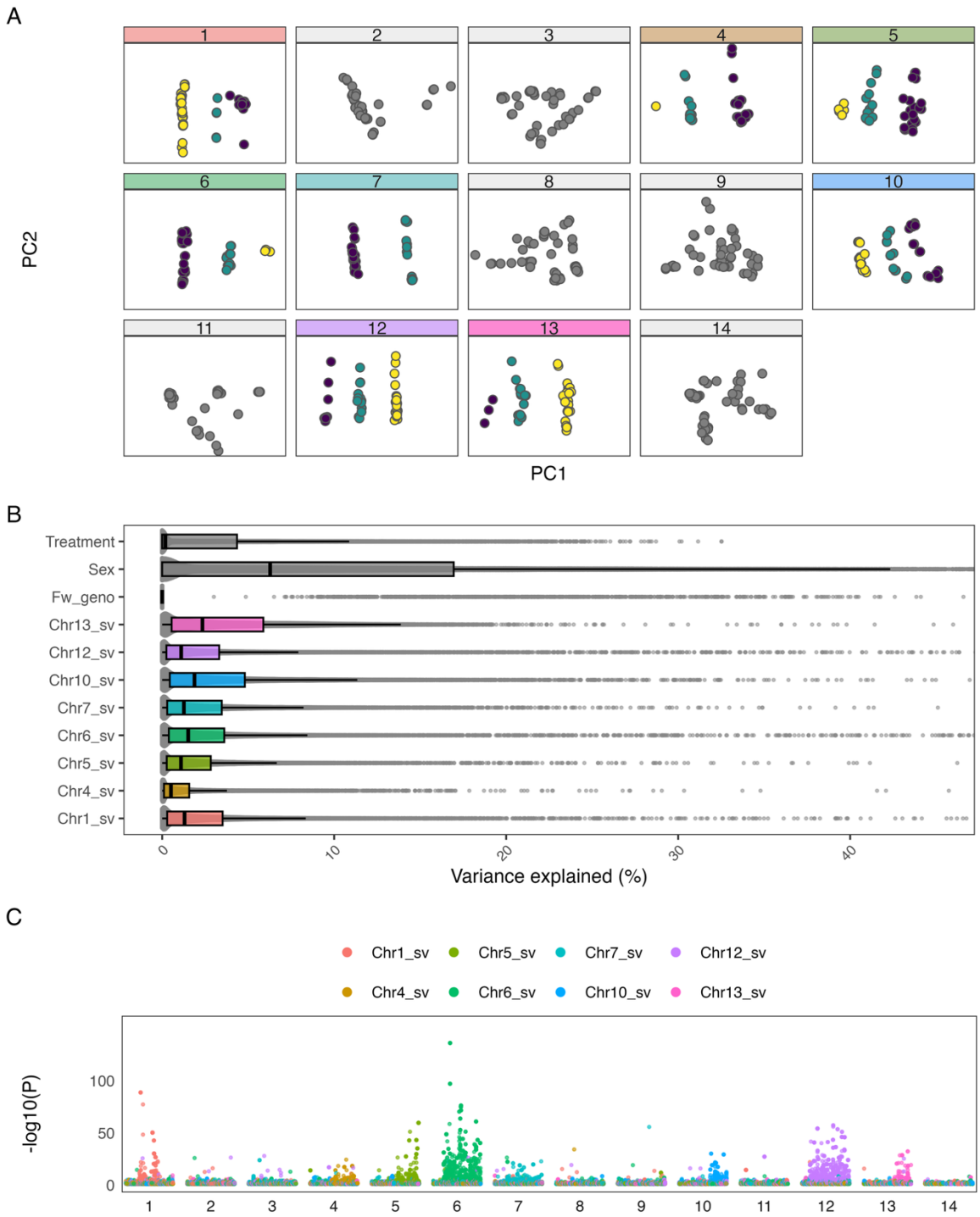

Figure S5. Large structural variants have substantial gene expression effects.

A) PCA of filtered variants from RNA-seq data separated by chromosome, with discrete clustering of samples indicative of one or more large structural variants. Samples are coloured based on assigned karyotype. **B)** Distributions of the proportion of variation in gene expression across 25,359 genes that is accounted for by structural variants on

835 different chromosomes (coloured), and other variables included in the original analysis by
836 (*36*). *Treatment* refers to the presence of male cricket song playback during rearing (Y/N),
837 and *Fw_geno* refers to genotype at the *flatwing* locus. **C)** The genomic distribution of genes
838 included in the analysis, with greater values on the Y-axis indicative of stronger evidence of
839 differential expression. Points are coloured according to the structural variant included as a
840 predictor variable in the differential expression analysis. For example, the cluster of strongly
841 differentially expressed green points on chromosome 6 indicate that the structural variant
842 identified on this chromosome had strong effects on gene expression.

843

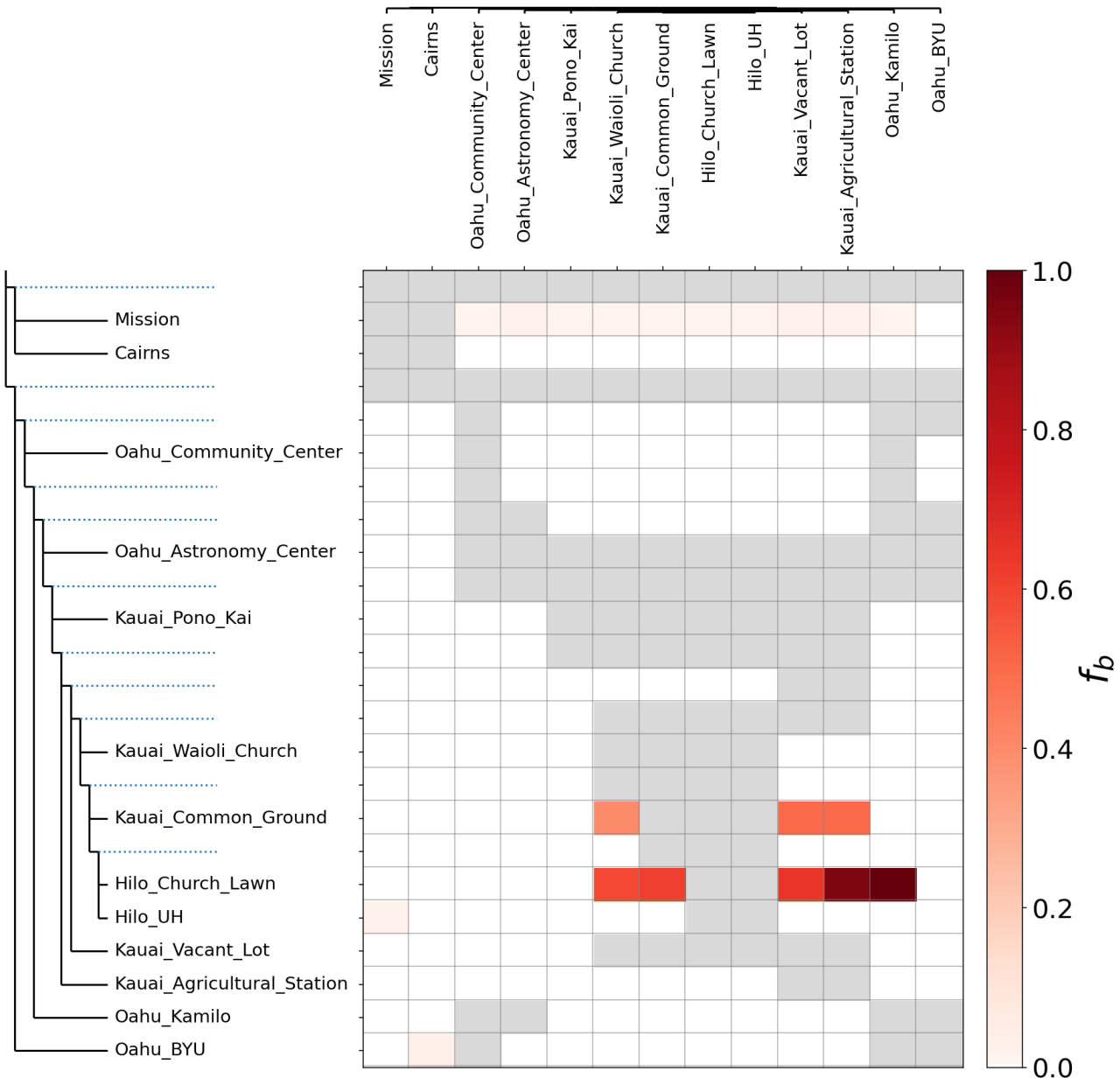

Figure S6. F-branch statistics showing evidence of excess allele-sharing between multiple populations, indicative of gene flow.

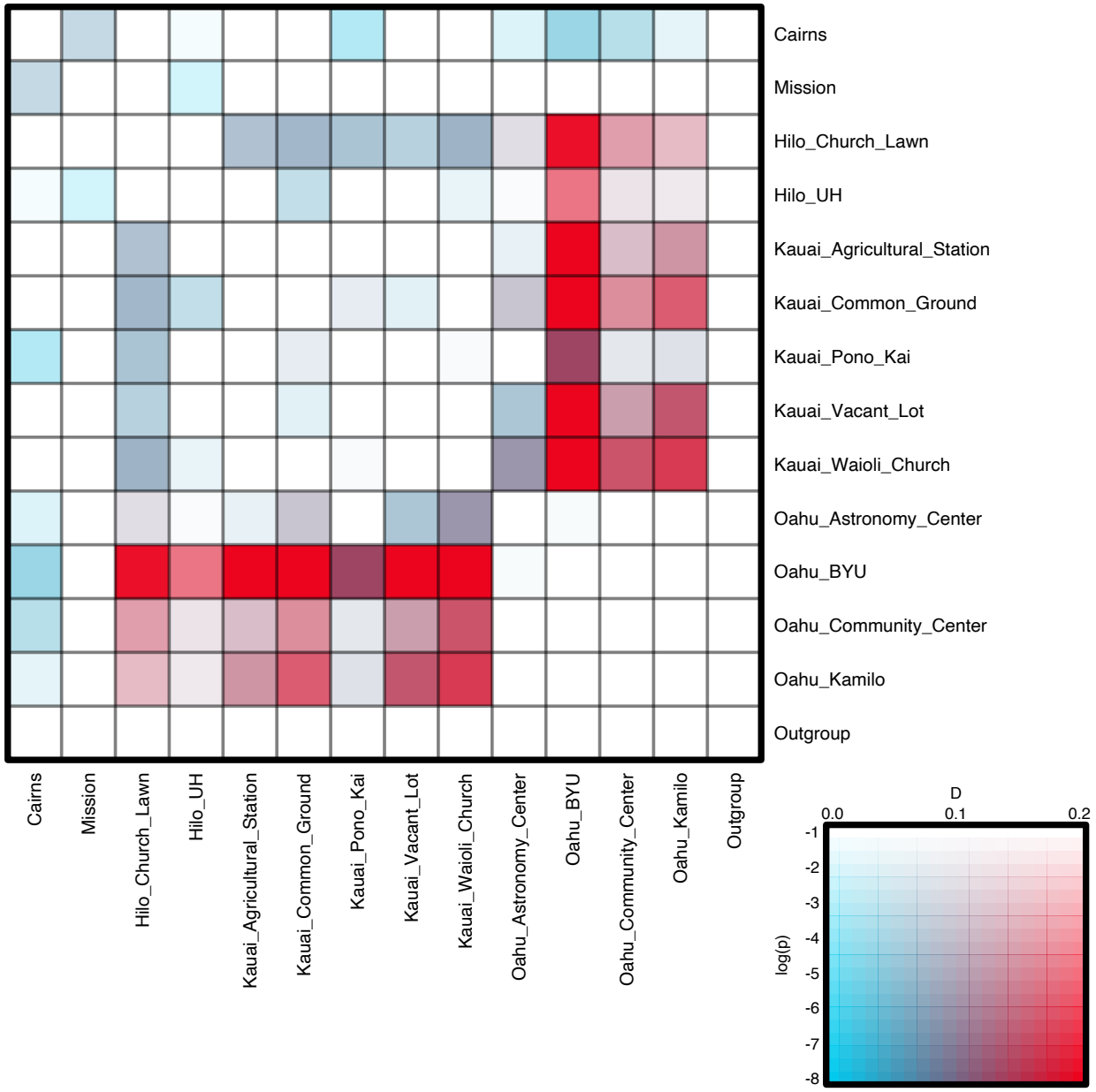

Figure S7. ABBA-BABA statistics summarised across population pairs, coloured by D (hue) and P-value (saturation).

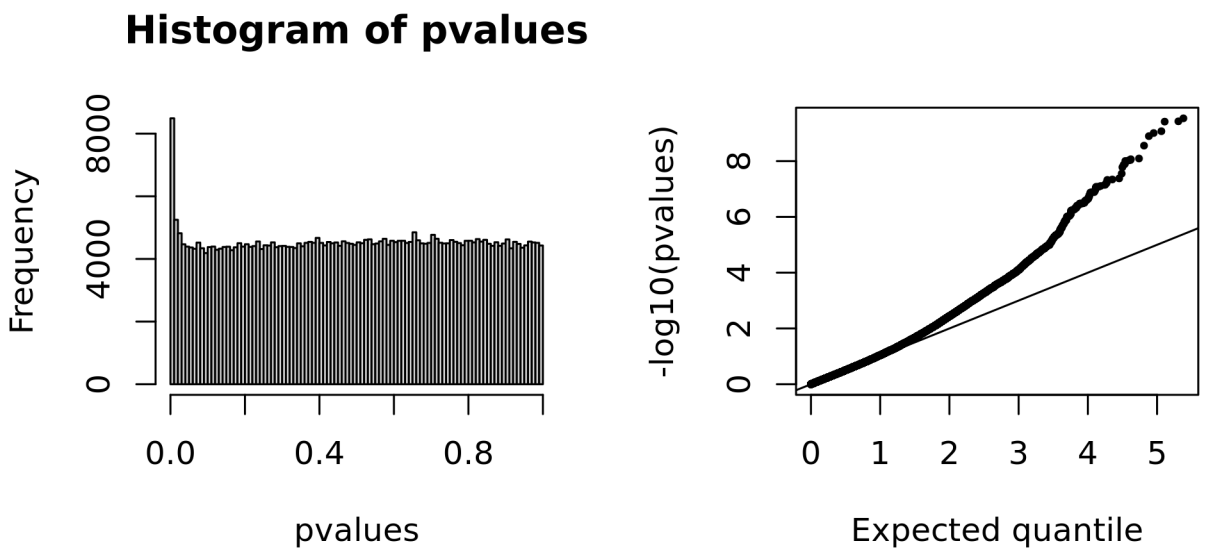

Figure S8. A histogram of calibrated P-values from the LFMM used to test evidence of fly selection on cricket genome variants, and a qqplot illustrating the inflation of low P-values relative to expectation.