Genetic variation associated with a geographical cline in invasive populations of Argentine Stem Weevil

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Goldson's dissection ppl?

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Abstract

The abstract should outline the purpose of the paper and the main results, conclusions and recommendations, using clear, factual, numbered statements

- context and need for the work
- approach and methods used
- main results (2-3 points)

Synthesis and applications

- wider implications and relevance to management or policy

Keywords

Naughty weevils, Invasive species, Molecular evolution, ???

Introduction

Materials and methods

Weevil sampling

From Goldson & co:

- weevil collection details for geographic survey
- collection and processing/dissection details for parasitised *vs.* unparasitised expt

Reduced-representation genome sequencing and processing

From AgResearch:

- details on DNA extraction, GBS pipeline and sequencing

We used a strict processing pipeline to prepare the raw GBS reads for locus assembly. Samples were demultiplexed with zero allowed barcode mismatches to 91–93 b reads, depending on barcode length. Reads were trimmed by searching for adaptors with a minimum match of 11 b. Reads shorter than 80 b after trimming were discarded. All remaining reads were truncated to 80 b to account for unmatched adaptor sequence < 11 b that may have been present at the end of reads. To remove overamplified samples, we calculated the GC content for each library and discarded samples with median read GC > 45%. We followed the recommended steps for optimising parameters [1,2] before assembling loci *de novo* using Stacks [3]. The code we used to process the raw reads, optimise parameters and assemble loci is hosted at github.com/TomHarrop/stacks-asw and github.com/MarissaLL/asw-para-matched.

Genome assembly

To produce the short read dataset, an Illumina TruSeq PCR-free 350bp insert library was generated from DNA extracted from a single, male Argentine stem weevil collected from endophyte-free hybrid ryegrass (*Lolium perenne* × *Lolium multiflorum*) at Lincoln, New Zealand. Library preparation and sequencing were performed by Macrogen Inc. (Seoul, Republic of Korea). A total of 158 GB of 100 b and 150 b paired-end reads were generated from the TruSeq PCR-free library. After removing common sequencing contaminants and trimming adaptor sequences using BBTools [4], the short-read-only genome was assembled with meraculous [5–7]. Reproducible code for assembling the short-read dataset and assessing the assemblies is hosted at github.com/tomharrop/asw-nopcr.

To produce long reads from a single individual, we produced high molecular weight DNA from a single, male ASW collected from Ruakura, New Zealand, using a modified QIAGEN Genomic-tip 20/G extraction protocol [8]. We amplified the DNA using Φ29 multiple displacement amplification (QIAGEN REPLI-g Midi Kit) and debranched the amplified DNA using T7 Endonuclease I (New England Biolabs) according to the Oxford Nanopore Technologies Premium whole genome amplification protocol version WGA_kit9_v1. Amplified DNA was sequenced on 6 R9.4.1 flowcells using a MinION Mk1B sequencer (Oxford Nanopore Technologies). We also extracted high molecular weight DNA from three pools, each of 20 unsexed individuals collected from Ruakura, New Zealand. We sequenced this pooled DNA on 5 R9.4.1 flowcells, following the Genomic DNA by Ligation protocol (SQK-LSK109; Oxford Nanopore Technologies). We removed adaptor sequences from the long reads with Porechop 0.2.4 (github.com/rrwick/Porechop) and assembled with Flye 2.6 [9]. Reproducible code for assembling and assessing the long-read ASW genomes is hosted at github.com/TomHarrop/asw-flye-withpool.

All genome assemblies were assessed by size and contiguity statistics and BUSCO analysis [10]. Redundant contigs were removed from the combined, long read assembly with Purge Haplotigs 0b9afdf [11] using a low, mid and high cutoff of 60, 120 and 190, respectively. We used the Dfam TE Tools Container v1.1 (github.com/Dfam-consortium/TETools) with RepeatModeler 2.0.1 [12] and RepeatMasker 4.1.0 [13] to estimate the repeat content of the long read genomes.

Genome-based analyses, F_{ST}, etc. etc.

- Catalog mapping *e.g.* `bwa mem`

Reproducibility and data availability

Raw sequence data for the ASW genome are hosted at the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) under accession **TBA**. We used `snakemake` [14] to arrange analysis steps into workflows and monitor dependencies, and `Singularity` [15] to capture the computing environment. Using the code repositories listed in each methods section, the final results can be reproduced from the raw data with a single command using `snakemake` and `Singularity`. The source for this manuscript is hosted at github.com/TomHarrop/asw-gbs-genome-paper.

Results

Variation in NZ populations of Argentine stem weevil

To measure genetic variation in invasive New Zealand populations of ASW, we collected individuals from 7 sites in the North Island and 5 sites in the South Island of New Zealand (Figure 1A). We genotyped individuals with a modified genotyping-by-sequencing (GBS) protocol [16]. After strict filtering of the raw GBS data, we used *de novo* locus assembly with the Stacks pipeline [3]. Our final dataset comprised 10–16 individuals per location (total 112), genotyped at more than 22 thousand loci. Principal components analysis (PCA) of the genotypes revealed overlapping populations of ASW, with only 12.2% of total variance explained by the first two components (Figure 1B). **Something about the general amount of variation e.g. heterozygosity measurement**. These results suggest that there is a large amount of unstructured variation across New Zealand populations of ASW.

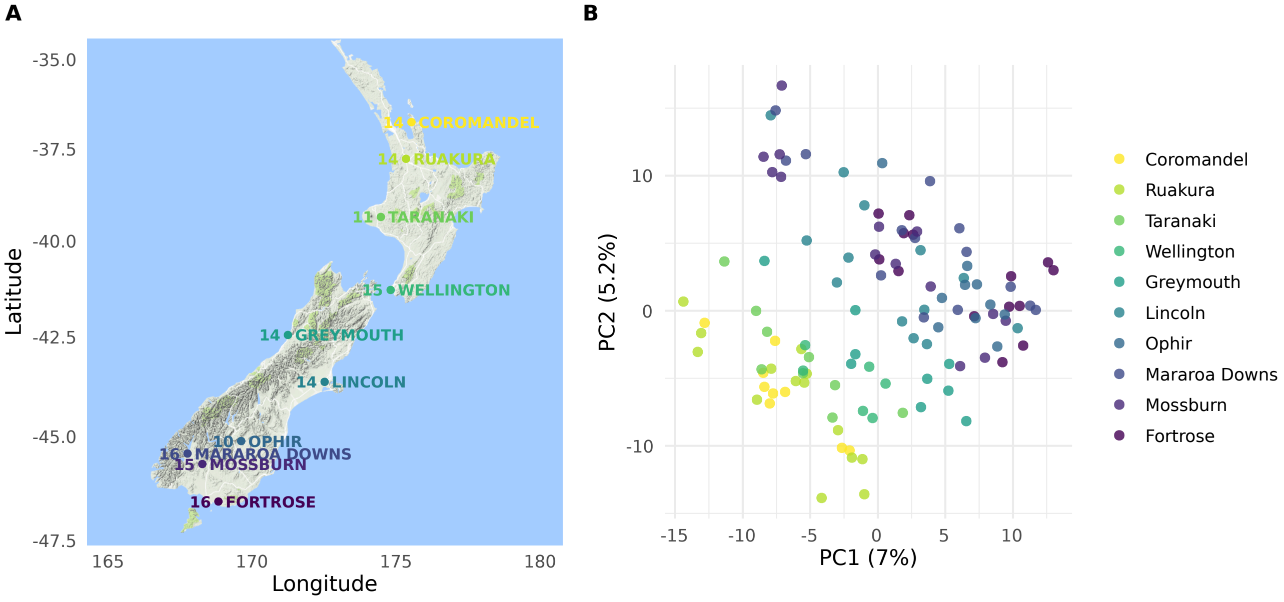

Figure 1. A Weevil sampling locations. We collected Argentine stem weevils from 4 locations in the North Island and 6 locations in the South Island of New Zealand. The number of weevils genotyped from each location is show on the map. The map was plotted with the ggmap package for ggplot2 [17]. **B** Pricipal components analysis (PCA) of 112 individuals genotyped at 22,397 loci. The first two principal components (PC1 and PC2) are shown. The populations overlap on PC1 and PC2, but weevils sampled from higher latitudes tend to have lower scores on PC1 and PC2. PC1 and PC2 together explain 12.2% of variance in the dataset, indicating a high level of unstructured genetic variation in weevil populations.

Genetic variation is not associated with parasitism by a biocontrol agent

To try to detect variation associated with parasitism by *Microctonus hyperodae* (*i.e.* selection exerted by the biocontrol agent), we genotyped weevils that had also been tested for the presence of a parasitoid larva. These weevils were collected from **Lincoln, New Zealand?** and **Ruakura, New Zealand?**, because of the decline in parasitism rate recorded at these locations [18]. After filtering and assembly, we genotyped **X** parasitised weevils and **Y** weevils without a detected parasitoid at **Z** loci. **We did not detect SNPs that were associated with the presence of a parasitoid larva, although we were able to detect SNPs that were associated with the location the weevil was collected. (Figure to show this).** This suggests that the developing resistance of the weevil to biocontrol [18] is not related to within-population genetic variation that allows some weevils to avoid parasitism or its effects.

The draft Argentine stem weevil genome

To find genomic loci associated with between-population variation, we constructed a draft assembly of the ASW genome. We initially attempted assembly from a single individual using PCR-free, short read sequencing. This resulted in a fragmented assembly with low BUSCO scores (Table 1). *k*-mer analysis on the raw short reads suggested 2.1% heterozygosity and a genomic repeat content of at least 28% (**Supporting Information**). We then attempted to produce a long-read genome assembly using whole-genome amplification (WGA) of high molecular weight (HMW) DNA from a single individual, followed by sequencing on the Oxford Nanopore Technologies (ONT) MinION sequencer. We produced 29.8 GB of quality-filtered reads with an N_{50} length of 9.0 KB. The low read N_{50} length was caused by debranching of the amplified DNA by T7 Endonuclease I, which is necessary following multiple displacement amplification (see methods). Assembling the single individual, long read genome resulted in improved contiguity and BUSCO scores (Table 1). Consistent with the raw short read data, we detected an **extreme level (how much?)** of repeats in the single individual, long read genome (Table 1). To improve assembly across long repeats, we produced a second ONT dataset with longer reads from HMW DNA from two pools of 20 individuals each. Sequencing these samples on the MinION sequencer produced a total of 12.0 GB of quality-filtered reads with an N_{50} length of 19.5 KB. Assembling the long reads from the pooled sample alone resulted in a more contiguous genome, but with lower BUSCO scores (Table 1). We constructed a combined, long-read genome using the pooled, long-read dataset for contig construction, and the single-individual, long-read dataset for assembly polishing. This improved the BUSCO scores, but produced a large number of redundant contigs (Table 1), presumably because of the high rate of heterozygosity in the pooled, long-read dataset. Finally, we used the PCR-free, short read sequencing data from a single individual with the Purge Haplotigs pipeline to remove redundant contigs from the combined long read assembly [11]. This resulted in a final draft assembly of 1.1 GB with an N_{50} length of 122.3 kb and a BUSCO completeness of 83.9%.

Short read assembly was not possible with this genome because of the extreme repeat content. The final draft assembly had a repeat content of **67.8%** (Table 1), with a maximum repeat size of 17.7 kb and a repeat N_{50} length of 485 bp. The non-repetitive regions (*i.e.* the gaps between repeats) had an N_{50} length of 1066 bp. Third generation (long read) sequencing enabled us to assemble a draft genome, but we expect gaps in the assembly to exist at larger repeat regions that were not covered by long reads.

Table 1. Assembly statistics for the final draft genome and intermediate assemblies. n.d.: not determined.

	Short read	Single individual, long read	Pooled, long read	Combined, long read	Final draft
Assembly length (Gb)	1.3	1.2	1.2	1.7	1.1
N_{50}	53046	4523	2958	5281	2681
N_{50} length (kb)	7.1	74.4	112.6	86.4	122.3
Complete single-copy BUSCOs (%)	32.7	72.2	71.0	69.2	78.8
Complete multiple-copy BUSCOs (%)	17.2	7.5	5.9	17.4	5.1
Repeat content (%)	n.d.	x	x	x	~67.8

Genetic variation between NZ weevils associates with a geographical cline

- `dapc` of weevil populations showing N-S cline

- SNPs associated with difference between groups
- signs of selection in the genome

New Zealand population of Argentine stem weevils is large and diverse, with multiple introductions

- lack of genetic structure
- historical Ne, if we can

Discussion

Authors' contributions

Acknowledgements

Data availability

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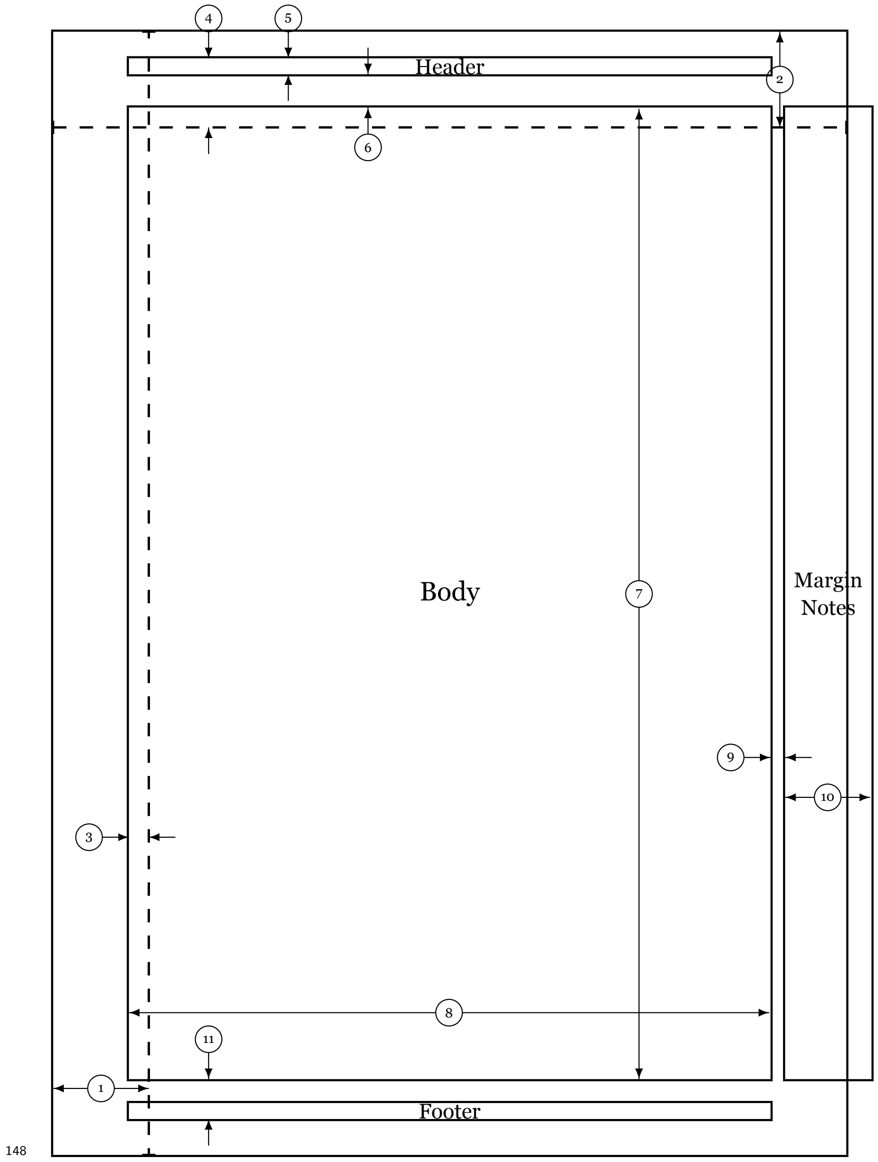

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